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Original article

Nontuberculous mycobacteria in cystic fibrosis patients on the Island of Gran Canaria. A population study

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ABSTRACT

Objective: To determine the prevalence of nontuberculous mycobacteria (NTM) colonization and disease in cystic fibrosis (CF) patients.
Patients and methods: All the CF patients followed-up from 2002 to 2012 with three acid-fast bacilli (AFB) cultures were included. The American Thoracic Society (ATS) criteria for NTM lung disease were applied.
Results: Forty-four of the 53 patients being followed-up were included. The mean time of follow-up was 7.0 years. A total of 18 patients (40.9%) were NTM positive. The NTN mean annual prevalence was 14.1%. The risk of *Mycobacterium abscessus* complex was higher in the group of 10–14 years-old ($p < 0.001$). Ten patients (22.7% of the entire cohort) met the ATS microbiological criteria. The mean annual prevalence of NTM disease was 10.4%. Seven patients (four with *Mycobacterium simiae* and three with *M. abscessus* complex) with multiple positive cultures, positive AFB smears and clinical worsening were treated. Three patients with *M. simiae* and none of those with *M. abscessus* were cured.
Conclusions: Overall NTM prevalence of colonization and disease were high in our CF patients. Patients <15 years old had a higher risk of *M. abscessus* complex colonization. Multiple positive cultures or positive AFB smears were associated with disease.

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disorder. Defects in the cystic fibrosis transmembrane conductance regulator gene product result in abnormally thickened airway secretions leading to infection and inflammation with chronic lung infection being the principal cause of morbi-mortality. The increase in survival rates has been accompanied by the appearance of drug-resistant strains of traditional pathogens and the emergence of new potential pathogens such as nontuberculous mycobacteria (NTM).

Very few colonization and NTM disease studies have been published in Spain in the last 20 years on patients diagnosed with CF [1–4] and the data vary enormously for both prevalence of colonization (4.4–25%) and for disease (0–8%). The objective of this study was to determine the prevalence of NTM colonization and disease among CF patients in our health area.

2. Patients and methods

All patients diagnosed with CF and under follow-up in the C.H.U.I. Materno-Infantil or the H.U.G.C. Doctor Negrín (460,000 inhabitants) on the island of Gran Canaria, were retrospectively studied. Patients with a minimum of three respiratory samples processed for mycobacterial investigation between 2002 and 2012 were included. Written consent was not obtained because study was retrospective, data were anonymous and analysis of results did not influence physicians' clinical management decisions.

Samples for mycobacterial detection were decontaminated with N-acetyl-L-cysteine-sodium hydroxide and placed on liquid BACTEC MGIT 960 medium (Becton-Dickinson; Maryland, USA) and on solid Löwenstein-Jensen medium. Acid-fast bacilli (AFB) smears were

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stained by auramine O. Identification of NTM isolates to the species-level was done using conventional phenotypic tests and/or molecular techniques: nucleic acid hybridization (Accuprobe, GenProbe Inc; California, USA) or PCR solid phase reverse hybridization assay (INNO-LiPa Mycobacteria v1 and v2, Innogenetics; Ghent, Belgium). *In vitro* susceptibility testing of rapidly growing mycobacteria to antibiotics recommended by the CLSI [5] was performed using agar elution, agar diffusion with E-test (bioMérieux; Marcy-l'Étoile, France) and/or broth microdilution (Sensititre RAPMYCOI, TREK Diagnostic Systems; Ohio, USA).

Annual NTM prevalence was defined as the percentage of patients who had at least one positive NTM culture during each calendar-year and, annual NTM incidence, as the percentage of patients with a NTM positive culture for the first time during each calendar-year. Criteria for NTM lung disease were those recommended by the American Thoracic Society (ATS) in 2007 [6].

PASW Statistics 18.0 (SPSS Inc.; Chicago, USA) was used for the statistical analysis. The bivariate relationships between qualitative variables were assessed with the Chi square test or the Fisher exact test. P values < 0.05 were considered statistically significant. The odds ratios (OR) and incidence rate ratios were estimated with a confidence interval of 95% (CI95%). The Mann–Whitney U test was used for comparing means. The survival curves were calculated using the Kaplan–Meier estimator.

3. Results

Fifty three patients diagnosed with CF were followed-up during the study period. Nine patients did not meet the inclusion criterion and were excluded: four children under three years-old with no suitable respiratory samples, and five patients with less than three samples processed for mycobacteria. Thus, 44 patients (83%) who had, at least, three respiratory samples were studied, 95.5% of them with more than five specimens received for mycobacterial culture.

There were 27 children (17 male, 10 female) and 17 adults (11 male, 6 female). The median ages at CF diagnosis were 5 (range 0–13) and 19 (2–57) years for children and adults respectively and the median ages at the time of inclusion in the study (when the first sample for mycobacterial culture was received) were 8 (range 3–14) and 24 (range 16–62) years for children and adults respectively. Fifteen patients, 10 children and 5 adults, were homozygous for the ΔF508 mutation.

The average follow-up time was 7.5 ± 3.8 years. Four patients died and nine patients were lost to follow-up.

A total of 1667 samples (median 30, range 3–121 per patient) were cultured for mycobacteria. Fifty percent of patients had 15–50 processed samples. The proportion of contaminated cultures were 7.6% and 3.8% in solid and liquid medium respectively, but the number of valid cultures for mycobacteria never was less than three per patient. Positive NTM cultures were obtained from 213 samples (12.8%), and positive AFB smears from 81 samples (4.9%). *Mycobacterium tuberculosis* complex was not isolated. The main colonizing microorganisms and their mean annual prevalence were *Staphylococcus aureus* (64.3%), *Pseudomonas aeruginosa* (48.2%), *Aspergillus* spp. (17.0%) and *Haemophilus influenzae* (16.1%).

NTM were isolated in 18 patients. Characteristics of these patients are summarized in Table 1.

The overall NTM prevalence was 40.9% and the mean annual prevalence 14.1 ± 8.8% (range 0–33.3%). Annual NTM prevalence and incidence over time are shown in Fig. 1. The mean annual prevalence by age groups was 18.5% in under-15s and 10.4% in older patients.

There was a significant association of NTM isolation with a lower frequency of *S. aureus* and a higher frequency of *Aspergillus* spp. (Table 2). Six patients (33.3%) had concomitantly *Aspergillus* spp. colonization, in four cases by *Aspergillus* section *Fumigati* (three *Aspergillus fumigatus* and one *Aspergillus fumigatiaffinis*), one of them with allergic bronchopulmonary aspergillosis (ABPA). NTM colonization preceded *Aspergillus* isolation in four subjects. No association was found between the presence of *P. aeruginosa* and NTM isolation.

The most frequent NTM species were: *Mycobacterium abscessus* complex (n = 7, 37%), *Mycobacterium simiae* (n = 7, 37%). Two different species were isolated in one patient: *M. simiae* and *Mycobacterium fortuitum*. The mean annual prevalence of NTM species by age groups is shown in Fig. 2. An increased risk for the first colonization episode by *M. abscessus* complex was observed in patients aged less than 15 years old when compared with older patients ($P = 0.039$; incidence rate ratio: 7.34; CI95%: 1.084–170). Patients with *M. abscessus* complex had a mean age at CF diagnosis

Table 1
Summary of characteristics of patients with NTM colonization.

Patient	Sex	Age at CF diagnosis (years)	Age at NTM colonization (years)	NTM species	2007 ATS microbiological criteria	Positive AFB smear	Positive NTM culture (n)	Clinical significance	Antimycobacterial treatment	Time to start treatment[a] (months)	Response after treatment
1[b]	F	6	12	*M. simiae*	Yes	Yes	>10	Disease	Yes	56	Cured
2	M	57	59	*M. simiae*	Yes	Yes	>10	Disease	Yes	35	Cured
3	F	2	7	*M. simiae*	Yes	Yes	>5	Disease	Yes	9	*Exitus*
4	M	0	10	*M. simiae*	Yes	Yes	>10	Disease	Yes	61	Cured
5	F	39	53	*M. simiae*	No	No	1	Colonization	No	–	–
6	M	2	9	*M. simiae*	No	No	1	Colonization	No	–	–
7	M	5	13	*M. simiae*	No	No	1	Colonization	No	–	–
8	F	1	9	MABC	Yes	Yes	>10	Disease	Yes	11	Failure
9	F	7	9	MABC	Yes	Yes	>5	Disease	Yes	7	Improvement
10	M	0	12	MABC	Yes	Yes	>5	Disease	Yes	41	Failure
11	F	5	5	MABC	Yes	No	2	Colonization	No	–	–
12	M	0	11	MABC	Yes	No	2	Colonization	No	–	–
13	F	18	25	MABC	Yes	No	2	Colonization	No	–	–
14	M	2	12	MABC	No	No	1	Colonization	Yes	–	–
15	M	20	25	*M. fortuitum*	No	No	1	Colonization	No	–	–
16	M	25	26	*M. fortuitum*	No	No	1	Colonization	No	–	–
17	M	0	8	*M. peregrinum*	No	No	1	Colonization	No	–	–
18	M	17	31	MAC	No	No	1	Colonization	No	–	–

CF: cystic fibrosis; NTM: nontuberculous mycobacteria; ATS: American Thoracic Society; AFB: acid-fast bacilli; F: female; M: male; MABC: *Mycobacterium abscessus* complex; MAC: *Mycobacterium avium* complex.
[a] Elapsed time from the first positive culture for NTM.
[b] Patient with two different species of NTM.