Nontuberculous Mycobacterial Infection in Patients With Cystic Fibrosis: A Multicenter Prevalence Study

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OBJECTIVE: To determine the prevalence of nontuberculous mycobacterial infection in patients with cystic fibrosis.

PATIENTS AND METHODS: We performed a prospective study in which patients with cystic fibrosis were followed for 2 years; the patients were recruited from specialized units and were all over 6 years old. Sputum samples collected every 6 months were stained with auramine-rhodamine and cultures were prepared with a liquid and a solid medium. When stains or cultures were positive for nontuberculous mycobacteria, 1 or 2 additional sputum samples were obtained from the patients, who were monitored closely to assess the need for specific treatment. We assessed the following clinical variables: age, sex, presence of pancreatic insufficiency, use of aerosol antibiotic therapy, and long-term azithromycin and inhaled or oral corticosteroid therapies.

RESULTS: A total of 220 patients (119 women) with a mean age of 22.62 years (range, 6-74 years) were enrolled; of these 23.6% were receiving azithromycin. We prepared 1303 sputum samples for mycobacterial growth (range per patient, 4-68 samples); 65 samples from a total of 17 patients (7.72%) were positive: 17 by auramine-rhodamine staining and 48 by culture. Eighty-eight culture samples were contaminated and *Mycobacterium tuberculosis* was not isolated in any of the cases. The mycobacteria isolated were *avium complex* (n=10), *abscessus* (n=6), and *fortuitum* (n=1). Two or more positive cultures were obtained in 9 patients, 5 of whom experienced clinical deterioration and were prescribed specific treatment. No significant differences in clinical variables were found between patients with nontuberculous mycobacteria and those without.

CONCLUSIONS: The prevalence of nontuberculous mycobacterial infection in patients with cystic fibrosis was not very high (7.72%), perhaps because azithromycin interfered with the growth of these bacteria. Patients with repeat isolations of mycobacteria should be monitored closely.

Key words: *Cystic fibrosis. Nontuberculous mycobacteria.* Mycobacterium avium. Mycobacterium abscessus.

Estudio multicéntrico de prevalencia de micobacterias ambientales en pacientes con fibrosis quística

OBJETIVO: Evaluar la prevalencia de micobacterias ambientales (MA) en pacientes con fibrosis quística (FQ).

PACIENTES Y MÉTODOS: Se ha realizado un estudio prospectivo de 2 años de duración en pacientes con FQ mayores de 6 años, procedentes de 6 unidades monográficas. En las muestras respiratorias, recogidas cada 6 meses, se efectuó una tinción de auramina-rodamina, además de cultivos en medios sólido y líquido. Si se detectaba una tinción o cultivo positivo para MA, se recogían 1 o 2 esputos más y se hacía un seguimiento estricto para valorar la necesidad de tratamiento específico. Se consideraron las siguientes variables clínicas: edad, sexo, insuficiencia pancreática, diabetes, uso de aerosolterapia antibiótica y de azitromicina de forma continua, tratamientos con corticoides inhalados o por vía oral de forma prolongada.

RESULTADOS: Participaron en el estudio 220 pacientes (119 mujeres), con una edad media de 22,62 años (rango: 6-74). El 24% recibía azitromicina. Se cultivaron para la detección de micobacterias 1.303 muestras de esputo (rango por paciente: 4-68), de las que la tinción de auramina fue positiva en 17 casos y el cultivo en 48, correspondientes a 17 pacientes (7,72%). En 88 muestras el cultivo estaba contaminado. En ningún caso se aisló *Mycobacterium tuberculosis*. Las MA aisladas fueron: *M. avium complex* (n = 10), *M. abscessus* (n = 6) y *M. fortuitum* (n = 1). Cinco de los 9 pacientes que presentaron más de un aislamiento tuvieron deterioro clínico y se les indicó tratamiento específico. No hubo diferencias entre las variables clínicas de los pacientes con y sin aislamientos de MA.

CONCLUSIONES: La prevalencia de MA en pacientes con FQ no fue muy alta (7,72%), quizá debido a la interferencia de la azitromicina sobre el crecimiento de MA. Hay que vigilar estrechamente a los pacientes con aislamientos repetidos.

Palabras clave: *Fibrosis quística. Micobacterias ambientales.* Mycobacterium avium. Mycobacterium abscessus.

Introduction

Cystic fibrosis is an autosomal recessive disease whose incidence in early neonatal detection studies varies according to country and to race.[1] Over the past 50 years, patients with cystic fibrosis have come to live increasingly longer lives thanks to advances in chronic lung infection treatments and improvements in specialized care. These

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patients have a respiratory system defect in the cystic fibrosis transmembrane conductance regulator protein, which leads to the accumulation of thick secretions in the airways. This allows bacteria such as *Staphylococcus aureus*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* to cross the damaged epithelium and to trigger a neutrophil-mediated inflammatory response that further increases tissue damage.[2]

Multiresistant bacteria begin to appear in patients (mostly adults) who have received repeated courses of antibiotics to treat respiratory infections associated with the disease. *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*, and nontuberculous mycobacteria (NTM) are particularly prevalent, although their pathogenic significance is still unclear.[3,4]

Water and earth act as reservoirs for NTM, which are widely distributed throughout nature. Various studies have indicated that aerosolization is the main route of acquisition, that person-to-person transmission is rare,[5] and that the prevalence of NTM—which in recent years have tended to be classified as emerging pathogens—is on the rise.[6,7] There have also been recent reports of a rising number of NTM isolations in patients with cystic fibrosis, perhaps because of the confluence of several factors such as longer survival, improved diagnostic methods, and increased awareness of NTM-related disease among clinicians.[3,8] Another point to consider is that an increasingly large number of patients with cystic fibrosis are now being prescribed azithromycin, a macrolide whose immunomodulatory properties have been seen to improve lung function and decrease exacerbations.[3] Continuous, long-term treatment with macrolides such as azithromycin, however, might interfere with the growth of NTM or increase resistance to these bacteria.

The aim of the present study was to assess prevalence of NTM isolations in sputum samples from patients with cystic fibrosis recruited over a period of 2 years and to compare the characteristics of these patients (in terms of symptoms, lung function, chest radiographs, clinical course, and treatment received) to those of patients without such mycobacterial findings.

Patients and Methods

Patients

We performed a prospective study of patients aged over 6 years with cystic fibrosis seen in 6 university hospital units in the Community of Madrid, Spain, between January 2004 and December 2005. The participating hospitals were Hospital de la Princesa (adults), Hospital del Niño Jesús (children), Hospital Ramón y Cajal (adults), Hospital La Paz (children and adults), Hospital 12 Octubre (adults), and Hospital Maternal Gregorio Marañón (children). All the patients seen in adult units were over the age of 18 years and those seen in children's units were under that age.

The following data were collected for each patient: age, sex, lung function, presence of pancreatic insufficiency, diabetes, use of antibiotic aerosol therapy, long-term oral azithromycin as part of immunomodulatory therapy, or long-term treatment with inhaled or oral corticosteroids, and bacterial colonization of the airway. Patients were considered to have pancreatic insufficiency when they needed pancreatic enzymes to digest food and when fecal fat concentrations were abnormal at 72 hours or as measured by the van de Kamer test. Bronchial colonization was considered to be present when the same microorganism was isolated in more than 3 consecutive respiratory samples taken at intervals of 1 month. Absolute values and percentages of predicted values for the following lung function parameters were recorded at the beginning and end of the study: forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and FEV_1/FVC. A Mantoux test using purified protein derivative (Evans RT-23; Celltech Group, Berkshire, UK) was also performed at the end of the study. Skin reactions were considered positive when indurations had a diameter of over 5 mm after 48 to 72 hours.

Microbiology

All the sputum samples collected in the study period were stained with auramine-rhodamine and cultures were prepared with Coletsos (BioMériux, Lyon, France) and MGIT 960 liquid (Becton-Dickinson, Maryland, USA) with modified Middlebrook 7H9 broth (BioMériux, Lyon, France); routine cultures for bacteria and fungi were also performed. Bacterial contamination was analyzed and samples decontaminated in 3 of the participating hospitals using the method described by Kubica et al,[9] whereby samples are digested and decontaminated with 2% sodium hydroxide and N-acetylcysteine as the mucolytic agent. An additional step with 5% oxalic acid was used in the other 3 hospitals.

Samples were collected every 6 months during outpatient visits.

Protocol

One or 2 additional sputum samples were collected from patients with positive NTM stains or cultures; these patients were then closely monitored to assess the possibility of clinical, functional, or radiologic deterioration and evaluate the need for specific treatment against the bacteria detected (Figure). Computed tomography (CT) of the chest was performed in patients whose chest radiograph did not clearly establish a diagnosis or whenever the attending clinician requested CT.

All other patients were evaluated every 3 months or in the event of exacerbation. Tests included evaluation of symptoms, sputum analysis, and spirometry.

Clinical deterioration was defined as the co-occurrence of 3 or more of the following signs and symptoms: increased cough, changes in the amount and appearance of sputum, onset of dyspnea, hemoptysis, asthenia, nocturnal sweating, weight and appetite loss, fever, changes in usual lung sounds,[10] and a lack of clinical response to antibiotics used to treat typical bacterial infections. Functional deterioration, in turn, was defined as a decrease in FEV_1 of greater than 10% with respect to the previous value, and radiologic deterioration was assessed by analyzing signs of the development or growth of alveolar consolidation, cavitation, or lung nodules.

All the patients used respiratory physiotherapy techniques as part of their standard treatment; adherence requirements and intensity were greater in patients with repeat NTM isolations.

Statistical Analysis

Quantitative variables were expressed as means (SD) and qualitative variables as percentages in different categories. For between-group comparisons, the former were examined using analysis of variance and the latter using the χ^2 test. All calculations were made using version 11.5 of the SPSS statistical software package (SPSS Inc, Chicago, Illinois, USA) and *P* values of less than .05 were considered significant.

Results

The study enrolled 220 patients (119 women and 101 men) with cystic fibrosis and a mean (SD) age of 22.62 (11.17) years (range, 6-74 years); 140 were from adult units and 80 from children's units. Eighty percent of the patients had pancreatic insufficiency and 14.5% had diabetes. The most frequently isolated bacteria were *S aureus* (in 62.7% of patients), *P aeruginosa* (48.6%), *H influenzae* (18.6%), *A xylosoxidans* (5.4%), *Burkholderia cepacia* (2.7%), and *S maltophilia* (10%). Fifteen patients were not colonized.

Of the 1303 sputum samples prepared for mycobacterial growth (range per patient, 4-68), 17 were positive by auramine-rhodamine staining and 48 by culture. All the positive samples were from 17 patients. Eighty-eight culture samples were contaminated and *Mycobacterium tuberculosis* was not isolated in any. The species isolated in the 17 patients were *Mycobacterium avium* complex (n=10), *Mycobacterium abscessus* (n=6), and *Mycobacterium fortuitum* (n=1). Twenty-six of the 170 patients for whom a Mantoux result was available tested positive; of these, 4 had NTM isolations. There were no Mantoux results for 3 of the patients with NTM isolations. Of the 9 patients with more than 1 isolation, 5 experienced clinical, functional, and radiologic deterioration; specific treatment was prescribed for all 9 patients. CT scans of the chest were performed in 8 patients. NTM were isolated in 2 of the 52 patients on long-term azithromycin (23.6% of the 220 patients studied).

Table 1 compares the age and initial lung function parameters of patients with and without NTM isolations. No significant differences were observed for any of the

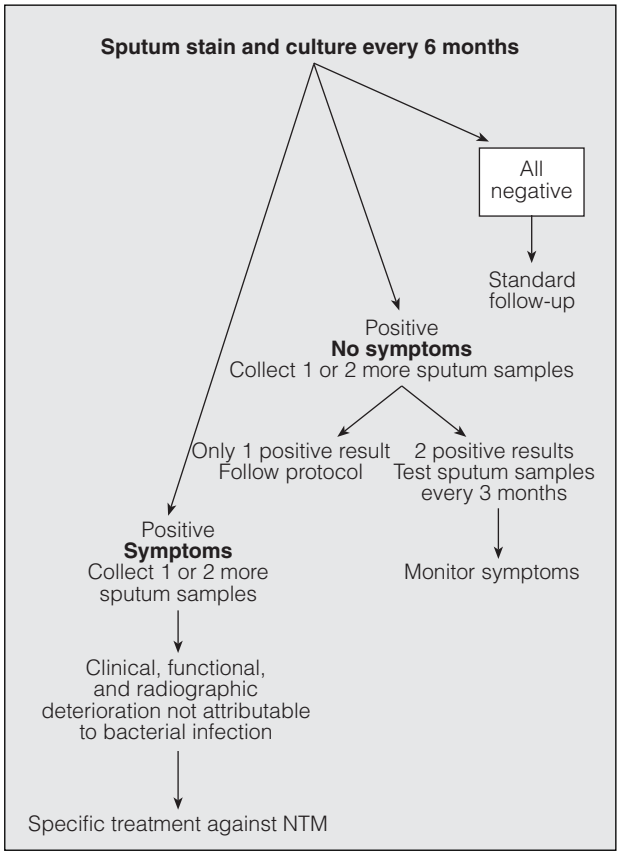

Figure. Diagnostic algorithm to apply after isolating nontuberculous mycobacteria (NTM) in sputum.

TABLE 1
Baseline Clinical Variables in Patients With and Without Isolation of Nontuberculous Mycobacteria (NTM)[a]

Clinical Variables	Patients With NTM (n=17)	Patients Without NTM (n=203)	*P*
Mean age, y	26.59 (15.83)	22.28 (10.67)	.128
Women/men	9/8	110/93	.55
Pancreatic insufficiency	12 (70.58)	164 (80.78)	.198
Staphylococcus aureus	12 (70.58)	126 (62.06)	.347
Pseudomonas aeruginosa	7 (41.17)	100 (49.26)	.343
H influenzae	5 (29.41)	36 (17.17)	.192
Strenotrophomonas maltophilia	2 (11.76)	20 (9.85)	.529
Burkholderia cepacia	0	6 (2.95)	.612
Achromobacter xylosoxidans	2 (11,76)	10 (4,92)	.236
Azithromycin therapy	2 (11.76)	50 (24.63)	.17
Antibiotic aerosol therapy	10 (58.8)	117 (57.63)	.586
Inhaled corticosteroids	6 (35,29)	107 (52,70)	.160
Oral corticosteroids	4 (23.52)	23 (11.33)	.144
Diabetes	1 (5,8)	31 (15,27)	.245
BMI, kg/m^2	20.0 (3.36)	20.7 (3.36)	.445
Initial FVC, L	2641.87 (1022.93)	2967.31 (1225.50)	.303
Initial FVC, % of predicted	75.14 (19.53)	81.0 (21.59)	.281
Initial FEV_1, L/min	1910.0 (869.28)	2167.10 (964.96)	.313
Initial FEV_1, % of predicted	63.17 (21.16)	70.46 (25.5)	.264
Final FVC, L	2515.0 (1042.88)	3068.1 (1185.87)	.076
Final FVC, % of predicted	75.94 (23.30)	80.43 (24.17)	.463
Final FEV_1, L/min	1736.87 (899.46)	2252.10 (1142.48)	.081
Final FEV_1, % of predicted	62.02 (25.68)	68.86 (29.03)	.362

Abbreviations: BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.
[a]Data are presented as mean (SD) or number of patients (%).

TABLE 2
Characteristics of Patients Who Received Treatment for Nontuberculous Mycobacteria (NTM) Infection

Patient	Sex	Age, y	Diabetes	Inhaled Corticosteroids	Aerosol Antibiotic Therapy	No. of Samples	Positive Stains	Positive Cultures	Type of NTM	Treatment
1	Man	18	No	Yes	Yes	6	0	1	*Mycobacterium avium*	Ciprofloxacin, azithromycin
2	Woman	17	No	No	Yes	10	3	6	*Mycobacterium abscessus*	Clarithromycin, amikacin, cefoxitin
3	Woman	11	No	Yes	No	6	0	1	*M abscessus*	Azithromycin
4	Woman	34	No	Yes	Yes	8	4	4	*M avium*	Isoniazid, ethambutol, clarithromycin
5	Man	16	No	No	No	9	0	4	*M avium*	Clarithromycin, rifampicin, ethambutol, streptomycin
6	Woman	66	No	No	No	68	6	6	*M avium*	Clarithromycin, rifampicin, ethambutol
7	Woman	44	No	No	Yes	58	1	6	*M avium*	Clarithromycin, rifampicin, ethambutol

parameters analyzed. The characteristics of the patients who were prescribed treatment by the attending clinician are shown in Table 2.

Discussion

We found that 8% of the patients with cystic fibrosis seen in the participating hospital units in the Autonomous Community of Madrid during the study period had NTM in sputum samples. Three percent of the whole group and approximately two thirds of patients with more than 1 NTM isolation presented related signs and symptoms, which improved with specific treatment. The most frequent mycobacterium isolated was *M avium*. Seven percent of the culture samples were contaminated. Almost a quarter of the patients (23.6%) were on long-term azithromycin (taken every 3 days or on a daily or alternate-day basis).

Since cystic fibrosis was first described by Wood in 1976,[11] an increasing number of studies have documented the presence of NTM in respiratory secretions of these patients, the majority of whom have been adults; the clinical significance of this finding, however, is disputed, particularly in cases in which only a single isolation is made.[12-24] On analyzing 2955 sputum samples, a recent study of 986 patients with cystic fibrosis from 21 hospitals in the USA found an NTM isolation prevalence of 13%,[25] which was higher than the prevalence we observed. Cystic fibrosis patients with NTM isolations have been seen to be older, to have better lung function, and to be more likely to be colonized by *S aureus* than *P aeruginosa* than patients without such isolations.[25] While we did not detect these differences in our study, we did find higher trends for some variables in patients with NTM.

NTM infection is difficult to diagnose in cystic fibrosis. Annual screening is recommended in adult and adolescent patients and in those who experience clinical deterioration, including children. The diagnostic criteria for NTM infection, which were recently revised by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA), are based on clinical, radiologic, and microbiologic findings. Most of the changes made to the previous guidelines, published in 1997, affected criteria for microbiology; it is now possible, for example, to diagnose NTM infection on the basis of just 2 positive sputum cultures or 1 positive bronchoalveolar lavage fluid culture. The above criteria, however, are still difficult to apply in cystic fibrosis[26] because NTM infection can produce signs and symptoms that are similar to those caused by bronchiectasis and persistent airway colonization in cystic fibrosis patients without infection. As Olivier et al[20] have stated, the presence of persistent fever or nocturnal sweating might warn of possible infection. Overlapping signs can also be a problem when assessing radiographic images in patients with NTM infections. Typical findings such as nodules, alveolar consolidations, and small cavities are also usual in cystic fibrosis and are of little use in establishing a diagnosis. The fact that the nodules associated with bronchiectasis are usually found in the upper lobes in cystic fibrosis, whereas those caused by NTM are in the lower and middle fields, may suggest a diagnosis. As argued by the authors of the US multicenter study, serial CT imaging and analysis of symptoms may help to establish a diagnosis and determine the need for treatment for mycobacterial infection.[25] Some authors have noted that a lack of response to conventional antibiotics in patients with several NTM isolations strongly suggests the need to start specific treatment.[20,25]

The NTM most frequently reported to be isolated from patients with cystic fibrosis are *M avium complex*, *M abscessus*, *Mycobacterium kansasii*, and *M fortuitum*.[25] We also found this to be the case in our series. An autopsy study published in 1996 found no clinical or histologic evidence of NTM infection in 12 of 18 cystic fibrosis patients with a single NTM isolation; in contrast, half

of the patients with repeated positive cultures for NTM had lung lesions and a third had histologic features of granulomatous infection.[27] The authors concluded that patients with several cultures positive for the same NTM species should be watched closely and treated in the event of clinical deterioration. The situation was similar in our study and, as in the US study,[25] two thirds of patients with repeated isolations of NTM required treatment.

High levels of bacterial overgrowth, especially by *Pseudomonas* species, can complicate mycobacterial culture in patients with cystic fibrosis. Accordingly, it is advised to decontaminate samples in 2 steps (0.25% N-acetylcysteine and 1% sodium hydroxide followed by 5% oxalic acid) before culturing in selective media as this reduces bacterial contamination by between 3% and 5%.[28] In our case, the percentage of contamination (7%) was close to the recommended figure.[16,20]

The specificity of skin tests for the different mycobacteria tested in our patients was very low, with cross-reactivity occurring between species, including *M tuberculosis*. Four of the patients in our series in whom NTM were isolated had a positive Mantoux test, possibly due to cross-reactions. In a study by Pinto-Powell et al,[29] skin testing with *M avium* antigen displayed good sensitivity, although such testing was unable to distinguish between simple isolation and true infection. The role of skin tests in this setting needs to be studied in greater detail.[29]

In our series, 23.6% of patients were receiving long-term azithromycin therapy. The antimicrobial effect that this macrolide has on NTM may have contributed to the fact that the overall prevalence of NTM infection in our series was lower than in previous reports.[20] Our findings therefore suggest that the prevalence of NTM infection will not increase in patients with cystic fibrosis in coming years, thanks to the increasing use of azithromycin. Most of the studies on the prevalence of NTM infection in cystic fibrosis are based on figures from before 2000, that is, before the immunomodulatory properties of macrolides were demonstrated and these drugs began to be widely prescribed. A group from our center reported a very high prevalence (25%) of NTM isolations in a group of 28 adults with cystic fibrosis in whom 251 sputum samples were analyzed.[30] One particularly worrying problem related to the increasing use of macrolides is the possible appearance of resistant strains in years to come.[26] According to the latest cystic fibrosis registry data from the United States (2005), 53.6% of patients are on long-term azithromycin[3]; this figure is much higher than the percentage in our series. The latest recommendations issued by the ATS/IDSA[26] state that maintenance doses of macrolides as monotherapy should only be prescribed if cultures have become negative for NTM. This drug should not be prescribed to patients with repeated isolations of these mycobacteria.

To conclude, the prevalence of NTM isolations in our series of patients with cystic fibrosis was not very high but this was perhaps due to the antimicrobial action of macrolides. Although the role played by NTM in respiratory decline in patients with cystic fibrosis is unclear, it is important to closely monitor those with repeated isolations.

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