ORIGINAL ARTICLES

Nontuberculous Mycobacteria in Patients With Cystic Fibrosis

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OBJECTIVE: Patients with cystic fibrosis are at great risk of infection by nontuberculous mycobacteria from the environment because of certain predisposing factors such as bronchiectasis, malnutrition, and diabetes. The aim of this study was to analyze the mycobacterial content of sputum smears and cultures from adult patients with cystic fibrosis attended at a specialized unit for adults from March 1997 through December 2001.

PATIENTS AND METHODS: Sputum samples were collected prospectively according to a protocol applied at each visit, and during most exacerbations staining and culture for mycobacteria were ordered in addition to the usual cultures for bacteria and fungi. A tuberculin test was performed at the end of the study.

RESULTS: Twenty-eight patients (16 men) with cystic fibrosis were enrolled. The mean (SD) age was 25.3 (6.7) years. A total of 251 samples were cultured (range in number of samples per patient, 1-31). The mean period of follow up was 40.3 (22.1) months. The sputum smear was positive in 29 cases (4 patients); the culture was positive in 7 patients. More than 3 samples were positive in only 4 patients. *Mycobacterium abscessus* was isolated in 3 cases, *Mycobacterium avium* complex in 2 and *Mycobacterium simiae* in 1 and other an unidentified rapid growth *Mycobacterium* species. The Mantoux test was positive in 5 patients. Two of the 4 patients in whose samples mycobacteria were isolated repeatedly required treatment.

CONCLUSIONS: The prevalence of nontuberculous mycobacterial infection is high in patients with cystic fibrosis. Staining and culture for mycobacteria should be carried out regularly and whenever exacerbation of pulmonary symptoms cannot be attributed to bacteria usually found in such patients. Patients with recurrent isolations of mycobacteria should be monitored closely.

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

Micobacterias ambientales en pacientes adultos con fibrosis quística

OBJETIVO: Los pacientes con fibrosis quística (FQ) presentan un mayor riesgo de infección por micobacterias ambientales en relación con ciertos factores predisponentes como bronquiectasias, desnutrición y diabetes. El objetivo del presente estudio es analizar los resultados de las baciloscopias y cultivos de micobacterias de esputos de pacientes con FQ de una unidad de adultos, entre marzo de 1997 y diciembre de 2001.

PACIENTES Y MÉTODOS: Las muestras de esputo se recogieron de forma prospectiva y protocolizada en cada visita y en la mayoría de las exacerbaciones, en las que, además de los cultivos bacterianos habituales y de hongos, se solicitaron tinción y cultivo para micobacterias. Se realizó la prueba de la tuberculina al final del estudio.

RESULTADOS: Se incluyó a 28 pacientes con FQ, 16 varones, con una edad media (± DE) de 25,3 ± 6,7 años. Se cultivaron un total de 251 muestras (rango por paciente de 1 a 31). El tiempo medio de seguimiento fue de 40,3 ± 22,1 meses. En 29 casos (4 pacientes) la baciloscopia fue positiva y se obtuvieron cultivos positivos en 7 pacientes, sólo en 4 en más de 3 muestras. Se aislaron: *Mycobacterium abscessus* en 3 casos, *M. avium complex* en 2 y *M. simiae* en uno y en otro una especie de crecimiento rápido no identificada. En 5 pacientes el Mantoux fue positivo. Dos de los 4 pacientes con aislamientos reiterados presentaron deterioro clínico y requirieron tratamiento.

CONCLUSIONES: Hay una alta prevalencia de micobacterias ambientales en pacientes con FQ. Habría que realizar tinción y cultivo para micobacterias de forma periódica y en caso de exacerbación pulmonar no atribuible a infección bacteriana habitual. Hay que vigilar estrechamente a los pacientes con aislamientos repetidos.

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

Introduction

Cystic fibrosis is a genetic, autosomal recessive disease whose incidence in early neonatal detection studies varies from country to country and race to race.[1] Over the past 50 years as treatments for chronic lung infection have advanced and as specialized care units

This study was financed by Red Respira (RTIC C03/011)-SEPAR (Spanish Society of Pulmonology and Thoracic Surgery).

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Manuscript received August 24, 2004. Accepted for publication May 17, 2005.

have emerged, patients with cystic fibrosis have come to live increasingly longer lives. A defect in the cystic fibrosis transmembrane conductance regulator (CFTR) protein affecting the respiratory system leads to the accumulation of thick secretions in the airways. This allows bacteria such as *Staphylococcus aureus, Haemophilus influenzae,* or *Pseudomonas aeruginosa* to cross the damaged epithelium. Bacterial invasion in turn stimulates a neutrophil-mediated inflammatory response that further increases tissue damage.[2]

Multiresistant microorganisms begin to appear in cultures after the repeated courses of antibiotic treatment these patients receive, particularly if they live to adulthood. Among the microorganisms whose pathogenic potential remains to be determined, but that are isolated with increasing frequency, are *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia,* and nontuberculous mycobacteria from the environment.[3,4]

Nontuberculous mycobacteria are widely found in nature, mainly in water and soil, which act as reservoirs. Various studies indicate that person-to-person transmission is rare; aerosolization is the main route of acquisition.[5] The incidence of infection by this type of mycobacteria has generally been seen to be on the rise and they tend to be grouped under the heading of emergent pathogens.[6,7] Nontuberculous mycobacteria are increasingly found in cystic fibrosis patients, as factors such as longer survival, improved diagnostic techniques, and clinicians' greater awareness of disease caused by these pathogens come together.[3,8]

The aim of the present study was to analyze the content of nontuberculous mycobacteria in sputum smears and cultures from adult patients with cystic fibrosis, and to compare the characteristics of these patients (symptoms, lung function, chest radiographs, clinical course, and treatment received) to those of patients without such mycobacterial findings.

Patients and Methods

Patients

A prospective study of all patients treated at the specialized adult cystic fibrosis unit of Hospital Universitario de la Princesa was carried out from March 1997 through December 2001. The specialized unit coordinates its work with the pediatric department of Hospital Universitario del Niño Jesús, from which patients are referred once they reach the age of 18 years.

Clinical Variables

The following information was collected for each patient: age, sex, presence of pancreatic insufficiency, diabetes, use of antibiotic aerosol therapy, and bacterial contamination of the airway. A patient was diagnosed with pancreatic insufficiency if there were abnormal stool fat findings in samples collected over a period of 72 hours or in samples tested by the van de Kamer method (mild steatorrhea, 6-10 g of fat/24 h; moderate steatorrhea, 10-20 g/24 h; and severe steatorrhea, >20 g/24 h),[9] and the patient required pancreatic enzymes to digest food. Bronchial colonization was defined when the same microorganism was isolated in more than 3 consecutive respiratory samples taken at intervals of 1 month. At each check-up we also recorded forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), and calculated the ratio FEV_1/FVC. Each parameter was also expressed as the percentage of predicted values. A Mantoux test with purified protein derivative (Evans RT23, Celltech Group, Berkshire, UK) was performed at the end of the study. An induration greater than 5 mm after 48 to 72 hours was considered positive.

Clinical severity was assessed with the Shwachman scoring system at the start of the study and at 1 year, on a date near the patient's birthday. In Shwachman scoring, a maximum of 25 points is given for each of the following categories: physical activity, physical examination, nutrition, chest radiographs. The ideal score is 100. Patient status in this system is classified as excellent (86-100 points), good (71-85), mild (56-70), moderate (41-55), or severe (≤40).[10]

Radiographic Assessment

Chest x-rays were taken at the start of the study, once a year (around the patient's birthday), upon signs of clinical or lung function deterioration with lack of clinical response to antibiotic therapy covering colonizing bacteria, and upon suspicion of noninfectious respiratory complications. The chest films were scored by the system of Brasfield et al.[11] In this system a score is assigned from 0 to 5 (with a higher score denoting greater involvement) to describe 5 radiographic signs: air trapping, linear shadows, nodular-cystic lesions, segmental or lobar consolidation, and overall impression of severity. The sum of the scores is then subtracted from 25. Thus, a lower final score indicates a higher degree of radiographic severity. Chest radiography and Brasfield scoring were carried out annually.

Microbiology

Sputum samples were stained with auramine-rhodamine and cultures were prepared with Coletsos (BioMériux, Lyon, France) and liquid MGIT 960 (Becton-Dickinson, Sparks, Maryland, USA) with modified Middlebrook 7H9 broth (BioMériux), in addition to the usual cultures for bacteria and fungi. Bacterial contamination was analyzed. Decontamination of respiratory samples was accomplished with the method of Kubica et al.[12] The sample was digested and decontaminated with 2% sodium hydroxide and N-acetyl-L-cysteine as the mucolytic agent, and the final sample concentration of sodium hydroxide was 1%, which is less toxic for the mycobacteria.

Samples were taken at each outpatient visit, including examinations during respiratory disease exacerbations.

Protocol

If a sample was positive for nontuberculous mycobacteria by staining or culture, sputum was then collected monthly and follow-up was strict to assess the possibility of clinical, functional, or radiographic deterioration and to evaluate the need for specific treatment against the mycobacteria detected (Figure 1).

If a sample was negative by staining or culture, the patient was usually reassessed in 3 months (symptoms, sputum tests, and spirometry) or in case of exacerbation.

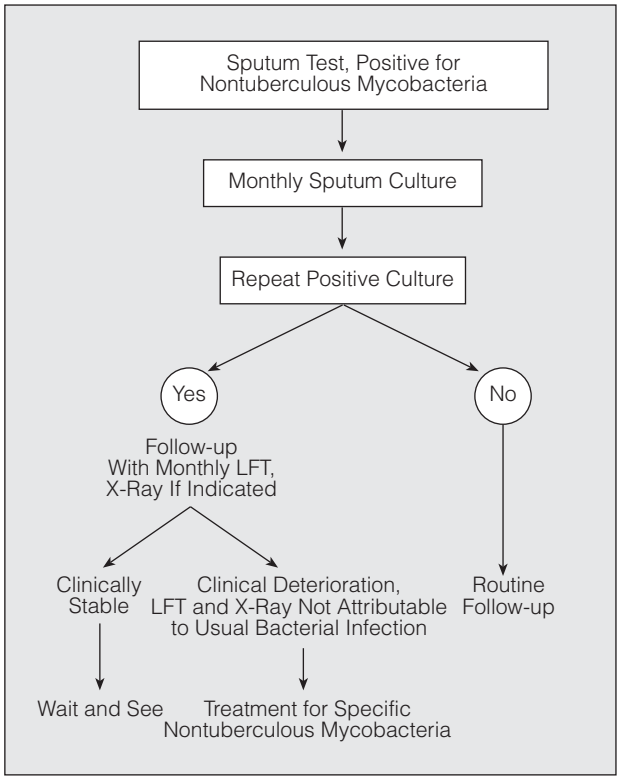

Figure 1. Diagnostic algorithm to apply after isolating nontuberculous mycobacteria in sputum. LFT indicates lung function tests.

Clinical deterioration was defined by the co-occurrence of 3 or more of the following signs and symptoms: increased coughing, change in amount or appearance of sputum, presentation of dyspnea, hemoptysis, asthenia, nighttime sweating, weight or appetite loss, fever, and changes in usual lung sounds.[13] Functional deterioration was defined by a decrease in FEV_1 of more than 10% of the previous value and by radiographic worsening indicated by such signs as the development or progression of alveolar consolidation, cavitation, or pulmonary nodules.

Statistical Analysis

For quantitative variables results are expressed as means (SD). Results for qualitative variables are shown as categorized percentages. The Student *t* test was used to compare means. All calculations were performed with SPSS version 11.5 and correlations were considered statistically significant if the value of *P* was less than .05.

Results

A total of 28 patients diagnosed with cystic fibrosis[14] (12 women, 16 men) were enrolled for study. The mean age was 25.3 (6.7) years. A total of 81% of the patients had pancreatic insufficiency and 7.1% had diabetes. The patients were treated at our specialist unit for a period ranging from 1 to 58 months, the mean being 40.3 (22.1) months. The most frequently isolated colonizing microorganism was *S aureus* (67.9%), followed in frequency by *P aeruginosa* (53.6%), *H influenzae* (4.3%), and other gram-negative bacilli (10.7%). One patient had a history of nontuberculous mycobacterial infection (*Mycobacterium abscessus*) and had received treatment with isoniazid and clarithromycin for 14 months and rifampicin for 9 months.

A total of 251 sputum cultures (range per patient, 1-31) were established for mycobacterial growth; 29 of them (4 patients) were positive by auramine staining. The culture was positive for 7 patients, although only 4 patients had more than 3 positive cultures. The cultures of 47 samples (18.8%) were contaminated. *Mycobacterium tuberculosis* was not isolated in any of the cases. The strains isolated were as follows: *M abscessus* in 3 cases, *Mycobacterium avium* complex in 2, *Mycobacterium simiae* in 1, and an unidentified rapid-growing mycobacterial species in 1. The Mantoux skin test was positive in 5 patients, and nontuberculous mycobacteria were isolated for 4 patients (Table 1). Clinical, functional, and radiographic deterioration was observed in only 2 of the 4 patients who had recurrent isolations; both were given germ-specific treatment.

TABLE 1
Characteristics of Patients With Isolations of Nontuberculous Mycobacteria*

Cases	Sex	Age, Years	Diabetes	Inhaled Corticosteroids	Antibiotic Aerosols	No. of Samples	Positive Stains	Positive Cultures	Type of Nontuberculous Mycobacteria	Treatment
1	Man	25	No	Yes	Yes	16	6	9	*M abscessus*	No
2	Woman	19	No	No	No	6	3	6	*M avium*	No
3	Man	26	No	No	Yes	12	0	1	*M abscessus*	No
4	Man	24	No	No	Yes	9	0	1	Rapidly growing mycobacteria	No
5	Man	22	No	Yes	No	31	12	10	*M abscessus*	Imipenem, amikacin, and clarithromycin
6	Woman	24	No	No	No	8	0	2	*M simiae*	No
7	Woman	21	No	Yes	Yes	19	8	13	*M avium*	Rifampicin, azithromycin, and ethambutol

*M refers to the genus *Mycobacterium*.

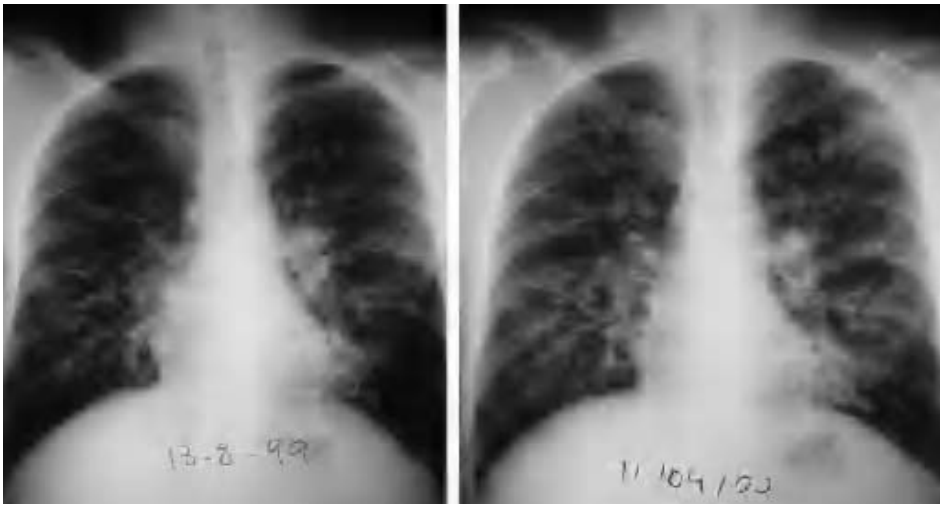

Figure 2. Posteroanterior radiographs of the chest for a patient who required specific treatment against nontuberculous mycobacteria. Nodular patterns can be seen to have increased in the middle and lower fields of both lungs in the second x-ray (right).

Table 2 shows the mean age, clinical and radiographic scores, and lung function findings at the start of the study for patients with and without isolations of nontuberculous mycobacteria. There were no significant differences between the 2 sets for any of the parameters analyzed.

Patient number 5, chronically colonized by *S aureus*, had stable lung function with a FEV_1 of 2.30 L (57% of predicted) and an oxygen saturation (SaO_2) of 95%. Six months after the first isolation of *M abscessus*, increased coughing and sputum production, low-grade fever, dyspnea, serious weakness, and increased pulmonary crackles were noted; SaO_2 was 91%, FEV_1 had fallen to 1.64 L (40% of predicted), and the chest radiograph revealed a larger area of nodular images in the middle and lower lung fields (Figure 2). A cycle of oral amoxicillin–clavulanic acid was prescribed but no improvement was noted. This patient had already been treated twice before for *M abscessus* infection; therefore, given the similarity of the present and previous clinical pictures, intravenous treatment with amikacin was given for 3 weeks, along with oral clarithromycin for 36 months. One month after starting that treatment, clinical signs and lung function were improving gradually. Sputum smear stains and cultures became negative at 3 and 5 months, respectively.

Patient number 7, colonized chronically by *P aeruginosa* and receiving continuous antibiotic aerosol therapy with colimycin, had stable lung function with FEV_1 of 1.76 L (57% of predicted) and SaO_2 of 96%. Six months after the first isolation of *M avium* complex, the clinical picture included high fever, increased productive coughing, changes in sputum characteristics, dyspnea, loss of appetite, decrease in SaO_2 to 92% and of FEV_1 to 1.50 L (44% of predicted). Intravenous treatment with ceftazidime and tobramycin for 14 days led to clinical and functional improvement. Two months later, symptoms returned and specific antibiotic treatment against *Pseudomonas* species was started. The patient did not respond, FEV_1 fell to 1.20 L (38% of predicted), and a chest x-ray showed alveolar consolidation in the upper left lobe. Treatment with azithromycin, rifampicin, and ethambutol for 17 months was prescribed. Improvement was seen after 2 weeks and radiographic and functional improvements were evident at 3 months. Sputum smear stains and cultures became negative at 2 and 6 months, respectively.

Discussion

This study demonstrates a high prevalence of nontuberculous mycobacterial colonization in adults with cystic fibrosis that approaches 25% among the patients treated in our practice. Sputum tests were negative for 42.8% of the patients in whom nontuberculous mycobacteria were found. Half of the patients in whom isolations were recurrent developed clinical signs related to nontuberculous mycobacterial infection; those patients improved when given treatment was directed against a specific germ. The most frequently isolated nontuberculous mycobacterial species was *M abscessus*. None of the patients colonized by nontuberculous mycobacteria were diabetic, 57.1% used aerosol

TABLE 2
Age, Brasfield and Shwachman Scores, and Spirometry at the Start of the Study of Patients With and Without Nontuberculous Mycobacterial Isolations*

Variables	With Isolations (n=7)	Without Isolations (n=21)	*P*
Age, years	23 (4.4)	26 (7.6)	.305
FVC, % pred	85.4 (21.1)	89.85 (24)	.670
FEV_1, % pred	78 (25.1)	76 (26.5)	.863
Brasfield score	17 (3.8)	18.6 (3.7)	.336
Shwachman score	77 (9)	80.1 (13.9)	.608

*Results are expressed as means (SD). FVC indicates forced vital capacity; FEV_1, forced expiratory volume in the first second; and % pred, the percentage of the theoretical reference value for the variable named.

antibiotics, and 42.8% used inhaled corticosteroids regularly. When we compared initial lung function parameters to Brasfield and Shwachman scores for patients with and without isolations of nontuberculous mycobacteria in sputum, there were no significant differences.

The most frequently isolated nontuberculous mycobacteria according to reports in the literature for patients with cystic fibrosis are *M avium* complex*, M abscessus, Mycobacterium kansasii*, and *Mycobacterium fortuitum.*[15] Since the earliest description of cases of cystic fibrosis by Wood et al[16] in 1976, subsequent reports have increasingly indicated the presence of nontuberculous mycobacteria in respiratory secretions, mainly in adult patients, although the clinical significance of the finding is disputed, particularly in cases in which only a single isolation is recorded.[17-30] A recent prevalence study of 986 cystic fibrosis patients at 21 hospitals in the United States of America analyzed 2955 sputum samples, finding the prevalence of nontuberculous mycobacteria to be 13%.[8] When patients with isolations were compared to the remaining patients, the former were seen to be older, have better lung function, and be more likely to be colonized by *S aureus* than by *P aeruginosa.* In our patients, however, we found no differences in age, lung function, or clinical and radiographic severity scores.

A diagnosis of nontuberculous mycobacterial infection is difficult to establish in cystic fibrosis. The diagnostic criteria established by the American Thoracic Society (ATS) in 1997 are based on clinical, radiographic, and microbiologic findings. These criteria are not readily applicable to patients with cystic fibrosis,[31] given that the usual clinical presentation of infection by nontuberculous mycobacteria overlaps with the manifestations of cystic fibrosis with regard to the presence of bronchiectasis and persistent airway colonization. It may be, as Olivier et al[25] have stated, that the presence of persistent fever or nighttime sweating can warn of possible infection. The same problem of overlapping signs arises when considering the usual radiographic images described for lesions caused by mycobacteria (nodules, alveolar consolidation, and small cavitations). Those are all lesions that are seen often in cystic fibrosis and finding them is of little use in establishing a diagnosis of mycobacterial infection. The fact that the nodules associated with bronchiectasis are usually found in the upper lobes in cystic fibrosis, whereas those caused by nontuberculous mycobacteria are in the lower and middle fields, may suggest a diagnosis. Probably, serial CT, as suggested by the authors of the US multicenter study,[8] alongside clinical symptoms, can help establish a diagnosis and the need for treatment for mycobacterial infection. In our study the patients who needed treatment had findings indicative of exacerbated lung disease due to bacteria, and 1 patient also had a low-grade fever. The finding that best suggested the need to start specific treatment against nontuberculous mycobacteria, and that was more evident in 1 of our patients, was a lack of response to antibiotics covering the usual colonizing pathogens.

A study published 8 years ago, based on autopsies of patients with cystic fibrosis who had cultures positive for nontuberculous mycobacteria, provided some indication of what can happen in the lungs of these individuals.[32] In 12 of the 18 patients in whom nontuberculous mycobacteria were isolated once, no clinical or histologic findings attributable to those germs were found, whereas half of the patients for whom there were multiple isolations of nontuberculous mycobacteria had pulmonary symptoms and only a third had histologic signs of granulomatous infection. Therefore, the authors concluded, the patient with several cultures positive for the same nontuberculous mycobacterial species should be watched closely and treated in case of clinical deterioration.

On the other hand, the ATS includes histologic confirmation of granulomas among the diagnostic criteria. Invasive procedures such as transbronchial or pulmonary biopsy would increase the risk of complications (bleeding) in these patients, given the frequent presence of pulmonary hypertension and enlarged bronchial arteries.

An additional problem is the difficulty of culturing mycobacteria because of the intense growth of other pathogens—particularly *Pseudomonas* species—in the airways of patients with cystic fibrosis. In our study the percentage of contamination was 18.8%, lower than that reported in the literature,[21,24] perhaps because of the low prevalence of colonization by *P aeruginosa* in our patients. That is the reason why decontamination of samples in 2 steps is recommended before culturing in selective media (0.25 N-acetylcysteine and 1% sodium hydroxide followed by 5% oxalic acid), as it reduces bacterial contamination from 3% to 5%.[33] Since we began to use a method of decontamination with oxalic acid to treat samples from cystic fibrosis patients in our laboratory in 2002, we have been able to reduce the rate of contamination considerably.

Results from skin tests with antigens for a specific mycobacterium have proven to have low specificity, as cross reactions have been observed among the different mycobacteria, including *M tuberculosis.* Among our patients for whom nontuberculous mycobacteria were isolated, 4 had a positive Mantoux test, possibly because of cross reactions. In a study by Pinto-Powell et al[34] with the *M avium* antigen, the skin test displayed good sensitivity, although it was unable to distinguish between simple isolation and true infection. Currently, therefore, the role of skin tests is uncertain.

Our study has certain limitations. The follow-up period was not the same for all the patients, as approximately 35% of them were enrolled gradually as they reached 18 years of age and were transferred from the pediatric hospital to our unit. Also, as occurred with 1 of our patients, a first sputum sample could be positive by auramine acid-fast staining but we would not know if there had already been a positive test earlier because the pediatric hospital did not routinely order specific microbiological tests to detect nontuberculous mycobacteria.

The prevalence of such bacterial infection in adults with cystic fibrosis is clearly high, and although the clinical implications remain to be established, it is important to detect it in order to identify patients in need of treatment. Given the difficulty of diagnosing this infection in the context of cystic fibrosis, we need to devise more concrete criteria than those proposed by the ATS. Thus, first, suspicion must arise when patients have several positive stains (particularly) or cultures; for suspicion to arise, we must order sputum tests for nontuberculous mycobacteria periodically (annually or semiannually). Second, the activity of nontuberculous mycobacteria should be suspected when patients have pulmonary symptoms, low-grade fever, weight loss, or increased sputum production accompanied by worsening lung function and/or evidence of progression of nodular lesions or cavitations on x-rays. Finally, infection by nontuberculous mycobacteria should be considered whenever a patient does not respond to conventional antibiotic treatment against colonizing bacteria.

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