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Multicenter Study of Prevalence of Nontuberculous Mycobacteria in Patients with Cystic Fibrosis in France▽

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We performed a multicenter prevalence study of nontuberculous mycobacteria (NTM) involving 1,582 patients (mean age, 18.9 years; male/female ratio, 1.06) with cystic fibrosis in France. The overall NTM prevalence (percentage of patients with at least one positive culture) was 6.6% (104/1,582 patients), with prevalences ranging from 3.7% (in the east of France) to 9.6% (in the greater Paris area). *Mycobacterium abscessus* complex (MABSC; 50 patients) and *Mycobacterium avium* complex (MAC; 23 patients) species were the most common NTM, and the only ones associated with fulfillment of the American Thoracic Society bacteriological criteria for NTM lung disease. The "new" species, *Mycobacterium bolletii* and *Mycobacterium massiliense*, accounted for 40% of MABSC isolates. MABSC species were isolated at all ages, with a prevalence peak between 11 and 15 years of age (5.8%), while MAC species reached their highest prevalence value among patients over 25 years of age (2.2%).

Nontuberculous mycobacteria (NTM) have emerged as "new" pathogens in cystic fibrosis (CF) patients over the last 2 decades (10). CF centers worldwide have reported isolation of NTM from the respiratory tracts of CF patients, with prevalence values ranging from 5% to 20% (5, 6, 8, 9, 13, 14, 16, 19, 22, 25). *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* complex (MABSC) species are the most frequently isolated NTM and together account for >95% of NTM lung diseases affecting CF patients. The MAC, a member of the subgroup comprising slowly growing mycobacteria, ranks first in North America (22), whereas the MABSC, a member of the subgroup comprising rapidly growing mycobacteria, seems to predominate in Western Europe (15, 23, 25) and is also more prevalent than the MAC in Israel (19).

Previous studies have reported isolation of NTM from 6.6 to 9.8% of French CF cohorts (9, 23, 25). These studies also reported a much higher isolation rate for MABSC than for MAC or other NTM species (23, 25). However, these studies were done exclusively in pediatric CF centers in Paris. This may have distorted the results since MABSC species are more prevalent than MAC species in children (23). Moreover, the epidemiology of NTM in Paris does not necessarily reflect the situation in other regions of France. For example, studies involving non-CF patients have reported higher rates of NTM disease in urban areas (20). Moreover, previous French studies were performed before *M. abscessus* (now *M. abscessus* sensu lato, or the MABSC) was shown to include at least three distinct species, *M. abscessus* (sensu stricto) (hereafter referred to as *M. abscessus*), *Mycobacterium massiliense*, and *Mycobacterium bolletii* (1, 3). The prevalences of these three species in CF patients in France were therefore unknown.

We thus conducted a large, prospective, nationwide study addressing NTM prevalence in CF patients in France. This study shows relatively low prevalence figures for French CF centers. It also provides evidence that MABSC species are currently the most prevalent NTM in the French CF population, far more frequent than MAC species, and that MABSC species mainly infect children and young adults.

The 49 French CF centers were asked to participate in the study. Each patient included was asked to provide at least three sputum samples (or other respiratory specimens) for NTM analysis between 1 January 2004 and 31 December 2004. Prior NTM isolation did not exclude subjects. Patients with a positive NTM sample had to submit three more sputum samples at monthly intervals and then every 3 months for a total of 18 months. All the patients, or their parents if they were chil-

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dren, gave their informed consent, and an internal review board approved the study. Samples were analyzed for NTM at each center, using approved techniques. Specimens were decontaminated with the two-step *N*-acetyl-L-cysteine-NaOH-oxalic acid method (31) as previously described (23). Acid-fast bacillus (AFB) smears were stained with auramine fluorescent dye and/or by using the Ziehl-Neelsen method. Liquid (MGIT [Becton Dickinson, Le Pont de Claix, France] and BacTAlert [bioMérieux, Marcy l'Etoile, France]) and/or solid (Löwenstein and Coletsos; bioMérieux) slants were used for culture. All NTM isolates (except *Mycobacterium gordonae* isolates) were sent to the Laboratory of Mycobacteria (Pasteur Institute, Paris, France) for identification using *hsp65* (*M. abscessus-Mycobacterium chelonae* group) and 16S-23S intergenic gene region (other NTM) sequencing (18, 24, 27). The different species of the MABSC were further identified by *rpoB* sequencing (2). The prevalence of NTM was defined as the proportion of studied subjects giving at least one positive NTM culture. The microbiological criteria for NTM lung infection were those recommended by the American Thoracic Society (ATS) (positive cultures from at least two separate expectorated sputum samples or from at least one bronchial wash or lavage) (11). Comparison assessments were performed using the chi-square test for qualitative variables and the Student test for quantitative variables (STATA software, version 9; StataCorp LP). Statistical significance was accepted for P values of <0.05.

Forty-one of the 49 French CF centers took part in the study: 17 adult centers, 20 pediatric centers, and 4 mixed adult-and-pediatric centers. Of the 2,912 CF patients registered at these 41 centers during 2004 (the year of the study), 1,582 (54.3%) were included in the study and screened for NTM. There were 813 male subjects and 769 female subjects (sex ratio, 1.06), ranging in age from 4 months to 82 years (mean age, 18.9 years). One hundred four of the 1,582 included patients (6.6%) were found to have at least one sample positive for NTM during the study period, of whom 19 (18.3%) were known to be positive for the same NTM species prior to their inclusion (15 patients with MABSC and 4 with MAC infections) (Table 1). Of these 104 patients, 101 were positive for a single species and 3 for two species (*M. massiliense* and *Mycobacterium peregrinum*, *M. abscessus* and *Mycobacterium simiae*, or *M. chelonae* and *M. gordonae*). The most prevalent NTM were MABSC species (50 patients [30 patients with *M. abscessus*, 9 with *M. bolletii*, and 11 with *M. massiliense*]), followed by MAC species (23 patients [15 patients with *M. avium* and 8 with *Mycobacterium intracellulare*]), *M. gordonae* (16 patients), and *M. chelonae* (8 patients). Other isolated NTM species included *Mycobacterium fortuitum* (2 patients), *Mycobacterium xenopi* (2 patients), *M. peregrinum* (2 patients), *Mycobacterium immunogenum* (1 patient), *Mycobacterium lentiflavum* (1 patient), "*Mycobacterium manitobense*" (1 patient), and *M. simiae* (1 patient). Of the 104 NTM-positive CF patients, 57 (54.8%) met the ATS bacteriological criteria for NTM pulmonary disease (Table 1), resulting in a prevalence of 3.6% in the studied population (47 patients met the 1997 ATS criteria [30] [prevalence, 3.0%]). These 57 patients were positive for MABSC ($n = 40$) or MAC ($n = 17$) infections. The prevalences of patients meeting the ATS bacteriological criteria for MABSC and MAC lung disease were thus 2.5% and 1.1%, respectively.

TABLE 1. Recovered NTM species

NTM group	No. (%) of patients with:			
	NTM positivity	Previous NTM positivity[c]	ATS criteria met	Positive AFB smear
MABSC	50	15 (30.0)	40 (80.0)	24 (48.0)
M. abscessus	30	9 (30.0)	23 (76.7)	15 (50.0)
M. bolletii	9	4 (44.4)	8 (88.9)	4 (44.4)
M. massiliense	11	2 (18.2)	9 (81.8)	5 (45.5)
MAC	23	4 (17.4)	17 (73.9)	11 (47.8)
M. avium	15	1 (6.7)	10 (66.7)	6 (40.0)
M. intracellulare	8	3 (37.5)	7 (87.5)	5 (62.5)
Other species[a]	34	0 (0)	0 (0)	0 (0)
Total	104[b]	19 (18.3)	57 (54.8)	35 (33.7)

[a] *M. gordonae* (16 patients), *M. chelonae* (8 patients), *M. fortuitum* (2 patients), *M. xenopi* (2 patients), *M. peregrinum* (2 patients), *M. immunogenum* (1 patient), *M. lentiflavum* (1 patient), *M. manitobense* (1 patient), and *M. simiae* (1 patient).
[b] Two different mycobacterial species were isolated from three patients (*M. massiliense* and *M. peregrinum*, *M. abscessus* and *M. simiae*, or *M. chelonae* and *M. gordonae*).
[c] Known positivity for the same NTM species prior to inclusion.

Thirty-five patients had at least one positive AFB smear (33.7% of NTM-positive patients). AFB positivity rates were similar for patients positive for MABSC (48.0%) and MAC (47.8%) species. There was also no noticeable difference between the three MABSC species.

NTM-positive patients (excluding patients positive for *M. gordonae* alone [$n = 89$]) had a significantly lower male/female ratio than NTM-negative patients (0.65 versus 1.08; $P = 0.02$); predominantly girls had MABSC (male/female ratio, 0.79), MAC (male/female ratio, 0.53), and "other NTM" (male/female ratio, 0.45) (not shown) infections. We analyzed the prevalence and distribution of the NTM species in relation to patients' ages (Table 2). The prevalence of NTM was lowest up to 10 years of age (4.5%), peaked between 11 and 15 years of age (10.4%), and then slightly declined (6.3%). However, there were marked differences between the NTM species. MABSC species were isolated at all ages, with a prevalence peak between 11 and 15 years of age (5.8%), while MAC species were not found before 9 years of age and reached their highest prevalence value in patients over 25 years of age (2.2%). MABSC species were more prevalent than MAC species up to 25 years (3.7 versus 1.2%) and less prevalent after 25 years (1.4 versus 2.2%) of age; however, the differences were significant only for patients aged 11 to 15 years (5.8 versus 1.9; $P = 0.02$). The prevalence values for other NTM species were relatively stable around 2% in the various age brackets, except between 16 and 20 years (0.6%).

The 104 NTM-positive patients were from 31 out of the 41 participating CF centers, which consisted of 15 adult, 14 pediatric, and 2 mixed adult-and-pediatric centers. The highest NTM prevalence values were observed for a pediatric center in Paris (14.5%) and an adult and pediatric center in Bordeaux (South West France) (14%) (not shown). Among the other large centers, the centers in Angers (western France) and Lille (northern France) also had relatively high prevalence values (9.4% and 8.1%, respectively), while prevalence was very low

TABLE 2. Prevalence of NTM in relation to age

NTM group	No. (%) of positive patients in indicated age group					
	≤10 yr (n = 377)	11–15 yr (n = 260)	16–20 yr (n = 321)	21–25 yr (n = 257)	>25 yr (n = 367)	Total (n = 1,582)
MABSC[a]	7[b] (1.9)	15 (5.8)[d]	14 (4.4)	9 (3.5)	5 (1.4)	50 (3.2)
MAC	1[c] (0.3)	5 (1.9)[d]	6 (1.9)	3 (1.2)	8 (2.2)	23 (1.5)
Other NTM species[a]	9 (2.4)	8 (3)	3 (0.9)	7 (2.7)	7 (1.9)	34 (2.1)
Any NTM[a]	17 (4.5)	27 (10.4)	22 (6.9)	18 (7)	20 (5.4)	104 (6.6)

[a] Two different mycobacterial species were isolated from three patients (*M. massiliense* and *M. peregrinum*, *M. abscessus* and *M. simiae*, or *M. chelonae* and *M. gordonae*).
[b] The youngest patient was 4 months old.
[c] This patient was 9 years old.
[d] $P = 0.02$ for MABSC versus MAC.

in Lyon (South East France) and Nancy (eastern France) (0.9% and 1.6%, respectively) (not shown). With respect to geographic region (Table 3), the highest NTM prevalence was in the greater Paris area (9.6%) and the lowest in eastern France (3.7%). Moreover, MABSC species were much more prevalent than MAC species (5.1 versus 1.0%; $P = 0.007$) in Paris and the greater Paris area; this region, which accounted for 26% of the included patients, represented 42% of all MABSC species-positive patients identified during the study, versus only 17% of all MAC species-positive patients (Table 3). The prevalence of NTM other than those included in the MABSC and MAC was also higher in Paris and the greater Paris area (3.6%).

This study is the first prospective NTM survey of CF patients carried out in France and Europe and the largest worldwide, with almost 1,600 CF patients included. Almost all the French CF centers throughout the country (41/49) took part in this study, and similar numbers of adult and pediatric centers were included. We included all patients capable of providing at least three sputum samples for NTM analysis, with no age limit, to prevent bias. However, the mean age of the recruited population was higher than expected from national registry data (18.9 versus 15.9 years [http://www.registredelamuco.org/]), mainly due to a smaller proportion of young children, as they have difficulty expectorating. This may have created a bias in our results for the youngest patients (i.e., selection of only the most severely ill subjects).

TABLE 3. NTM prevalence and geographic regions

Region of France	No. (%) of positive patients			
	MABSC[a]	MAC	Other NTM species[a]	Any NTM[a]
North (n = 164)	4 (2.5)	4 (2.5)	3 (1.8)	11 (6.7)
West (n = 247)	7 (2.8)	5 (2.0)	1 (0.4)	13 (5.3)
South West (n = 293)	6 (2.0)	4 (1.4)	7 (2.4)	17 (5.8)
South East (n = 274)	9[b] (3.3)	5 (1.8)	3 (1)	16 (5.8)
East (n = 189)	3 (1.6)	1 (0.5)	4 (2.1)	7 (3.7)
Greater Paris area (n = 415)	21 (5.1)[c]	4 (1.0)[c]	16 (3.9)[d]	40 (9.6)
Total (n = 1,582)	50 (3.2)	23 (1.5)	34 (2.1)	104 (6.6)

[a] Two different mycobacterial species were isolated from three patients (*M. massiliense* and *M. peregrinum*, *M. abscessus* and *M. simiae*, or *M. chelonae* and *M. gordonae*).
[b] Including five patients from Réunion Island.
[c] $P = 0.007$ for MABSC versus MAC.
[d] Including six patients positive for *M. chelonae*.

The overall NTM prevalence was 6.6%, and the prevalence of patients meeting the 2007 ATS bacteriological criteria for NTM lung disease was 3.6% (with 3% meeting the 1997 ATS criteria). These values are relatively low. An NTM prevalence of 13% was reported in the American multicenter study, but with a similar prevalence of subjects satisfying the ATS criteria (3%; 1997 ATS criteria) (22). More recently, an overall NTM prevalence of 22.6% was reported for Israel (in the only other nationwide study published to date), with 10.8% of patients meeting the 2007 ATS criteria (19). However, this study was a retrospective, observational study of patients tested for NTM as part of routine management, and there was therefore a high risk of selection bias: the study included only patients whose physicians had decided it was necessary to request mycobacterial culture, so the selection of patients with NTM was more likely. Moreover, almost half the study patients came from one center with an abnormally high prevalence of patients positive for *M. simiae* (19).

We observed a widespread geographic distribution, with prevalences of NTM varying from 3.7% in eastern France to 9.6% in the greater Paris area. Such widespread geographic distribution agrees with the U.S. NTM prevalence study, where NTM prevalences ranged from 7% in Boston, MA, to 24% in New Orleans, LA (22). The high prevalence observed in the Paris region confirms our data for the year 2000, based on the monitoring of 385 patients in three Parisian pediatric centers (NTM prevalence, 8.1%) (23). The urban nature of the Paris region may have influenced this relatively high prevalence. Similar prevalence figures were obtained for northern France (6.7%), which is also very urbanized (the Lille-Roubaix-Tourcoing conurbation). Previous studies involving non-CF patients have reported other NTM species, such as *Mycobacterium kansasii*, to be associated with residence in nonrural areas (20). Moreover, several studies have suggested that certain chemical compounds present in polluted water distribution systems stimulate the growth of NTM (28). Air pollution in cities may also contribute to NTM lung disease by interfering with the mucosal immune system. Apart from centers in the Paris area and the Lille-Roubaix-Tourcoing conurbation, the centers with the highest prevalences were in southwestern and western France, close to the Atlantic coast. Conversely, the prevalences in centers in eastern and southeastern France were very low.

These results are similar to those for the American multicenter study, in which the highest prevalence values were those reported for coastal states (22).

More than 80% of the NTM-positive patients in our study (excluding patients positive for *M. gordonae* only) were positive for MAC or MABSC species, and these were the only NTM associated with patients satisfying the ATS criteria. These results confirm that MAC and MABSC species are the main pathogenic NTM in CF patients and that other NTM make only a minor contribution, except in particular epidemiological situations, such as those described for *M. simiae* in Israel (19). Our study also showed that MABSC species were much more prevalent than MAC species in France, with twice as many patients positive for MABSC as for MAC species, regardless of whether ATS criteria were met. This result is particularly significant, as the opposite, a higher isolation rate for MAC than for MABSC species, has recently been reported in a French national study of respiratory NTM infection in non-human immunodeficiency virus-positive patients, mostly non-CF subjects (7). This reinforces the idea that CF subjects are particularly susceptible to MABSC infection.

Our result showing a clear predominance of MABSC over MAC infections is consistent not only with our previous studies of patients in France but also with those for other European countries and Israel (15, 19, 23, 25). However, they differ from the results of the American study, in which the prevalence of MAC species in CF patients was five times that of MABSC species (22). Two aspects of the large, multicenter American study may explain these discrepancies. First, the study was based on adult patients only (patients aged 10 years or above were included [mean age, 23 years]), which may partly explain the greater prevalence of MAC than of MABSC species. Second, the study was published in 2003 but related to patients included over 6 years, beginning in 1992 (21). This may also have increased the bias in favor of the MAC, given the relatively recent emergence of the MABSC (15).

As we reported previously (23), MABSC species are isolated from patients during the first years of life, with a maximum prevalence between 11 and 20 years of age. Conversely, MAC species are rarely isolated before 10 years of age and have a maximum prevalence after the age of 25 years, after which they are isolated more frequently than MABSC species. We previously suggested that these differences in profile could be linked to the greater virulence of the MABSC (10), because it does not necessarily require the presence of existing pulmonary lesions to become established (23). MABSC species could thus infect young CF patients more easily. The decrease in MABSC species prevalence after 25 years of age may also indicate that the MABSC became important in CF only in the 15 to 20 years before our study, in the late 1980s and early 1990s (e.g., with greater exposure to MABSC species and involvement of cofactors facilitating MABSC species implantation and development in the respiratory tract). Supporting this hypothesis of a recent emergence of the MABSC, the West Swedish CF Centre in Gothenburg, Sweden, also found an increase in the number of positive cases of MABSC infection in the middle of the 1990s, whereas the authors reported no change in laboratory techniques at their center (15).

We found a significantly higher proportion of girls than boys among NTM-positive patients. This sex ratio imbalance was particularly marked for the MAC and other-NTM subgroups, in which there were twice as many NTM-positive women as men. As far as we are aware, this has never previously been reported. Selection bias in our study is unlikely because of the way the study was designed. Moreover, similar observations have been reported for non-CF patients, with a predominance of women among patients with NTM infection (12). Differences in disease expression as a function of sex have also been reported for tuberculosis, with lung disease more frequent in men and other forms of the disease more frequent in women (32). Further study should confirm our observations and elucidate the mechanisms involved.

We aimed to determine the prevalences of *M. abscessus*, *M. bolletii*, and *M. massiliense* in our cohort. Several studies have implicated *M. bolletii* and *M. massiliense* in epidemics of skin and soft tissue infections similar to those described for *M. abscessus* (17, 29), but only a few respiratory infections have been reported, mostly for non-CF patients (4, 26). We show here that *M. bolletii* and *M. massiliense* play a significant epidemiological role in CF, accounting for almost 40% of MABSC isolates (representing a total number of patients only slightly smaller than the number of MAC species-positive patients). We also show that the proportion of cases satisfying the ATS criteria is similar to that for *M. abscessus* infection and thus that *M. bolletii* and *M. massiliense* appear to share the same pathogenicity as *M. abscessus*. However, different risk factors may be involved. A recent study of both CF and non-CF patients reported possible differences in characteristics between patients infected with *M. massiliense* and those infected with *M. abscessus* (33). We are currently investigating this occurrence in CF.

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