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# Chronic *Mycobacterium abscessus* infection and lung function decline in cystic fibrosis

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## Abstract

**Background—**Although nontuberculous mycobacteria (NTM) are recognized pathogens in cystic fibrosis (CF), associations with clinical outcomes remain unclear.

**Methods—**Microbiological data was obtained from 1216 CF patients over 8 years (481±55 patients/year). Relationships to clinical outcomes were examined in the subset ($n$=271, 203±23 patients/year) with longitudinal data.

**Results—**Five hundred thirty-six of 4862 (11%) acid-fast bacilli (AFB) cultures grew NTM, with *Mycobacterium abscessus* ($n$=298, 55.6%) and *Mycobacterium avium* complex ($n$=190, 35.4%) most common. Associated bacterial cultures grew *Stenotrophomonas* or *Aspergillus* species more often when NTM were isolated (18.2% vs. 8.4% and 13.9% vs. 7.2%, respectively, $p$<0.01). After controlling for confounders, patients with chronic *M. abscessus* infection had greater rates of lung function decline than those with no NTM infection (−2.52 vs. −1.64% predicted $FEV_1$/year, $p$<0.05).

**Conclusions—**NTM infection is common in CF and associated with particular pathogens. Chronic *M. abscessus* infection is associated with increased lung function decline.

### Keywords

Nontuberculous mycobacteria

## 1. Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous environmental mycobacteria that have the potential to cause respiratory disease [1], particularly in hosts with compromised lung defenses such as patients with cystic fibrosis (CF) [2,3]. Recovery of NTM from CF respiratory secretions is relatively common, with an overall prevalence of 13% reported in a large prospective study [4]. More recent studies have suggested that the NTM prevalence in CF may be increasing [5,6], though it is not clear if this represents increasing rates of infection or improved surveillance [7].



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While the potential for NTM to cause respiratory disease is well established, the clinical impact of chronic NTM infection in CF is not well defined [2]. Although associations between NTM infection and clinical deterioration in CF have been reported [8–10], the largest prospective study of NTM infection in CF to date did not demonstrate a relationship between NTM infection and worsening lung function [11]. Establishing a relationship between chronic NTM infection and clinical severity in CF is complicated by the many factors that influence respiratory deterioration in CF, as well as the overlap between features of CF and NTM respiratory disease [2].

We hypothesized that examination of a large database over a long interval would reveal relationships between chronic NTM infection and clinical disease in CF. Therefore, we retrospectively examined a microbiological database of all culture results, including acid-fast bacterial (AFB) cultures for NTM, from 1216 patients with CF at a single university CF center from 2000 to 2007. We then examined the relationships between NTM isolation and clinical outcomes through evaluation of a subset of 271 patients with at least 3 years of available longitudinal clinical data.

## 2. Methods

### 2.1. Microbiological data

Cultures for acid-fast bacilli, bacterial, and fungal pathogens were performed using standardized methods [12]. Culture results were obtained from a database maintained by the Clinical Microbiology–Immunology Laboratories at the University of North Carolina Hospitals and included results from 13,033 bacterial and fungal cultures and 4862 acid-fast bacterial (AFB) cultures performed on 1216 patients with CF from 2000 to 2007. On average, the database included 608±299 AFB culture results and 481±55 individual patients each year. In the last year of the study (2007), introduction of *hsp65* sequence analysis revealed that a subset of mycobacterial species previously identified as *Mycobacterium abscessus* could not be distinguished from another member of the *M. abscessus* group, *Mycobacterium massiliense*. Prior to this year, NTM speciation was based on standard biochemical methods [12,13]. For simplicity and consistency with previous publications, both *M. massiliense* and *M. abscessus* are referred to as *M. abscessus* within this manuscript.

### 2.2. Clinical data

Clinical data was abstracted from the PortCF database for adult and pediatric CF programs at the University of North Carolina. Demographic data were recorded for each calendar year, with age in each calendar year defined as age on the subject's day of birth. In the clinical correlation analyses, only patients with at least three AFB cultures in the microbiology database and at least three calendar years of lung function data at age 6 years or older were included. For this subset, data on lung function, body mass index (BMI), diagnosis of CF related diabetes mellitus, and diagnosis of allergic bronchopulmonary aspergillosis (ABPA), and care episodes (intravenous antibiotic treatment) were abstracted from Port CF. For clinical parameters in which multiple values were obtained throughout the calendar year (e.g., percent predicted $FEV_1$, and body mass index), the maximum value was recorded for each year. Within this cohort, chronic NTM infection was defined as three or more cultures positive for NTM over three or more quarterly visits.

This study was approved by the Office of Human Research Ethics at the University of North Carolina at Chapel Hill.

### 2.3. Statistical analysis

Descriptive statistics were calculated for year of first entry into the study for groups classified by NTM infection status. These are presented as means and standard deviations (SD) for continuous variables and proportions for categorical variables. Characteristics included gender; pancreatic insufficiency (≥2 years pancreatic enzyme use); chronic *Pseudomonas* infection (≥2 years with one or more cultures positive for *Pseudomonas aeruginosa*); nutritional failure (average BMI< 19 [adults] or percent predicted BMI< 10% [children]); ABPA (diagnosed on at least one occasion); and CF related diabetes (diagnosed on at least one occasion) were obtained from the data abstracted from PortCF.

Comparisons were made for continuous variables using the non-parametric Mann–Whitney test (bivariate) or the Krusal– Wallis test with Dunn's post test (multivariate). The chi-squared test of association (or Fisher's exact test, where necessary) was utilized for categorical comparisons; post hoc bivariate comparisons were conducted if there were more than 2 levels for each variable.

To examine the relationship between chronic NTM infection and changes in lung function over time, linear mixed models with a random intercept and slope were fit to determine the effect of NTM infection status on percent predicted $FEV_1$ over time (year of age) using maximum likelihood estimation. Each patient contributed a minimum of 3 and a maximum of 8 (1 per year) data points. The primary covariate of interest was the interaction between time (year of age) and infection status. In addition to age, infection status, and their interaction, models were adjusted for confounders established *a priori* as defined above: gender; pancreatic insufficiency, chronic *Pseudomonas* infection, nutritional failure, ABPA, and CF related diabetes.

Statistical significance was established at 0.05. All analyses were conducted in GraphPad Prism (La Jolla, CA) or SAS version 9.2 (SAS, Cary, NC).

## 3. Results

### 3.1. Prevalence of NTM and its relation to other pathogens

Of the 1216 CF patients included in the database, 829 (68%) had one or more AFB cultures obtained over the course of the study for a total of 4862 AFB cultures (average 5.9 AFB cultures per patient). NTM were isolated from 536 (11.0%) of these AFB cultures. *M. abscessus* was the most commonly NTM species cultured, representing 298 (55.6%) of the isolates; *Mycobacterium avium* complex was second with 190 (35.4%) isolates (Fig. 1A). Other mycobacterial species, including *M. gordonae* and *M. kansasii*, were identified at much lower frequencies (<5% for each).

The 536 NTM isolates were obtained from 166 individual patients (13.7% of the entire cohort, 20.0% of the cohort with at least one AFB culture). Among these NTM positive patients, 98 (59.0%) patients had at least one positive culture for *M. avium*, with an average of 1.9±1.9 *M. avium* isolates per patient; whereas 68 (41.0%) patients had at least one positive culture for *M. abscessus* with an average of 4.9±7.0 *M. abscessus* isolates per patient. *M. abscessus* represented the majority (55.6%) of NTM positive isolates but a smaller proportion (41.0%) of the NTM positive patients, reflecting the greater number of *M. abscessus* isolates per patient.

The relationships between NTM and other CF pathogens were determined by examining the 3789 instances in which an AFB culture for NTM and a standard culture for bacteria or fungi were obtained from the same patient on the same date. When NTM were recovered from AFB cultures, the associated bacterial/fungal cultures had a higher prevalence of

*Stenotrophomonas maltophilia* (18.2% vs. 8.4%, $p<0.01$) and *A. fumigatus* (13.9% vs. 7.2%, $p<0.01$) than when AFB cultures were negative for NTM (Fig. 1B). Conversely, *P. aeruginosa* was less commonly associated with NTM positive cultures (50.8% NTM positive vs. 57.9% NTM negative, $p<0.01$).

Because previous studies suggested that NTM infection was more common in older individuals with CF [4], we examined the relationship between NTM infection and age. In the very young (<5 years of age), the percentage of patients with NTM positive cultures was low (mean 1.3±0.4%). Prevalence slowly increased with age until age 14, then remained fairly constant (mean 11.0±3.3%, range 5.7–20.6%) (Fig. 2A). Although we did not observe a marked increase in NTM prevalence with age, overall prevalence was modestly increased in the older CF population (13.2±3.8% ages 40+) compared to adolescents and younger adults (9.9±2.6% ages 14–39, $p<0.05$). Although these findings are consistent with previous reports [4,5,14], interpretation of the data is limited by the fact that AFB cultures were primarily obtained from bronchoalveolar lavage fluid in the young (≤5 years old) and sputum from older individuals, which complicates comparison of prevalence estimates across ages.

To determine whether the prevalence of NTM changed over time, we assessed the prevalence of patients with NTM positive cultures in each calendar year. The number of patients with at least one NTM positive culture increased from 2000 to 2007, with an additional 4.8±0.9 NTM positive patients each year or 0.7±0.2% annual increase of the total cohort ($p<0.01$, Fig. 2B). However, the number of patients with at least one AFB culture attempt each year also increased significantly over time (4.9± 1.0% annual increase, $p<0.01$). Interestingly, the percentage of AFB screened patients who were NTM positive each year remained relatively unchanged throughout the study (range 11.5–16%, Fig. 2B).

### 3.2. Clinical correlates of chronic NTM infection

We next examined relationships between NTM infection and clinical parameters, using a subset of patients who had longitudinal clinical data available and were screened for AFB as defined in Section 2. A total of 271 patients were included (mean 203±23 patients per year, 6.0±1.9 years of data per patient). From this group, 95 patients (35% of entire cohort) had at least one NTM positive culture over the time frame of the study, with a mean annual prevalence of 10.8±1.6%. Of these 95 patients, 52 patients (55%) had at least one positive culture for *M. avium*, and 38 patients (40%) had at least one positive culture for *M. abscessus*. The percentage of NTM positive patients per year (both of the total group and of those with AFB screening) was similar to those measured in the total cohort (Fig. 3A, compare to Fig. 2A), although the overall prevalence of NTM was higher.

To determine if NTM infection was more common in patients with more severe clinical disease, we examined differences in specific clinical parameters between patients with chronic NTM infection (three or more AFB cultures positive for NTM, $n=38$) and those with no positive NTM isolates despite at least three AFB cultures ($n=178$). All of the patients with chronic NTM infection meet the recently revised ATS microbiological criteria for NTM infection [15]. The demographic characteristics of the groups were similar, although the chronic NTM infection group included fewer females (Table 1). Rates of ABPA and CF related diabetes were similar between groups, although an increased rate of CF related diabetes in the chronic NTM infection group (7/23) compared to the no NTM infection group (9/87, $p=0.04$) was observed in the pediatric group (age 18 or younger). Average lung function values (percent predicted $FEV_1$) at study entry were not different between groups.

Because the clinical impact of NTM infection may be worse with rapidly growing mycobacteria [8], we separately examined the subset of subjects with chronic *M. abscessus*

infection ($n$=23). The demographic features of this group were similar to those of the no NTM infection group except for higher rates of nutritional failure (Table 1). We observed that subjects with at least one positive culture for *M. abscessus* had a higher rate of chronic infection (23 with chronic infection among 38 with at least one *M. abscessus* positive culture, 61%) than those with at least one positive *M. avium* culture (10 with chronic infection among 53 with at least one positive *M. avium* culture, 19.2%, $p$<0.001). Of note, five patients met the definition of chronic NTM infection with more than one NTM species and did not meet the criteria for either chronic *M. abscessus* or chronic *M. avium* infection.

### 3.3. Lung function decline in chronic NTM infection

To determine the relationship between chronic NTM infection and longitudinal changes in lung function, we performed a linear mixed model analysis including predicted $FEV_1$, age, and several potential confounders including gender, chronic *Pseudomonas* infection, nutritional failure, CF related diabetes, and ABPA. As expected, we observed an overall relationship between lung function and year of age, reflecting an annual decline of −1.64% predicted $FEV_1$ per year (Table 2). The subjects with chronic *M. abscessus* infection had an excess decline of −0.78% predicted $FEV_1$ per year over the no NTM infection group, for a total annual decline of −2.42%/year ($p$=0.02 vs. no NTM infection, Table 2, Fig. 4). Interestingly, the rate of lung function decline in the group of patients who had chronic NTM infection but not chronic *M. abscessus* infection (excess decline −0.57%/year, total decline, total decline −2.21%/year) was intermediate between, but not statistically different from, the rate of decline in the no NTM infection and chronic *M. abscessus* infection groups. Overall, the group with chronic NTM infection (any species) had a higher rate of annual decline in percent predicted $FEV_1$ (−2.33%/yr, $p$<0.01 vs. no NTM infection, data not shown). Interestingly, patients with chronic *M. abscessus* infection received more intravenous antibiotic treatment (31.3±37.0 days treatment/year) than patients with no NTM infection (17.6± 9.4 days treatment/year, $p$<0.01). However, the impact of treatment directed against NTM was difficult to assess, since the clinical database does not distinguish between treatments directed against NTM vs. other CF pathogens.

## 4. Discussion

This study provides the strongest evidence to date that chronic NTM infection, particularly chronic infection with *M. abscessus*, is associated with worsening lung function over time in patients with CF. Although consistent with the view of NTM as respiratory pathogens in CF, the relationships between chronic NTM respiratory infection and clinical disease in CF have been difficult to establish [2,3]. One of the largest prospective studies of NTM infection to date [11] failed to uncover a statistically significant effect of chronic NTM infection on lung function decline. Interestingly, the authors of that study determined that observed differences in $FEV_1$ might become statistically significant if follow-up were extended to 6.4 years, a time frame similar to that of our study (mean 6.0±1.9 years of data per patient). Although substantial differences in methodology preclude a direct comparison between studies, the longer time frame available for our retrospective analysis may explain our greater ability to detect the impact of chronic NTM infection on lung function decline even after controlling for potential confounders.

Based on the known pathogenicity of NTM [1,2,15], we suspect that chronic NTM infection directly contributes to worsening lung function in patients with CF. Direct causation would also be consistent with the fact that excess lung function decline was most clearly observed in those patients infected with *M. abscessus*, generally considered to be a virulent species of NTM [16–18]. However, the data demonstrate an association, and we cannot exclude the possibility that NTM infection is a marker of worsening CF lung disease rather than a causative factor. Nevertheless, the fact that lung function (% predicted $FEV_1$) was similar in

the no NTM infection and chronic NTM infection groups at the start of the study suggests that NTM infection was not limited to patients with more severe preexisting lung disease. We did not observe a relationship between chronic NTM infection and pancreatic sufficiency, a marker of "mild" CF mutations [19]. Furthermore, while we observed some relationships between chronic NTM infection and other features of clinical severity such as nutritional failure and CF related diabetes, the association between infection and lung function decline remained significant even after controlling for these potential confounders. Thus, a causative relationship between NTM infection and lung function decline seems likely, although prospective studies with more universal screening will be necessary to address this issue. Interestingly, the decline in lung function occurred despite the fact that patients with chronic *M. abscessus* infection received greater treatment with intravenous antibiotics. Based on our clinical experience with NTM infected CF patients at our institution, we suspect that some of this treatment reflects antibiotics directed against NTM. However, assessing the impact of this treatment on lung function and other clinical parameters will require further, prospective study.

Although our study population was limited to a single center, our findings are similar to previous studies of NTM infection in CF. For example, overall prevalence of NTM infection in our CF population (mean annual prevalence 10.8%) and its relationship to age are similar to those previously described [4,5]. In addition, the association of NTM with other known CF pathogens including *Stenotrophomonas* and *Aspergillus* species is consistent with previous literature, as was the modest but statistically significant negative association between NTM and *P. aeruginosa* [4,5]. While this negative relationship could reflect an interaction between NTM and *Pseudomonas* infection within the airway, we cannot rule out the possibility that this association results from technical factors; e.g., the well recognized propensity of *Pseudomonas* species to overgrow NTM in a small fraction of AFB cultures despite decontamination procedures [7,20]. In our population, *M. abscessus* represented a higher fraction of NTM isolates than typically reported [4,9], although higher rates of infection with this organism have been observed [5,21]. We suspect that this greater number of *M. abscessus* positive cultures reflects its propensity for causing chronic infection relative to other NTM species [8,10], resulting in more positive cultures per patient over the course of this longitudinal study. It is also possible that this rapidly growing NTM may have a higher recovery rate in the presence of overgrowth with *Pseudomonas* species.

The relatively long time frame of our study allowed us to determine whether the prevalence of NTM infection was changing over time in our CF population. While we observed increasing prevalence of documented NTM infection over the 8 years of this study, much of this increase could be accounted for by higher rates of AFB screening, and the percentage NTM positive patients among AFB screened population was relatively constant. Our data suggest that increased prevalence of NTM could reflect improved surveillance, and universal screening may be needed to effectively identify all CF patients with NTM infection. However, further investigation is needed to determine the clinical benefit of universal screening.

In summary, our study demonstrates that NTM are common respiratory pathogens in CF, and that chronic NTM infection is associated with clinical deterioration as measured by an increased rate of decline in $FEV_1$. These findings further highlight the need for both effective NTM screening and further research on effective treatment modalities for those with chronic NTM infection.

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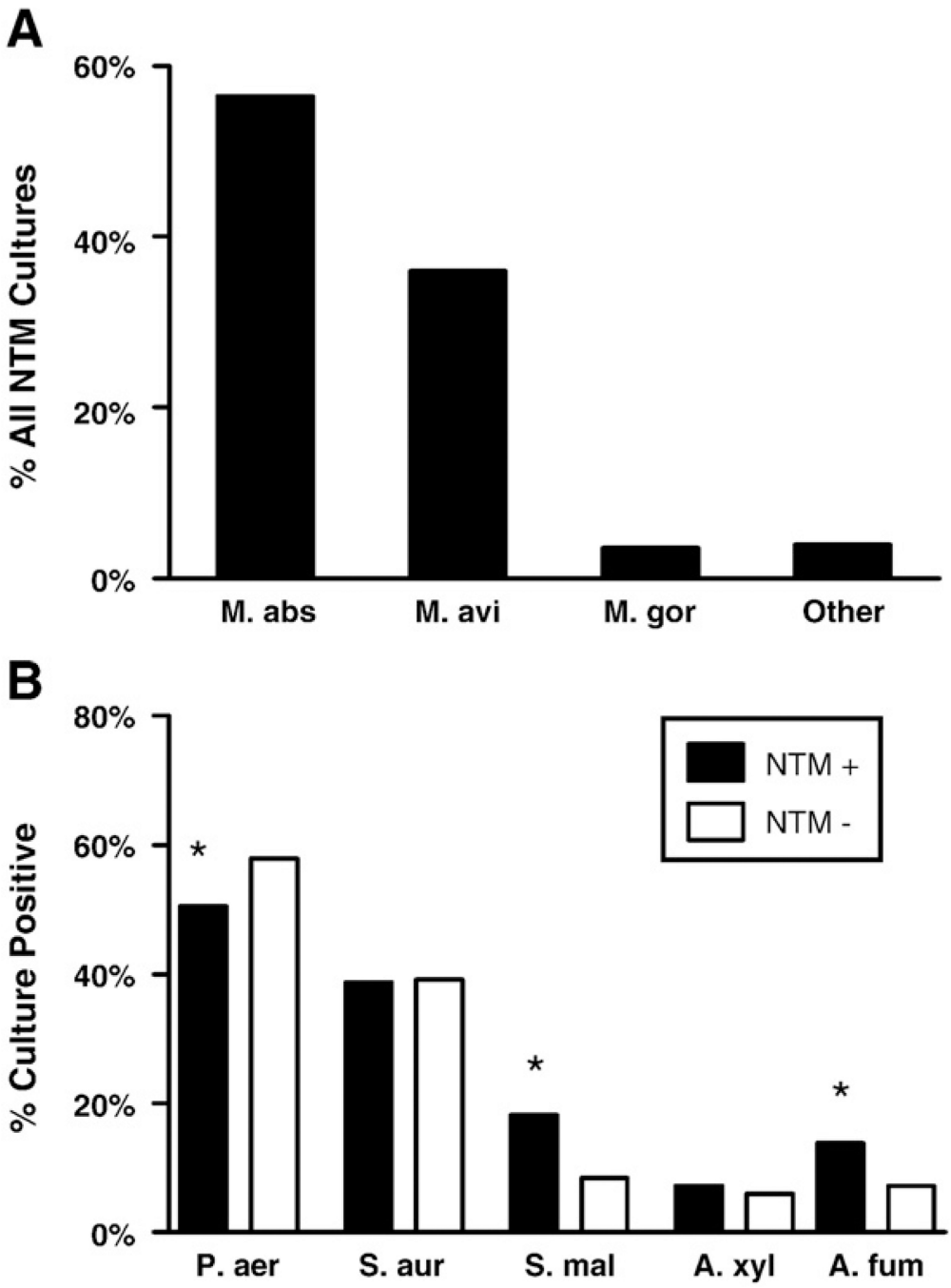


**Fig. 1.**

Prevalence of NTM species and associated pathogens. A) Relative distribution of NTM species ($n$=536). M. abs=*M. abscessus*, M. avi=*M. avium* complex, and M. gor=*M. gordonae*. Others included all other NTM, predominantly unidentified species ($n$=33) but small numbers of *M. fortuitum* ($n$=3), *M.* B) Frequency of culture positivity for various CF pathogens in bacterial/fungal cultures associated with NTM+ or NTM− AFB cultures. P. aer=*P. aeruginosa*, S. aur=*Staphylococcus aureus*, S. mal=*S. maltophilia*, and A. xyl=*Achromobacter xylosoxidans*. A. fum=*Aspergillus fumigatus*. *$p$<0.05 NTM+ vs. NTM − by Chi-squared analysis.

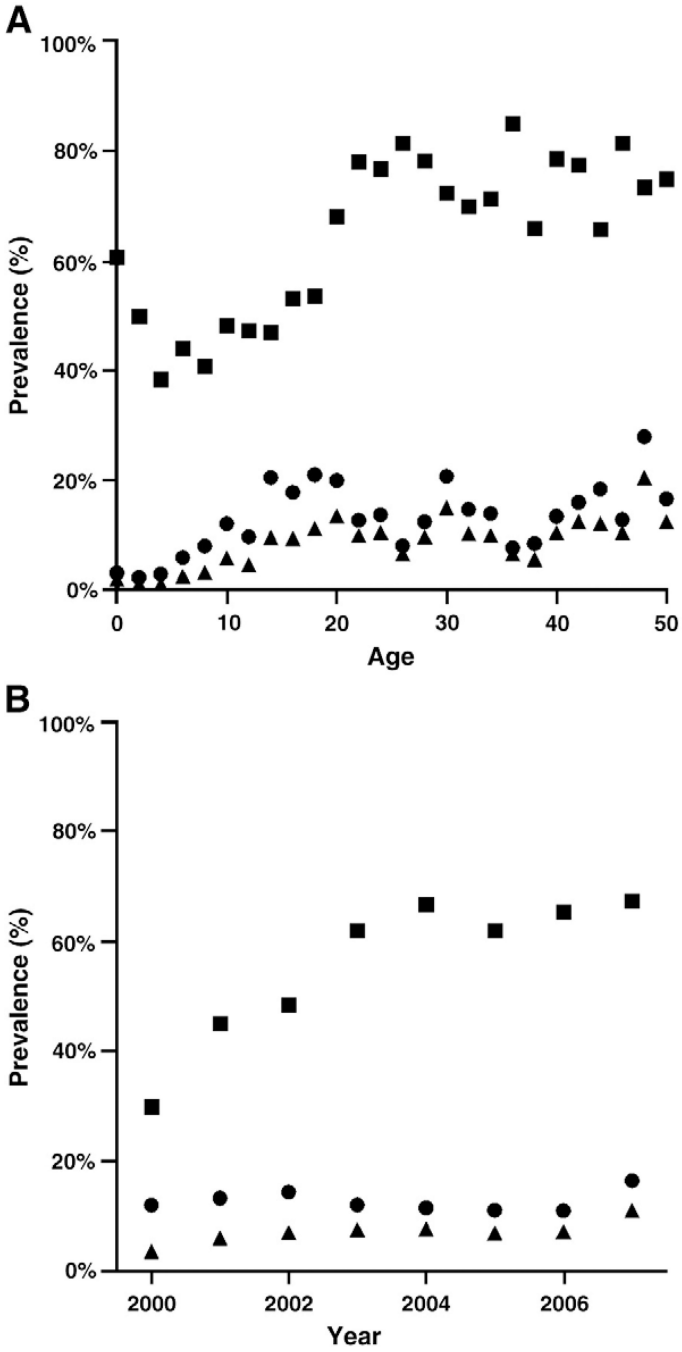


**Fig. 2.**

Age distribution and annual prevalence of NTM infection. Prevalence was calculated form records of 1216 CF patients with at least one microbiological culture obtained from a single university CF center from 2000 to 2007. A) For each year of age, the percentage of patients with at least one AFB culture attempt (squares) was determined, as was the percentage of patients with at least one culture positive for NTM among all patients (triangles) and AFB screened patients (circles). Older patients were more likely to have both AFB culture attempts and NTM isolated from AFB cultures. B) For each calendar year of the study, the percentage of patients with at least one AFB culture attempt (squares) and at least culture positive for NTM among all patients (triangles) and AFB screened patients (circles) was

determined. Among all patients, the percentage of AFB cultures and NTM positive cultures increased over time, although the percentage of NTM positive cultures among AFB screened patients remained relatively constant.

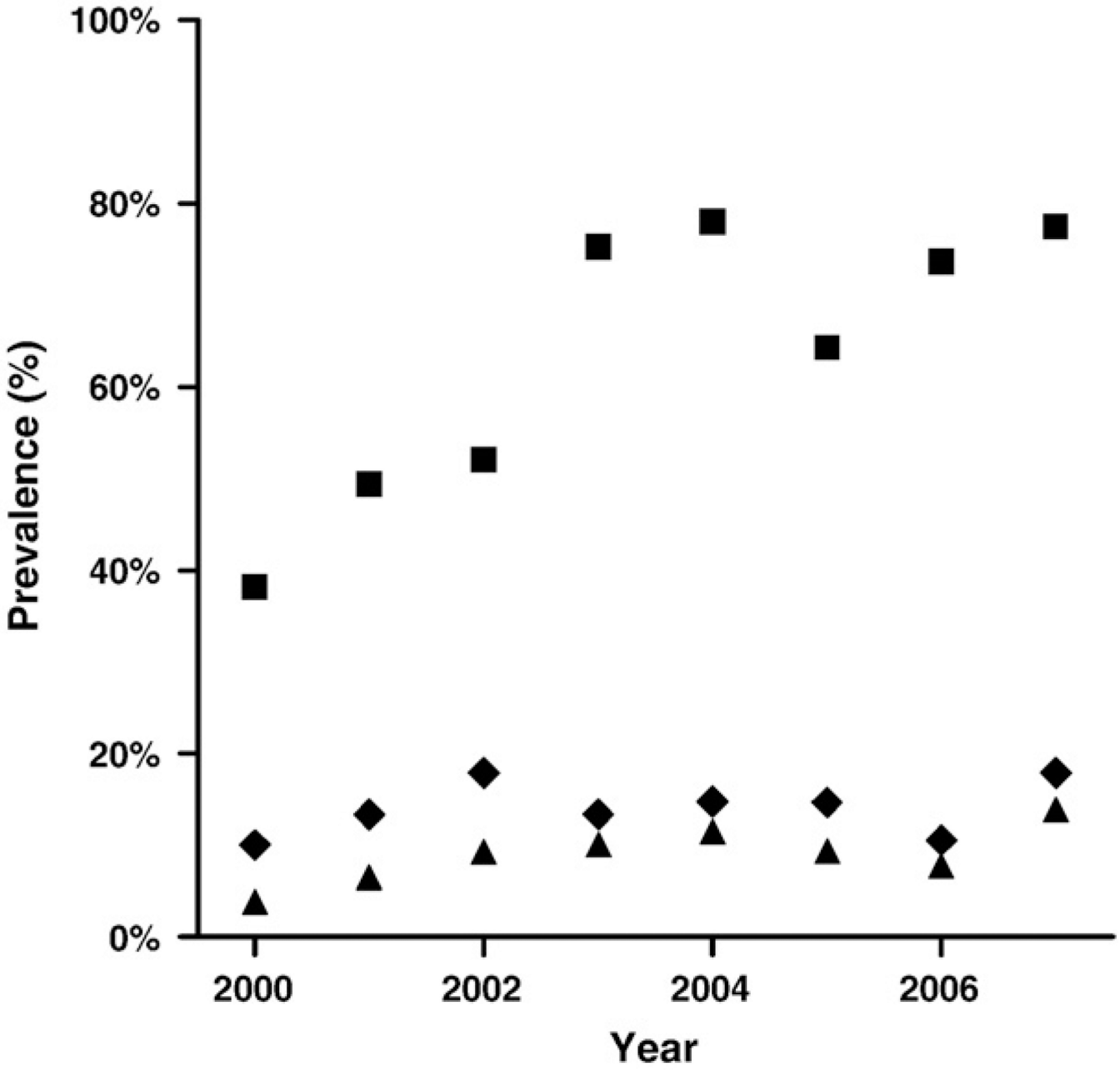


**Fig. 3.**

Prevalence of NTM in patients with longitudinal clinical data. The subset of CF patients with at least three AFB culture attempts and at least 3 years of lung function data was examined ($n$=271). Within this group, the percentage of patients with at least one AFB culture attempt (squares) and at least one positive culture for NTM among all patients (triangles) and those patients with least one AFB culture attempt (circles) was determined. As with the larger group, the percentage of patients with AFB cultures and NTM positive cultures increased over time, although the percentage of patients with AFB culture attempts that were NTM positive was relatively constant.

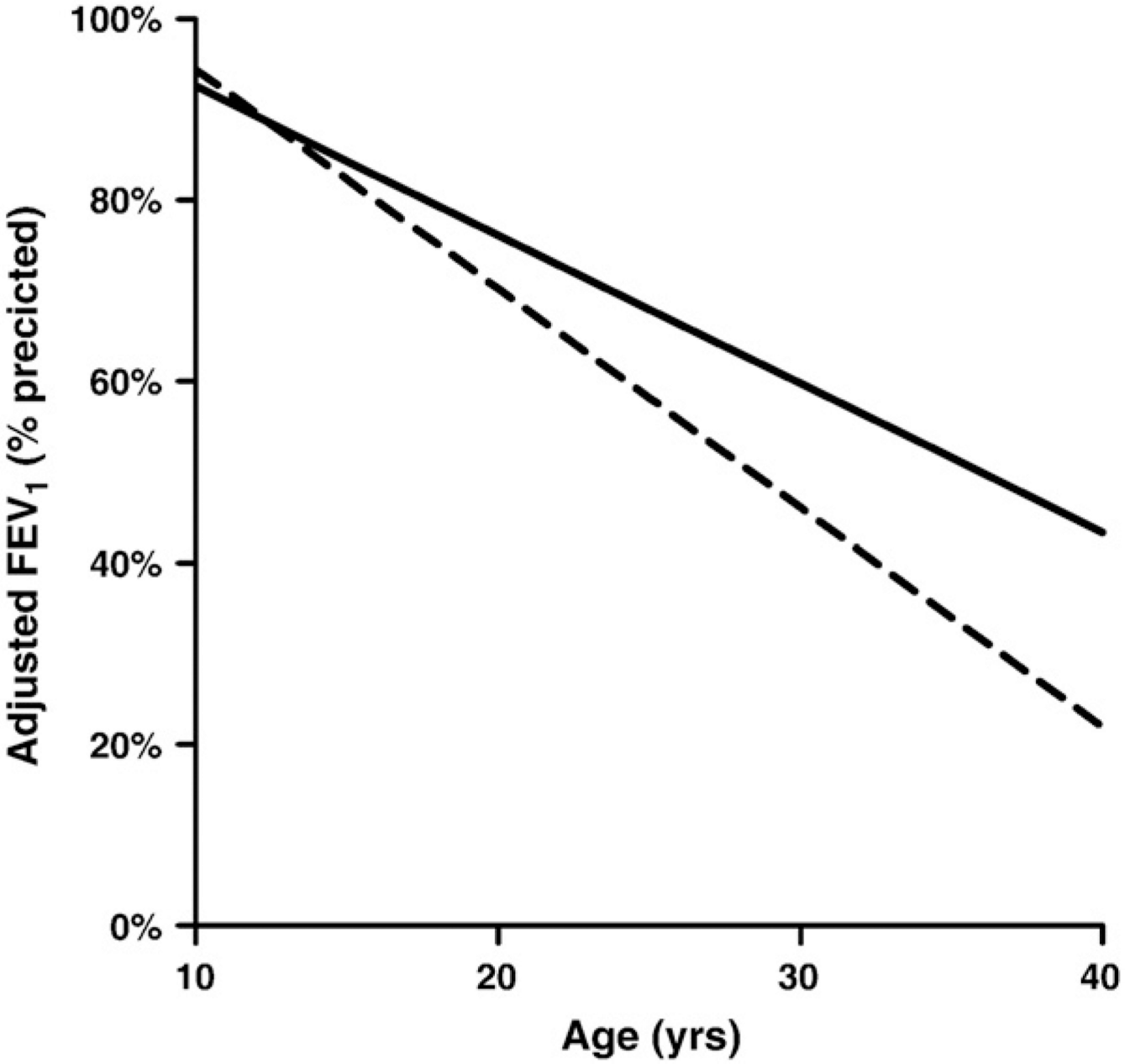


**Fig. 4.**

Model of changes in FEV1 % predicted over time in chronic NTM infection, adjusted for confounders. Using the data from Table 2, a model of lung function decline over time was generated for groups of patients with no NTM infection (solid line) and chronic *M. abscessus* infection (dashed line) after adjustment for potential confounders. This model demonstrates the worsening decline in lung function over time in the chronic *M. abscessus* infection group.

## Table 1

Characteristics of the longitudinal study cohort ($n$ = 271).

| Characteristic* | No NTM infection | Chronic NTM infection | Chronic *M. abscessus* infection |
|---|---|---|---|
| | ***n*=178** | ***n*=38** | ***n*=23** |
| Age, years | 18.8±11.5 | 17.1±11.5 | 16.4±10.4 |
| Gender, female | 93 (52.2) | 11 (28.9) ** | 8 (34.8) |
| $FEV_1$, % predicted | 76.7±27.6 | 79.4±24.1 | 79.4±21.4 |
| Pancreatic insufficient (%) | 170 (95.5) | 36 (94.7) | 21 (91.3) |
| Chronic *Pseudomonas* (%) | 154 (86.5) | 32 (84.2) | 18 (78.3) |
| Nutritional failure (%) | 40 (22.5) | 13 (24.2) | 10 (43.5) ** |
| ABPA (%) | 18 (10.7) | 4 (10.5) | 3 (13.6) |
| CFRD (%) | 43 (24.2) | 12 (31.5) | 7 (30.4) |
| Years in study | 5.9±2.0 | 6.2±1.9 | 6.2±2.0 |

NTM, nontuberculous mycobacteria; ABPA, allergic bronchopulmonary aspergillosis; CFRD, cystic fibrosis related diabetes.

* Characteristics were defined as described in the Section 2. Note that ABPA (%) included data from only 168 No NTM infection and 21 chronic *M. abscessus* infection patients.

** $p<0.05$ vs. no NTM infection by Chi-squared analysis.

## Table 2

Impact of chronic NTM infection on lung function changes over time, adjusted for confounders.

| Characteristic | Coefficient | 95% CI | *p* value |
|---|---|---|---|
| Age, years | −1.64 | −1.87 to −1.41 | <.0001 |
| Chronic NTM infection * age, years * | | | 0.0284 |
| Age * chronic *M. abs* infection | −0.78 | −1.43 to −0.12 | |
| Age * chronic NTM infection, no *M. abs* | −0.57 | −1.31 to 0.17 | |
| Infection status ** | | | 0.0372 |
| Chronic *M. abs* infection | 9.72 | −1.14 to 20.58 | |
| Chronic NTM infection, no *M. abs* | 13.64 | 0.73 to 26.55 | |
| Gender, female | 5.59 | 0.42 to 10.75 | 0.0340 |
| Pancreatic insufficient | −10.82 | −28.33 to 6.68 | 0.2251 |
| Chronic *Pseudomonas* | −9.69 | −16.92 to −2.46 | 0.0087 |
| Nutritional failure | −13.55 | −19.79 to −7.31 | <.0001 |
| ABPA | −3.73 | −11.65 to 4.20 | 0.3561 |
| CFRD | −10.54 | −17.22 to −3.86 | 0.0020 |

NTM, nontuberculous mycobacteria; *M. abs*, *M. abscessus*; ABPA, allergic bronchopulmonary aspergillosis; CFRD, cystic fibrosis related diabetes.

Characteristics are defined as in Table 1.

* Reference group = age * no infection.

** Reference group = no infection.