Original Article

High incidence of non-tuberculous mycobacteria-positive cultures among adolescent with cystic fibrosis

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Abstract

Background: We evaluated the prevalence of non-tuberculous mycobacteria (NTM)-positive cultures among our cystic fibrosis (CF) center patients, reviewed risk factors for NTM positivity, and determined its impact on lung function evolution.
Methods: From 2009 to 2014, CF adults and children attending the CF center of Lyon (France) and having at least one positive NTM isolate were included. Each case was matched by age and gender with two CF patients with no NTM isolate (controls).
Results: 48 CF patients with NTM-positive isolates were matched to 96 controls. The age group for whom incident NTM was higher was young adolescents aged 13 to 17. A significant association for NTM positivity was found with Staphylococcus aureus in multivariate analysis and with allergic bronchopulmonary aspergillosis, corticosteroid and itraconazole in univariate analysis. Mean annual FEV1 decline was faster for NTM-positive patients compared to controls.
Conclusion: These data highlight the high incidence of NTM-positive cultures among young adolescents with CF.

Keywords: Cystic fibrosis; Mycobacterium avium; Mycobacterium abscessus; Non-tuberculous mycobacteria; Pulmonary disease

1. Introduction

Among individuals with cystic fibrosis (CF), non-tuberculous mycobacteria pulmonary disease (NTM-PD) has emerged as a major threat [1]. In response, the United States CF Foundation and the European Cystic Fibrosis Society (ECFS) have updated guidelines for NTM-PD screening, diagnosis and treatment [2]. Still, the situation of NTM-positive cultures without PD remains challenging; its clinical impact and identifying indicators for initiating treatment have not been resolved.

The prevalence of NTM isolation from sputum within the CF population is rising [3] due to increasing survival of patients and better NTM recognition [4]. Recent multicenter studies have estimated NTM prevalence at 13% in USA [5] and 6.6% in France [6]. The main pathogenic species of NTM are Mycobacterium abscessus (MABSC) (including Mycobacterium abscessus subsp. abscessus and Mycobacterium abscessus subsp. abscessus).
bolletii) and Mycobacterium avium complex (MAC) (including Mycobacterium avium, Mycobacterium intracellularum and Mycobacterium chimaera) [7].

In the literature, associations with NTM positivity have already been highlighted: Staphylococcus aureus [5,8,9] and Stenotrophomonas colonization [10,11], as well as Aspergillus [3,8,10–12] and allergic bronchopulmonary aspergillosis (ABPA) [3]. Limited data are available concerning concomitant treatments such as steroids or antifungals. Only one study identified steroids as a risk factor for NTM acquisition [13]. Azithromycin was described both as a predisposing [14] and protective factor [8,11].

The impact of NTM positivity on the clinical course of CF has been evaluated in several studies but remains controversial [10,15–19].

The aim of our case–control study was first to evaluate the prevalence of NTM-positive cultures among a large cohort of both pediatric and adult French CF patients, and second, to review the risk factors associated with NTM-positive cultures and determine the impact of NTM identification on lung function evolution.

2. Materials and methods

2.1. Selection of cases and controls

We performed a longitudinal, retrospective case–control study in the CF center of Lyon that included a pediatric (n = 297) and an adult (n = 350) cohort. Diagnosis of CF was made by sweat test and genotype determination. Lyon is located at the south est of France. This CF center is one of the biggest French CF centers, following respectively around 10% and 15% of the adult and pediatric French CF population. All patients with regular follow-up at our CF center between 2009 and 2014 were included. The patients were routinely screened at least once a year for NTM as soon as they were able to expectorate. Transplanted CF patients before NTM identification were excluded. Cases had at least one isolate of NTM-positive culture, and all species and subspecies of NTM were considered. Cases were classified as transient (unique isolate of NTM-positive culture, and all species and subspecies of NTM were identified by hsp65 sequencing as previously described [21]. Antimicrobial susceptibility tests were performed with a Sensititre™ Mycobacteria plate (Trek diagnostic Systems) and interpreted according to CLSI recommendations [22].

2.2. Microbiological analysis of NTM specimens

For analysis of NTM specimens, we followed the recommendation of the French microbiology society [20]. Non-mycobacterial isolates were identified by Vitek-MS (bioMerieux). Acid-fast bacillus (AFB) smears were stained with acidine fluorescent dye and/or by using the Ziehl-Neelsen method. All NTM isolates were identified by hsp65 sequencing as previously described [21]. Antimicrobial susceptibility tests were performed with a Sensititre™ Mycobacteria plate (Trek diagnostic Systems) and interpreted according to CLSI recommendations [22].

2.3. Data collection

Using our local database, clinical data including age, gender, genotype, BMI, exocrine pancreatic insufficiency, CFTR-related diabetes (CFRD), ABPA (defined by clinic deterioration associated with elevated total or Aspergillus-specific serum immunoglobulin E levels or precipitating antibodies to Aspergillus in the serum [23]), hospitalization in the last 5 years, and treatment received in the 12 months preceding the index date (azithromycin, minocycline, oral corticosteroid therapy, non-steroid anti-inflammatory, oritraconazole) were obtained at the index date of first NTM identification for cases and controls. For cases whose NTM was identified before 2009, we traced back the time of the first identification.

Chronic colonization (defined by the presence of the pathogen microorganism in at least 3 sputum during the year preceding NTM identification) with S. aureus (SA methicillin-susceptible and methicillin-resistant), Pseudomonas aeruginosa, Haemophilus spp., Stenotrophomonas maltophilia, Burkholderia spp., and Aspergillus spp. were described. Data concerning NTM (species, presence of AFB) were also obtained.

Cases and controls were followed over a period of four years. We obtained the best FEV1 per year for all patients from the year preceding the index date until the end of follow-up.

2.4. Statistical analysis

Continuous variables were reported as the mean (with standard deviation, SD) and were compared using parametric t-test or non-parametric Mann–Whitney–Wilcoxon’s test to account for non-normality of the variables. Categorical variables were reported as count and percentage and were compared using Chi-squared or Fischer’s exact test as appropriate. Multivariate conditional logistic regression was used to examine which independent factors were associated with NTM-positive cultures. All variables with a p-value less than 0.20 in a univariate analysis were entered into the models following a stepwise backwards approach by removing non-significant variables at each step until the final model was reached. p < 0.05 was considered to have statistical significance. Analyses were performed using the SPSS program for windows (SPSS Inc. Released 2008, SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.).

3. Results

3.1. Prevalence of NTM-positive culture and description of cases

Among 401 CF patients followed in our center that had NTM screening at least once a year between 2009 and 2014, 48 had NTM-positive cultures (12%). MAC made up the majority (27 cases, 56.3%), and MABSC was isolated in 37.5% of cases (18 cases). Three patients were positive for other mycobacteria:
Mycobacterium gordonae (only one positive culture so we could not exclude a lab contamination), Mycobacterium lentiflavum and Mycobacterium paraffinicum. Six patients were positive for multiple NTM species: four had different subspecies of the same NTM complex (3 of MABSC, 1 of MAC), one had a MABSC and M. gordonae, and one had several samples with MABSC and only one with MAC. In these six cases, the first NTM identified was predominant, and the patient was classified in this NTM group.

NTM-positive individuals were 58.3% men. The mean age was 18.9 ± 7.4 years, and 28 NTM-positive cases (58.3%) were less than 18 years old. The number of NTM cases generally increased over time; we observed 2, 5, 8, 10, 9, and 9 cases in 2009, 2010, 2011, 2012, 2013 and 2014, respectively. Our youngest case was 8 at the index date, and the oldest was 35. The age group for whom incident NTM was higher was young adolescents aged 13 to 17 (Fig. 1). MABSC was predominant in children (67%; 12/18 patients) and the mean age was 17.1 years, while MAC was abundant equally in children (14/27 patients) and adults (13/27 patients) and the mean age was 20.2 years. However, a large part of MAC-positive cases (51.9%) acquired their NTM before 18 years.

Fifteen out of 48 NTM cases (31.3%) were defined as NTM-PD, 18/48 (37.5%) as persistent infection (more than one positive sample), and 15/48 patients (31.3%) as transient infection (only one positive sample). Among the NTM cases in children, 10 out of 28 (35.7%) were classified as NTM-PD, 13/28 (46.4%) as persistent infection, and 5/28 (17.9%) as transient infection. Only 13 patients within the 48 cases (27.1%) had detection of acid-fast bacillus at least in one sample: 9 with NTM-PD and 4 with persistent infection. In our study, MAC cases had more NTM-PD than MABSC, but this difference was not significant (37.3% vs 27.8%, p = 0.74), whereas in most studies NTM-PD concerned more patients with MABSC [3,8,24].

Among the 15 NTM-PD cases, seven patients had a low FEV1 at diagnosis, four had fevers, and two had hemoptysis. One patient had few symptoms but received appropriate treatment to obtain eradication before lung transplantation.

The 15 individuals with NTM-PD received a specific NTM treatment. Nine obtained durable remission (NTM eradication and clinical improvement), two were still under treatment at the end of the study, and three were still colonized after adequate treatment. The last case was transferred to another CF center. One patient underwent a surgical lobectomy to complete NTM eradication. Due to major clinical deterioration, one patient received a pulmonary transplant while he was still under NTM-specific treatment. Treatment initiation was delayed from 1 to 36 months followed by a duration of 6 months to 9 years. Delay for negative conversion of culture ranged from one month to 6 years.

At the time of the last data collection, all the NTM cases were still alive but seven of them received lung transplantation (four with NTM-PD and three with transient colonization).

3.2. Population characteristics

A total of 96 controls matched for age and sex at the index date to the 48 cases were included. The number of NTM samples cultured in controls was 4.7 ± 2.8 during the study period. Population characteristics are described in Table 1. No differences in age, sex, genotype, CFRD, pancreatic insufficiency or FEV1 were observed at the index date between the two groups. There were two patients colonized with methicillin-resistant SA out of 43 SA in NTM cases (4.7%) and 6 out of 65 SA in controls (9.2%). BMI was lower at inclusion in the NTM cases group than in the controls (p = 0.034).

3.3. Risk factors associated with NTM positivity

In univariate analysis, a significant association was found between NTM and S. aureus, showing a fourfold increased risk of NTM positivity (OR 4.1, 95% CI [1.47–11.38], p = 0.007). We observed a significant association between ABPA, corticosteroid, itraconazole and NTM positivity (OR 3.2, 95% CI [1.28–6.69]; p = 0.011; OR 9.4, 95% CI [1.91–46.24]; p = 0.006; OR 3.8, 95% CI [1.36–10.51]; p = 0.011; respectively). We found no significant association between azithromycin (prior to NTM infection) and NTM. Cases had been treated by azithromycin in 47.9% of NTM patients versus 52.1% for controls (p = 0.479). Mean exposure time to azithromycin was the same in both groups (2.4 years). We also found an association with hospitalization...
within the last five years (OR 2.17, 95% CI [1.07–4.41]; p = 0.031). Considering subgroups analysis by NTM species (MAC and MABSC), no other significant risk factors were identified.

After logistic regression, only *S. aureus* (OR 5.85, 95% CI [1.79–19.08]; p = 0.003) remained an independent risk factor associated with NTM.

### 3.4. Comparison of lung function evolution between the two groups

FEV₁ evolution obtained between the year preceding the index date until two years after NTM identification was compared between the two groups. FEV₁ remained stable in controls during all follow-up. We observed an FEV₁ decrease in cases in the year after NTM identification. Global FEV₁ decline was faster in cases in comparison to controls. Mean annual FEV₁ decline in cases was $-1.68 \pm 1.77$ versus $-0.58 \pm 1.77$ in controls (p = 0.047) (Fig. 2). Due to small subgroups sample size, no comparison of lung function degradation between MAC and MABSC was analyzed.

### 4. Discussion

The prevalence of NTM-positive cultures among patients in our center who were annually screened for NTM was 12%. Our data are in accordance with different studies that reported equivalent prevalence of NTM positivity [5,25,26]. The multi-center French study (that included patients of Lyon but not the patients of our study as it was a prospective study realized in 2004) published by Roux et al. [6] found a prevalence of 6.6%.

Incidence of NTM positivity in our population increased over time, as described by Bar-On [3]. Potential explanations for this included improved microbiological detection (double decontamination by oxalic acid [4,27]) and enhanced surveillance. But there is also a true rise in the frequency of NTM infection, with these possible reasons: increases in environmental exposure (shower aerosol), increased antibiotic usage and greater chronic use of medications [2].

We reported a higher number of MAC (56%) than MABSC (38%). These data are usually observed in North American CF populations [5,10,11] but not in European ones [8,24,25,28–30]. Cases with MAC had more NTM-PD than MABSC (37.3 vs 27.8%).

Whereas some studies reported a lower prevalence of NTM in children less than 15 years old [28,30], we observed that the prevalence of NTM-positive cultures was the highest in young adolescents aged from 13 to 17. This could be linked to poor disinfection of home nebulizers [31].

![Fig. 2. Evolution of FEV₁ from one year before to two years after NTM isolation.](https://example.com/fig2.png)
In accordance with Catherinot’s et al. study [24], we found that MAC-positive patients were older than MABSC-positive patients. However, a large part of MAC-positive cases (52%) acquired their NTM before 18 years and our youngest patients had MAC, whereas Catherinot found that MAC was only very rarely isolated from patients younger than 10 years of age and that nearly 75% of MAC-positive patients were 16 years old or younger. Our data were similar to other studies reporting higher MABSC positivity frequency in children [12,25,29], with 67% of MABSC and 52% of MAC patients identified as children.

We confirmed a risk associated with NTM positivity for S. aureus colonization [5,8,9], ABPA [3], corticosteroid treatment [13] and itraconazole treatment as described in other studies. Itraconazole was not previously described as a risk factor but was likely linked to ABPA as were corticosteroids. Intra-venous antibiotherapy was already described as having an association with NTM [24]. We found neither protective nor predisposing effects of azithromycin on NTM. The impact of this treatment is questioned in most studies [3,9,10,24].

We confirmed a slight clinical impact on pulmonary function of NTM identification [10]. This may be due to inclusion of all NTM-positive cases, without excluding transient infection. Although only NTM-PD cases received specific treatment, this category may have had higher FEV1 decline. Analysis of transient, persistent infection and NTM-PD in the same time could under estimate the degradation in lung function by two mechanisms: transient infected patients may have a slight lung function degradation than others, and due to better recognition and treatment burden NTM-PD may also have slighter impact of NTM on their lung function. The potentially poor clinical impact of NTM positivity without PD challenged the necessity of early treatment in this situation, but more data are necessary.

The present study was performed in a single CF center with homogeneous practices. Individual matching of each case with two controls reduced potential bias related to control selection. Cases and controls were annually checked for NTM, as recommended by recent guidelines. Moreover, we obtained a three-year period of follow-up for FEV1. But this study is subjected to several limitations. The first is the number of cases NTM, due to the monocentric characteristic of the study. The deterioration of lung function may not be immediate at NTM acquisition, and a three-year period of follow-up may be too short to observe any difference. Then, we analyze simultaneously patients with NTM-PD, with persistent and transient infection, which could underestimate the degradation of lung function since transient may have a slight degradation than others. It would have been also interesting to compare the slope of degradation of pulmonary function three years before NTM identification and three years after but, confusing factors other than NTM may have participated to lung function degradation before NTM (diabetes, ABPA ...) and the degradation may not be due to NTM.

In conclusion, our study brought to light a high incidence of NTM-positive cultures among young adolescents with CF. Moreover, MAC concerned more children and was responsive of more NTM-PD than in previous studies.

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**Conflicts of interest**

None of the authors have any relevant conflicts of interest to disclose.

**References**


