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Original Article

Reduced risk of nontuberculous mycobacteria in cystic fibrosis adults receiving long-term azithromycin

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Abstract

Background: Azithromycin reduces exacerbations in cystic fibrosis (CF) patients. Our aim was to investigate its association with nontuberculous mycobacteria isolation and macrolide susceptibility.
Methods: From 2006 to 2010, all adult CF subjects at Cochin Hospital (Paris, France) harboring at least one positive NTM isolate were identified (Cases). In a nested case–control study, each Case was individually matched for age and gender with up to 4 CF adults with no NTM isolate (Controls). Clinical data at the time of first NTM isolate (index date) in Cases were compared with those of Controls using multivariate conditional regression analysis.
Results: CF subjects with positive NTM isolates (Cases, $n = 41$) were matched to 155 Controls. Among Cases, 48.7% had isolates from *Mycobacterium avium* complex and 58.5% from *Mycobacterium abscessus* complex, and 31 Cases fulfilled the 2007 American Thoracic Society criteria for NTM infection (ATS+ Cases). Cases and ATS+ Cases were more likely to have low body mass index and colonization with *Aspergillus fumigatus*. Azithromycin was associated with a two-fold reduction in NTM isolates. Only one *M. avium* complex isolate had acquired macrolide resistance.
Conclusion: These data suggest that azithromycin is a primary prophylaxis for NTM infection in CF adults.

Keywords: Cystic fibrosis; Nontuberculous mycobacteria; Risk factors; Macrolides; *Aspergillus fumigatus*

1. Introduction

Nontuberculous mycobacteria (NTM) have emerged as recognized pathogens in patients with cystic fibrosis (CF) [1]. NTM have been isolated from the respiratory tract of CF patients with prevalence values ranging from 5% to 20% [1–4]. Although an initial large scale epidemiological study did not demonstrate an association between NTM infections and worsening of lung function [5], it was subsequently reported that NTM could cause significant morbidity and mortality in CF patients [6–8]. A recent single center analysis has further suggested that NTM infection, especially with *Mycobacterium abscessus*, was associated with increased rate of decline in FEV_1 [9]. These findings justify the growing interest in developing preventive and therapeutic interventions for NTM in CF patients.

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Several risk factors associated with isolation of NTM have been identified in CF subjects. NTM-positive sputum cultures were found more frequently in adolescents and in adult subjects than in young children [1,2,10], and *Aspergillus fumigatus* colonization was associated with NTM isolates in several studies [9–11]. An important unanswered question lies in the poorly understood interference of treatments used for CF lung disease with NTM. For example, Mussaffi et al. reported that systemic steroids, which were often used for the treatment of allergic bronchopulmonary aspergillosis (ABPA), were associated with severe NTM disease in CF subjects [11]. However, very little data exist on the impact of other potentially important therapies (e.g., inhaled steroids, long-term macrolides, inhaled and systemic antibiotics) on the incidence of NTM. Further, azithromycin may also have an impact on the incidence of macrolide-resistant NTM whether resistance is natural such as the one conferred by *erm(41)* gene in *M. abscessus* complex [12] or whether resistance is acquired such as the one conferred by *rrl* gene mutations in either *M. abscessus complex* and *Mycobacterium avium complex* [13].

In the present study, our goals were to examine the impact of the various drugs used in CF adults on NTM incidence and their macrolide susceptibility. We performed a case–control study, comparing adult CF patients who had NTM-positive isolates to individually matched CF patients who had no positive NTM sputum culture. Our results suggested that long-term azithromycin therapy was associated with a reduction in the incidence of NTM isolates.

2. Methods

2.1. Selection of cases and controls

The Pulmonary Department at Cochin University Hospital (Paris, France) hosts an accredited CF adult center. The CF population followed in our center comprised 311 patients in 2006 and progressively increased to 347 patients at the end of the study period (May 2010). Patients followed at our center were routinely (at least once a year and often more frequently) screened for NTM. From January 2006 to May 2010, all consecutive CF adults followed in this center and who had at least one respiratory sample positive for NTM (Cases) were identified. Four patients with positive samples for *Mycobacterium gordonae*, which is generally considered a contaminant and not usually a clinically relevant cause of lung disease [14], were excluded from the analyses. Because NTM can be isolated due to environmental contamination, more than one culture-positive specimen for NTM is considered necessary to establish diagnosis of NTM infection [14]. The 2007 American Thoracic Society (ATS) guidelines for the diagnosis of NTM lung disease provide with compatible clinical, radiographic and bacteriologic criteria for NTM infection (i) 2 positive sputum cultures or 1 positive culture obtained through bronchial wash, lavage or lung biopsy; or (ii) at least 1 sputum or bronchial washing culture positive for NTM and evident mycobacterial histopathological features [14]. Thus, our study identified both subjects who fulfilled the 2007 ATS criteria (ATS + Cases) and those who did not (ATS − Cases).

The Control group was composed of CF subjects followed in the same institution. For each Case, we selected up to 4 Controls who had no positive respiratory sample for NTM. Controls were individually matched with Cases for gender and closest age (maximum age difference: ±3 years) at the time of first NTM identification in the index case. All patients who were identified as Cases had previously documented negative cultures and were considered incident Cases at index date.

The study conformed to the Declaration of Helsinki and was approved by the Institutional Review Board of the French learned society for respiratory medicine—Société de Pneumologie de Langue Française (approval #, 2012/013). Information was provided to patients, but written consent was not required due to the observational design of this study in accordance with French laws.

2.2. Data collection

CF was diagnosed on the basis of clinical manifestations with a sweat chloride concentration exceeding 60 mM/L and/or two disease-causing mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene [15]. Using our local database, we collected clinical and microbiological data that were obtained at the time of first positive respiratory sample for NTM (index date) in Cases and in their Controls. Clinical data included age, gender, age at CF diagnosis, *CFTR* genotype, body mass index (BMI), exocrine pancreatic insufficiency, diabetes mellitus, liver cirrhosis and pulmonary function tests (forced expiratory volume in one second (FEV_1, % predicted), forced vital capacity (FVC, % predicted)). Data on treatment (long-term oxygen therapy, azithromycin use, inhaled antibiotic therapy, inhaled or oral corticosteroid therapy, intravenous antibiotic therapy, and quinolones) in the 12 months before the index date were also obtained. Chronic colonization with major Gram negative (*Pseudomonas aeruginosa*, *Burkholderia cepacia*, or *Haemophilus influenza*) or Gram positive (methicillin-susceptible *Staphylococcus aureus* or methicillin-resistant *S. aureus*) bacteria found in CF patients were recorded. Chronic *A. fumigatus* colonization (defined as three or more positive respiratory samples in the past two years) and a history of ABPA were also recorded.

2.3. Processing of sputum samples for identification of NTM

Sputum samples were successively processed with N-acetylcysteine/NaOH (BBL MycoPrep, BD, Franklin Lakes, NJ) and 5% oxalic acid, as previously described [16]. Before inoculation onto growing media, vancomycin, tobramycin, colistin and amphotericin B were added to each specimen at final concentrations of 2.5 mg/L, 4.0 mg/L, 4.10^5 UI/L and 2.5 mg/L, respectively. Specimens were inoculated onto two Coletsos (Bio-Rad, Marne-la-Coquette, France) and one Lowenstein–Jensen (LJ) slants, and one mycobacteria growth indicator tube (BBL MGIT, BD). Coletsos and LJ media were supplemented with vancomycin and amphotericin B (0.8 mg/L and 3 mg/L final concentration, respectively), and the MGIT medium was supplemented with Middlebrook OADC enrichment, as well as with polymyxin B, amphotericin B, nalidixic acid, trimethoprim,