Journal of Cystic Fibrosis 16 (2017) 579 – 584

www.elsevier.com/locate/jcf

Original Article

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

Zoé Cavalli [a,b], Quitterie Reynaud [a,b,c,*], Romain Bricca [a,b], Raphaële Nove-Josserand [a], Stéphane Durupt [a], Philippe Reix [d,e], Marie Perceval [a], Michèle Pérouse de Montclos [b,f], Gérard Lina [b,f], Isabelle Durieu [a,b,c]

[a] Cystic Fibrosis Adult Care Center, Department of Internal Medicine, Hospices Civils de Lyon, Lyon, France
[b] Université Claude Bernard Lyon 1, France
[c] EA HESPER 7425, Université Claude Bernard Lyon 1, France
[d] Cystic Fibrosis Children Care Center, Department of Pediatric Pneumology, Hospices Civils de Lyon, Lyon, France
[e] UMR 5558 (EMET), Université Claude Bernard Lyon 1, France
[f] Department of Bacteriology, Hospices Civils de Lyon, Lyon, France

Received 23 August 2016; revised 11 November 2016; accepted 30 January 2017
Available online 12 February 2017

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.

* Corresponding author at: Centre de référence de la mucoviscidose, Département de Médecine Interne, Hospices Civils de Lyon, Centre Hospitalier Lyon Sud, 69495 Pierre-Bénite, France.
E-mail address: quitterie.reynaud@chu-lyon.fr (Q. Reynaud).

http://dx.doi.org/10.1016/j.jcf.2017.01.017
1569-1993/

bolletii) and *Mycobacterium avium complex* (MAC) (including *Mycobacterium avium*, *Mycobacterium intracellulare* 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 during the study period), persistent (at least two isolates with the same NTM without clinical or radiological sign of NTM-PD during the study period), or NTM-PD (presence of NTM-PD clinical, radiological and bacteriological criteria, according to ATS guidelines) retrospectively [2,7]. All NTM-persistent cases had a chest radiography.

For each case, we selected two controls with no history of positive NTM sputum, matched for age and gender at the time of the first isolation (maximum age difference: ±2 years).

This study was approved by our local Institutional Review Board.

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 acridine 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, or itraconazole) 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: