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Original Article

Increasing nontuberculous mycobacteria infection in cystic fibrosis

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Abstract

Background: Nontuberculous mycobacteria (NTM) are emerging infections in the CF population.
Aims: To assess NTM infection prevalence and associated features in our CF clinic population.
Methods: Patient records, 2002–2011, were reviewed for NTM infection. FEV_1, pancreatic function, sputum microbiology, and serum cytokines were compared in patients with and without NTM infection.
Results: Incidence rate of NTM infection increased from 0 in 2002 to 8.7% in 2011 ($p < 0.001$). NTM infection prevalence increased 3-fold from 5% (4/79) in 2003 to 14.5% (16/110) in 2011 ($p = 0.05$). Prevalence of chronic NTM lung disease has decreased somewhat since a peak in 2009, with institution of aggressive triple therapy. Of NTM-infected compared to uninfected patients, 88.2% vs. 60.3% had a known 'severe' CFTR genotype ($p = 0.04$), 88.2% vs. 58.9% were pancreatic insufficient ($p = 0.02$); 70.6% vs. 43.8% had chronic *Pseudomonas aeruginosa* ($p = 0.06$); 75% vs. 32% had *Aspergillus* infection ($p = 0.007$) and 23.5% vs 2.7% had allergic bronchopulmonary aspergillosis ($p = 0.01$). Patients infected with *Mycobacterium abscessus* had increased TGF-β, TNF-α, IL-1β, IL-2, IL-4 and IL-5 levels ($p < 0.05$). There was no difference in cytokine levels for all NTM infected compared to uninfected patients. *M. abscessus* comprised 46% of all NTM infections. Comparing *M. abscessus* versus other NTM, duration was 10.5 (1–118) months versus 1 (1–70) month, median (range) ($p = 0.004$); lung disease occurred in 69% versus 17% ($p = 0.0004$), with sputum conversion in 4/11 versus 5/6, respectively (NS).
Conclusions: NTM incidence and prevalence have increased dramatically in our CF clinic, associated with a severe CF genotype and phenotype. *M. abscessus*, the most prevalent NTM, caused prolonged infection despite therapy. There has been some decrease in the prevalence of NTM lung disease since 2009.

Keywords: Cystic fibrosis; Nontuberculous mycobacteria; *Mycobacterium abscessus*; Allergic bronchopulmonary aspergillosis

1. Introduction

As survival in cystic fibrosis (CF) increases, the emergence of new and resistant bacterial infections, including nontuberculous mycobacteria (NTM) is an increasing concern [1]. NTM infection was first described in CF patients in the 1980s [2,3] but was considered rare and of unknown pathogenicity. In the 1990s an increasing number of CF centers reported NTM infection, with various single-site studies describing about 1300 CF patients, and an estimated NTM prevalence of 2–28% [4]. In 2002, a cross-sectional multi-center study of CF patients in the United States, reported an overall NTM prevalence of 13% [4], but no data was given regarding changing prevalence over time.

Indeed, the prevalence of NTM as a cause of significant pulmonary disease has been increasing globally [5]. Although variable, prevalence within the CF population is also rising and could be associated with increasing survival as well as prolonged antibiotic therapy [6–8]. In addition, the infection may be

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diagnosed more often due to increasing awareness by both clinicians and microbiologists of the consequences of NTM lung disease. A previous study of NTM in Israeli CF patients showed that the prevalence was highest (up to 29%) in the center and south of the country and in the coastal cities where the weather is hot and humid, rather than the dry mountains of Jerusalem [9].

Species diversification of NTM within the CF population appears to vary with geographical distribution. In the United States, *Mycobacterium avium* complex (MAC), followed by *Mycobacterium kansasii* and *Mycobacterium abscessus* are the most frequently recognized pulmonary pathogens [4]. In Europe, however, *M. abscessus* appears to be the major pathogen in CF [6].

NTM are ubiquitous and are readily recovered from environmental sources, such as soil, water, plants and animals. Tap-water is considered the major reservoir for most NTM species pathogenic to humans, and bacteria can be isolated from the solid–liquid interface biofilm, especially within piping systems [10]. This renders the mycobacteria less susceptible to disinfectants and antimicrobial therapy.

Although in the past NTM was not considered a major pathogen, descriptions of fulminant NTM infections, particularly with *M. abscessus*, are increasingly evident in the CF population [5,11,12]. Other NTM species are of undetermined and variable clinical importance [13]. Nosocomial spread of NTM infection in CF was previously considered unlikely [4]. Recently however, whole-genome sequencing revealed frequent transmission of multidrug resistant *M. abscessus*, subspecies *massiliense* [14]. This may have been by indirect means, and occurred despite conventional cross-infection measures.

Normal host defenses against NTM include a well-orchestrated inflammatory response. Initially, mycobacteria bind Toll-like-Receptor (TLR)2 on macrophages which produce TNF-α and IL-12, up-regulating a TH1 response and IFN-gamma production, activating NK-cells and resulting in intra-cellular mycobacteria killing. Multiple cytokines are involved, including GM-CSF, IL1β, IL2 and IL8. Deficiency of leptin as in malnutrition increases susceptibility to rapidly growing mycobacteria, as does increased IL10. Similarly, immune dysregulation, with increased TH2 or decreased TH1 response, may enhance NTM infection [15–17].

We have had the impression that NTM lung disease has been increasing steadily in our CF clinic. As a result, aggressive, prolonged triple therapy has been instituted in recent years. We therefore decided to review and analyze the incidence and prevalence of NTM infection and lung disease in the past decade, and to correlate this with demographic, clinical and immunologic patient data.

2. Methods

This was an observational, longitudinal, retrospective study conducted at a single CF center in Israel. The study was approved by the ethics committee of SCMCI, approval no. 7043 0295-12.

The study population included CF patients attending the Graub CF Center at Schneider Children's Medical Center of Israel (SCMCI) from 2002 till 2011, and diagnosed with CF according to accepted criteria [18].

As part of the routine protocol, sputum was cultured for nontuberculous mycobacteria (NTM) as well as other bacteria and fungi, every 3–6 months. In patients previously diagnosed with NTM infection, sputum was sent for NTM culture at every clinic visit (every 1–2 months). In addition, sputum culture for NTM was performed when clinical deterioration in pulmonary disease was not clearly explained by the presence of other bacteria or fungi.

3. NTM laboratory diagnostic protocol

Expectorated sputum was transferred immediately and analyzed at the mycobacteria laboratory within the Department of Microbiology, Rabin Medical Center, adjacent to SCMCI. Specimens underwent mucolysis using AlphaTec NAC-PAC-Red (N-Acetyl-Cysteine) and then decontamination using 3% NaOH for 15 min followed by addition of buffer (AlphaTec NPC67, Vancouver, Washington, USA) to neutralize the NaOH. Several drops of the resultant fluid were used for Ziehl–Neelsen (ZN) staining, and the rest was inoculated onto Loewenstein–Jensen (LJ) slanted agar (Loewenstein–Jensen + Glycerol + PACT, Heipha, Germany) and BD BACTEC MGIT incubator tubes. LJ tubes were placed in a 37 °C incubator and inspected weekly for growth till 8 weeks. MGIT tubes were placed in computerized incubators at 37 °C. Once growth was seen in either media, a repeat ZN stain was performed and further identification was performed by biochemical PCR using Mycobacteria Genotype kits (Hain Life Science, Germany).

Susceptibility testing was performed at the Mycobacterium Reference Laboratory, the Public Health Laboratory of the Ministry of Health, Abu-Kabir, Israel.

Both the clinical and the laboratory diagnostic protocol were consistent and did not change throughout the study period.

4. Patient data collection

CF patient charts were reviewed and data recorded from January 2002 to December 2011. Microbiologic data included results of NTM culture, ZN staining, species of NTM and results of culture for other bacteria and fungi.

Annual incidence rate and prevalence were assessed throughout this period as was the use of azithromycin.

In 2008 a cross-sectional assay for cytokines was performed for all CF patients aged >2 years at the Graub center, while in a stable pulmonary state. We now reviewed levels of cytokines considered to have a role in host defense against NTM (IL1β, IL2, IL-4, IL-5, IL-10, IL-12, IL-17, TNF-α, INF-γ, GM-CSF and leptin). Demographic, genetic and clinical data for all clinic patients are presented at the time of cytokine testing in 2008, including gender, age, height, weight, CFTR mutations, sweat chloride, FEV_1 (best value measured during that year), pancreatic enzyme therapy, azithromycin therapy (number of years with at least 3 months of therapy), presence of CF related diabetes treated with insulin, and 25-OH vitamin D levels and were compared between patients with at least one positive