Journal of Cystic Fibrosis 15 (2016) 366–371

www.elsevier.com/locate/jcf

Original Article

Streptococcus pneumoniae oropharyngeal colonization in children and adolescents with cystic fibrosis

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Received 5 March 2015; revised 18 May 2015; accepted 19 May 2015
Available online 3 June 2015

Abstract

Background: This study was designed to evaluate *Streptococcus pneumoniae* (*S. pneumoniae*) carriage rates in patients with cystic fibrosis (CF).
Methods: An oropharyngeal swab was obtained from 212 CF children and adolescents enrolled during routine clinical visits. DNA from swabs was analyzed by real-time polymerase chain reaction.
Results: A total of 42 (19.8%) CF patients (mean age ± standard deviation [SD], 12.0 ± 3.3 years) were colonized by *S. pneumoniae*. Carriage was more common in younger patients and tended to decline with age. Administration of systemic and/or inhaled antibiotics in the last 3 months significantly correlated with a reduced carrier state [odds ratio (OR) 0.23, 95% confidence interval (CI) 0.07–0.69, and OR 0.26, 95% CI 0.08–0.77, respectively]. Vitamin D serum levels ≥30 ng/mL were less common in carriers than that in non-carriers (OR 0.35; 95% CI 0.08–1.49). In both the vaccinated and unvaccinated subjects, serotypes 19F, 5, 4, and 9V were the most commonly carried serotypes.
Conclusions: S. pneumoniae carrier state of school-age children and adolescents with CF is more prevalent than previously thought, and pneumococcal conjugate vaccination administered in the first year of life does not reduce the risk of re-colonization in later childhood and adolescence.

Keywords: Cystic fibrosis; Pneumococcal carrier; Pneumococcal colonization; Pneumococcal conjugate vaccine; Pneumococcal vaccination; *Streptococcus pneumoniae*

1. Introduction

Cystic fibrosis (CF) is considered a clinical condition at increased risk of pneumococcal invasive disease (IPD) for which pneumococcal vaccination is recommended [1]. However, the clinical relevance of *Streptococcus pneumoniae* (*S. pneumoniae*) in CF patients as well as the pathological importance of the different pneumococcal serotypes and the preventive efficacy offered by the presently available vaccines are poorly understood. This could explain why the vaccination coverage of CF patients is frequently suboptimal [2].

Pharyngeal colonization by *S. pneumoniae* is considered the basis of pneumococcal transmission among humans and a

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http://dx.doi.org/10.1016/j.jcf.2015.05.008
1569-1993/

prerequisite for both invasive (IPD) and non-invasive pneumococcal diseases [3]. Pneumococcal conjugate vaccines significantly influenced pneumococcal colonization by the reduction of acquisition rates and density of serotypes included in each preparation [4]. The evaluation of pneumococcal carriage before and after vaccine administration has been considered an effective measure to estimate potential coverage and real efficacy of these preventive measures [5,6]. This study was designed to evaluate *S. pneumoniae* carriage rates in a group of school-age children and adolescents with CF for two reasons: 1) to gain information about the potential risk of *S. pneumoniae* infections in these patients, and 2) to evaluate the potential coverage offered by the pneumococcal conjugate vaccine including the greatest number of serotypes, the 13-valent preparation (PCV13). Moreover, because in Italy the first conjugate pneumococcal vaccines [those containing seven serotypes (PCV7)], was administered until 2008 to no more than 50% of infant population [7], our study could permit a comparison of *S. pneumoniae* carriage in vaccinated and unvaccinated subjects and evaluation of the long-term impact of PCV7 on colonization.

2. Material and methods

2.1. Enrolment of patients and swab collection

The study was conducted at four Regional Fibrotic Cystic Centers situated in Italy: Milan, (Lombardia), Verona (Veneto), Rome (Lazio), and Naples (Campania) from January 1, 2014, to June 30, 2014. The study was approved by the Ethics Committee of all the hospitals in which each center is located. Patients aged 6–17 years with documented diagnosis of CF regularly followed in each center were screened and enrolled during a routine clinic visit. Subjects with an active respiratory infection at the time of sampling, and those with a further chronic underlying disease different from CF were excluded from the study.

The children were enrolled after parental consent and subject assent had been obtained. After enrollment, clinical and laboratory data for each child collected during the previous 3 months were retrieved from the clinical records of the hospital and recorded in an electronic file specifically prepared for the study. Pneumococcal vaccination status was established by consulting the official vaccination chart issued by the Vaccination Services of each region.

The pneumococcal immunization schedule recommended by the Italian Ministry of Health for children born before 2008 included three options: 1) three doses of PCV7 in the first year of life, 2) two doses in the second year, or 3) a single dose after the second year until the fifth [8]. Children were considered fully vaccinated if one of these recommendations had been met by the time of enrolment, and not fully vaccinated if they had started but not completed the vaccination schedule. The latter group comprised only 1% of the enrolled subjects and was not compared with the groups of fully vaccinated or unvaccinated children.

The oropharyngeal samples were obtained using an ESwab kit containing a polypropylene screw-cap tube filled with 1 mL of liquid Amies medium (Brescia, Copan, Italy). The sampling was conducted by pressing the tongue downward to the floor of the mouth with a spatula and swabbing both tonsillar arches and the posterior nasopharynx, without touching the sides of the mouth. All of the swabs were immediately refrigerated at −20 °C, transported to the central laboratory within a week, and processed within 2 h from arrival.

2.2. Identification of S. pneumoniae

Bacterial genomic DNA was extracted from the samples using a NucliSENS easyMAG automated extraction system (BioMeriéux, Bagno a Ripoli, Florence, Italy), a 250 μL sample input, and a generic protocol. The DNA was analyzed for the autolysin-A-encoding gene (*lytA*) and the *wzg* (*cpsA*) gene of *S. pneumoniae* by real-time polymerase chain reaction (PCR) as previously described [9]. Each sample was tested in triplicates and was considered positive if at least two of the three tests revealed the presence of both genes. The levels of detection of the test were 16 genome copies. In order to maximize sensitivity, no internal amplification control was used in the reaction, but there was an external control. The real-time PCR negative specimens were also tested for the presence of an RNase P-encoding gene to exclude PCR inhibition and DNA extraction failure. All of the positive cases were serotyped using primers and probes designed on the basis of the GenBank database sequences (www.ncbi.nlm.nih.gov) of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (i.e., those in the 13-valent pneumococcal conjugate vaccine, PCV13), and synthesized by TIB Molbiol (Genoa, Italy) as previously described [9]. Analytical specificity was pre-evaluated by means of computer-aided analyses using Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast) and BLAST (www.blast.ncbi.nlm.nih.gov/Blast.cgi) software to compare the sequences with all of the sequences listed under bacteria and *Homo sapiens*.

2.3. Statistical analysis

The groups were compared using the X^2 or Fisher's exact test, when appropriate. The ordered categorical data were compared using a Cochran–Armitage trend test. Multivariate odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional multiple logistic regression models to measure the association between the following: i) pneumococcal vaccination and pneumococcal carrier status and ii) selected demographic and clinical characteristics and pneumococcal carrier status. Adjustment was made for a priori defined covariates such as age, gender, number of siblings, and parental smoking habits. Stratified analyses for the two major age subgroups (<10 and 10–14) were also performed. All of the analyses were two tailed, and p-values <0.05 were considered statistically significant. All analyses were conducted using the SAS version 9.2 (Cary, NC, USA).

3. Results

A total of 212 CF children and adolescents (mean age ± SD, 12.0 ± 3.3 years) were enrolled. Their demographic and