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ORIGINAL ARTICLE

Increased prevalence of *Mycoplasma pneumoniae* serological positivity in Chilean young children

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Abstract
Background: *Mycoplasma pneumoniae* is a frequent cause of respiratory infections in school children and adolescents. Epidemiological suspicion is important, since there are no specific symptoms or signs to help in diagnosing infection caused by this agent.
Objective: To determine the variation in prevalence over the last 10 years of *M. pneumoniae* IgM seropositivity according to age, particularly in pre-schoolers.
Method: The results of *M. pneumoniae* IgM serological testing between January 2004 and December 2013 were analysed. Variables such as gender and month and year of sample processing were studied according to age groups (<5, 5–18, 19–50, 51–70 and >70 years of age).
Results: Of a total of 20,020 serological samples, 31.9% proved positive for *M. pneumoniae*. All age groups showed increases in percentage seropositivity over the last 10 years, although the most significant increase corresponded to the 5–18 years group (from 15.8% to 54%), followed by children <5 years of age (from 8.6% to 30%). Seropositivity was significantly higher in women in all age groups, except in those over 50 years of age.
Conclusion: Children under five years of age were the group with the second highest increase in seropositivity.

Introduction

Mycoplasma pneumoniae is a common cause of respiratory infections in children, and is recognised as having been one of the aetiologies of atypical pneumonia over the last 50 years.[1,2] In children, *M. pneumoniae* is responsible for 10%–40% of all cases of community-acquired pneumonia.[2,3] Although the infection is mild and self-limiting, some patients of all ages can develop severe and fulminating disease. *M. pneumoniae* can also manifest with extra-pulmonary symptoms.[4] Since there are no specific clinical symptoms or signs of help in diagnosing community-acquired pneumonia caused by this agent, epidemiological suspicion

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proves very important.[5] The early diagnosis of this microorganism allows specific antimicrobial therapy and limits its spread within the community.[6]

Up until the early 1990s it was thought that *M. pneumoniae* was a pathogen that causes pneumonia in patients over five years of age. Since then, however, specialised diagnostic techniques have shown that *M. pneumoniae* may play an important role as a cause of upper and lower respiratory tract infections also in children under five years of age.[2,7] A study has shown that in the last 16 years, the average age of *M. pneumoniae* seropositivity in patients with prolonged respiratory infections has decreased from 7 years to 2.9 years.[8] The more recent outbreaks in Korea have shown a peak in the early years of life in comparison to the situation found in the past.[9] Another study has reported the peak incidence of *M. pneumoniae* pneumonia in children to be in the 4–6 years age range.[2] Furthermore, the prevalence of *M. pneumoniae* pneumonia among hospitalised children under five years of age has been found to be 17%.[10]

All these changes in the prevalence of *M. pneumoniae* should have inferences in the current treatment guidelines. However, there is little information on the relationship between this infection and age distribution in Latin America,[8,11] and no previous epidemiological studies have been made in the concrete case of Chile. A single study conducted in ambulatory clinics in Santiago (Chile) reported a 2% prevalence of *M. pneumoniae* pharyngeal carrier status in 185 asymptomatic children.[6]

The aim of this study is to determine the variation in prevalence over the last 10 years of *M. pneumoniae* IgM seropositivity according to age, particularly in preschoolers. Our working hypothesis is that seropositivity in children under five years of age has increased over the last 10 years in Santiago (Chile).

Methods

A cross-sectional study was made between 2004 and 2013 in our clinical university laboratory (UC-Christus C.M. San Joaquin, Santiago, Chile). Inclusion criteria: all samples collected for *M. pneumoniae* IgM serological testing requested by the attending physician were analysed. A browser was added with the program Query Builder (v. 6.0.7.0.0) to highlight all the results of *M. pneumoniae* IgM serological testing performed during this period. The result of such serological testing was considered to be positive based on the cut-off value used in our laboratory employing enzyme immunoassay (ELISA IgM M. pneumoniae Test System®, Zeus Scientific, Raritan, NJ, USA) testing during 2004–2008, or indirect immunofluorescence (IFI M. pneumoniae IgM IFI Antibody Test System®, Zeus Scientific, Raritan, NJ, USA) during 2009–2013. The sensitivity and specificity of these techniques were 89.1% and 92.8% for the ELISA, and 100% and 97.5%, respectively, for the IFI. The IFI technique was reported positivity with a cut-off point >1/16 dilutions, and indeterminate when the solution could not be clearly defined as either positive or negative.

The following patient parameters were analysed: age, gender, year and month of processing of the test, and the origin of the test sample (ambulatory or in-hospital). For the purposes of this study, the results were stratified according to five age groups: patients under 5 years of age, 5–18 years of age, 19–50 years of age, 51–70 years of age, and over 70 years of age.

The Ethics Committee of the Pontificia Universidad Catolica de Chile approved the study protocol (# 13-052).

Statistical analysis

In assessing the differences between positive versus negative *M. pneumoniae* serology, the chi-squared test was used for categorical variables and the Student *t*-test for continuous variables. A *p*-value of <0.05 using a two-tailed test was taken to indicate significance. Logistic regression analysis was performed to discard confounding variables in the analysis of IgM seropositivity over time. The SPSS® version 17.0 statistical package (IBM, Armonk, NY, USA) was used throughout.

Results

During the 10-year study period, a total of 20,020 *M. pneumoniae* serological tests were retrieved from the database (54.3% corresponding to tests in females). Ambulatory patients were more frequently involved (83.2%) than hospitalised patients. A total of 29.4% ($n = 5886$) of the study sample corresponded to children under the age of five years; while 35.6% ($n = 3824$) corresponded to children between 5 and 18 years of age; 19.1% ($n = 7127$) to patients between 19 and 50 years of age; 10.4% ($n = 2082$) to patients between 51 and 70 years of age; and 5.4% ($n = 1081$) to patients >70 years of age. The largest annual number of requested tests corresponded to the year 2011 with 2866 tests (13.3%) and the lowest to the year 2004 with 1481 tests (7.4%).

Of the total serological tests, 31.9% yielded a positive result ($n = 6385$), while 64.5% proved negative ($n = 12,915$) and 3.6% were indeterminate ($n = 720$). The age group with the highest percentage *M. pneumoniae* seropositivity was the 5–18 years age group (45.6%), followed by the patients under five years of age (31.4%). The lowest percentage seropositivity corresponded to patients over 70 years of age (7.4%). From the age of 18 years to the oldest patients, percentage seropositivity was found to decrease with increasing age (from 24.3% to 7.4%).

Over the 10-year study period, all age groups showed an increase in percentage seropositivity, although the two groups with the highest increments were the 5–18 years age group (from 15.8% to 54%), followed by the patients under five years of age (from 8.6% to 30%) ($p < 0.001$) (Fig. 1). Among the children under five years of age, those over two years of age showed a greater increase than those under two years of age (33% versus 17%, respectively; $p < 0.001$). The group with the lowest increment corresponded to the patients over 70 years of age.

In all the age groups women showed the highest percentage seropositivity ($p < 0.05$), except in the 51–70 years age group, where no significant gender differences were observed ($p = 0.47$) (Fig. 2).

As regards seasonal distribution in the course of the year, increased seropositivity was recorded in May, June, August, September and October. In general terms, a seropositivity