

Comparison of *Mycoplasma pneumoniae* Infections in Asthmatic Children Versus Asthmatic Adults

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Background: *Mycoplasma pneumoniae* has been implicated in asthma exacerbations and chronic asthma. A 2-year longitudinal study has been conducted to investigate the role of *M. pneumoniae* infections in 168 and 20 hospitalized children and adults, respectively, with asthma exacerbation compared with outpatients (88 children and 48 adults) with chronic asthma (without an exacerbation). The prevalence of *Chlamydia pneumoniae* and respiratory viruses was also assessed in these 2 populations.

Methods: Lung function testing, blood sampling and microbiological testing (polymerase chain reaction, culture and serology) were performed for 256 children and 68 adults followed by a 7-week, follow-up visit with repeated blood sampling for serological testing and phone interviews at 6 and 12 months later.

Results: *M. pneumoniae* infection was more prevalent in children with chronic asthma (13.6%) compared with children with exacerbation (7.1%), while the reverse was true in adults (6.3 vs. 10.0%, respectively). However, these differences were not statistically significant. Acute *C. pneumoniae* infection was identified in 3.9% of children and 7.4% adults. Children seen for chronic asthma were significantly more likely to be infected with *C. pneumoniae* than children hospitalized for an asthma exacerbation. Viruses were the most prevalent microorganisms detected in children with an asthma exacerbation. No differences in the outcome parameters were identified between *M. pneumoniae*-infected and noninfected patients.

Conclusions: The present study suggests that *M. pneumoniae* does not play a direct role in the pathogenicity of acute or chronic asthma in most children.

Key Words: asthma, exacerbation, chronic asthma, *M. pneumoniae*, *C. pneumoniae*, virus

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Asthma is a prevalent disease worldwide with a marked effect on quality of life. However, the causes and pathogenesis of this syndrome are not completely understood. A major share of the burden of asthma involves acute exacerbations. Exacerbations have been strongly associated with respiratory infections in children and

adults. Viral infections, such as rhinovirus, coronavirus, influenza, parainfluenza virus, respiratory syncytial virus and human metapneumovirus, are the most prevalent with regard to asthma exacerbations.^{1–5} Atypical bacteria, such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, have also been implicated in asthma exacerbations in many studies in both children and adults.^{1–12} However, other studies have not demonstrated an association between these 2 bacteria and asthma exacerbations.^{13–20} According to more recent studies, *M. pneumoniae* and *C. pneumoniae* might be involved in chronic asthma rather than disease exacerbations.^{1,4,21,22} *M. pneumoniae* has also been associated with severe persistent asthma in children.^{6,12}

A 2-year longitudinal observational study was conducted to investigate the role of *M. pneumoniae* infection in children and adults hospitalized for an asthma exacerbation compared with outpatients seen for chronic asthma (without an exacerbation). The prevalence of *C. pneumoniae* and respiratory virus infections in these 2 populations was also estimated.

PATIENTS AND METHODS

Study Design

Patients

The Regional Institutional Ethics Committee approved this observational multicentered cohort study. Written informed consent was obtained from the parents and all children over 6 years of age. The study was conducted from March 14, 2007, to October 30, 2010, in France at the Bordeaux University Hospital (Pediatrics: Pulmonology Unit, Emergency Unit, Intensive Care Unit (ICU); Adults: Pulmonology Unit, Emergency Unit, ICUs), the Bayonne General Hospital (Pediatrics and Pulmonology units) and the Libourne General Hospital (Pediatrics).

Preschool children <3 years of age who had experienced more than 3 wheezing episodes and children >3 years diagnosed with asthma according to the “Global Initiative for Asthma (GINA) Guidelines” were included in the study. Adults (18 to < 70 years of age) with asthma according to the American Thoracic Society were also included. Patients with X-ray evidence of congenital or anatomic lung disorders were excluded. Patients were grouped according to their asthma status. Patients with asthma exacerbation (Group 1) included individuals hospitalized for an acute asthma exacerbation in the emergency room or pulmonary and/or ICU. The chronic asthma group (Group 2) included outpatients seen for chronic asthma without an exacerbation for 1 month. In this group, only 2 children and no adults reported acute respiratory events associated with asthma during the 7 weeks after inclusion.

At inclusion, the clinical and asthma control statuses (GINA in children, Juniper score in adults)^{23,24} were assessed, and the following information was gathered: history of atopy, previous hospitalizations for asthma exacerbations, asthma medication use and active or passive smoking. Current asthma was based on reported physician-diagnosed asthma with symptoms and/or use of asthma medications within the past 12 months. Asthma diagnosis, severity

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and control were assessed according to the GINA guidelines.²³ Lung function testing, blood sampling and microbiological testing were performed as reported below. The follow-up examination included blood sampling for repeat serology testing at 7 weeks and a phone interview at 6 and 12 months to assess for residual symptoms. The outcome parameters in Group 1 patients included the GINA asthma exacerbation grade, duration of oxygen therapy and duration of hospitalization. In Group 2, the asthma severity grade, control, clinical signs and lung function were evaluated. In both groups, the residual symptoms during the following year were compared between the infected and the noninfected groups.

Lung Function Testing

Lung function testing included spirometry, plethysmography, exercise challenge and bronchodilator testing.²⁵ The baseline lung function data were expressed as percentages of the predicted values calculated using Zapletal reference equations²⁶ according to the recommendations of the European Respiratory Society 1993.²⁷

Microbiological Investigations

A baseline blood sample and a throat swab for adults and a nasopharyngeal aspirate for children were collected at inclusion for serological and microbiological testing. At the 7-week, follow-up visit, a second blood sample was collected. The initial blood samples were tested via enzyme immunoassay for IgM and IgG antibodies specific to *M. pneumoniae* and *C. pneumoniae* (Diasorin, Antony, France). These tests were repeated on a second blood sample obtained at 7-week follow up. The blood samples were stored at -20°C , and the throat swabs and nasopharyngeal aspirates were stored at -80°C .

DNA and RNA were extracted from 200 μL of throat and nasopharyngeal secretions in the Universal Transport Medium (Copan, Brescia, Italy) using the MagNA Pure LC kit I (Roche Diagnostics, Meylan, France) according to the manufacturer's instructions. A *M. pneumoniae* in-house, real-time polymerase chain reaction (PCR) reaction targeting the P1 adhesin gene²⁸ and a *C. pneumoniae* in-house, real-time PCR reaction targeting the *PMP4* gene,²⁹ both validated by the use of an internal control, were performed on the DNA extracts as previously described. Nucleic acids for the following respiratory viruses were amplified from the throat samples using the RV5 and RV15 ACE kits from Seegene (Seoul, Korea) according to the manufacturer's instructions: rhinovirus, respiratory syncytial virus, influenza A and B virus, parainfluenza virus, metapneumovirus, coronavirus, adenovirus, bocavirus and enterovirus. All the throat swabs and nasopharyngeal aspirates were grown in Hayflick modified broth and agar for *M. pneumoniae* as previously described.³⁰

Acute *M. pneumoniae* and *C. pneumoniae* infections were diagnosed when the patient showed a significant antibody response and/or the PCR of respiratory specimens was positive. The serology testing corresponding to a recent *M. pneumoniae* or *C. pneumoniae* infection was accepted when IgM antibodies were detected or a seroconversion or a 4-fold increase in IgG antibodies was identified between both sera. A diagnosis of acute viral infection was confirmed with a positive PCR result.

Statistical Analysis

The results are expressed as the medians [interquartile range, q1–q3], means (standard deviation), or n (%) as appropriate. Differences between subgroups were assessed using χ^2 tests or Fisher's exact tests for categorical variables and Student's *t*-tests or Wilcoxon test were used for continuous variables. In each clinical category subgroup of asthma and for adults and children separately, the prevalence of infections was estimated as the number

of patients with a positive result over the number of patients with available samples, and their 95% confidence intervals were calculated. Univariable logistic regression analyses were performed to explore the association of infection status (Wald test), and other factors associated with asthma exacerbation [antibiotics during the previous month, smoking status, overweight, age (<18 years/ ≥ 18 years) and personal atopy]. Variables with $P < 0.20$ in the univariable analyses were included in a full model. A multivariable model was constructed using a backward stepwise modeling procedure, including interaction terms when appropriate and retaining variables with $P \leq 0.05$. Infection status was maintained in the final model, regardless of the significance level. A $P \leq 0.05$ was considered statistically significant for all tests. All statistical analyses were performed using SAS software version 9.1 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

The study included 256 children [167 males and 89 females; median age = 4.4 years (interquartile range: 2.4; 8.4)] and 68 adults [16 males and 52 females; median age = 44.1 years (28.9; 54.3)]. Children in Group 1 acutely infected with *M. pneumoniae* compared with noninfected children were older [5.4 (3.3; 10.4) vs. 3.3 (1.5; 5.8) years], with a trend towards increased exposure to second-hand smoke [n/N (%), 8/12 (67) vs. 62/154 (40)], had a later age of onset of asthma [27 (12; 36) vs. 13 (7; 24) months] and more frequently had positive allergy tests [4/7 (67) vs. 57/122 (34)]. Among the 12 children and 2 adults infected with *M. pneumoniae* from Group 1, only 1 child visited the emergency room alone and left the hospital quickly. All others were hospitalized in Pediatrics or Pulmonology units.

Children with chronic asthma presenting with an acute *M. pneumoniae* infection were less exposed to second-hand smoke [2/12 (17) vs. 32/76 (42)] compared with noninfected children. In adults, the small number of patients infected with *M. pneumoniae* precluded such comparisons. Asthma severity, control and baseline treatments were similar across all groups.

Prevalence of *M. pneumoniae*, *C. pneumoniae* and Viral Infections

The prevalence of acute *M. pneumoniae* infection was similar in both age groups. There was a tendency for more frequent *M. pneumoniae* in Group 2 (13.6 %) compared with Group 1 (7.1 %) children, while the inverse was true in adults (6.3 vs. 10.0 %, respectively). However, these differences were not statistically significant. Infection was serologically determined in 28/29 *M. pneumoniae*-infected patients (13 seroconversions, IgM positive in 14 cases and 1 case with a significant increase in IgG titers) and confirmed through PCR in 5 patients. Only 1 infected child did not present antibodies against *M. pneumoniae*, but exhibited a positive PCR for *M. pneumoniae*. One strain of *M. pneumoniae* was obtained through culture from a hospitalized child. Among *M. pneumoniae*-infected patients, only 1 adult had received antibiotics active against this microorganism <1 month before infection (Table 1).

Acute *C. pneumoniae* infection was identified in 3.9% (10/256) of children and 7.4% (5/68) of adults (Table 1). There was a significant difference ($P = 0.006$) in *C. pneumoniae*-infected children, as children seen for chronic asthma were more likely to be infected with *C. pneumoniae* than children hospitalized for asthma exacerbation (Table 1). In adults, 2/20 (10%) Group 1 and 3/48 (6.3%) Group 2 patients were infected. Compared with *C. pneumoniae*-infected patients, there was no significant difference between

TABLE 1. Prevalence of *M. pneumoniae*, *C. pneumoniae* and Viral Infections in Children and Adults With an Asthma Exacerbation (Group 1) or Chronic Asthma (Group 2)

Microbe	All	Children	Adults
<i>M. pneumoniae</i>			
All (%)	29/324 (9.1)	24/256 (9.4)	5/68 (7.4)
Group 1 (%)	14/188 (7.4)	12/168 (7.1)	2/20 (10.0)
Group 2 (%)	15/136 (11.0)	12/88 (13.6)	3/48 (6.3)
P-value (Group 1 vs. 2)	0.27	0.096	0.59
<i>C. pneumoniae</i>			
All (%)	15/324 (4.6)	10/256 (3.9)	5/68 (7.4)
Group 1 (%)	3/188 (1.6)	1/168 (0.6)	2/20 (10.0)
Group 2 (%)	12/136 (8.8)	9/88 (10.2)	3/48 (6.3)
P-value (Group 1 vs. 2)	0.006	0.006	0.59
Viruses			
All (%)	93/304 (30.6)	90/237 (38)	3/67 (4.5)
Group 1 (%)	69/169 (40.8)	67/149 (45.0)	2/20 (10.0)
Group 2 (%)	24/135 (17.8)	23/88 (26.1)	1/47 (2.1)
P-value (Group 1 vs. 2)	<0.0001	0.004	0.19

Statistically significant differences are indicated in bold.

adults during asthma exacerbation and adults with chronic asthma (Table 1). The infection was serologically determined in 13/15 *C. pneumoniae*-infected patients (9 seroconversions and 4 cases with a significant increase in IgG titers), but no case was confirmed through PCR. However, 1 adult and 1 child seen for chronic asthma and serologically negative for *C. pneumoniae* exhibited a positive PCR result for this microorganism. None of the *C. pneumoniae*-infected patients received active antibiotics within 1 month before infection.

Viruses were the most prevalent microorganisms detected, particularly in children [38 % overall (90/237)] in Group 1 [45 % (67/149)]. There was a significant difference ($P = 0.004$) in children infected with viruses; children hospitalized for asthma exacerbation were more likely to be infected with viruses than children seen for chronic asthma (Table 1). Incidentally, the viral prevalence remained high in Group 2 children [26 % (23/88)]. Rhinovirus was detected in 70 patients (69 children and 1 adult), while respiratory syncytial virus was detected in 15 patients (15 children), influenza A was detected in 11 patients (9 children and 2 adults) and metapneumovirus and adenovirus were both detected in 2 children, separately. Additionally, the latter 4 children were coinfecting with a rhinovirus.

Among the *M. pneumoniae*-infected patients, 7 children with an asthma exacerbation (Group 1) and 2 children with chronic asthma (Group 2) demonstrated an *M. pneumoniae* and viral coinfection. No adult was coinfecting with *M. pneumoniae* and a virus. Among the *C. pneumoniae*-infected patients, 1 adult in Group 1 and 2 children in Group 2 were coinfecting with *C. pneumoniae* and viruses. Only 2 children seen for chronic asthma were coinfecting with both *M. pneumoniae* and *C. pneumoniae*.

Outcome Parameters

No significant differences were detected with respect to the severity of exacerbations, duration of hospitalization [3 (2;5) vs. 3(3;4) days - acute infections vs. none], oxygen therapy [0 (0;2) vs. 0 (0;1)], chest X-ray findings [lung consolidation n/N (%); 1/10 (10) vs. 19/145 (13)], requirement for ICU transfer or death (Group 1) or asthma severity, asthma control, lung function and recovery at 6 months [3/10 (30) vs. 68/120 (57)] and 12 months [6/10 (60) vs. 98/124 (79); Group 1] between *M. pneumoniae*-infected and noninfected children. Findings in adults were similar.

Furthermore, there was no significant difference in the prevalence of *M. pneumoniae* infection in children with controlled

asthma (5/38, 13.1%) versus partially controlled or uncontrolled asthma (7/44, 15.9%).

DISCUSSION

In this 2-year longitudinal comprehensive study, *M. pneumoniae* infection was not detected significantly more often during an asthma exacerbation than in chronic asthma in children. *C. pneumoniae* infection was significantly more frequent in children seen for chronic asthma than children hospitalized for an asthma exacerbation. For adults, prevalence of *M. pneumoniae* or *C. pneumoniae* infections was similar in both groups. An acute *M. pneumoniae* or *C. pneumoniae* infection did not modify disease morbidity during asthma exacerbations or chronic disease. Furthermore, evidence of an ongoing viral infection was detected at a significantly higher proportion in children with asthma exacerbations (45%) versus chronic asthma (26.1%).

Previous studies have reported variable prevalence rates for *M. pneumoniae* and *C. pneumoniae* infections in patients with asthma exacerbations, ranging from 2.2% to 38% in children^{1,3-6,12} and from 3.5% to 33% in adults.^{7,11,18} Most of the pediatric studies reported a low 2.5–5% rate suggesting that *M. pneumoniae* and *C. pneumoniae* play minor roles in asthma exacerbations. Other studies, conducted in India and France, reported an *M. pneumoniae* infection rate of 38% and 20%, respectively, in children experiencing asthma attacks.^{6,12} An Italian study also showed an association between atypical bacteria and wheezing (13% *M. pneumoniae* and 20% *C. pneumoniae* infections).⁸

Fewer studies have been performed in outpatients seen for chronic asthma. In this population, infection rates of 5.3–18% and 2.5–7% have been reported for *M. pneumoniae* and *C. pneumoniae* in children, respectively.^{6,32} In a study conducted in adults, a much higher rate of infection was observed, that is, 45.5% *M. pneumoniae* and 10.9% *C. pneumoniae*.²²

Two studies compared patients with asthma exacerbations to patients with chronic asthma.^{5,6} However, in these studies, either there was no control group or the control group was composed of patients without asthma. However, Biscardi et al⁶ described the second highest rate of *M. pneumoniae* infection in children with asthma exacerbations (20%) compared with the 5.3% rate observed in children with stable asthma and observed a significant association between *M. pneumoniae* infection and acute asthma exacerbations. Such an association was not described for *C. pneumoniae*.⁶ Thumerelle et al. observed much lower rates of *M. pneumoniae* or *C. pneumoniae* infections in children with acute asthma (total 10%; *M. pneumoniae* 5%, *C. pneumoniae* 5%) but did not screen for these bacteria in their control group of asymptomatic children.⁵ Surprisingly, in the present study, a significantly higher rate of *C. pneumoniae* infection was observed in children seen for chronic asthma than in children hospitalized for an asthma exacerbation. However, the number of *C. pneumoniae*-infected children in the 2 groups (10) was too low to obtain a valid interpretation.

As in other studies,¹⁻⁵ viral infections resulting from rhinoviruses were significantly more frequent in children hospitalized with an acute exacerbation than those seen in an outpatient asthma clinic. This association was not observed in adults; only 4.5% of all the adult patients were infected with respiratory viruses. Interestingly, Martin et al.²² did not observe any difference in the viral infection rates between adults with chronic stable asthma and normal control subjects. However, in contrast to our study, the specimens used in their study were obtained from the lower respiratory tract. Coinfection, including a virus and *M. pneumoniae* (37.5%) or *C. pneumoniae* (20%), was a common phenomenon. A high level of coinfections has been previously described in children.^{3,33} In adults, at least a quarter of the patients experiencing an asthma

exacerbation were coinfecting with *M. pneumoniae* and an additional pathogen, a virus in most cases.¹¹ In contrast, in our study, only 1 adult was coinfecting with a virus and an atypical bacterium.

The pathogenicity of *M. pneumoniae* in asthma is widely debated. In vitro studies in mice have shown that ovalbumin-induced allergic airway inflammation impairs TLR2 expression and host defense cytokine (eg, IL-6) production and subsequently delays lung *M. pneumoniae* clearance.³⁴ Similarly, asthmatic children showed significantly reduced plasmatic IL-18 and CXCL10 responses and had more severe pneumonia symptoms compared with nonasthmatic patients.³⁵ Biscardi et al⁶ and Thumerelle et al⁵ reported more persistent symptoms, more recurrent asthma symptoms and a reduced recovery after 3 weeks in patients infected with *M. pneumoniae*. Cosentini et al. described a more severe functional impairment in patients with atypical infections upon admission for exacerbation of asthma.⁷ However, Thumerelle et al⁵ also showed that there was no correlation between the severity of chronic asthma or asthmatic exacerbations and the diagnosis of *M. pneumoniae* or *C. pneumoniae* infection in children. Another study did not report any differences regarding the asthma control status and lung function parameters between adult chronic asthmatic patients with or without remote *M. pneumoniae* infection.³⁶ In the present study, in the acute phase of the disease, we were unable to demonstrate any significant deleterious effects of *M. pneumoniae* infection on subjective (GINA severity score) and objective parameters (chest X-ray findings, duration of oxygen therapy, ICU-requirement and hospital stay). During the subsequent 12-month, follow-up period, the recurrence of asthma symptoms and exacerbation rates were similar, regardless of the presence of an acute *M. pneumoniae* infection upon enrolment. In the chronic setting, there was no significant difference regarding clinical characteristics, asthma control, pulmonary function tests and long-term, follow-up visits regardless of *M. pneumoniae* infection status. Thus, acute *M. pneumoniae* infection does not seem to be an aggravating factor in pediatric asthma.

However, this statement has to be interpreted with caution. During recent years, in contrast to *C. pneumoniae*, significant progress has been made in the microbiological diagnosis of *M. pneumoniae* infections. Serology is the most common laboratory method used. EIAs have become the most widely used commercial methods for detection of *M. pneumoniae* and can be comparable with PCR in sensitivity whether paired sera are obtained by using an assay with appropriate sensitivity and specificity performances.^{31,37} However, serology remains a retrospective diagnosis and has significant limitations and combined use of PCR and serology may be a useful approach for diagnosis of *M. pneumoniae* respiratory infection. Indeed, PCR, which is more sensitive than serological testing for the detection of *M. pneumoniae* infection, especially in the early stages of the disease,^{38,39} has to be used in combination with serology to determine the difference between *M. pneumoniae* infection and colonization. Moreover, some infections that are well-documented by seroconversion are negative by the PCR assay. Furthermore, if antibiotics have been administered, PCR results may be negative even though serology is positive.³¹ In our study, most of the *M. pneumoniae* infections were diagnosed through serological EIAs performed on paired sera and confirmed in some patients with a real-time PCR assay validated with an internal control on a respiratory sample suitable for *M. pneumoniae* detection.²⁸ Only 1 child did not present antibodies against *M. pneumoniae*, but exhibited a positive PCR for *M. pneumoniae*. Culture led to the isolation of only 1 strain of *M. pneumoniae*, confirming the low sensitivity of this technique.²² Biscardi et al⁶ and Esposito et al⁸ also reported similar efficacy with serology compared with PCR for the diagnosis of *M. pneumoniae* infection in asthmatic patients.

Reliable diagnosis of respiratory infection due to *C. pneumoniae* and investigation of its role in chronic diseases remains difficult because of the absence of well-standardized diagnostic tests. In recent studies, the prevalence of *C. pneumoniae* respiratory infections was reported to be much lower (<1.5%) than previously described (6–22%).⁴⁰ Only 2 of 1583 patients were identified by real-time PCR with *C. pneumoniae* infection during a 10-year period.⁴¹ We reported similar low real-time PCR detection rates of *C. pneumoniae* in our study.

The presence of a viral infection might influence the outcome parameters. In our study, a viral coinfection was present in 58.3% (7/12) of Group 1 and 16.6% (2/12) of Group 2 asthmatic children infected with *M. pneumoniae*.

Another limitation of our study is the small sample size of *M. pneumoniae*-infected and particularly *C. pneumoniae*-infected patients. At the time of the study, there was no ongoing *M. pneumoniae* epidemic. Regarding the adult cohort in our study, we were not able to include a large number of patients, further limiting the interpretation of our data. Finally, although this study was observational, antibiotics active against *M. pneumoniae* and *C. pneumoniae* were administered up to 1 month before inclusion to only 5.4% (16/295) of noninfected patients, which might not have significantly reduced the impact of infection on the outcome.

In conclusion, the present study suggests that *M. pneumoniae* infections do not play a direct role in the pathogenicity of acute or chronic asthma in most children and adults; the high prevalence of viral infections, particularly rhinovirus, during asthma exacerbations in children was confirmed.

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Authors' contributions: C.B. and M.F. conceived of the study, participated in its design and coordination and drafted the article. C.R. conceived of the study, participated in its design and coordination and helped to draft the article. B.d.B. and S.P. participated in the conception of the study, its design, coordination and microbiological results analysis. F.N. participated in the design of the study, its coordination and the results analysis and helped to draft the article. G.C. conceived of the study, participated in its design and helped to draft the article. P.O.G. and S.D. participated in the design and coordination of the study. H.R. participated in the coordination of the study and microbiological results analysis. F.M. performed the statistical analysis and helped to draft the manuscript. All authors read and approved the final article.

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