

Prevalence of *Mycoplasma pneumoniae* infection in pediatric patients with acute asthma exacerbation

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ABSTRACT

Introduction. *Mycoplasma pneumoniae* may be involved in refractory asthma exacerbation.

Objective. To determine the prevalence of *Mycoplasma pneumoniae* infection in patients with acute asthma exacerbation.

Material and method. A prospective, cross-sectional, observational, case-control study was carried out in patients older than 2 years old and younger than 12. Immunoglobulin M (IgM) antibodies were serologically determined for *M. pneumoniae*, using the NovaLisa[®] NovaTeckit for enzyme-linked immunosorbent assay (ELISA). Test results ≥ 11 NTU (NovaTec units) were regarded as positive. The statistical analysis was performed by means of the analysis of variance (ANOVA) and the χ^2 test, with a significance level of $p < 0.05$.

Results. One hundred and eighty children were studied, of which 130 had asthma and 50 comprised the control group. Specific IgM was positive for 60 patients, that is 46.15% of the asthmatic children ($p < 0.001$). The severity of the exacerbation was directly related to IgM levels ($p < 0.001$). Hospitalization rate was 75%, and it was significantly associated to specific IgM levels ($p < 0.001$).

Conclusion. Our data suggest that children with acute asthma show a high prevalence (46%) of *Mycoplasma pneumoniae* infection and that there is a close relation between severe acute asthma exacerbation and the presence of *Mycoplasma pneumoniae* infection. These findings might result in therapeutic implications centered in the use of specific antibiotics to fight this atypical organism.

Keywords: acute asthma, exacerbation, *Mycoplasma pneumoniae*.

<http://dx.doi.org/10.5546/aap.2018.eng.179>

To cite: Kassisse E, García H, Prada L, et al. Prevalence of *Mycoplasma pneumoniae* infection in pediatric patients with acute asthma exacerbation. *Arch Argent Pediatr* 2018;116(3):179-185.

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Funding:
None

Conflict of interest:
None.

Received: 12-20-2016
Accepted: 10-10-2017

INTRODUCTION

The relationship between respiratory infections and asthma development or exacerbation in both children and adults has been well established. Many of these events are basically caused by viral infections.¹⁻³

Contrary to what happens with viruses, the relationship with atypical respiratory infections has not been completely established and is under investigation, not only in the case of exacerbation, but also as regards asthma development, persistence and severity.⁴⁻⁷

On the one side, acute or previous infection by *M. pneumoniae* (MP) is more frequent in children and adults with asthma than in patients without asthma. On the other side, MP may be involved in refractory asthma exacerbations, and failure to identify it increases morbidity and, potentially, mortality.⁸

The purpose of this study was to establish the prevalence of MP infection in patients with acute asthma exacerbation.

MATERIALS AND METHODS

A prospective, cross-sectional, observational, case-control study was carried out in the pediatric emergency department of the Autonomous Service of the "Hospital Universitario Antonio Patricio de Alcalá", Cumaná, Sucre, Venezuela, from February, 2015 to July, 2016.

In a consecutive fashion, patients > 2 and < 12 years old with asthma exacerbation seen in our hospital were included in the study.

Patients who had been previously diagnosed with asthma or who had suffered at least three exacerbations, and whose condition had improved

with the use of bronchodilators, were considered asthmatic.

Children with heart, kidney or immune conditions, as well as concomitant hematologic conditions, were excluded; and so were those patients who had received macrolids within the previous 4 weeks.

Asthma exacerbation was defined as the patient presenting one or a combination of the following signs and symptoms: a sudden or progressive event of cough, respiratory distress, wheezing or chest pain.

Subjects selected as controls were healthy children > 2 and < 12 years old, without respiratory symptoms, not asthmatic, and who had not received macrolids for at least 4 weeks. They were selected from the Hospital's pediatric office.

The study was assessed and approved by the Hospital's Postgraduate Education Commission, which ensured the ethical aspects were met in accordance with the Declaration of Helsinki standards.

All the study subjects were requested to sign an informed consent.

Asthmatic exacerbation severity was measured using the Wood-Downes scale, as modified by Ferrés, including 6 parameters. A score of < 3 points described mild asthma exacerbations; between 4 and 7 points, moderate exacerbations; and > 7 points indicated severe exacerbations.⁹

A sudden-onset crisis was one that presented within the first three hours of a medical visit, and a late-onset crisis, that which required more time.

The investigators did not participate in therapeutic decision-making.

A case report card specially designed for the collection of demographic, clinical and lab data was used.

The socioeconomic stratification of the study population was determined using the Graffar-Méndez level scale, with stratum I being the highest level, and strata IV/V, the lowest and, therefore, the lowest socioeconomic status.¹⁰

A 2 cm³ blood sample from a superficial vein of the forearm was obtained from each patient and each control in order to perform a serological study of MP immunoglobulin M (IgM) antibodies. The samples were analyzed by means of the qualitative immunoenzymatic determination of specific MP antibodies using the NovaLisa[®] NovaTec Immundiagnostica GmbH kit for the enzyme-linked immunosorbent assay (ELISA). Positive test results were those ≥ 11 NTU (NovaTec units) obtained in a single

determination, as per the manufacturer's specifications, with 95% sensitivity and specificity.

The primary outcome measure (purpose of the study), were specific IgM levels and their relationship with the exacerbation severity, and secondary outcome measure was the hospitalization rate.

To determine the significance of the relationship between the categorical outcome measures and the positivity of the specific IgM test, a statistical analysis was performed through the χ^2 test.

In order to determine the relationship between the different levels of specific IgM and the presence of fever, the severity of the exacerbation, time since asthma diagnosis, time of crisis onset and hospitalization, the one-way analysis of variance (ANOVA) was used. Once the differences were found, the analysis was followed by the Student-Newman-Keuls (SNK) test at 95%.

Statistical significance was set at $p < 0.05$.

RESULTS

One hundred and eighty subjects were studied, of which 130 had asthma and 50 comprise the control group. Average age (\pm standard deviation [SD]) was 4.9 ± 1.2 years old. Fifty three percent were females. Ninety seven percent were on Graffar's strata III-IV (87 [48.3%] and 88 [48.9%] subjects, respectively). Serum level of specific IgM was 8.66 ± 5.77 NTU.

Table 1 summarizes the general characteristics of the population of children with acute asthma exacerbation and children in the control group. No differences between the average age (\pm SD) and the distribution by gender were observed. Ninety two percent of the children in the control group and 99% of the asthmatic children were in strata III and IV. Specific IgM levels were much higher among asthmatic children than among children in the control group (10.79 ± 5.406 vs. 3.11 ± 1.262 NTU, respectively).

Table 2 describes the characteristics of the 130 asthmatic children studied.

Specific IgM was positive in 60 patients, which implies a 46% prevalence. Prevalence in children 2-6 years old (37.69%) was higher than prevalence in children > 6 years old (8.46%). However, no significant differences were observed between specific IgM positivity and age ($\chi^2 = 0.00$, not significant [NS]; $\chi^2_{(1, 0.05)} = 3.841$ [with Yates' correction]).

The ANOVA and the subsequent analysis

(SNK at 95%) revealed highly significant differences between the asthmatic patients and the control group ($p < 0.001$). Asthmatic children showed much higher specific IgM levels (Figure 1).

Asthmatic children who developed fever and cough during the exacerbation course presented higher, statistically significant IgM levels ($p = 0.0000$, both for fever and cough).

The relationship between the severity of the exacerbation and the specific IgM levels could be observed through the ANOVA and the SNK

95% analyses-acute asthma patients showed the highest levels ($p = 0.0000$) (Table 3).

Seventy six percent of study children had been diagnosed with asthma less than 2 years ago. The ANOVA did not show any statistical significance between the time since diagnosis and serum levels of specific IgM ($p = 0.3430$).

Both the crisis onset (sudden vs. late) and the hospitalization rate were related to specific IgM levels. The existing relationship between specific IgM and the outcome measures previously described are summarized in Table 3.

TABLE 1. General characteristics of the study population

Outcome measures*	All subjects (n= 180)	Control subjects (n= 50)	Asthmatic (n= 130)
Age (years)	4.9 ± 1.92 (2–12)	4.6 ± 1.65 (3–10)	5.0 ± 2.01 (2–12)
Gender (M/F)	84/96	24/26	60/70
Graffar#			
II	4 (2.2)	3 (6.00)	1 (0.7)
III	87 (48.3)	20 (40.0)	67 (51.5)
IV	88 (48.9)	24 (48.0)	62 (47.7)
V	1 (0.6)	1 (2.00)	-
IgM (NTU)**	8.66 ± 5.77 (1.0–25.2)	3.11 ± 1.26 (1.0–6.0)	10.79 ± 5.406 (1.4–25.2)

* Age and IgM data are presented as means, standard deviation and ranges; n (%)

#: Correspond to socioeconomic levels. ** NTU: NovaTec units.

M: masculine; F: feminine; IgM: immunoglobulin M.

TABLE 2. Characteristics of patients with acute exacerbation

Outcome measures*	Age groups		
	All subjects 2-12 years old (n= 130)	2-6 years old (n= 106)	7-12 years old (n= 24)
Fever (yes/no)	20/110 (15.38/84.62)	16/90 (12.31/69.23)	4/20 (3.08/15.38)
Cough (yes/no)	51/79 (39.23/60.77)	36/70 (27.69/53.85)	15/9 (11.54/6.92)
Severity			
Mild	26 (20.00)	22 (16.92)	4 (3.08)
Moderate	98 (75.38)	79 (60.77)	19 (14.62)
Severe	6 (4.62)	5 (3.85)	1 (0.77)
IgM (NTU)**	10.79 ± 5.406 (1.4–25.2)	10.84 ± 5.312 (1.4–25.2)	10.58 ± 5.919 (2.4–22.2)
Positive	60 (46.15)	49 (37.69)	11 (8.46)
Negative	70 (53.85)	57 (43.85)	13 (10.0)
Time since diagnosis			
< 1 years	13 (10.00)	6 (4.62)	7 (5.38)
2 years	73 (56.15)	65 (50.00)	8 (6.15)
≥ 3 years	44 (33.85)	35 (26.92)	9 (6.92)
Crisis onset			
≤ 3 hours	87 (66.92)	70 (53.85)	17 (13.08)
≥ 3 hours	43 (33.85)	36 (27.69)	7 (5.38)
Hospitalization			
Yes	97 (74.62)	80 (61.54)	17 (13.08)
No	33 (25.38)	26 (20.00)	7 (5.38)

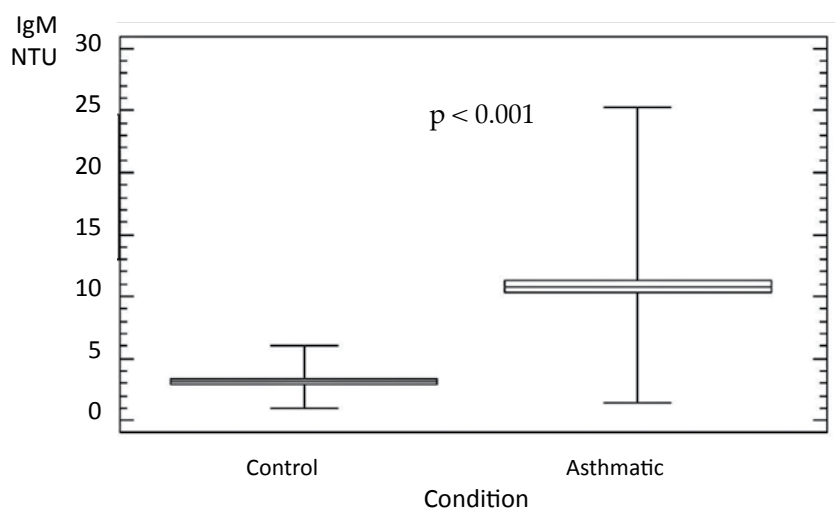
* Data are presented as means, standard deviation and ranges; n (%)

** NTU: NovaTec units.

The outcome measures analysis by means of the χ^2 test did not show any statistical relationship between positivity and age, though there were differences for gender distribution, since

positivity was higher for females. The distribution by Graffar's method also yielded statistical significance. These analyses are described in Table 4.

FIGURE 1. Immunoglobulin M levels. Comparison between asthmatic patients and control



* Statistical significance $p < 0.001$. NTU (NovaTec units).
IgM: immunoglobulin M.

TABLE 3. Statistically significant outcome measures related to specific immunoglobulin M level

Outcome measures	N	Specific IgM levels (NTU)*	P**
Control group	50	3.1	0.0000*
Asthmatic	130	10.79	
Fever			
No	110	10.01	0.0001*
Yes	20	15.03	
Cough			
No	79	8.34	0.0000*
Yes	51	14.58	
Severity			
Mild	26	6.08	0.0000*
Moderate	98	11.41	
Severe	06	21.12	
Crisis onset			
< 3 hours	87	12.94	0.0000*
> 3 hours	43	6.43	
Hospitalization			
No	33	5.91	0.0000*
Yes	97	12.45	

*: Average IgM. **: Highly significant, $p < 0.001$. NTU (NovaTec units).
IgM: immunoglobulin M.

DISCUSSION

The association existing between acute MP infection and the severity of asthma exacerbation was observed in this study.

The relationship between respiratory infections and asthma has been described both in children and in adults.^{11,12}

In a revision study, Metz et al. concluded that these agents were involved both in the exacerbations and in chronic asthma; therefore, these organisms were able to modify the severity of the condition, although their role in the development of asthma was not entirely identified.¹³

Biscardi et al. observed that MP infection was related to the onset and recurrence of exacerbations in asthmatic children. However, they were not able to establish a clear relationship nor the role of MP colonization in this occurrence.¹⁴

The prevalence found in this study was considered high (46%), lower than Wood et al.'s report of 64% and much higher than that of other reports, which ranged between 3% and 20%.^{13,15-17}

Another important finding of our study was the highly significant association between asthma severity and MP infection, since prevalence was much higher in more severe cases than in cases of moderate exacerbation or in the controls.

Not all the studies agree on this association between exacerbation severity and IgM levels for MP. For example, in their study, Duenas et al. did not find significant differences when comparing the severity of the exacerbations with the positive MP result.¹⁸

In a prospective, case-control study performed on 82 patients, Iramain et al. showed a positive relationship between MP infection

and exacerbation severity, and concluded that MP infection played an important role in the development of severe acute asthma.¹⁹

Likewise, in the prospective assessment of 58 patients with acute exacerbation, they found that specific IgM levels were much higher in patients with severe exacerbations both for MP and *Chlamydomphila pneumoniae*.²⁰

Studies associating the presence of MP in acute exacerbations with the risk of hospitalization were also contradictory.

Duenas et al. did not find differences between hospitalization risk and hospitalization days with MP positivity; hospitalization rate was 24.5%.¹⁸

In their study, Lieberman et al. analyzed the hospitalization risk among several atypical agents and concluded that only MP infected patients were actually at a higher risk.¹¹

Another study found that children infected with MP or *Chlamydomphila pneumoniae* who were not treated with macrolids presented higher recurrence and admission rates than those who received macrolids.²¹

The results of our research allowed us to determine that hospitalization rates were higher in children with high IgM levels.

MP is a tiny organism that is transmitted from one person to another through respiratory secretion drops which scatter in the air when coughing, and a close contact is needed for transmission to occur. Despite being asymptomatic, many individuals can keep on transmitting the organism for long periods of time.²²

The socioeconomic status classification of the population studied suggests, with statistical significance, an association between poverty, over-crowding and IgM positivity. There are no

TABLE 4. Outcome measures analysis as regards specific immunoglobulin M positivity or negativity

Outcome measures	Specific IgM result		P*
	Positive	Negative	
Age			
2-6 years old	49 (37.69)	57 (43.85)	NS
7-12 years old	11 (8.46)	13 (10.00)	
Sex			
Male	20 (15.38)	40 (30.77)	1
Female	40 (30.77)	30 (23.08)	
Graffar			
II	-	1 (0.77)	2
III	22 (16.92)	45 (34.62)	
IV	38 (29.23)	24 (18.46)	

n (%); NS: not significant. 1. $X^2 = 6.44$; $X^2_{(1,0.05)} = 3.841$ with Yates' correction. 2. $X^2 = 11.35$ $X^2_{(2,0.01)} = 9.210$.
IgM: immunoglobulin M.

studies relating this outcome measure during acute asthma exacerbation, as it was done in this research.

An MP seroprevalence study evaluating overcrowding did not find any relationship between this outcome measure and seropositivity.²³

The socioeconomic status of both populations (asthmatic vs. control) was exactly the same. Therefore, in addition to the socioeconomic factor, there must be some other factor in the asthmatic population, probably a higher level of asymptomatic carriers who increase the risk.

It has been mentioned that chronic MP infection increases asthma incidence, which is related to a poor disease control. The existing relationship between atopic MP infected patients has also been mentioned; this combination may contribute not only to the early development of asthma, but also to its persistence during adulthood.^{24,25}

This study was not able to confirm a relationship between the time since asthma diagnosis and specific IgM levels. There were no differences between patients with recently diagnosed asthma (< 1 year) and those who had been diagnosed a longer time ago (> 3 years).

An important finding in our results is the significant association between the time from the onset of the exacerbation and the presence of MP. No studies have related MP specific IgM with sudden-onset crisis and, opposite to the statement that infections were related to a slow onset, findings might suggest the likelihood of a mixed phenotype, including predominant bronchospasm followed by severe and potentially persistent inflammation.

It is known that toxins can be released during the course of an MP acute infection causing a toxic syndrome known as community-acquired respiratory distress syndrome (CARDS) that conditions epithelial damage and the activation of the immune response. Among MP infected patients, bronchial hyperresponsiveness can even be observed in non-atopic patients. Severe inflammation in the initial stages of the infection implies such infection has a role in the development of acute exacerbation.²⁶⁻²⁸

Some of this study's weaknesses are that it wasn't a multicenter study, which might have allowed for a larger sample size, and that it was not possible to investigate other infectious etiologies as exacerbation causes. For these reasons, prevalence cannot be generalized. Other

studies in larger population groups should be carried out in order to obtain a microbiologic assessment and determine how the use of specific antibiotics affects acute asthma exacerbation during childhood.

To conclude, data suggest, in the first place, a high prevalence of MP disease within the sample of children with asthmatic exacerbations and, in the second place, a relationship between severe acute asthma exacerbation and MP infection. This might have therapeutic implications regarding the use of specific antibiotics against this atypical organism during the development of a severe exacerbation. ■

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