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Mycoplasma pneumoniae in children with acute and refractory asthma

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ABSTRACT

Background: The presence of *Mycoplasma pneumoniae* has been associated with worsening asthma in children. Sensitive assays have been developed to detect *M pneumoniae*-derived community-acquired respiratory distress syndrome (CARDS) toxin.

Objectives: To identify the frequency and persistence of *M pneumoniae* detection in respiratory secretions of children with and without asthma and to evaluate antibody responses to *M pneumoniae* and the impact of *M pneumoniae* on biological markers, asthma control, and quality of life.

Methods: We enrolled 143 pediatric patients (53 patients with acute asthma, 26 patients with refractory asthma, and 64 healthy controls; age range, 5–17 years) during a 20-month period with 2 to 5 follow-up visits. We detected *M pneumoniae* using CARDS toxin antigen capture and polymerase chain reaction and P1 adhesin polymerase chain reaction. Immune responses to *M pneumoniae* were determined by IgG and IgM levels directed against CARDS toxin and P1 adhesin. pH was measured in exhaled breath condensates, and asthma control and quality of life were assessed using the Asthma Control Test and Pediatric Asthma Quality of Life Questionnaire.

Results: *M pneumoniae* was detected in 64% of patients with acute asthma, 65% with refractory asthma, and 56% of healthy controls. Children with asthma had lower antibody levels to *M pneumoniae* compared with healthy controls. Exhaled breath condensate pHs and asthma control and quality of life scores were lower in *M pneumoniae*-positive patients with asthma.

Conclusion: The results suggest that *M pneumoniae* detection is common in children, *M pneumoniae* detection is associated with worsening asthma, and children with asthma may have poor humoral immune responses to *M pneumoniae*.

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Introduction

Asthma is a prevalent and heterogeneous disease that not only has a marked effect on the quality of life of affected patients but also imparts a significant economic burden on society. The term *refractory asthma* (RA) is used for patients with persistent asthma symptoms in whom comorbidities have been treated, triggers addressed, adherence with treatment evaluated, and alternative diagnoses excluded.¹ Various phenotypes of chronic asthma with persistent inflammation have been recognized, and a link between bacterial infections and RA has emerged.^{2–4} Some studies have

implicated *Mycoplasma pneumoniae* in the initiation and persistence of asthma, although its precise role and pathogenic mechanisms remain elusive.^{5,6} Prior studies on the role of *M pneumoniae* in asthma have been limited by the inability to consistently culture this organism, the poor performance of *M pneumoniae* serologic testing in defining active infection, and variable sensitivities of polymerase chain reaction (PCR) assays in detecting *M pneumoniae*.

Our group has identified a 68-kDa protein unique to *M pneumoniae* called the community-acquired respiratory distress syndrome (CARDS) toxin that possesses adenosine diphosphate-ribosyltransferase activity similar to pertussis toxin.⁷ We have developed assays to detect CARDS toxin by PCR and antigen capture (AC) and to detect IgM and IgG antibodies directed against CARDS toxin.^{8–10} CARDS toxin gene sequences are more sensitive for the detection of *M pneumoniae* by PCR than other sequences, such as P1 adhesin (P1) and adenosine triphosphatase.^{11,12}

The purpose of this study was to identify the frequency and persistence of *M pneumoniae* in respiratory secretions by

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measuring CARDS toxin protein by AC, as well as both CARDS toxin and P1 by PCR. In addition, we evaluated IgG and IgM antibody levels to CARDS toxin and P1, exhaled breath condensate (EBC) pH, and asthma control and quality of life.

Methods

Study Participants

This single-center, prospective cohort study was conducted from December 2009 through June 2011 and was approved by the institutional review board. We obtained written informed consent from the parent or legal guardian of each participant. We enrolled 3 groups of children 5 to 17 years of age: (1) children hospitalized for an acute exacerbation of asthma; (2) children with RA (poorly controlled moderate-severe persistent asthma who met RA criteria); and (3) healthy children without asthma (controls). We enrolled children from the inpatient setting (acute asthma) or from pulmonary or general pediatric clinics during routine visits (groups 2 and 3, respectively). The diagnosis of asthma was based on physician assessment according to national guidelines.¹³ Exclusion criteria included the following: pneumonia, bleeding disorders, pulmonary disease other than asthma, other serious medical conditions (eg, cerebral palsy, pregnancy, malignancy, and cystic fibrosis), and, for healthy control subjects only, any infection within the month before enrollment. Patients with asthma were monitored for 2 to 5 follow-up visits for a 12-month period. We examined data at enrollment by study group and over time.

Specimens were collected from the throat and nasopharynx using nylon-tipped swabs (Copan, Murrieta, California) and from blood to measure CARDS toxin by AC and PCR, P1 by PCR, and serum IgG and IgM antibody responses to CARDS toxin and P1. We examined the persistence of *M pneumoniae* and corresponding antibody responses in children with at least 3 visits. We collected EBC using the RTube (Respiratory Research, Austin, Texas) and measured EBC pH with an Orion Ross Micro pH electrode (Thermo Scientific, Beverly, Massachusetts) after argon degassing as described.¹⁴ Throat and nasopharyngeal swabs were placed in transport media immediately after specimen collection. All samples were stored at 4°C, transported within 24 hours for processing, and subsequently stored at –20°C (serum, throat, nasopharyngeal) or –80°C (EBC) until analyzed.

Surveys

For children with asthma, we measured asthma symptoms (Asthma Control Test [ACT])^{15,16} and quality of life (Pediatric Asthma Quality of Life Questionnaire [PAQLQ]).¹⁷ Patients with asthma returned for 2 to 5 follow-up visits. The Childhood Asthma Control Test (C-ACT) was administered to children younger than 12 years; older children completed the ACT. Scores below 20 on either questionnaire indicate poor asthma control in the past 4 weeks. Patients 7 years of age and older completed the PAQLQ. Scores on this questionnaire range from 1 to 7. Higher scores indicate better quality of life in the past 7 days; the minimal clinically important difference in scores is 0.5. We examined asthma symptom control (ACT) and quality of life at enrollment by group. In addition, we examined these variables by combining all visits for children with asthma, excluding the enrollment visit for the acute asthma group. We recorded exposure to environmental tobacco smoke (parent report) for all study participants and hospital length of stay (medical record review) for the acute asthma group.

Detection of *M pneumoniae* DNA, Protein, and Antibodies

DNA from airway and serum samples was purified using the QIAmp DNA Mini Kit (Qiagen, Valencia, California). Real-time PCR for CARDS toxin (MPN372) and P1 (MPN141) was performed as

described.^{11,12} CARDS toxin protein was detected and quantified using AC enzyme-linked immunosorbent assay (ELISA) methods as previously described.¹⁰ Because CARDS toxin shares limited amino acid sequence identity with the S1 subunit of *Bordetella pertussis* toxin,⁷ we examined the cross-reactivity of CARDS toxin-specific reagents to pertussis toxin, and none was detected (data not shown). The detection of antibodies against recombinant CARDS toxin and recombinant P1 immunodominant carboxy domain was performed using ELISA as described.^{7,9,10,18} *M pneumoniae*–positive patients were defined as those with any PCR or AC value in a detectable range, and *M pneumoniae*–negative patients were defined as those in whom all test results were negative.

Statistical Analyses

Data were analyzed using SPSS statistical software, version 19.0 (SPSS Inc, Chicago, Illinois). We examined data for normality and for other specific test assumptions, such as homogeneity of variance. If variables did not meet the assumption of normality, a nonparametric test was used. Statistical differences between group means were determined using parametric (ie, 2-tailed *t* test or 1-way analysis of variance) or nonparametric analyses (ie, Mann-Whitney test). Differences in categorical data were determined using the Pearson χ^2 tests. Correlation analyses (ie, Pearson *r* and Kendall τ) were performed to test for significant relationships between variables. For categorical variables, the Kendall τ rank correlation coefficient was used. Results for normally distributed variables were expressed as mean $\pm$ SD. We used nonparametric test for nonnormally distributed variables and reported results as median and 25th to 75th percentile interquartile range (IQR). $P \leq .05$ was considered to indicate statistically significant differences and associations.

Results

We enrolled 143 patients (mean [SD] age, 10.33 [3.13] years; range, 5–17 years; 62% male) with and without asthma from an ethnically diverse population (57% white, 14% black, 2.8% Asian, 2.1% American Indian, 24.5% other, and 81% Hispanic ethnicity). We enrolled 53 patients with an acute exacerbation of asthma requiring hospitalization, 26 patients with RA (poorly controlled moderate to severe persistent asthma), and 64 healthy controls with no history of asthma and no symptoms of a respiratory tract infection within the prior 4 weeks. Baseline demographic characteristics were compared across groups. Significant differences were found in sex and body mass index (Table 1).

Using CARDS toxin AC and CARDS toxin and P1 PCR, *M pneumoniae* was detected in 64% of patients with acute asthma, 65% of those with RA, and 56% of healthy controls (Table 1), with no significant differences among the 3 groups. The proportion of positive samples varied by method (AC vs PCR) and sampling site (nasopharynx, throat, and serum) (eTable 1). In all study participants, CARDS toxin AC was more sensitive than any PCR (55% vs 16%, $P = .04$), and CARDS toxin PCR was more sensitive than P1 PCR (14% vs 5.1%, $P < .001$). Regardless of test, nasopharyngeal specimens were more likely to be positive than other sites (eg, comparing nasopharyngeal vs throat samples by AC) (51.8% vs 17%, $P < .001$). We have previously observed discordance between the PCR and AC assays and between different sites of collection (nasal, throat, sputum).¹⁹ In addition, in a separate study, we identified a patient with confirmed detection of *M pneumoniae* by immunofluorescence to both CARDS toxin and P1 in a sputum sample but who was PCR negative (unpublished observations).

Patients with asthma were monitored for 2 to 5 follow-up visits, whereas controls had a single visit. Although no differences were found in the proportion of patients testing positive for *M pneumoniae* among the 3 groups, there was a distinct seasonal variation

Table 1
Baseline characteristics of the study participants^a

Characteristic	Acute Asthma Patients (n = 53)	Refractory Asthma Patients (n = 26)	Healthy Controls (n = 64)	P value
Age, y	9.53 ± 3.02	10.58 ± 2.64	10.90 ± 3.3	.057
Race, No. (%)				.15
White	29 (54.7)	10 (38.5)	42 (65.6)	
Black	10 (18.9)	6 (23.1)	4 (6.3)	
American Indian	1 (1.9)	0 (0)	2 (3.1)	
Asian	2 (3.8)	0 (0)	2 (3.1)	
Other	11 (20.8)	10 (38.5)	14 (21.9)	
Hispanic ethnicity, No. (%)	40 (75.5)	19 (73.1)	57 (89.1)	.09
Male sex, No. (%)	38 (71.7)	20 (76.9)	31 (48.4)	.008
BMI percentile	62.81 ± 36.8	79.14 ± 28.4	76.49 ± 26.9	.04
ETS exposure, No. (%)	19 (35.8)	6 (23.1)	14 (22.2)	.22
Mycoplasma positive, No. (%)	34 (64.2)	17 (65.4)	36 (56.3)	.60
ACT scores <20, No. (%)	41 (64.2)	25 (51.0)	N/A	.05
PAQLQ score	3.9 ± 1.2	4.7 ± 1.5	N/A	.01

Abbreviations: ACT, asthma control test; BMI, body mass index; ETS, environmental tobacco smoke; PAQLQ, Pediatric Asthma Quality of Life Questionnaire.

^aData are presented as mean ± SD unless otherwise noted.

in the detection of *M pneumoniae*, with the highest rates occurring from December to March and the lowest from June to October (Fig 1). The proportion of patients with asthma who tested positive was slightly higher during the summer months compared with controls. Many asthmatic patients, who were initially positive for CARDS toxin, had positive test results on follow-up visits (Fig 2). Some individuals were persistently positive for CARDS toxin AC throughout the study, whereas some had negative test results during the summer months but again tested positive during the following winter/spring season. Despite this persistence of CARDS toxin detection, most patients with asthma did not demonstrate significant IgM or IgG antibody levels to CARDS toxin. However, a few patients clearly demonstrated robust antibody responses that paralleled the CARDS toxin levels measured by AC (Fig 2).

Overall, most patients with asthma had low serum IgM and IgG levels to *M pneumoniae* (Fig 3 and Fig 4) that were not significantly different between *M pneumoniae*–positive and –negative patients for either visit 1 (Fig 3 and Fig 4) or all visits combined. In healthy controls, IgM levels directed against P1 were significantly higher in *M pneumoniae*–positive vs *M pneumoniae*–negative patients (0.049 [IQR, 0.027–0.09] vs 0.026 [IQR, 0.014–0.052], $P = .048$). IgG levels to CARDS toxin were also slightly higher in *M pneumoniae*–positive compared with *M pneumoniae*–negative patients but not significantly (0.080 [IQR, 0.029–0.282] vs 0.059 [IQR, 0.033–0.104], $P = .10$).

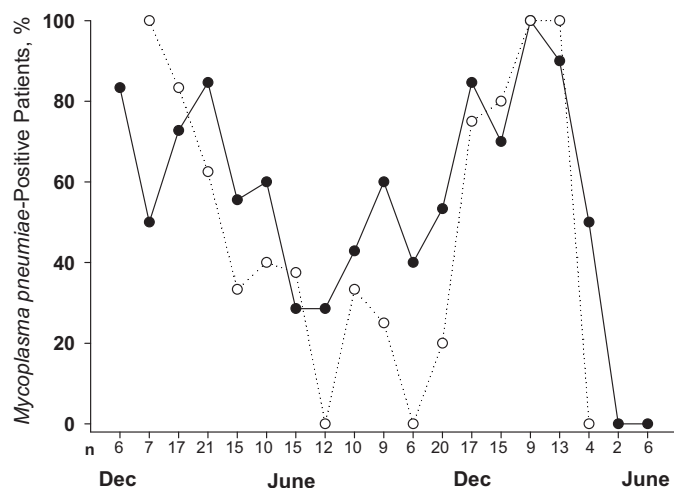


Figure 1. Seasonal variation in the frequency of *Mycoplasma pneumoniae* detection in children. Monthly percentages of *M pneumoniae*–positive patients (any site) from December 2009 through June 2011 for asthmatic patients (solid line, filled circles) and healthy controls (dotted lines and open circles).

In patients with an acute asthma exacerbation, the hospital length of stay was slightly longer in *M pneumoniae*–positive compared with *M pneumoniae*–negative patients but not significantly (3.09 vs 2.21; $P = .07$). The EBC pH was lower in patients with acute asthma compared with healthy controls (7.55 [IQR, 7.08–8.05] vs 8.07 [IQR, 7.9–8.22], $P < .001$). The EBC pH was also lower in *M pneumoniae*–positive asthma patients when analyzing all visits (7.88 [IQR, 7.26–8.17] vs 8.12 [IQR, 7.76–8.25], $P = .02$) even when the initial hospitalization visit for the acute asthma group was excluded ($P = .03$) and was lower in *M pneumoniae*–positive patients with RA (7.93 [IQR, 7.68–8.17] vs 8.15 [IQR, 7.99–8.30], $P = .007$) (eFig 1). This relationship remained significant ($P = .03$) even when pH values less than 6 were excluded from the analysis (values <6 could represent contamination from gastric acid).

The mean (SD) ACT score was 16.1 (4.7) in patients with an acute asthma exacerbation and 16.9 (5.0) in RA (scores <20 indicate poor asthma control in the past 4 weeks). In all visits, except the hospitalization visit for acute asthma, patients who were *M pneumoniae*–positive were more likely to score less than 20 on the ACT than those who tested negative (70% vs 30%, $P = .001$). In addition, poor asthma control (ACT score <20) correlated with *M pneumoniae* status ($r = -0.35$, $P = .001$) and with the CARDS toxin antigen level ($r = -0.27$, $P = .01$).

The quality-of-life mean scores at enrollment were lower in patients during an acute exacerbation of asthma compared with RA (3.9 ± 1.2 vs 4.7 ± 1.5, $P = .01$; lower scores indicate poorer quality of life in the past 7 days). To examine the effect of *M pneumoniae* in chronic asthma, we examined all visits except acute asthma exacerbations. The PAQLQ scores trended lower for *M pneumoniae*–positive vs *M pneumoniae*–negative patients (4.9 ± 1.4 vs 5.5 ± 1.2, $P = .06$). In RA, *M pneumoniae*–positive patients scored significantly lower on the PAQLQ than *M pneumoniae*–negative patients (4.9 ± 1.4 vs 5.7 ± 1.1, $P = .03$) (eFig 2).

Discussion

M pneumoniae infects the upper and lower respiratory tract,²⁰ causing pneumonia, bronchitis, and possibly asthma.²¹ Prior evidence has linked *M pneumoniae* to new-onset asthma,²² exacerbations of asthma,²³ chronic worsening of asthma, and long-term decrements in pulmonary function.^{24–26} Biscardi et al²⁷ reported that *M pneumoniae* is associated with the initiation and recurrence of asthma exacerbations in children. However, defining a clear cause-and-effect relationship between infection and asthma has been elusive, and the role of colonization by *M pneumoniae* is unclear.

In the past, the detection of *M pneumoniae* has been difficult because *M pneumoniae* is a fastidious organism that is capable of

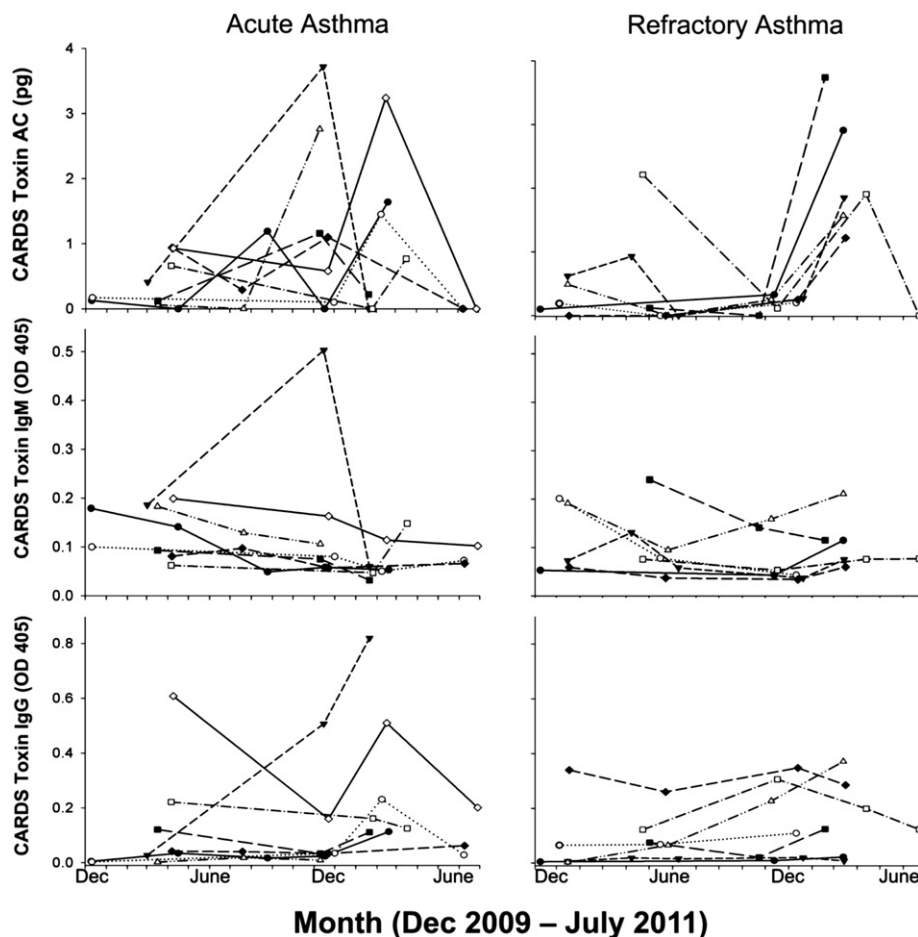


Figure 2. *Mycoplasma pneumoniae* detection in children with asthma over time. Longitudinal data for community-acquired respiratory distress syndrome (CARDS) toxin antigen capture (AC) levels (any site); IgM and IgG levels for 8 patients with acute asthma and 7 patients with refractory asthma who had at least 3 study visits.

extracellular and intracellular growth and persistence in vivo; even in laboratory conditions, growth is difficult.²⁸ *M pneumoniae* may be more easily cultured in cases with severe pneumonia²⁹ and more difficult to culture in states of colonization, where organism burdens are presumably lower. In an outbreak of *M pneumoniae* community-acquired pneumonia, the Centers for Disease Control and Prevention demonstrated that a PCR assay to CARDS toxin was the most sensitive assay tested.¹¹ Our results in the current study confirm that the CARDS toxin PCR assay is superior to detection by P1 PCR but that the CARDS toxin AC assay was more sensitive than either PCR assay. We further observed that CARDS toxin antigen detection in samples derived from the nasopharynx was higher than in samples from the throat or serum.

In our study, we have found high rates of *M pneumoniae* detection in the winter/spring seasons. Other recent reports have found similar seasonal patterns and a notably increased incidence of *M pneumoniae* in 2010 and 2011,^{30,31} suggesting that our study may have coincided with a worldwide epidemic of *M pneumoniae*. In addition, a high rate of *M pneumoniae* was detected in healthy children (54%), despite no reported respiratory symptoms within 4 weeks of study enrollment. Although some investigators have found low rates of *M pneumoniae* detection in healthy children (4%–8%),^{32,33} others have identified higher rates. One large study identified that 30% of household contacts were *M pneumoniae* positive, half of whom were asymptomatic.³⁴ Another study found 21% of asymptomatic children were *M pneumoniae* positive.³⁵ On the basis of our findings, as well as those of other investigators, it would appear that *M pneumoniae* may persist for long periods in

asymptomatic children. This finding prompts the question of whether healthy children possess protective factors that allow them to recover from or resist *M pneumoniae* infections without sequelae. Indeed, higher levels of *M pneumoniae* antibodies in our healthy control group compared with the asthma groups may provide insight into a protective immune response.

In the current study, we have found a higher rate of *M pneumoniae* detection in acute asthma (64%) than previous studies. In hospitalized asthmatic children older than 2 years, Freymuth et al³⁶ identified only 3% *M pneumoniae*–positive subjects. Maffey et al³⁷ found 6.6% positive in children 4 to 16 years old. Biscardi et al²⁷ found 20% positive in children 2 to 15 years old. Some studies have reported higher rates of 29% in children with acute wheezing³⁸ and 48% in acutely ill children with recurrent wheezing or asthma.³⁹ In most of these studies, *M pneumoniae* was detected using P1 PCR or by acute and convalescent antibody titers, which are not as sensitive as our assays. In our study, the rate of *M pneumoniae* detection was higher in patients with asthma than in healthy controls, although this difference was not significant. The higher rate of *M pneumoniae* detection in children with asthma compared with healthy controls may be because we selected children with ongoing asthma symptoms (acute asthma or RA) and *M pneumoniae* may have contributed to these symptoms.

We have previously found that detection of *M pneumoniae* in adults with RA is more frequent (52%) and persists for longer than previously recognized.¹⁹ In the current study, we found that children with RA also have high rates of *M pneumoniae* detection (65%) and tend to be persistently positive on multiple follow-up visits.

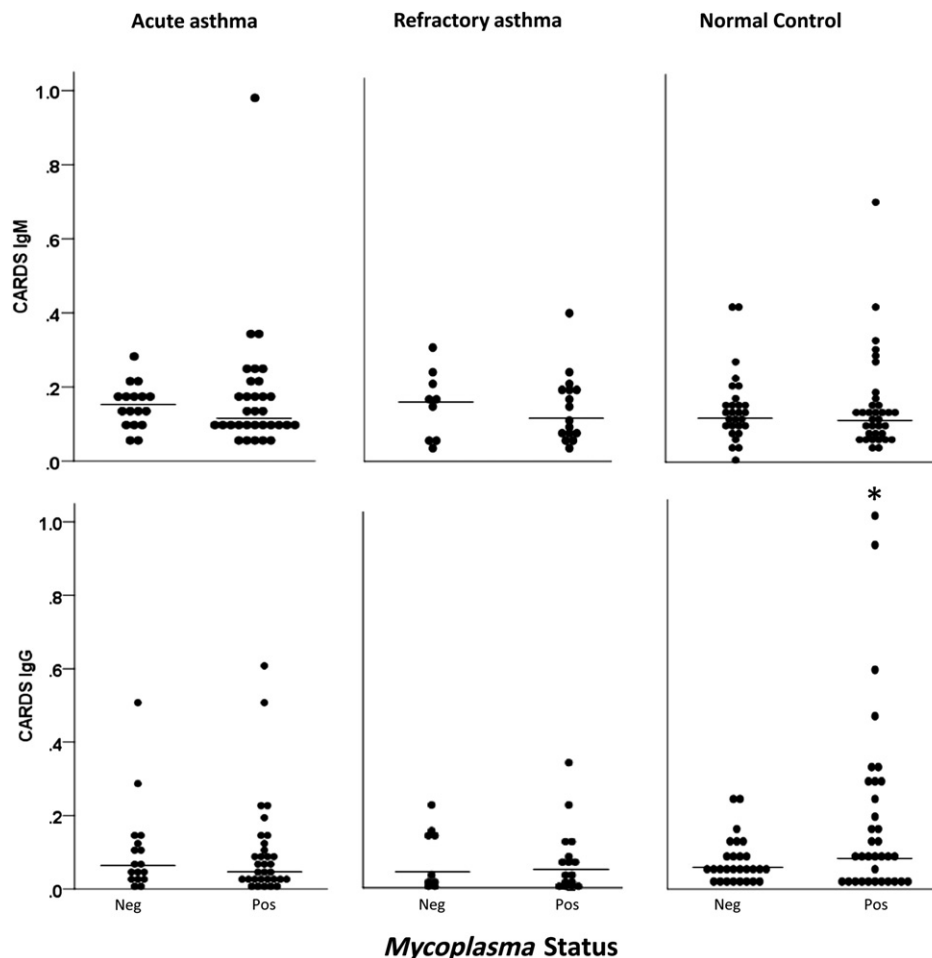


Figure 3. Community-acquired respiratory distress syndrome (CARDS) toxin antibody levels by *Mycoplasma pneumoniae* status. Antibody levels to CARDS toxin at enrollment by *M pneumoniae* status (neg indicates negative; pos, positive). Horizontal lines indicate median for each group. *IgG in healthy controls, *M pneumoniae* pos vs *M pneumoniae* neg, $P = .10$.

Whether this represents true persistence, reinfection, reactivation, or colonization is unclear because many patients also had at least one visit at which they were *M pneumoniae* negative.

We found lower EBC pH values in patients with acute and chronic asthma. Although it was not surprising that patients with acute asthma had lower EBC pH, as has been reported previously,¹⁴ lower EBC pH correlated with *M pneumoniae* detection in RA patients who were not experiencing an acute exacerbation. Lower EBC pH was recently reported to correlate with higher scores on the Asthma Predictive Index in children and thus may represent a biological marker of chronic asthma.⁴⁰ In addition, patients with RA who were positive for *M pneumoniae* had lower symptoms scores (ACT) and lower quality-of-life scores (PAQLQ). Taken together, these data indicated that *M pneumoniae* detection is associated with worsening chronic asthma.

The direct effect of *M pneumoniae* infection is unknown, but we speculate that immune modulation mediated by CARDS toxin may occur. Animal studies report that low-dose *M pneumoniae* infection enhances lung eosinophilia and interleukin 4.⁴¹ We recently reported that CARDS toxin alone recapitulates an “asthma-like” phenotype in mice with promotion of T_H2 cytokines⁴² and induces pulmonary inflammation, mucous hypersecretion, and hyperreactivity in mice and baboons.⁴³ Differences between *M pneumoniae* strains may also contribute to the severity of the resultant pathologic phenotypes.¹²

Serologic responses involving IgM and IgA can be an early indicator of *M pneumoniae* infection. The IgG antibody response in

humans and primates is reported to peak 4 to 6 weeks after the onset of infection.⁴⁴ The importance of an intact adaptive humoral immune response for protection against *M pneumoniae* infection is evidenced by the increased rate of severe infections and systemic complications in patients with humoral immune deficiency.^{45,46} One possible clue to the pathophysiology of *M pneumoniae* infection in children with asthma may be the IgM and IgG responses. Although healthy control children in our study who were *M pneumoniae* positive appeared to mount somewhat higher antibody responses, patients with acute asthma or RA had low IgM and IgG levels to *M pneumoniae*. Other investigators have reported reduced or absent IgG levels to *M pneumoniae* during acute infections and in chronic infection/colonization states.^{3,47–49} Thus, the inability to mount an appropriate immune response to *M pneumoniae* may be germane to understanding the effect of *M pneumoniae* on asthma.

The salient findings of the current study are that the detection of *M pneumoniae* in children with asthma (1) had a seasonal pattern with the highest prevalence in the winter/spring, (2) tended to be recurrent or persistent, (3) was associated with lower quality of life and ACT scores, (4) was associated with lower EBC pH, and (5) was associated with poor antibody responses. However, this study had several limitations. We did not test for viral respiratory pathogens or other bacterial pathogens and thus are unable to comment on the role of other organisms, either alone or in combination with *M pneumoniae*, on asthma morbidity. Most patients had a limited number of follow-up visits, and thus future studies should examine

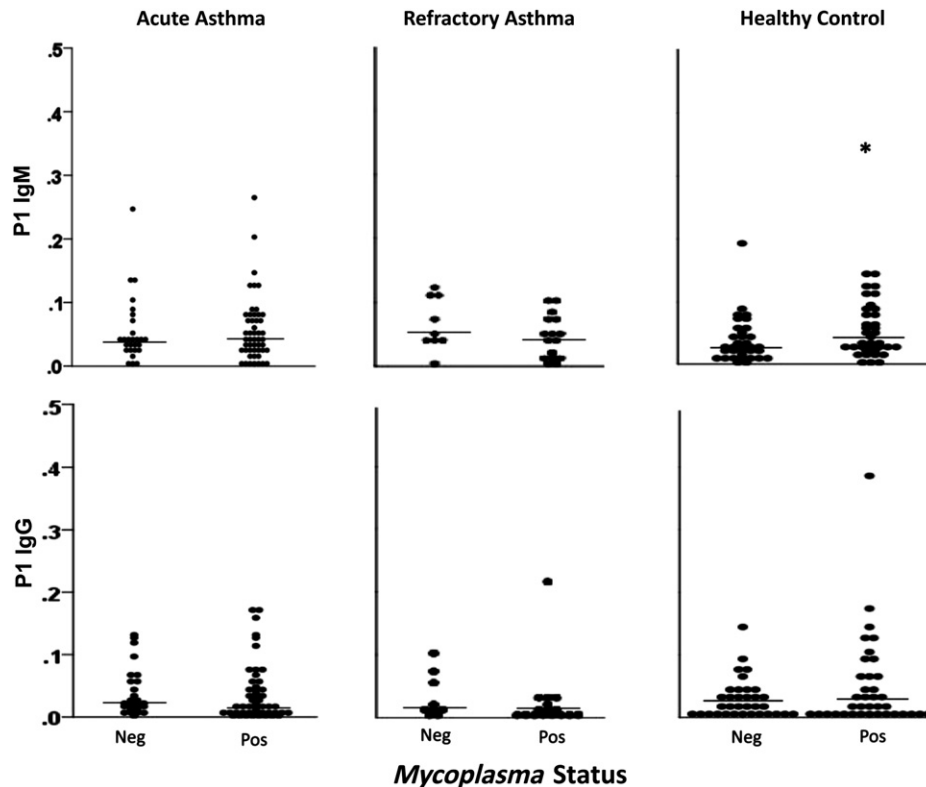


Figure 4. P1 adhesin antibody levels by *Mycoplasma pneumoniae* status. Antibody levels to P1 adhesin at enrollment by *Mycoplasma pneumoniae* status (neg indicates negative; pos, positive). Horizontal lines indicate median for each group. *IgM in healthy controls, *M pneumoniae* pos vs *M pneumoniae* negative, $P = .048$.

the role of *M pneumoniae* on asthma control and morbidity over time by monitoring patients longitudinally.

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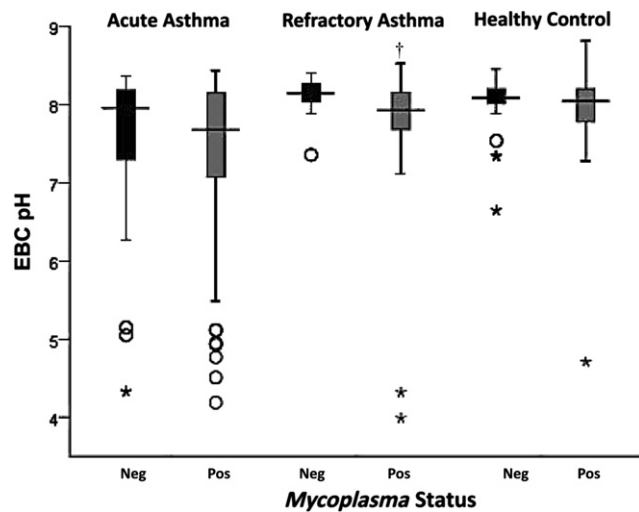
Supplementary Data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.anai.2013.01.022>

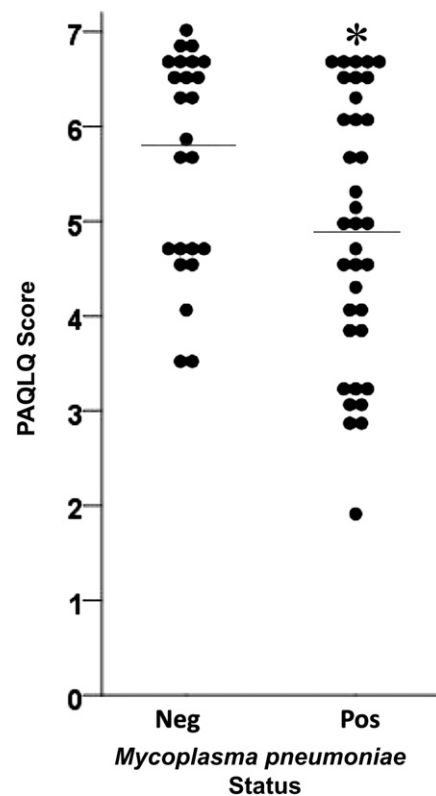
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eFigure 1. Exhaled breath condensate (EBC) pH by *Mycoplasma pneumoniae* status. EBC pH by group and *M pneumoniae* status (neg indicates negative; pos, positive). Solid bars indicate interquartile range (IQR) 25th to 75th percentile; central line, medians; whiskers, highest (lowest) value within 1.5 IQR from end of boxes; circles and asterisks, outliers. †*M pneumoniae* pos vs *M pneumoniae* neg (refractory group), $P = .007$.



eFigure 2. Quality of life in refractory asthma. Pediatric Asthma Quality of Life Questionnaire (PAQLQ) scores in refractory asthma group according to *Mycoplasma pneumoniae* status (neg indicates negative; pos, positive). Horizontal lines indicate means for each group. * *M pneumoniae* pos vs *M pneumoniae* neg, $P = .03$.

eTable 1
Correlations of the sampling sites^a

	P1-P-N (2.8%) 6/214	P1-P-T (2.8%) 6/214	P1-P-S (2.9%) 6/205	C-P-N (9.8%) 21/214	C-P-T (6.5%) 14/214	C-P-S (6.8%) 14/205	C-AC-N (51.8%) 113/218	C-AC-T (17.1%) 37/217
P1-P-N	1.000	.143^b 1 (0.5%)	.142^b 1 (0.5%)	.420^c 5 (2.3%)	.070 1 (0.5%)	.182^c 2 (1.0%)	.049 4 (1.9%)	-.075 0 (0.0%)
P1-P-T		1.000	-.030 0 (0.0%)	.325^c 4 (1.9%)	.299^c 3 (1.4%)	.068 1 (0.5%)	.105 5 (2.3%)	-.075 0 (0.0%)
P1-P-S			1.000	.419^c 5 (2.4%)	.182^c 2 (1.0%)	.297^c 3 (1.5%)	.112 5 (2.4%)	-.078 0 (0.0%)
C-P-N				1.000	.294^c 6 (2.8%)	.419^c 8 (3.9%)	.095 14 (6.5%)	.023 4 (1.9%)
C-P-T					1.000	.310^c 5 (2.4%)	.139^b 11 (5.1%)	.036 3 (1.4%)
C-P-S						1.000	-.045 6 (2.9%)	-.017 2 (1.0%)
C-AC-N							1.000	.267^c 30 (13.8%)

Abbreviations: AC, antigen capture; C, community-acquired respiratory distress syndrome toxin; N, nasopharyngeal; P1, P1 adhesin; P, polymerase chain reaction; S, serum; T, throat.

^aHeading numbers indicate percentage positive in parentheses and number positive/number tested on the second line below. Within the table, numbers in bold list the correlation coefficients, and second line below indicates the number (%) positive for both tests. Headers indicate target for assay—type of assay—site.

^b $P < .05$ level (2-tailed).

^c $P < .01$ level (2-tailed).