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Original Article

The significance of a rapid cold hemagglutination test for detecting mycoplasma infections in children with asthma exacerbation

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Background and Purpose: *Mycoplasma pneumoniae* infection is a frequent cause of community-acquired respiratory infections in children and adults. However, standardized, rapid, specific methods for its diagnosis are lacking. The relationship between *M. pneumoniae* infection and asthma exacerbation has been recently discussed in the literature. We investigated the accuracy of rapid detection of mycoplasma infection by cold hemagglutination test compared to conventional enzyme immunoassays. The clinical characteristics of mycoplasma infection seen during emergent visits in asthmatic children were reviewed.

Methods: We retrospectively reviewed medical records of patients with asthma exacerbation visiting the Department of Pediatric Emergency, National Taiwan University Hospital, over a 12-month period. Subjects 2-18 years of age diagnosed with asthma at our outpatient clinic were included in this study. Patients with immunodeficiency, congenital anomalies, neurological diseases and irregular follow-up were excluded.

Results: A total of 269 children (174 males and 95 females) with a mean (± standard deviation) age of 6.15 ± 3.08 years were included. The prevalence of asthma exacerbation in regular follow-up patients was 13.4%, and as many as 19.6% of cases (74/378 person-times) required hospitalization. Asthma attacks were most prevalent during December. 126 patients had both rapid cold hemagglutination testing and mycoplasma immunoglobulin M titers determined using acute blood samples drawn in the emergency room; 46 (36.5%) of these patients demonstrated mycoplasma infection. Sensitivity and specificity of the rapid cold hemagglutination test was 78.3% and 41.3%, respectively. The positive predictive value was 43.4%. Comparison of patients with or without mycoplasma infection revealed no differences in gender, age, chest X-ray findings, and most symptoms/signs and laboratory data, except that more signs of fever and auscultatory rales were seen in the non-mycoplasma infection group.

Conclusions: Mycoplasma infections could be an exacerbating factor for asthma, and the rapid cold hemagglutination test should not be a guideline for prescribing macrolides in the emergency room.

Key words: Asthma, bacterial antigens, hemagglutination tests, immunoenzyme techniques, *Mycoplasma pneumoniae*

Introduction

Mycoplasma pneumoniae is a frequent cause of community-acquired respiratory infections in children and adults. An estimated 30% or more of *M. pneumoniae* infections in children 5-15 years of age result in pneumonia, and as many as 18% of cases require hospitalization [1,2]. *M. pneumoniae*, a small, cell wall-deficient bacterium, is insensitive to the activity of beta (β)-lactam antimicrobials, and cannot be detected by Gram staining. Proper treatment of infected patients with macrolide antibiotics shortens the duration of overt infection and decreases the severity of respiratory symptoms [3,4].

However, the diagnosis of *M. pneumoniae* infection is hampered by the lack of standardized, rapid, specific

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methods. Most of the current diagnostic methods are perhaps better suited for use in epidemiological studies because of their turnaround time, limited availability and cost. Current methods for the specific diagnosis of *M. pneumoniae* infection include culture, serology and polymerase chain reaction (PCR). Culture methods are insensitive, require special techniques, and need at least 2-3 weeks of incubation. PCR is a promising diagnostic tool, but is available only in specialized laboratories. No standardized commercial PCR kit has gained widespread use in clinical laboratories. Therefore, reliable serology remains the mainstay for the diagnosis of *M. pneumoniae* infections [4,5].

The concept that *M. pneumoniae* infection may be a cofactor in the pathogenesis of asthma was first considered over 30 years ago [6]. Owing to advanced detection (with methods such as PCR), the potential for this organism to initiate or exacerbate asthma has come under new scrutiny in the past few years [5-9]. Asthma exacerbation is a critical and sometimes lethal event that needs immediate treatment. The aim of this study was to investigate the accuracy of rapid detection of mycoplasma infection by cold hemagglutination test compared with conventional enzyme immunoassays. Additionally, the clinical characteristics of mycoplasma infection seen during emergent visits in asthmatic children were reviewed.

Methods

We retrospectively reviewed the medical records of patients with asthma exacerbation visiting the Department of Pediatric Emergency, National Taiwan University Hospital from January to December 2002. The charts of all patients 2-18 years of age diagnosed with asthma at our outpatient clinic were included in this study. Patients with immunodeficiency, congenital anomalies, neurological diseases and irregular follow-up were excluded.

A clinical case of asthma exacerbation was defined as episodes of rapidly progressive increases in shortness of breath, cough, wheezing, chest tightness, or some combination of these symptoms. Respiratory distress was common. An acute blood sample was drawn in the emergency room for serologic testing. The rapid cold hemagglutination test was performed on a chilled (4°C) slide that was placed on ice in advance. A few drops of heparinized blood and an equal volume of sodium citrate were placed on the slide. A positive test showed coarse granular agglutination within 30 sec; no agglutination or fine granularity was read as negative. If a positive agglutination occurred, it was resolved at room temperature and re-agglutinated on ice. The *M. pneumoniae* immunoglobulin M (IgM) antibody was assayed by our central laboratory using the semi-quantitative enzyme-linked immunosorbent assay (Savyon, Ashdod, Israel) according to the manufacturer's instructions. An IgM titer >10 BU/mL was considered negative; a positive finding was defined as IgM titer ≥10 BU/mL, which indicated current infection. Data including previous asthma history, routine control medications, clinical symptoms and signs, laboratory results, chest X-ray findings (based on the radiology report) and disease management and outcome were collected.

Statistical analysis

Calculations and statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software package (SPSS 9.0, Chicago, IL, USA). Continuous variables were analyzed with independent Student's *t* test. Chi-squared, Fisher's exact 2-tailed, and Wilcoxon rank-sum tests were used to examine the nominal data. Data are presented as mean ± standard deviation (SD) or number (percentage). A p value less than 0.05 was considered statistically significant.

Results

A total of 269 children were included in this study. Asthma attacks were most prevalent during the month of December. The prevalence of asthma exacerbation in regular follow-up patients was 13.4%. The age, previous asthma history and emergency visits are shown in Table 1. Nearly 40% of children who visited the emergency room for asthma exacerbation did not have a prior asthma diagnosis. Children regularly followed in our clinics averaged slightly less than 3 emergency department visits in a year. In the

Table 1. Fundamental characteristics of patients

	Male n = 174	Female n = 95
Age (years)[a]	6.46 ± 3.28	6.20 ± 3.16
Previously diagnosed asthma [no. (%)]	110 (63.2)	59 (62.1)
Frequency of emergency visits		
1-3 times [no. (%)]	169 (97.1)	92 (96.8)
>3 times [no. (%)]	5 (2.9)	3 (3.2)

[a]Mean ± standard deviation.

Table 2. Relation of cold hemagglutinin test to mycoplasma immunoglobulin M (IgM) titer

Rapid test	IgM		Total
	<10 BU/mL	≥10 BU/mL	
–	33[a]	10[b]	43
+	47[c]	36[d]	83
Total	80	46	126

Abbreviations: + = positive; – = negative
[a]True negative.
[b]False negative.
[c]False positive.
[d]True positive.

children with a prior diagnosis of asthma, about 20% took their medication compliantly. Of those children who suffered from an asthma attack, only 29.6% had used a short-acting β_2 agonist agent before visiting the emergency room. In the 378 person-times of visits, fever was detected 20.4% of the time (77/378), and the ratio of asthma exacerbation requiring hospitalization was 19.6% (74/378).

126 children (mean age, 5.35 ± 2.90 years) had both a rapid cold hemagglutination test and a mycoplasma IgM titer. Of these 126 children, 46 (36.5%) demonstrated mycoplasma infection. Correlation of cold hemagglutination test with mycoplasma IgM titer is summarized in Table 2. The sensitivity and specificity of the rapid cold hemagglutination test was 78.3% and 41.3%, respectively. A comparison between the 2 groups (asthma exacerbation with or without mycoplasma infection) is shown in Table 3. Surprisingly, the group without mycoplasma infection had a higher number of patients that presented with fever as the chief complaint and rales in auscultation. There were no significant differences between the groups in age, chest X-ray findings, and most symptoms/signs and laboratory data. There were also no statistically significant differences in the frequency of prescribed medications and emergency room hospitalizations.

Discussion

We describe the characteristics of patients who visited a medical center emergency department for treatment of asthma exacerbation. Although September has been described in the literature as an epidemic factor for

Table 3. Comparison of clinical characteristics between patients with and without mycoplasma infection

	Immunoglobulin M		*p*
	Negative n = 80	Positive n = 46	
Fever [n (%)]	42 (52.5)	14 (30.4)	0.016[a]
BT (°C) [mean ± SD]	37.3 ± 1.0	37.2 ± 1.0	0.552
Heart rate (/min) [mean ± SD]	131 ± 22	129 ± 22	0.666
Physical examination			
Coarse [n (%)]	22 (27.5)	16 (34.8)	0.391
Rales [n (%)]	38 (47.5)	12 (26.1)	0.018[a]
Wheeze [n (%)]	73 (92.4)	43 (93.5)	0.823
Retraction [n (%)]	63 (78.8)	37 (80.4)	0.822
Nasal flaring [n (%)]	33 (41.3)	17 (37.0)	0.635
Laboratory data (mean ± SD)			
WBC (/μL)	11,806 ± 4094	11,206 ± 3958	0.43
Segment (%)	74.0 ± 13.5	71.5 ± 18.0	0.433
Lymphocyte (%)	17.2 ± 10.3	19.0 ± 14.6	0.489
Monocyte (%)	5.7 ± 2.8	6.6 ± 4.1	0.215
Eosinophil (%)	2.7 ± 3.2	2.7 ± 2.2	0.974
RBC (M/μL)	4.58 ± 0.48	4.62 ± 0.52	0.671
Platelet (K/μL)	287.37 ± 86.19	305.00 ± 97.38	0.301
CRP (mg/dL)	2.14 ± 2.04	1.64 ± 1.93	0.209
Chest X-ray	n = 76	n = 44	
Infiltration [n (%)]	72 (94.7)	42 (95.5)	0.862
Patchy [n (%)]	13 (17.1)	6 (13.6)	0.616

Abbreviations: BT = body temperature; SD = standard deviation; WBC = white blood cells; RBC = red blood cells; CRP = C-reactive protein
[a]$p<0.05$.

asthma exacerbation [10,11], the weather in December was a risk factor in our study. Although it is possible that the geographic and temporal variations between our study and previous studies could account for this difference, additional research would be needed to verify these points. The prevalence of asthma exacerbation in regular follow-up patients was 13.4%, and the ratio of asthma exacerbation requiring hospitalization was 19.6%. These results are similar to those seen in other countries [12-15]. About 20% of children visiting the emergency room were medication compliant, and only 30% had used short acting β_2 agonists before visiting the emergency room. Exacerbations were characterized by a decrease in expiratory airflow that could be quantified by measurement of lung function (peak expiratory flow [PEF] or forced expiratory volume in 1 sec). These measurements are more reliable indicators of the severity of airflow limitation than is the degree of symptoms.

Daily PEF monitoring is useful for evaluating a child's response to therapy and it can also help detect early signs of worsening before symptoms occur. If we can educate children and families effectively in asthma care programs, the frequency of emergency visits might decline in the future. Furthermore, recording arterial oxygen saturation and blood pressure readings at the initial visit could be useful in monitoring the severity of an acute exacerbation [16].

Chlamydia pneumoniae or *M. pneumoniae* respiratory tract infections are thought to be a trigger for 5 to 30% of wheezing episodes and asthma exacerbations. It also appears that acute infections with *C. pneumoniae* and *M. pneumoniae* can initiate asthma in some previously asymptomatic patients; however, the quantitative role of these atypical bacteria as asthma initiators is presently unknown. Previous studies have demonstrated that some cytokines (e.g., IL-2, IL-6, IL-5, TNF-alpha) may play an important role in the pathophysiological mechanisms of these bacterial infections [17,18].

In our study, 126 patients with asthma exacerbation were tested for mycoplasma IgM serum levels in the emergency room, and the mycoplasma infection rate was found to be 36.5%. It is known that a 4-fold or greater increase in immunoglobulin G (IgG) antibody titer between acute and convalescent serum specimens, using the complement fixation assay, demonstrates mycoplasma infection. However, the IgG antibody titer peaks at approximately 3 to 6 weeks and persists for 2 to 3 months after infection, which might delay timing of treatments [19]. Besides, it is also difficult to have children return to the clinic to collect blood samples 3 or more weeks after treatment for asthma exacerbation; IgG antibody testing is not practical when rapid diagnosis is needed.

A number of commercially available serologic assays use the complement fixation test as a reference standard. However, sensitivity of these assays is low because the glycolipid antigen mixture used is not specific for *M. pneumoniae* and may be found in other microorganisms, as well as in human and plant tissues; therefore, assay specificity is reduced due to possible cross reactions. Furthermore, the presence of increased specificity of IgM against *M. pneumoniae* in a pediatric or college-age young adult patient during acute respiratory tract illness defines *M. pneumoniae* infection; however, rapid diagnostic methods for detecting IgM are not routinely available in the emergency room. The rapid cold hemagglutination test for detecting *M. pneumoniae* has been discussed since 1990. Definite diagnosis by this method was based on a combination of an elevated complement fixation titer (≥1:256) with a positive cold agglutinin titer (≥1:64). Sensitivity and specificity of the rapid cold hemagglutination test was 100% and 97%, respectively. The positive predictive value was about 70% [20]. Previous studies also demonstrated that the rapid cold hemagglutination test was successful in detecting approximately 65 to 85% of patients proven to be infected with *M. pneumoniae* by increasing complement fixing antibody [21-23].

In our study, the sensitivity and specificity of the rapid cold hemagglutination test was 78.3% and 41.3%, respectively. The positive predictive value was 43.4%. Results of the rapid cold hemagglutination test may be more reliable when the mycoplasma IgM antibody enzyme-linked immunosorbent assay titer method is used as a reference standard.

Fever, cough, malaise and rales on chest auscultation are the most commonly encountered symptoms and signs of acute mycoplasma infection, as demonstrated in multiple studies [5,24]. Comparing 2 groups (asthma exacerbation with or without mycoplasma infection) in our study, there was no difference in gender, age, chest X-ray findings, and most symptoms/signs and laboratory data. However, signs of fever and rales on chest auscultation were noted significantly more often in the non-mycoplasma infection group than in the mycoplasma infection group. Additionally, virus, and most notably rhinovirus, was attributed primarily to the non-mycoplasma infection group. It is possible that

cytokine production and lymphocyte activation of *M. pneumoniae* infection minimized disease through the enhancement of host defense mechanisms or exacerbated disease through immunologic lesion development. Therefore, unapparent fever and rales in breath sounds implicate mycoplasma infection-triggered asthma exacerbation.

M. pneumoniae infections usually result in asthma exacerbation and severe pneumonia. In order to adequately prescribe β-lactam antibiotics, a rapid serologic test is needed to reliably diagnose *M. pneumoniae* infection during the initial evaluation. However, because there are no diagnostic tests currently available that reliably and rapidly detect *M. pneumoniae*, prescribed therapy must usually be empirical.

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