Asthma and Atypical Bacterial Infections: *Chlamydia pneumoniae, Mycoplasma pneumoniae* and *Legionella pneumophila*

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Abstract

Background: Respiratory infections with *M. pneumoniae* and *C. pneumoniae* are well-defined causes of acute exacerbation of asthma. This study was undertaken to investigate the association between infections with these pathogens, as well as *L. pneumophila*, and expression of asthma symptoms.

Materials and Methods: A single throat swab specimen was taken from 72 asthma patients (39 presented with an acute exacerbation and 33 had stable asthma) and 46 controls. Bacterial infection with *C. pneumoniae, L. pneumophila,* and *M. pneumoniae* were detected by polymerase chain reaction (PCR).

Results: *C. pneumoniae* infection was related with exacerbation of symptoms in asthmatic patients (23% in asthma exacerbation, 3.3% in stable asthma, 2.2% in controls). *L. pneumophila* infection was higher in asthmatic group than in the control group (5.5% *vs* 0%). *M. pneumoniae* infection was not associated with asthma (7% *vs* 4.3%). There was a statistically significant positive correlation between asthma severity and PCR positivity, a finding that is consistent with the dose-response relationship and may be considered as a positive evidence for the causality of association.

Conclusions: *C. pneumoniae* infection should be sought in an acute asthma attack and especially if the patient has severe asthma, and ampirical antibiotic treatment may be considered.

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Key words: *asthma; atypical bacterial infections; PCR*

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Introduction

Although viruses remain as the most common etiological agents in respiratory infections, M. *pneumoniae* and C. *pneumoniae* are also well defined causes of acute exacerbation of asthma. However, current evidence associating these infections with asthma attacks is still insufficient. It is suggested that C. *pneumoniae* can achieve a state of dormancy and in that state may synthesize a 60-kDa heat shock protein, the so-called "stress protein", which is able to elicit a strong inflammatory response at its sites of production and is involved in tissue injury and scarring process (1). This study was undertaken to investigate the association between infections with these pathogens, as well as L. *pneumophila*, and expression of asthma symptoms.

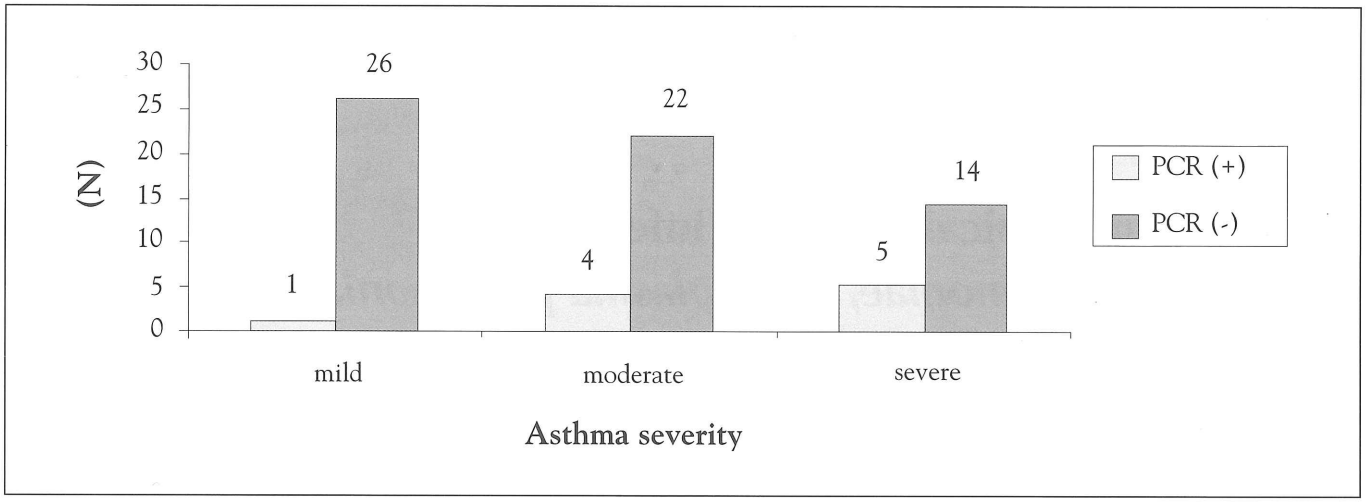

Figure 1a. Chlamydia pneumoniae *PCR positivity according to asthma severity.*

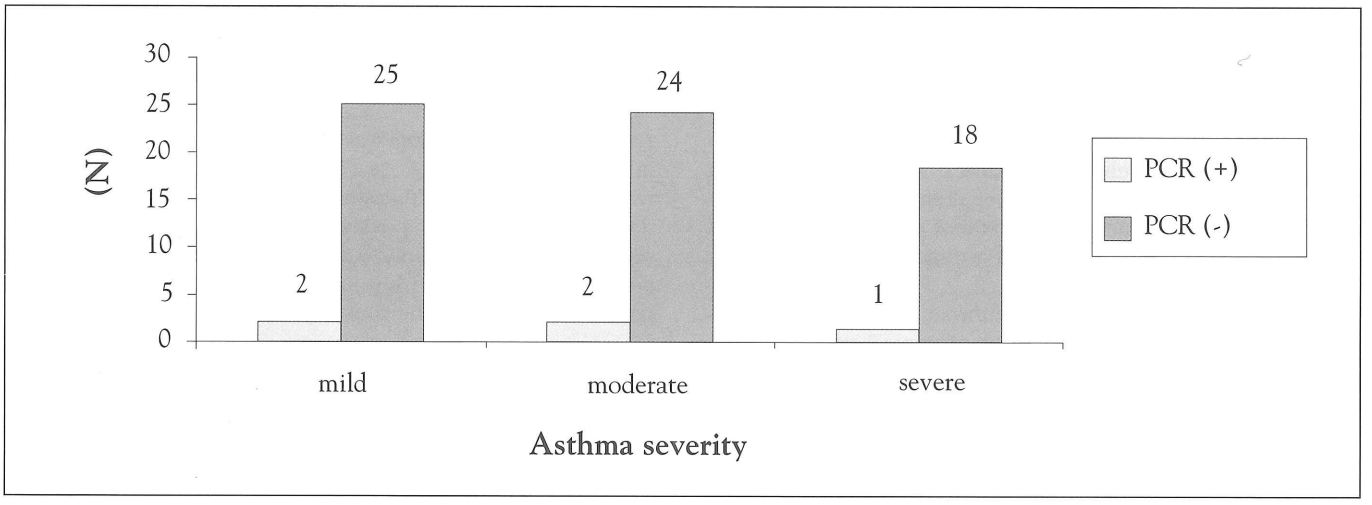

Figure 1b. Mycoplasma pneumoniae *PCR positivity according to asthma severity.*

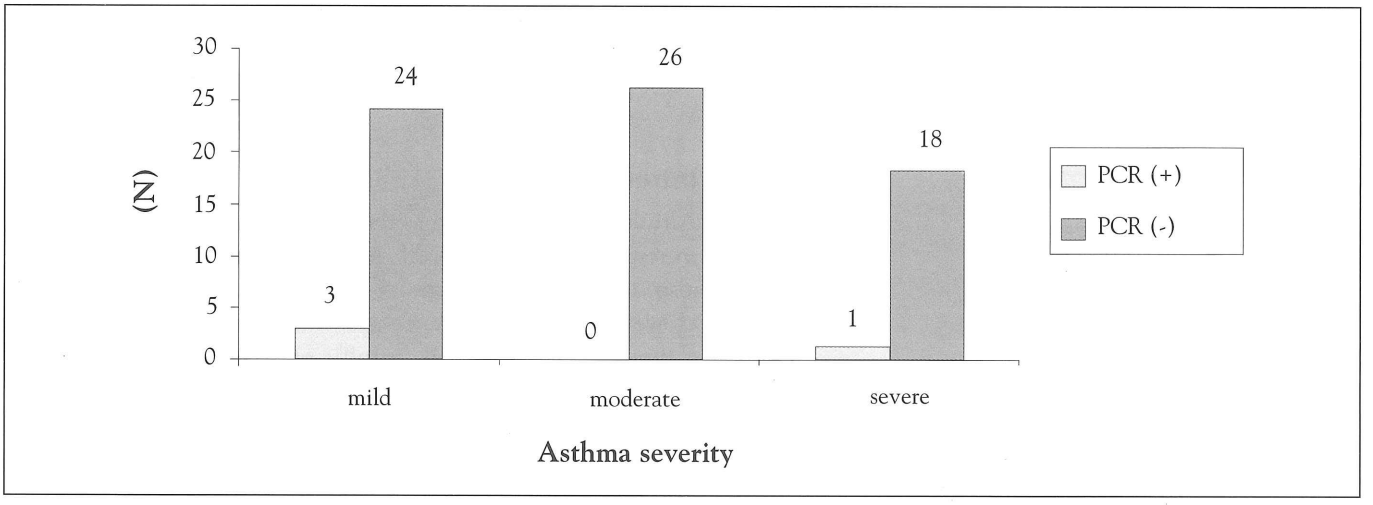

Figure 1c. Legionella pneumophila *PCR positivity according to asthma severity.*

Table 1. PCR findings in asthma patients and in controls

	C. pneumoniae PCR (+) n (%)	*M. pneumoniae* PCR (+) n (%)	*L. pneumophila* PCR (+) n (%)
All asthma cases, n:72	10 (14%)*	5 (7%)	4 (5.5%)*
Acute exacerbation cases, n:39	9 (23%)**	2 (5.1%)	3 (7.6%)
Stable asthma cases, n:33	1 (3.3%)	3 (9%)	1 (3.3%)
Control group, n:46	1 (2.2%)	2 (4.3%)	0

* Statistically significant difference ($p<0.05$) for asthma vs control group.
** Statistically significant difference ($p<0.05$) for asthma acute exacerbation vs stable asthma.

Materials and Methods

We enrolled to the study 72 asthma patients and 46 controls who were free of respiratory or cardiovascular disease. The mean age of the control group was 45.10±2.61 yrs with a female/male ratio of 1.55. In the asthma group mean age in 39 patients who presented with an acute exacerbation was 45.21±2.40 yrs, with a female/male ratio of 2.45, and the mean age of 33 patients who had stable asthma was 45.60±1.61 yrs (female/male ratio 1.70). The presence of atypical bacterial infection was detected by polymerase chain reaction (PCR) from a single throat swab specimen. PCR reaction is reported as a rapid, highly specific and sensitive method that greatly simplifies the diagnosis of infection (2). The study group consisted of patients who presented to the outpatient clinic of the Chest Diseases Department of Hacettepe University Hospital, Ankara-Turkey, during January 1999-January 2000 time period. All of the asthmatic patients fulfilled the diagnostic criteria for asthma and chest radiographs were taken to rule out infiltrations. Asthma patients were grouped as mild (27), moderate (26) and severe (19) asthmatics according to the Asthma Guidelines of the Turkish Thoracic Society.

Throat swab samples were obtained by a dacron swab and put into sterile tubes containing 2 ml Tris-EDTA (TE) solution (2M Tris HCL+0.5 M EDTA) and transported quickly to the laboratory for DNA extraction. A simple and sensitive method for DNA extraction similar to the method previously described by us for *M. tuberculosis* was used (3). Briefly, the samples were vortexed for a few seconds, transported to 1.5 ml microcentrifuge tubes and centrifuged for 5 minutes. The supernatant was removed and debris was washed with TE solution twice. The debris then was suspended in 100 µl TE solution and boiled for 20 minutes. Samples were centrifuged for 5 minutes at 12 000 rpm and the supernatant containing the DNA was transferred to a clean tube and stored at -20°C until it was used for amplification.

The primers used in the study were described for *C. pneumoniae*, *M. pneumoniae*, and *L. pneumophila* by Capbell *et al*, Buck *et al* and Starnbach *et al* (4-6).

The association between asthma and atypical infections was assessed by *Chi*-square testing. Spearman non-parametric correlation was used to evaluate the association between asthma severity and atypical infections. Statistical significance was defined as a p value less than 0.05.

Results

C. pneumoniae:

PCR was found as positive in 10 of 72 asthmatics (13.8%) and 1 of 46 controls (2.2%). The difference was statistically significant ($p<0.05$). Nine of 39 patients (23%) with acute exacerbation and 1 of 33 (3%) stable asthmatics (this one patient was also positive for *M. pneumoniae*) were found to be PCR positive. The difference was statistically significant ($p<0.05$). The proportion of PCR positivity for *C. pneumoniae* was 3.7% (1/27) in the mild asthmatic group, 15.3% (26/4) in the moderate asthmatic group and 26.3% (19/5) in the severe asthmatic group. There was a statistically significant positive correlation between asthma severity and PCR positivity ($p=0.027$) (Figure 1a).

M. pneumoniae:

M. pneumoniae PCR was positive in 5 of 72 asthmatics (6.9%) and 2 of 46 (4.3%) controls. The difference was not statistically significant. Two of 39 patients with acute exacerbation (5%) and 3 of 33 stable asthmatics (9%) were PCR positive, but this difference did not reach statistical significance. The proportion of PCR positivity for *M. pneumoniae* was 7.4% (2/27) in the mild asthmatic group, 7.2% (2/16) in the moderate asthmatic group and 5.2% (1/19) in the severe asthmatic group. PCR positivity for *M. pneumoniae* was not significantly correlated with severity of asthma (Figure 1b).

L. pneumophila:

L. pneumophila PCR was positive in 4 of 72 asthmatics (5.5%) and none of the control patients. The difference was statistically significant ($p<0.05$). Three of 39 (7.6%) patients with acute exacerbation and 1 of 33 (3%) stable asthmatics were PCR positive, but this difference did not reach statistical significance. Three of 27 (11%) mild, none

of the 26 moderate (0%) and 1 of 19 (5.2%) severe asthmatics were PCR positive for *L. pneumophila*. PCR positivity for *L. pneumophila* was not significantly correlated with severity of asthma (Figure 1c).

A summary of PCR findings is given in Table 1.

Discussion

In our study C. *pneumoniae* PCR positivity was significantly higher in the asthmatic (13.8%) than in the non-asthmatic group (2.2%). These results are similar to those reported by Martin *et al*, who found C. *pneumoniae* PCR positivity from throat swabs in 7 of 55 (12.8%) asthmatic patients (7). But in our study this marked difference was observed only in patients with acute asthma exacerbation (9/39 *vs* 1/33 in acute exacerbation and stable asthma, respectively). There was a positive correlation between asthma severity and PCR positivity. This finding is consistent with a previous study of Hahn and coworkers who reported a higher detection rate of C. *pneumoniae* in severe steroid dependent asthmatics. Our findings support these authors' recommendation to evaluate C. *pneumoniae* infection and in selected cases to administer an empirical antibiotic treatment to patients with persistent severe asthma that is poorly controlled despite conventional anti-inflammatory asthma therapy (8).

In contrast to some studies which suggest a link between M. *pneumoniae* infection and exacerbation of asthma as well as initiation of the disease (9), we did not find any statistically significant difference for M. *pneumoniae* PCR between asthmatics and non-asthmatics (6.9% *vs* 4.3%) or between acute exacerbation and stable asthma (5% *vs* 9%). Actually, findings on this issue are not consistent and while C. *pneumoniae* seems to be more important for asthma pathogenesis and exacerbation than M. *pneumoniae* in some studies, the role of M. *pneumoniae* was found to be more significant in other studies (8,11).

Few studies investigated the association between asthma and *L. pneumophila*. Boldur et al found a higher seroprevalance in asthmatic children compared to healthy controls (10). In our study, we found a higher *L. pneumophila* PCR positivity in the asthmatic group (5.5% *vs* 0%), but there was no correlation between the asthma severity score and the titer of serum antibodies to *L. pneumophila*. Three of 4 patients who were PCR positive presented with acute exacerbation and 1 had stable asthma. Legionella infections, causing an intracellular infection similar to that of potential initiators or exacerbators of asthma such as viruses and C. *pneumoniae*, may also be associated with asthma symptoms. In this study we did not use the "golden standard" diagnosis of *Legionella* infections namely, culture of the microorganism, so this possibility remains to be investigated.

In summary, infection with C. *pneumoniae* is related with exacerbation of asthma symptoms and is strongly correlated with the severity of the disease. This finding, namely, correlation of PCR positivity with asthma severity, is consistent with the dose-response relationship and may be considered as a positive evidence for the causality of this association. The possibility of C. *pneumoniae* infection should be considered in acute exacerbations and severe, difficult to treat asthmatics. Empirical antibiotic treatment targeting this pathogen may be administered in selective cases. (8,11)

References

1. Von Hertzen LC. Role of persistent infection in the control and severity of asthma: Focus on C. *pneumoniae*. *Eur. Respir J* 2002;19:546-556
2. Ramirez JA, Ahkee S, Tolentino A. Diagnosis of *Legionella pneumophila*, *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* lower respiratory infection using the polymerase chain reaction on single throat specimen. *Diag Microbiol Infect Dis* 1996: 4;7-14
3. Kocagöz T, Yılmaz E, Özkara S, *et al.* Detection of M. *tuberculosis* in sputum samples by polymerase chain reaction using a simplified procedure. *J Clin Microbiol* 1993;31:1435-1438
4. Capbell LA, Melgosa MP, Hamilton DJ, *et al.* Detection of C. *pneumoniae* by polymerase chain reaction. *J Clin Microbiol.* 1992:30;434-39
5. Buck GE, O'Hara LC, Summersgill JT, *et al.* Rapid, sensitive detection of M. *pneumoniae* in simulated clinical specimens by DNA amplification. *J Clin Microbiol* 1992:30;3280-3
6. Starnbach MN, Falkow S, Tomkins LS. Species specific detection of *L. pneumophila* in water by DNA amplification and hybridization. *J Clin Microbiol* 1989:27;1257-61
7. Martin RJ, Kraft M, Chu HW, *et al.* A link between chronic asthma and chronic infection. *J Allergy Clin Immunol.* 2001:107;595-601
8. Hahn DL. C. *pneumoniae*, asthma and COPD: What is the evidence? *Ann Allergy Asthma Immunol.* 1999:83;271-88
9. Cunnigham AF, Johnston SL, Julious SA, *et al.* Chronic C. *pneumoniae* infection and asthma exacerbations in children. *Eur Respir J.* 1998:11;345-49
10. Boldur I, Beer S, Kazak R, *et al.* Predisposition of the asthmatic child to legionellosis?. *Isr J Med Sci.* 1986:22;732-36
11. Kraft M. The role of bacterial infections in asthma. *Clin Chest Med.* 2000:21;301-13