ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 1, 19 SECTIO D 2004

Department of Clinical Microbiology, Department of Pulmonology and Rheumatology
Department of Otolaryngology, Phoniatry and Audiology, Skubiszewski Medical University of Lublin

AGNIESZKA SZCZEPANIK, MARIA KOZIOŁ-MONTEWKA,
EWA TUSZKIEWICZ-MISZTAL, GRAŻYNA NIEDZIELSKA,
GRAŻYNA GÓRNICKA, JUSTYNA NIEDŹWIADEK, ARTUR NIEDZIELSKI

Evaluation of the association between atypical bacteria infections and respiratory tract diseases with emphasis on bronchial asthma exacerbations in children

A growing body of evidence is suggestive of an important role of respiratory tract infections in the pathogenesis of asthma (6). Viral respiratory tract infections are recognized to exacerbate established asthma in both adults and children. It has also been speculated that viruses may be associated with the initiation and maintenance of this chronic inflammatory disease process (2, 4). Recently, nonviral respiratory pathogens such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have received much attention as possible etiological agents involved in the initiation and promotion of asthma in both children and adults because of their tropism to the human respiratory tract and their ability to produce chronic respiratory tract infection and inflammation (4). Atypical bacteria are also emerging as the causative agents in respiratory infections such as rhinitis, pharyngitis, sinusitis and otitis, as well as bronchitis and atypical pneumonia (2, 4, 8, 11, 12). However, a reported link between these microorganisms and the exacerbation of asthma, as well as asthma development is not conclusive; further studies evaluating atypical bacteria as possible agents involved in asthma pathogenesis are required. Most of the published information regarding association between atypical bacteria and asthma pathogenesis is derived from studies of adults (6, 8, 10, 16).

The aim of the study was to investigate the presence of *M. pneumoniae* and *C. Pneumoniae*-specific antibodies in children suffering from respiratory tract disorders with emphasis on bronchial asthma exacerbations.

MATERIAL AND METHODS

30 children aged 3–14 years suffering from bronchial asthma exacerbations, 10 children aged 5–11 years with pneumonia, 22 children aged 1–15 years with sinusitis, and 28 children aged 2–14 years with chronic otitis media with effusion (COME) were enrolled in our study.

20 children of similar age without any respiratory symptoms but admitted to the same hospital for stomach disorders were evaluated as control group for *M. pneumoniae* serological studies.

Serum samples for determination of levels of *M. pneumoniae* and *C. pneumoniae*-specific antibodies were collected and frozen at –20°C. Serological studies were performed with an enzyme-linked immunosorbent assay (ELISA, Pointe Scientific, Poland) for IgM and IgG directed against *M. pneumoniae* and with a microimmunofluorescence test (MIF, Labsystems, Helsinki, Finland) for

C. pneumoniae-specific IgG and IgA antibodies. All tests were performed according to the manufacturers' instructions. The serological evidence of infection with these bacteria was based on criteria published by Principi et al. and Esposito et al. (12, 4).

Acute *M. pneumoniae* or *C. pneumoniae* infection was diagnosed if a significant antibody response was shown in patient's serum sample – *M. pneumoniae*: IgM specific antibody ≥ 1:100, IgG specific antibody ≥ 1:400, *C. pneumoniae* IgG ≥ 1:512. Past *M. pneumoniae* infection was diagnosed if the patient had an IgG antibody titer ≥ 1:100 but less than 1:400; past *C. pneumoniae* infection was diagnosed if the patient had an IgG antibody titer ≥ 1:16 but less than 1:512 and/or an IgA specific antibody titer ≥ 1:16.

Statistical analysis. Depending on the results of the Normality test Student's t-test or Mann-Whitney U-test was employed to analyse significance of differences between patients and controls.

RESULTS

C. pneumoniae serology results were positive relatively rarely in the analysed groups of patients (Table 2). Specific anti-chlamydial antibodies were detected in four children (13.3%) with asthma exacerbations and in one child (10%) with pneumonia. According to the criteria adopted from the literature data the mentioned patients had serological profile consistent with past chlamydial infection. Two children (7.1%) with COME met serological criteria suggestive of a possible acute *C. pneumoniae* infection. Additionally, one patient with COME had high *C. pneumoniae* and *M. pneumoniae*-specific IgG titers – 1:512 and 1:800, respectively. It suggested a possible co-infection with both pathogens. Chlamydial antibodies were not detectable in any patient with sinusitis.

Specific antibodies directed against *M. pneumoniae* were detected more frequently than C. pneumoniae-antibodies in all examined groups of patients (Table 1). *M. pneumoniae*-specific antibodies were detected in 18 patients (60%) with asthma, in five (50%) children with pneumonia, in 11 subjects (39.2%) with COME, and in 10 (45.4%) of sinusitis cases. Eight (26.6%) patients with asthma exacerbations, five (50%) patients with pneumonia, nine (32.1%) children with COME and 10 (45.4%) sinusitis patients met serological criteria indicative of a possible acute mycoplasmal infection. Positive serological status was confirmed in six (30%) of 20 control subjects including two children (10%) with possible acute infection with *M. pneumoniae* (Table 1). Differences between the patients and control group did not achieve statistical significance.

Table 1. *M. pneumoniae* serology results

Antibody titer	Number of positive samples				
	Asthma (n=30)	Pneumonia (n=10)	COME (n=28)	Sinusitis (n=22)	Controls (n=20)
IgG ≥ 1:100 < 1:400	10	0	1	0	3
IgM ≥ 1:100 and IgG < 1:400	0	0	1	0	1
IgM ≥ 1:100	1	2	2	1	1
IgG ≥ 1:400	6	1	5	5	1
IgM ≥ 1:100 and IgG ≥ 1:400	1	2	2	4	0

Table 2. *C. pneumoniae* serology results

Antibody titer	Number of positive samples			
	Asthma (n=30)	Pneumonia (n=10)	COME (n=28)	Sinusitis (n=22)
IgG ≥ 1:512	0	0	2	0
IgG ≥ 1:16 < 1:512	3	1	0	0
IgA ≥ 1:16	1	0	0	0

DISCUSSION

Microorganisms responsible for respiratory tract infections, among which there are atypical bacteria such as *C. pneumoniae* and *M. pneumoniae*, are suggested to take part in the etiopathogenesis of bronchial asthma.

Chlamydiae are obligate intracellular pathogens mainly infecting epithelial cells and macrophages (6). One of the most characteristic features of these microorganisms is a unique biphasic life cycle which occurs during infection. The infectious chlamydial particle is the elementary body that attaches itself to and enters a susceptible cell by phagocytosis. Elementary body changes to the larger metabolically active reticulate body within the phagosome. Reticulate bodies multiply using the host--cell energy stores and form characteristic cytoplasmic inclusions. The reticulate bodies revert to the elementary body form prior to cell lysis (1, 11).

Intracellular growth is one of the mechanisms preventing recognition of chlamydial infections by the host immune system. It favors persistent infection being a key concept in the pathogenesis of *Chlamydia* (6).

M. pneumoniae is a microorganism distinguished phenotypically from other bacteria by its minute size and lack of a rigid cell wall. Adhesion of *Mycoplasma* to host cells is a prerequisite for colonization and for infection. This pathogen colonizes the bronchial passages, localizing to the base of the cilia where it interacts directly with the host cell surface. *M. pneumoniae* attaches to ciliated epithelial cells by a specialized terminal organelle. Adhesion is a multifactorial process, involving action of adhesins and a number of accessory membrane proteins. The intimate contact of bacteria with the host cell membrane may result in local, perhaps transient fusion of the two membranes or exchange of membrane components and hence, in direct "injection" of mycoplasma cytoplasmic content, including hydrolytic enzymes into the host cell cytoplasm (14). Metabolic and ultrastructural alterations in the affected cell are observed and these result in epithelial cell damage and ciliostasis (1). Oxidative damage to host cells is caused by mildly toxic by-products of mycoplasma metabolism, such as hydrogen peroxide and superoxide radicals (14).

M. pneumoniae and *C. pneumoniae* are also responsible for numerous interactions with the host immune system.

Most patients develop *M. pneumoniae*-specific antibodies of IgM and IgG classes. It has been also reported that these bacteria induce production of specific IgA both in serum and in respiratory secretions (15).

The level of serum total IgE, *M. pneumoniae*-specific IgE and IgE specific to common allergens unrelated to the infectious agent have also been detected during the course of mycoplasmal infection. As *M. pneumoniae* infections lead to the destruction of the respiratory mucosal cells, they may facilitate the penetration of allergens into the mucosa. Favourable condition for an IgE response may result in the release of chemical mediators leading to bronchospasm, airway inflammation and airway hyperresponsiveness (9, 15, 16).

Infection with *C. pneumoniae* induces serum immunoglobulin IgM, IgA and IgG responses (11). As Hahn et al. reported *C. pneumoniae*-specific IgA immunoglobulins may be useful as markers for persistent infection in asthma. The half life of serum IgA is less than one week. Hence, its continuous presence may indicate persistent antigenic stimulation to the immune system. It is also possible that *C. pneumoniae*-specific IgE antibodies play a role in asthma pathogenesis, since their presence suggests that the infection is capable of triggering allergic symptoms which could include asthma (8).

Although some evidence has drawn attention to these atypical bacteria as possible infectious agents in asthma pathogenesis, available data are not conclusive.

In the study undertaken by Cunningham et al. to investigate the association between *C. pneumoniae* and *M. pneumoniae* infection and the expression of asthma-related symptoms in children suffering from this disorder, the most common pathogen in nasal aspirates obtained during acute exacerbations and when patients were well was *C. pneumoniae*. The authors also found a relationship between the local production of secretory IgA antibodies for *C. pneumoniae* and the numbers of asthma exacerbations. *M. pneumoniae* was not found to be an important pathogen as it was detected using PCR assay only in two of 292 reports and in two of 65 asymptomatic samples (2).

Emre et al. examined association of *C. pneumoniae* infection and reactive airway disease in children and isolated this from 11% children with wheezing. However, serologic evidence of acute infection was present in only 25% of asthmatics with positive cultures (3).

Gil et al. studied 77 patients with asthma (aged 8 months to 31 years) and found that *M. pneumoniae* colonized a higher percentage of patients suffering from asthma than controls and concluded that this pathogen could have been responsible for inducing the wheezing (7).

Esposito et al. reported that *M. pneumoniae* and *C. pneumoniae* were significantly related to wheezing in children, particularly in subjects with a history of recurrent episodes. According to these authors the finding of a relationship between wheezing episodes and acute infections caused by *M. pneumoniae* and *C. pneumoniae* suggests a potential role for these pathogens in the exacerbation of bronchial asthma. Acute *M. pneumoniae* and *C. pneumoniae* infections were demonstrated in 16 (22.5%) and in 11 (15.5%) of the 71 children with wheezing, respectively (4).

The incidence of acute *M. pneumoniae* and *C. pneumoniae* infections in children hospitalized for community-acquired lower respiratory tract infections such as bronchitis, wheezing and pneumonia on the basis of serological and PCR findings was evaluated by Principi et al. Acute *M. pneumoniae* and *C. pneumoniae* infections were diagnosed in 34.3% and 14.1% of cases, respectively (12).

In the light of our present study *M. pneumoniae* seems to be an important etiological agent in children with asthma since the frequency of occurrence of anti-*M. pneumoniae* antibodies (60%) was twofold higher than in control subjects and it was the highest among the studied groups of patients. Evidence for acute infection on the basis of serological findings was shown in 26.6% of asthma cases. Hence, we can expect that there exists a possible association between infections caused by this pathogen and bronchial asthma exacerbations. We cannot also exclude its possible role in the previous initiation of asthma symptoms, since the rate of serological profile consistent with past mycoplasmal infection was 33.3% among asthma patients.

It is conceivable that this microorganism may be involved in subsequent asthma development as the effects of mycoplasmal infection, which can persist for months, result in decreased expiratory flow rates and increased airway hyperresponsiveness in nonasthmatic individuals [10]. Yano et al. described

a patient in whom a previous acute mycoplasmal respiratory infection led to an initial onset of bronchial asthma (16).

The results of our study suggest that *M. pneumoniae* could also be involved in the etiology of other diseases involving the upper and lower respiratory tract such as COME, sinusitis and pneumonia.

Both *C. pneumoniae* and *M. pneumoniae* have been linked to the pathogenesis of common childhood diseases including otitis media in other reports but their role has not been fully elucidated (5, 13).

Although patients suffering from COME did not show other respiratory symptoms, we do not exclude the possibility of asymptomatic infections affecting other parts of respiratory system. Hence, our data suggest but do not prove that *M. pneumoniae* could be involved in the etiology of middle ear inflammation and our speculations regarding its causative role in this disease process must be taken with caution. The same conclusion can be obtained in case of 45.4% of the sinusitis patients, who met serological criteria indicative of a possible current infection with *M. pneumoniae*.

Infection with *C. pneumoniae* is the most common among children 5 to 14 years of age. The seroprevalence continues to increase among older age groups reaching approximately 75% in the elderly. It suggests that reinfection with this pathogen is common and widespread as antibody response to first infection is only time-limited (11).

However, we observed low frequency of occurrence of immunological response directed against *C. pneumoniae* in the patients compared with the rate of antibody response against *M. pneumoniae*.

This phenomenon can be explained by an immature ability to produce a *C. pneumoniae*-specific humoral response or poor antigenic stimulation (12). Hence, the use of serologic testing alone underestimates the prevalence of *C. pneumoniae* infections in children (3).

In the present study evidence of acute chlamydial infection was observed only in two (7.1%) COME patients including one patient with a possible co-infection with *C. pneumoniae* and *M. pneumoniae*. These results show that infections with both atypical bacteria are frequently encountered in children suffering from middle ear diseases. However, their significance in the pathogenesis of otitis media still remains a contentious point and requires further investigation.

CONCLUSIONS

The results of our study corroborate the hypothesis that *M. pneumoniae* is an important etiological agent in children with asthma exacerbations.

In addition to the suggested possible role of *M. pneumoniae* in childhood asthma pathogenesis we observed higher rates of specific antibody response indicative of a possible acute mycoplasmal infection in patients with other respiratory tract disorders, such as pneumonia, COME and sinusitis compared to the controls.

Hence, it can be assumed that this atypical microorganism remains an important infectious agent probably responsible for a broad spectrum of respiratory diseases in children.

REFERENCES

1. Andersen P.: Pathogenesis of lower respiratory tract infections due to *Chlamydia, Mycoplasma, Legionella* and viruses. Thorax, 53, 302, 1998.
2. Cunningham A. F. et al.: Chronic *Chlamydia pneumoniae* infection and asthma exacerbations in children. Eur. Respir. J., 11, 2, 345, 1998.

3. Emre U. et al.: The association of *Chlamydia pneumoniae* infection and reactive disease in children. Arch. Pediatr. Adolesc. Med., 148, 7, 727, 1994.
4. Esposito S. et al.: Importance of acute *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infections in children with wheezing. Eur. Respir. J., Vol. 16. Issue, 6, 1142, 2000.
5. Falck G. et al.: Association of *Chlamydia pneumoniae* with otitis media in children. Scand. J. Infect. Dis., 30, 4, 377, 1998.
6. Gencay M. et al.: Increased frequency of *Chlamydia pneumoniae* antibodies in patients with asthma. Am. J. Respir. Crit. Care Med., 163, 5, 1097, 2001.
7. Gil J. C. et al.: Isolation of *Mycoplasma pneumoniae* from asthmatic patients. Ann. Allergy, 70, 1, 23, 1993.
8. Hahn D. L. et al.: Serologic markers for *Chlamydia pneumoniae* in asthma. Ann. Allergy Asthma Immunol., 84, 227, 2000.
9. Koh Y. Y. et al.: Levels of Interleukin-2, Interferon-gamma and Interleukin-4 in bronchoalveolar lavage fluid from patients with *Mycoplasma* pneumonia: implication of tendency toward increased Immunoglobulin E production. Pediatrics, 107, 3, 39, 2001.
10. Kraft M. et al.: Detection of *Mycoplasma pneumoniae* in the airways of adults with chronic asthma. Am. J. Respir. Crit. Care Med., 158, 998, 1998.
11. Kuo C. et al.: *Chlamydia pneumoniae* (TWAR). Clin. Microbiol. Rev., 8, 4, 451, 1995.
12. Principi N. et al.: Role of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in children with community-acquired lower respiratory tract infections. Clin. Infect. Dis., 32, 1281, 2001.
13. Räty R., Kleemola M.: Detection of *Mycoplasma pneumoniae* by polimerase chain reaction in middle ear fluids from infants with acute otits media. Pediatr. Infect. Dis. J., 19, 7, 666, 2000.
14. Razin S. et al.: Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev., 62, 4, 1094, 1998.
15. Seggev J. S. et al.: Isotype-specific antibody responses to acute *Mycoplasma pneumoniae* infection. Ann. Allergy Asthma Immunol., 77, 67, 1996.
16. Yano T. et al.: Association of *Mycoplasma pneumoniae* antigen with initial onset of bronchial asthma. Am. J. Respir. Crit. Care Med., 149, 1348, 1994.

SUMMARY

Mycoplasma pneumoniae and *Chlamydia pneumonie* are important etiological agents responsible for human respiratory tract diseases. Recently, these atypical microorganisms received much attention regarding their role in bronchial asthma pathogenesis, which is one of the most frequent chronic diseases in children. The aim of the study was to investigate the association between infections caused by these pathogens and respiratory tract diseases in children. Levels of *M. pneumoniae* and *C. pneumoniae* - specific antibodies were determined in serum samples obtained from 30 patients suffering from bronchial asthma exacerbations, 10 patients with pneumonia, 28 patients with chronic otitis media with effusion (COME) and 22 sinusitis patients. Specific anti-*M. pneumoniae* antibodies were detected more frequently in the patients enrolled in the study than in control subjects. The highest percentage of the serum samples, which demonstrated the presence of *M. pneumoniae*-specific antibodies was demonstrated in patients with asthma (60%) and it was twofold higher than in control subjects. Serologic profile of 26.6% patients with asthma, 50% of patients with pneumonia, 39.2% of patients with COME, 45.4% of patients with sinusitis and 10% of control subjects was consistent with a possible acute infection caused by *M. pneumoniae*. The presence of specific anti-*C. pneumoniae* antibodies was demonstrated in a smaller percentage of patients – in 13.3% of children with asthma, 10% of children with pneumonia and in 7.1% of patients with COME; the level of specific antiobodies

was suggestive of acute chlamydial infection only in COME patients. Analysis of serologic markers for atypical bacteria infections indicates a possible association between infections caused by *M. pneumoniae* and bronchial asthma exacerbations and other respiratory tract disorders including pneumonia, sinusitis and COME.

Ocena związku pomiędzy infekcjami wywoływanymi przez atypowe bakterie a chorobami dróg oddechowych ze szczególnym uwzględnieniem zaostrzeń astmy oskrzelowej u dzieci

Mycoplasma pneumoniae i *Chlamydia pneumonie* są ważnymi czynnikami etiologicznymi chorób układu oddechowego człowieka. W ostatnich latach wiele uwagi poświęca się roli tych atypowych mikroorganizmów w patogenezie astmy oskrzelowej, którą zalicza się do najczęściej występujących przewlekłych chorób u dzieci. Celem badania było określenie związku pomiędzy infekcjami wywoływanymi przez te patogeny a chorobami układu oddechowego, poprzez oznaczenie poziomu przeciwciał swoistych wobec *M. pneumoniae* i *C. pneumoniae* w próbkach surowicy pobranych od 30 pacjentów cierpiących na zaostrzenie astmy oskrzelowej, 10 pacjentów z zapaleniem płuc, 28 pacjentów z przewlekłym wysiękowym zapaleniem ucha środkowego (*wzuś*) oraz 22 pacjentów z zapaleniem zatok. Przeciwciała skierowane przeciwko *M. pneumoniae* występowały częściej u pacjentów poddanych badaniu niż u osób z grupy kontrolnej. Najwyższy odsetek próbek surowicy, wykazujących obecność przeciwciał swoistych wobec *M. pneumoniae,* występował u pacjentów z astmą (60%) i był dwukrotnie wyższy niż dla osób z grupy kontrolnej. Profil serologiczny 26,6% pacjentów z astmą, 50% pacjentów z zapaleniem płuc, 39,2% dzieci z *wzuś,* 45,4% osób z zapaleniem zatok oraz 10% dzieci z grupy kontrolnej wskazywał na prawdopodobną ostrą infekcję wywołaną przez *M. pneumoniae.* Obecność przeciwciał anty-*C. pneumoniae* stwierdzono u mniejszego odsetka pacjentów – u 13,3% dzieci z astmą, u 10% dzieci z zapaleniem płuc oraz u 7,1% pacjentów z *wzuś*; jedynie u pacjentów z *wzuś* poziom swoistych przeciwciał sugerował ostrą infekcję. Analiza występowania serologicznych markerów zakażenia atypowymi bakteriami wskazuje na możliwy związek między infekcjami wywołanymi przez *M. pneumoniae* a zaostrzeniami astmy oskrzelowej oraz innymi chorobami układu oddechowego, włączając zapalenie płuc, zatok oraz wysiękowe zapalenie ucha środkowego.