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Importance of acute *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infections in children with wheezing

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Importance of acute Mycoplasma pneumoniae *and* Chlamydia pneumoniae *infections in children with wheezing. S. Esposito, F. Blasi, C. Arosio, L. Fioravanti, L. Fagetti, R. Droghetti, P. Tarsia, L. Allegra, N. Principi. ©ERS Journals Ltd 2000.*

ABSTRACT: In order to evaluate the role of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in reactive airway disease, 71 children aged 2–14 yrs with an acute episode of wheezing and 80 age-matched healthy children were studied.

Sera for the determination of specific antibody levels and nasopharyngeal aspirates for the detection of *M. pneumoniae* and *C. pneumoniae* deoxyribonucleic acid were obtained on admission and after 4–6 weeks. All children with wheezing received a standard therapy with inhaled corticosteroids and bronchodilators for 5–7 days; when antibiotic was added on the basis of the judgement of the paediatrician in charge, clarithromycin 15 mg·kg body weight^{-1}·day^{-1} for 10 days was used.

Acute *M. pneumoniae* and *C. pneumoniae* infections were detected significantly more often in children with wheezing than in controls. In patients infected with one of the two pathogens, a history of recurrent wheezing was significantly more frequent than in those without either infection. During a 3-month follow-up period, among nonantibiotic-treated children, those with acute *M. pneumoniae* and/or *C. pneumoniae* infection showed a significantly higher recurrence of wheezing than those without acute *M. pneumoniae* and/or *C. pneumoniae* infection (p=0.03).

These results highlight the apparently significant relationship of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* with wheezing in children, particularly in subjects with a history of recurrent episodes, and the possible improvement in the course of reactive airway disease within paediatric patients with acute *Mycoplasma pneumoniae* and/or *Chlamydia pneumoniae* infection.

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During the late 1900s, wheezing became one of the most frequent causes of consultation in paediatric practice [1, 2]. A number of epidemiological and clinical studies have highlighted most episodes of wheezing occurring in early life as being associated with viral infections, the most frequently encountered agents being respiratory syncytial virus, adenovirus, parainfluenza viruses 1, 2 and 3, influenza virus types A and B, and rhinovirus [3, 4]. The possibility that viruses may interact with the immune and respiratory systems in early life to initiate the complex pathogenetic mechanism leading to asthma has been the matter of considerable study and debate [5]. It is generally recognized that viral respiratory infections often exacerbate established asthma, and there is speculation that they may be associated with the initiation and maintenance of asthma.

Nonviral respiratory pathogens such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have also been associated with the possible initiation and promotion of asthma [6–9]. Both *M. pneumoniae* and *C. pneumoniae* are the causative agents in a number of respiratory diseases, including upper respiratory tract illnesses such as rhinitis, pharyngitis and otitis, as well as bronchitis and atypical pneumonia [10]. These pathogens are plausible candidates for being aetiological agents in asthma because of their tropism in regard the human respiratory tract and their demonstrated ability to produce chronic respiratory tract infection and inflammation. Further evidence for the role of these pathogens in asthma comes from the observation of improvement in asthma symptoms after antimicrobial therapy active against *M. pneumoniae* and *C. pneumoniae* [11, 12].

Most of the published information linking *M. pneumoniae* or *C. pneumoniae* infection to asthma is derived from studies in adult patients [6, 7, 11, 12]. Few data are available regarding childhood [8, 9]. The aim of the present study was to evaluate the role of *M. pneumoniae* and *C. pneumoniae* in paediatric patients with reactive airway disease.

Subjects and methods

Study subjects

Between December 1997 and May 1999, 71 children aged 2–14 yrs, presenting to the Paediatric Emergency Department with an acute episode of wheezing (defined by cough and/or dyspnoea with expiratory rales and wheezes) associated with fever and signs or symptoms of

upper respiratory tract infection, were studied. During the same time period, 80 healthy subjects of similar sex and age, without any history of respiratory tract infection in the 3 months before enrolment, seen at Paediatric Department for minor surgical problems, were evaluated as control group.

All children with wheezing received a standard therapy with inhaled corticosteroids and bronchodilators for 5–7 days; when antibiotic was added on the basis of the judgement of the paediatrician in charge, clarithromycin 15 mg·kg body weight^{-1}·day^{-1} for 10 days was used. The standard treatment with steroids and bronchodilators was equivalent in clarithromycin-treated and nonantibiotic-treated children as regards the drugs used and the length of therapy. Clarithromycin was chosen based on the *in vitro* susceptibility to macrolides of *M. pneumoniae* and *C. pneumoniae* [13, 14]. The duration of therapy was based on previous clinical observations from use of this antibiotic in children [15, 16]. Parents and legal guardians were informed of the empirical nature of the therapy. They were told that it represented an unapproved indication for an approved antibiotic likely to be effective against *M. pneumoniae* and *C. pneumoniae* infection, if present. To mitigate expectation bias, they were not informed of the time course of improvement reported by other patients. After admission, children with recurrent or worsening signs and symptoms were asked to return immediately to the study centre for evaluation. The effect of the different therapies on paediatrician-diagnosed wheezing relapses was analysed; positive response to treatment was defined as control of wheezing-related symptoms (*i.e.* cough, dyspnoea, expiratory rales and wheezes) for the following 3 months.

The study was approved by the Institutional Review Board of the University of Milan, and written informed consent was obtained from the parents or legal guardians of all participants.

Methods

On admission and after 4–6 weeks, sera for determination of levels of antibodies directed against *M. pneumoniae* and *C. pneumoniae* and nasopharyngeal aspirates for *M. pneumoniae* and *C. pneumoniae* deoxyribonucleic acid (DNA) detection were obtained from all of the participants in the study.

Serum samples were collected and frozen at -20°C. Serological studies were performed with an enzyme-linked immunosorbent assay (Pantec, Turin, Italy) for immunoglobulin (Ig) M and IgG directed against *M. pneumoniae* and a microimmunofluorescence test (Labsystems, Helsinki, Finland) for IgM, IgG and IgA directed against *C. pneumoniae*. Nasopharyngeal aspirate samples in 2 mL of transport medium containing a sucrose phosphate buffer were frozen at -70°C. Nested polymerase chain reaction (PCR) was performed for both pathogens with validated methods, as previously described [17, 18]. To avoid the risk of contamination, sample preparation, PCR amplification and product analysis were performed in separate rooms. In each assay, positive and negative controls were included. The primer set MP-1 and MP-2 was used for *M. pneumoniae*-specific amplification [17]. The reaction volumes for the first and second rounds of amplification were 50 μL with 0.1 μM (each) primer. Amplification was carried out for 40 cycles. For *M. pneumoniae* nested PCR, the primers MUH-1 and MUH-2 were used. Nested amplification was performed, using 5 μL 1:10-diluted PCR product (5 μL in 45 μL sterile water) from the first round of amplification under identical conditions. Touchdown nested PCR for detection of *C. pneumoniae* DNA was performed using primers designed to detect the major outer membrane protein [19]. Extracted DNA solution (10 μL in a total volume of 50 μL) was used in the first PCR round; 5 μL of the PCR products amplified by the outer primers was then transferred to a new 50-μL PCR reaction mix for a second amplification using the inner primers [18]. The first round consisted of 40 cycles and the second 35.

Acute *M. pneumoniae* and/or *C. pneumoniae* infection was diagnosed if the patient showed a significant antibody response to one of the pathogens in paired sera (*M. pneumoniae*: specific IgM ≥1:100, specific IgG ≥1:400, or a four-fold increase in IgG titre; *C. pneumoniae*: specific IgM ≥1:16, specific IgG ≥1:512, or a four-fold increase in IgG titre) and/or if the PCR on nasopharyngeal aspirates was positive for DNA from one of the two organisms [20].

In children with wheezing and in controls, skin-prick tests for common allergens were performed on the arm to demonstrate allergen sensitization [21]. Children were to have refrained from taking antihistamines or antidepressants for ≥72 h and from topical or systemic corticosteroids for ≥7 days. Both positive (10 mg·mL^{-1} histamine) and negative (saline/glycerol 50/50) controls were included. A standard battery of extracts were tested. These were all Soluprick SQ 10 HEP (ALK A/S, Hörsholm, Denmark): hen′s egg white, cow′s milk, house dust mite (*Dermatophagoides pteronyssinus* and *D. farinae*), dog and cat dander, birch and timothy grass pollen, mugwort (*Artemisia vulgaris*), *Aspergillus* mix and *Alternaria tenuis*. The size of the reactions was measured 15 min after testing. The histamine weal size was recorded as the sum of the longest plus the midpoint orthogonal diameters divided by 2. A child was considered atopic to a specific allergen if the mean diameter of the weal was at least half that produced by a 10-mg·mL^{-1} solution of histamine, and with a mean diameter of the weal due to histamine of ≥3 mm [21]. Atopy was defined by at least one positive skin-prick test.

Statistical analysis

Comparisons between the groups were performed using Fisher′s exact test or the Chi-squared test. Age comparison was performed using an unpaired t-test. A p-value of <0.05 was considered significant.

Results

Study subjects

Table 1 shows the demographic characteristics of the study population. No significant difference was observed in sex, age, prevalence of atopy or eczema, and family history of atopic diseases and asthma between children with wheezing and controls. Among children with wheezing, 31 (43.7%) had a history of paediatrician-diagnosed recurrent episodes (*i.e.* at least four acute episodes of wheezing in the 12 months preceding enrolment),

Table 1. – Demographic characteristics of the study population

	Children with wheezing	Controls
Subjects n	71	80
Males	32 (45.1)	38 (47.5)
Age yrs	4.5 (2–14)	5.4 (2–14)
Recurrent episodes of wheezing	31 (43.7)$^{+}$	0
Atopy	22 (30.9)	14 (17.5)
Eczema	7 (9.8)	3 (3.7)
Family history of atopic diseases	11 (15.4)	6 (7.5)
Family history of asthma	7 (9.8)	4 (5.0)

Data are presented as absolute numbers with percentages in parentheses or median (range). $^{+}$: p<0.0001 *versus* controls (Chi-squared test).

whereas it was the first episode of wheezing for 40 (56.3%) children. None of the controls showed a history of recurrent episodes of wheezing.

Incidence of acute Mycoplasma pneumoniae *and* Chlamydia pneumoniae *infection*

Acute *M. pneumoniae* infection was demonstrated in 16 of the 71 (22.5%) children with wheezing: it was serologically determined in all 16 infected patients (specific IgM ≥1:100 in 12 children and IgG titre ≥1:400 in four), and confirmed by PCR in one subject (who presented with an IgG titre of ≥1:400); in none of the patients was *M. pneumoniae* DNA detected without any evidence of seroconversion. Among the controls, six of the 80 (7.5%) children showed evidence of acute *M. pneumoniae* infection without any respiratory symptom (children with wheezing *versus* controls, p=0.01 (Chi-squared test): all of the six subjects presented serological evidence of acute infection (specific IgM ≥1:100 in two children and IgG titre ≥1:400 in four); in none of the controls was *M. pneumoniae* DNA detected.

Acute *C. pneumoniae* infection was shown in 11 of the 71 (15.5%) patients with wheezing: it was serologically determined in nine of the 11 infected children (specific IgG ≥1:512 in one child and a four-fold rise in IgG titre in eight), and confirmed by PCR in four of the nine (in the child with specific IgG ≥1:512 and in three of those with a four-fold rise in IgG litre); in two further patients, *C. pneumoniae* DNA was detected without any evidence of seroconversion. Among the controls, two of the 80 (2.5%) children showed evidence of acute *C. pneumoniae* infection without any respiratory symptom (children with wheezing *versus* controls, p=0.01 (Chi-squared test)): both subjects showed *C. pneumoniae* DNA without seroconversion.

Among the infected children, three patients with wheezing and none of the controls showed *M. pneumoniae* and *C. pneumoniae* coinfection.

Considering studies that have shown the highest incidence of atypical bacterial infections in children of >5 yrs [16, 22], a sub-analysis was performed, comparing subjects with wheezing and controls aged 2–4 yrs and those of >5 yrs. Table 2 summarizes the incidence of acute *M. pneumoniae* and *C. pneumoniae* infection in the study population in different age groups. Despite the higher incidence of infection due to either pathogen in children with wheezing than in controls in both age groups, the difference was significant only in subjects aged >5 yrs.

Fifteen of the 16 (93.7%) wheezing patients with acute *M. pneumoniae* infection had a history of recurrent wheezing, as against only 16 of the 55 (29.1%) without (p<0.0001 (Chi-squared test)); results were similar for acute *C. pneumoniae* infection: eight of 11 (72.7%) as against 23 of 60 (38.3%) (p=0.04 (Chi-squared test)).

Table 3 details the incidence of recurrent episodes in children with wheezing in different age groups. In both age groups, a history of recurrent wheezing was significantly more frequent in the patients infected by one of the two pathogens than in those without either infection.

No significant difference in the prevalence of atopy was found between the wheezing subjects with and without infections due to these pathogens. In the group of 16 patients with wheezing and acute *M. pneumoniae* infection, six (37.5%) appeared to be atopic, whereas, among the 55 with wheezing but no *M. pneumoniae* infection, 16 (29.1%) were atopic (p=0.54 (Fisher's exact test)). Similarly, among the 11 patients with wheezing and acute *C. pneumoniae* infection, four (36.7%) were atopic, whereas, among the 60 with no *C. pneumoniae* infection, 18 (30.0%) subjects showed evidence of atopy (p=0.72 (Fisher's exact test)).

Table 2. – Incidence of acute *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infection in the study population in different age groups

	Children with wheezing	Controls	p-value
M. pneumoniae			
2–4 yrs	4/40 (10.0)	2/34 (5.9)	0.68*
≥5 yrs	12/31 (38.7)	4/46 (8.7)	0.0003^{+}
C. pneumoniae			
2–4 yrs	4/40 (10.0)	2/34 (5.9)	0.68*
≥5 yrs	7/31 (22.6)	0/46 (0)	0.001^{+}

Data are presented as absolute values with percentages in parentheses. *: Fisher's exact test; $^{+}$: Chi-squared test.

Table 3. – Incidence of recurrent episodes in children with wheezing in different age groups

Age group yrs	Infected$^{\#}$	Not infected$^{\#}$	p-value
2–4 yrs	8/8 (100.0)	9/32 (28.1)	0.003*
≥5 yrs	12/16 (75.0)	2/15 (13.3)	0.002^{+}

Data are presented as absolute numbers with percentages in parentheses. $^{\#}$: with *Mycoplasma pneumoniae* and/or *Chlamydia pneumoniae*; *: Fisher's exact test; $^{+}$: Chi-squared test.

Antibiotic treatment

Twenty-two of the 71 (30.9%) children with wheezing, in addition to standard therapy with steroids and bronchodilators, received clarithromycin, irrespective of serological and PCR results. Among the antibiotic-treated patients, 11 (50.0%) showed evidence of acute *M. pneumoniae* and/or *C. pneumoniae* infection. In 13 of the 49 (26.5%) nonantibiotic-treated subjects, evidence of acute *M. pneumoniae* and/or *C. pneumoniae* infection was found.

Table 4. – Clinical outcome of wheezing in nonantibiotic-treated children during the 3-month follow-up, according to diagnosis of infection

	Infected*	Not infected*
Clinical resolution	4 (30.8)	25 (69.4)$^{+}$
Recurrence	9 (69.2)	11 (30.6)$^{+}$

Data are presented as absolute numbers with percentages in parentheses. *: with *Mycoplasma pneumoniae* and/or *Chlamydia pneumoniae*. $^{+}$: p=0.03 (Chi-squared test).

During the 3-month follow-up period, among children with evidence of acute *M. pneumoniae* and/or *C. pneumoniae* infection, nine of the 13 (69.2%) nonantibiotic-treated subjects showed recurrence of wheezing; conversely, none of the clarithromycin-treated patients showed a new episode of wheezing (p=0.0005, (Fisher′s exact test)).

Table 4 summarizes the clinical outcome of wheezing in nonantibiotic-treated children during the 3-month follow-up, according to diagnosis of infection. Significantly more recurrence of wheezing was found in children with acute *M. pneumoniae* and/or *C. pneumoniae* infection than in those without acute *M. pneumoniae* and/or *C. pneumoniae* infection (p=0.03 (Chi-squared test)).

Follow-up PCR at 4–6 weeks in patients for whom it was positive on admission was negative for *M. pneumoniae* DNA in one antibiotic-treated child, whereas it was still positive for *C. pneumoniae* DNA in two of three clarithromycin-treated children.

Discussion

The present study indicates that *M. pneumoniae* and *C. pneumoniae* are significantly related to wheezing in children, particularly in subjects with a history of recurrent episodes, and that clarithromycin therapy may improve the course of reactive airway disease in paediatric patients with acute *M. pneumoniae* and/or *C. pneumoniae* infection.

The finding of a relationship between wheezing episodes and acute *M. pneumoniae* or *C. pneumoniae* infection is intriguing and suggests a potential role for these pathogens in the exacerbation of childhood asthma. It is likely that *M. pneumoniae* and *C. pneumoniae* can trigger the "wheezing process" in subjects who are predisposed by either their genetic background or events that have "primed" their immune systems and lungs.

In agreement with previous reports, the present results also show that, in children with wheezing, the incidence of acute *M. pneumoniae* and *C. pneumoniae* infection increases with age and occurs mainly after 5 yrs of age [8, 9]. Moreover, in the present study population, the incidence of asymptomatic infection in healthy subjects seemed to be low and was similar to that recently reported in adults [23, 24].

The present data support the concept that measurement of the antibody response in paired sera represents an accurate diagnostic test for determining the aetiology of acute *M. pneumoniae* or *C. pneumoniae* infection. In the present study, there was a relationship between serological evidence of acute infection with the two pathogens and wheezing status. All children with acute *M. pneumoniae* infection met accepted serological criteria, and in none of them was *M. pneumoniae* DNA detected without any evidence of seroconversion. Only in two children with wheezing and two controls was *C. pneumoniae* DNA detected without any evidence of seroconversion. However, results with PCR positive for *C. pneumoniae* in the absence of diagnostic antibody have been reported in paediatric age by other authors and may be caused by an immature ability to produce a humoral response or poor antigenic stimulation after *C. pneumoniae* infection [22].

Transient elevation of total serum IgE levels has been demonstrated during the acute phase of viral as well as *M. pneumoniae* infections, even in the absence of wheezing [25]. Moreover, EMRE *et al*. [26] demonstrated the presence of anti-*C. pneumoniae* IgE by immunoblotting in 85.7% of culture-positive children with wheezing, in contrast to only 9.1% of culture-positive patients with community-acquired pneumonia who were not wheezing. Thus the IgE response seems to be an integral part of the host response to a variety of infections.

The present report highlights the link between wheezing, atopy defined by positive skin-prick tests and atypical bacterial infections. Interestingly, no significant difference in the prevalence of atopy was found between wheezing children with and without infections due to either pathogen. However, further studies of the relationship between *M. pneumoniae* and *C. pneumoniae* infection and atopy are needed to provide a more comprehensive understanding of how these triggers for wheezing interact.

Regarding antimicrobial therapy, macrolides are the only drugs active against *M. pneumoniae* and *C. pneumoniae* that can be safely used when treating paediatric patients [15, 16]. Previous studies have demonstrated the efficacy of macrolides in the treatment of atypical bacterial infections in children with community-acquired pneumonia [15, 16]. EMRE *et al*. [8] observed that nine of 12 children with asthma and positive cultures for *C. pneumoniae* demonstrated clinical and laboratory improvement of their symptoms following antibiotic therapy with clarithromycin for 10 days or erythromycin for 14 days. However, in children with wheezing, the efficacy of antibiotic treatment and the optimal length of therapy have not been established. Furthermore, it is well known that macrolides have anti-inflammatory activities due to an interaction with the natural effectors involved in antimicrobial defences and inflammation [27]. This interaction has been shown to affect bronchial hyperresponsiveness and to improve clinical status in patients with asthma, but the importance of this property has not been fully elucidated [28].

In the present study population, clarithromycin, 15 mg·kg body weight^{-1}·day^{-1} for 10 days, seemed to be effective in the resolution of wheezing-related symptoms in subjects with atypical bacterial infections. Respiratory symptoms frequently recur in the absence of antimicrobial treatment of acute *M. pneumoniae* and/or *C. pneumoniae* infection. On the contrary, all 11 patients with acute *M. pneumoniae* and/or *C. pneumoniae* infection treated with clarithromycin appeared to benefit from the macrolide even though, in some patients, PCR results remained still positive. Considering that therapy with inhaled steroids was equivalent in both groups with regard to drugs used and the length of treatment, this benefit seems to be related to clarithromycin′s activity. Preliminary experience in adults suggests that *M. pneumoniae* and *C. pneumoniae* may prove difficult to eradicate with the currently available antibiotic regimens [29]. HAMMERSCHLAG *et al*. [29] described

a number of subjects who remained culture-positive for *C. pneumoniae* despite treatment with one or more courses of tetracycline or doxycycline of up to 3 weeks′ duration. Even when a subject becomes culture-negative, this does not necessarily mean that the organism has been eradicated; it may persist in the body in a latent form [30].

A limitation of the present report is the lack of respiratory virus testing. It is possible that *M. pneumoniae* and *C. pneumoniae* infections act as cofactors, possibly rendering subjects more susceptible to other stimuli such as viruses.

In conclusion, the present preliminary results show a possible association of infection with *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* and wheezing, particularly in children with recurrent episodes. Clarithromycin-treatment of paediatric patients with wheezing and atypical bacterial infection appears to be associated with clinical improvement. Further studies are needed to clarify the actions of macrolides in subjects with asthma-like manifestations. However, in children whose wheezing-related symptoms remain poorly controlled, a careful search for evidence of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* infection may be indicated. The present results also support the argument that definitive randomized double-blind trials of antibiotics in various wheezing children populations should be carried out, since, if these preliminary findings can be confirmed, the benefit to patients could be significant.

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