CONCISE ARTICLE

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Role of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* as Causative Agents of Community-Acquired Pneumonia in Hospitalised Children and Adolescents

Abstract The aim of the study presented here was to determine the prevalence of *Chlamydia pneumoniae* versus *Mycoplasma pneumoniae* infections in paediatric patients with community-acquired pneumonia. A total of 50 patients (mean age, 5.5 years; median, 3.9 years) with community-acquired pneumonia were enrolled. Four patients were found to have *Chlamydia pneumoniae* infection (1 culture positive, 1 PCR positive and 2 serology positive) and 16 patients had *Mycoplasma pneumoniae* infection (2 PCR positive, 4 PCR and serology positive, 10 serology positive), including three patients with coinfection. The rates of *Mycoplasma pneumoniae* infection were 22%, 35% and 40% in children aged 1–3, >3–7 and >7 years, respectively. Acute *Chlamydia pneumoniae* infection was substantially less common than *Mycoplasma pneumoniae* infection in our study cohort.

Introduction

Little is known about the frequency of community-acquired pneumonia (CAP) caused by *Chlamydia pneumoniae* in paediatric patients in Europe, and the results published so far are conflicting. Prevalence rates of between 6% and 23% have been reported so far for *Chlamydia pneumoniae* found in the nasopharynx of healthy children using the polymerase chain reaction (PCR) [1, 2]. Furthermore, in studies using combinations of PCR, culture and serology, *Chlamydia pneumoniae* has been identified as the presumptive causative agent of CAP in children to various degrees [3, 4, 5, 6, 7].

The goal of the present study was to estimate the prevalence of *Chlamydia pneumoniae* infections in comparison with *Mycoplasma pneumoniae* infections in paediatric patients with CAP admitted to the University Children's Hospital, which provides basic paediatric care for the region of Basel, Switzerland.

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Patients and Methods

The study was performed from March 1999 to February 2000, and all patients aged 1–18 years who were hospitalised with a diagnosis of radiographically proven CAP were eligible for participation. To avoid interference of maternally derived IgG antibodies with the serological diagnosis, infants were not enrolled. Subjects were also excluded for the following reasons: known immunodeficiency, treatment with immunoglobulins during the previous 3 months, or chronic underlying disease. The diagnosis of pneumonia required characteristic clinical findings as proposed by the World Health Organization [8], i.e. tachypnoea, respiratory distress, cyanosis, feeding difficulty, absence of wheezing, and radiological confirmation. Chest radiographs were assessed by the consultant radiologist who was unaware of the microbiological diagnosis. The study protocol was approved by the ethical commission of the University of Basel Medical Faculty.

Nasopharyngeal aspirates and oropharyngeal specimens were obtained for PCR using Dacron swabs from each patient on admission, and one further oropharyngeal specimen was obtained for culture (*Chlamydia pneumoniae*). Nasopharyngeal aspirates and oropharyngeal specimens were placed into transport medium (0.9% sodium-chloride solution) and stored at 4°C until PCR was performed (within 48 h). DNA was extracted and purified by proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation. For *Chlamydia pneumoniae*, a semi-nested PCR was performed using the primer pair HL/HR-1 and HM/HR-1 as described previously [9]. PCR of *Mycoplasma pneumoniae* was performed using the method described by Ieven et al. [10] with the primer pair MP P1-1 and MP P1-2. In-house validation tests have been performed for both PCR methods.

Cycloheximide-treated HEp 2 cells were used for culture of *Chlamydia pneumoniae*. Confirmation of suspicious cells was performed using fluorescent antibody staining with an in-house *Chlamydia pneumoniae*-specific monoclonal antibody [11].

Acute (on admission) and convalescent (4–6 weeks post-admission) serum samples were collected. Antibody titres (IgG, IgM and IgA) for *Chlamydia pneumoniae* were measured using a microimmunofluorescence assay that includes positive and negative controls (Serofia; Savyon Diagnostics, Switzerland). For *Mycoplasma pneumoniae*, a semiquantitative enzyme-linked immunosorbent assay (SeroMP; Savyon Diagnostics) was performed (IgG and IgM). Antibody values were determined using the reference line method, which allows the calculation of elevations in antibody levels. The antigen used was the P1 membrane protein. Serum samples that were positive for IgM but negative for IgG by enzyme-linked immunosorbent assay were also analyzed using the particle agglutination test and immunoblotting (Virotech, Germany).

Statistical analysis was performed using SPSS version 10.0 for Windows. Independent proportions were compared using Fisher's exact test or the Mantel-Haenszel chi-square analysis, as appropriate. A *P* level<0.05 was considered significant.

Results and Discussion

During the 12-month study period, 64 patients hospitalised with CAP were eligible for study participation and the parents of 50 of these patients provided informed consent for them to participate. Patients were aged 1.2–15.3 years (mean, 5.5 years; median, 3.9 years) and 29 were female. Nasopharyngeal aspirates and oropharyngeal specimens were available from all study participants and acute and convalescent serum specimens were obtained from 49 and 46 subjects, respectively. Five patients had received antibiotic treatment for at least 48 h before hospitalisation. Mean temperature on admission was 39.2°C (range, 36.4–40.2°C). Mean peripheral leucocyte count on admission was 18.0×10^9/l (median, 16.7×10^9/l; range, $4.6-44\times10^9$/l) and the mean C-reactive protein level was 140 mg/l (median, 124 mg/l; range, 3–567 mg/l). One of 40 blood cultures was positive and grew *Streptococcus pneumoniae*.

Overall, four patients were found to have *Chlamydia pneumoniae* infection (Table 1). Interestingly, three of the four patients with *Chlamydia pneumoniae* infection also had signs of concomitant *Mycoplasma pneumoniae* infection. In addition, three study subjects (aged 3.6, 11.1 and 12.4 years) had serological evidence of past *Chlamydia pneumoniae* infection. Three of the four

patients with acute *Chlamydia pneumoniae* infection, including two with *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* coinfection, showed lobar consolidation involving several adjacent segments. Their C-reactive protein values on admission were 152 mg/ml, 139 mg/ml and 258 mg/ml. Of those three patients, two (both with *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* coinfection) had pneumonia with effusion. One patient with *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* coinfection had an interstitial pneumonia and a C-reactive protein value of 42 mg/ml.

Overall, 16 patients, including three with concomitant *Chlamydia pneumoniae* infection, were diagnosed as having acute *Mycoplasma pneumoniae* infections. The incidence of *Mycoplasma pneumoniae* as the cause of CAP increased according to the age of the patients. Thirteen cases were diagnosed during the cold season (October–March) without any apparent clusters. Using enzyme-linked immunoassay, 17 patients were found to have signs of acute *Mycoplasma pneumoniae* infection. In eight of these subjects, the diagnosis relied on the presence of IgM antibodies only. Confirmatory particle agglutination test and immunoblot assay could be performed in seven cases; all samples tested positive with the particle agglutination test ($\geq$1:40) and four were also confirmed by immunoblot. This decreases the total number of serologically confirmed cases of acute *Mycoplasma pneumoniae* infection to 14 (Table 2). All patients recovered without significant sequelae, including a 14-month-old girl with *Mycoplasma pneumoniae* infection who required 2 days of intensive care treatment for imminent respiratory failure.

Radiographic patterns of pneumonia did not correlate with aetiological diagnosis nor did the clinical characteristics, such as maximum body temperature, C-reactive protein values and leucocyte counts (data not shown).

In this study of 50 children and adolescents with CAP examined for signs of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* infection, we found *Mycoplasma pneumoniae* infection to be frequent (32%) and its relative frequency tended to increase according to age. PCR and serology findings were concordant in 25% of cases and discordant in 75%; PCR missed 10 of the 16

Table 1 Detection of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in children and adolescents with CAP according to patient age

Age in year	No. with *C. pneumoniae* [a]				No. with *M. pneumoniae* [b]			
	PCR + (Sero/cult −)	Sero + (PCR/cult −)	Cult + (Sero/PCR −)	Any +	PCR + (Sero −)	PCR + (Sero +)	Sero + (PCR −)	Any +
1–3 (*n*=18)	1	1	–	2	2	1	1	4
>3–7 (*n*=17)	–	–	1	1	–	1	5	6
>7–16 (*n*=15)	–	1	–	1	–	2	4	6
Total (*n*=50)	1	2	1	4	2	4	10	16

PCR, polymerase chain reaction; Sero, serology; Cult, culture

[a] Acute *C. pneumoniae* infection: positive culture or PCR or specific serological criteria, i.e. a single positive IgM ($\geq$1:20), IgA ($\geq$1:20) or IgG ($\geq$1:512) titre or a $\geq$4-fold IgG titre rise in paired serum specimens [14]

[b] Acute *M. pneumoniae* infection: positive PCR (NP or OP) or positive IgM antibodies ($\geq$10 U/ml) in acute and/or convalescent serum specimens or a significant IgG antibody increase ($\geq$4-fold) in paired serum specimens

Table 2 Antibody values (IgM/IgG) against *Mycoplasma pneumoniae* detected in acute and convalescent serum specimens of 50 paediatric patients with community-acquired pneumonia

Antibody values[a]		No. of patients	Diagnosis[b]
Acute specimen	Convalescent specimen		
IgM pos/IgG pos	IgM pos/IgG pos		
21/29	30/32	4	acute infection
40/12	37/10		
139/24	93/54		
48/37	61/42		
IgM pos/IgG neg	IgM pos/IgG neg		
32/–	27/–	4	acute infection
37/–	111/–		
42/–	52/–		
13/–	11/–		
IgM pos/IgG neg	IgM pos/IgG pos		
106/–	83/42	4	acute infection
94/–	126/28		
122/–	114/38		
117/–	110/26		
IgM pos/IgG neg			
20/–	n.a.	1	acute infection
IgM neg/IgG neg	IgM pos/IgG neg		
–/–	13/–	1	acute infection
IgM neg/IgG pos	IgM neg/IgG pos		
9/22	7/22	2	previous infection
–/40	–/28		
IgM neg/IgG neg	IgM neg/IgG neg	33	no infection

pos, positive; neg, negative; n.a., not available
[a] Antibody levels determined by enzyme-linked immunosorbent assay in binding units/ml and confirmed by particle agglutination test and immunoblot
[b] Acute infection: see Table 1; Previous infection: positive IgG/negative IgM, no significant IgG antibody rise and negative PCR

total cases. Similarly, Waris et al. [12] were unable to demonstrate the presence of *Mycoplasma pneumoniae* by PCR in 10 of 20 children with serologically confirmed *Mycoplasma pneumonia*. Interestingly, only patients with negative IgG antibodies in the acute phase had a positive PCR result. In accordance with this observation, three of our four PCR-positive patients were positive for IgM but negative for IgG antibodies against *Mycoplasma pneumoniae* in their acute serum specimens. This observation suggests that *Mycoplasma pneumoniae* organisms may be harboured in the upper respiratory tract preferentially during the very early phase of infection.

It has been shown previously that a subset of patients with pneumonia and a corresponding positive culture or PCR result for *Mycoplasma pneumoniae* lack an antibody response to the organism [3]. The reasons for this are unknown. It is notable that both of our patients who were positive for *Mycoplasma pneumoniae* by PCR but lacking an antibody response were rather young (2.8 and 1.9 years). This suggests that the serological antibody response to *Mycoplasma pneumoniae* may mature with age.

In contrast to *Mycoplasma pneumoniae*, the rate of infection with *Chlamydia pneumoniae* was low in our study cohort (8%). Moreover, two of the four cases were diagnosed based on demonstration of the organism in the upper respiratory tract without a concomitant antibody response in acute or convalescent serum specimens. This raises doubts about the quality of the PCR technique we used [13] and/or the aetiological role of *Chlamydia pneumoniae* in two of our cases, decreasing the rate of *Chlamydia pneumoniae* infection to 4%. Similarly, in a previous study conducted in Germany, a rate of 5.6% was found for *Chlamydia pneumoniae* infection [2]. In contrast, several other studies identified *Chlamydia pneumoniae* as the cause of CAP at a higher rate, i.e. in 15–30% of children [3, 5, 6, 7]. The significant differences among the relative frequencies of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in association with CAP from study to study may be attributable to variable age groups, the kinds of diagnostic procedures used and the presence or absence of local outbreaks in the respective communities during the investigation period [2]. No such outbreak occurred during the course of our study.

Interestingly, three of our four cases with demonstrable *Chlamydia pneumoniae* colonization and/or infection had evidence of simultaneous *Mycoplasma pneumoniae* coinfection. This observation has been described before [3, 4, 6]. It raises the question of whether one pathogen simply facilitates the penetration of the other or whether both organisms act independently to cause the respiratory tract infection.

No specific radiological feature or laboratory parameter was found that allowed us to distinguish patients with *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* infection from those without a known cause for pneumonia, which is in accordance with previous studies [4, 6]. This highlights the need for specific microbiological tests capable of identifying patients with *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* infection who may benefit from specific antibiotic treatment.

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