Frequency of Mycoplasma pneumoniae among atypical pneumonia of childhood

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We aimed to investigate the frequency of Mycoplasma pneumoniae among atypical pneumonia of childhood that is acquired from the community and to determine a practical approach to the diagnosis of these patients.

In this prospective study, 55 patients (31 male and 24 female) with atypical pneumonia were investigated with conventional laboratory and radiological methods as well as culture and polymerase chain reaction (PCR) on throat swab. In addition, serum of the patients was tested for M. pneumoniae specific IgM. The patients were reevaluated clinically at 3-5 days and 3-4 weeks and serologically at 3-4 weeks. The data on patients with M. pneumoniae pneumonia were compared with the other patients with atypical pneumonia and controls. All patients were treated with macrolide antibiotics. The mean age of the patients was 7.8±2.9 years. The frequency of M. pneumoniae by this method was 34.5%. Neither clinical, laboratory, or epidemiological data nor response to macrolide antibiotics was useful in detecting the etiology of atypical pneumonia. Diagnostic sensitivity and specificity of IgM+IgG antibodies plus PCR on throat swab were estimated as 100%. M. pneumoniae was an important microorganism in the etiology of atypical pneumonia of childhood in our community. In order to prevent loss of time with beta-lactamase antibiotics, which are usually started in severe pneumonia, serologic tests and PCR must be done during the initial evaluation of the patient for the reliable diagnosis of M. pneumoniae, which will increase the chance of early and appropriate therapy.

Key words: childhood, infection, Mycoplasma pneumoniae, pneumonia.

Mycoplasma pneumoniae infections have been commonly reported over the past 25 years[1]. Laryngotracheitis is the most common clinical presentation of M. pneumoniae infections. The infection is asymptomatic in 15 to 55% and especially during infancy. Pneumonia develops in 3 to 10% of the patients[1,2]. M. pneumoniae, which was the etiologic factor in 5 to 10% of community-based pneumonia cases in the 1980s, has accounted for 20 to 30% of cases in the past few years[3-6]. M. pneumoniae infections may not be diagnosed in the majority of the patients in our country, and there are only a few studies in the pediatric age group. In this investigation, we tried to determine the incidence of M. pneumoniae infections among patients with community-based pneumonia that presents like atypical pneumonia with its clinical and radiological features. Atypical pneumonia was defined as clinical diagnosis of pneumonia with poor correlation between the clinical findings and chest x-ray[7]. The chest x-ray of these patients showed typical patchy infiltration for atypical pneumonia. In addition, we compared the clinical, radiological, and epidemiological features as well as the response to macrolide antibiotics of M. pneumniae-positive and –negative patients with atypical pneumonia in order to propose a practical approach and management strategy for patients with community-based pneumonia in an outpatient setting.

Material and Methods

This prospective study was carried out in the outpatient clinic of the Department of Pediatrics at İstanbul University Faculty of Medicine

between April 1995 and May 1996. It was conducted with 55 patients with atypical pneumonia and 20 healthy children as the control group. M. pneumoniae-positive patients were named Group 1, and negative patients were named Group 2. A third group served as the control group and consisted of 20 healthy children who had no respiratory complaints during the past month. The history and physical findings as well as the family history and epidemiological features, such as seasonal variations of respiratory symptoms in the family, were recorded. A complete blood count, differential leukocyte count, AST, ALT, and a chest X-ray were obtained from all patients. M. pneumoniae was investigated with culture and polymerase chain reaction (PCR) of the throat swab. We used Hayflick biphasic medium in culture technique[8]. Dot-blot hybridisation method was applied to the PCR products. In addition, M. pneumoniae specific IgM was measured by immunocapture ELISA (Platelia, Sanofi Diagnostica Pasteur, France), and M. pneumoniae specific IgG was measured by ELISA (Platelia, Sanofi Diagnostica Pasteur, France) in the sera of all patients during the acute phase and convalescent phase) (3^{rd}-4^{th} week of follow-up period)[9]. Inclusion criteria of the patients in Group 1 were defined as follows. 1) All patients had atypical pneumonia. Atypical pneumonia was defined as clinical diagnosis of pneumonia with poor correlation between the clinical findings and chest X-ray[7]. The chest X-ray of these patients showed typical patchy infiltration for atypical pneumonia. 2) Those who had used antibiotic therapy over the past two weeks were excluded. 3) Patients were considered as having M. pneumoniae when a significant increase in IgM and IgG+IgM levels occurred between the two serum samples, and when PCR was positive with the presence of accompanying clinical evidence. Patients who had only IgM antibodies against M. pneumoniae were not accepted as M. pneumoniae pneumonia because this positive result of IgM might have been due to a silent M. pneumoniae infection in the past 3-4 weeks[10]. The patients in Group 2 were also diagnosed as atypical pneumonia, but their infections were caused by agents other than M. pneumoniae. Sera in Group 2 did not show any rise of M. pneumoniae specific antibody. In the control group (Group 3), we were able to analyze IgM and IgG antibodies against M. pneumoniae at the beginning of the study. The frequency of M. pneumoniae positivity was compared between Groups 1 and 2 and the control group. In addition, Groups 1 and 2 were compared with respect to clinical and radiological findings as well as other laboratory findings and the response to therapy. All patients in Groups 1 and 2 were treated with macrolide antibiotics: erythromycin 40 mg/kg for 10 days, clarithromycin 15 mg/kg/day for 10 days, or azithromycin 10 mg/kg/day for 3 days. The patients were reevaluated clinically at 3-5 days and 3-4 weeks and serologically at 3-4 weeks. Recovery was accepted as total absence of the clinical symptoms. Student's t test, chi-square or Fisher's exact test was utilized for statistical evaluation. A p value of <0.05 was considered statistically significant.

Results

The mean age of the patients was 7.8 ± 2.9 (1.3-14) years. Seven patients (12.7%) were <5 years of age, 32 (58.2%) were 5-9 years, and 16 (29.1%) were >9 years old. The age distribution among M. pneumoniae-negative patients was 6 (16.6%), 17 (47.3%), and 13 (36.1%), and among M. pneumoniae-positive patients was 1 (5.2%), 15 (78.9%), and 3 (15.7%), respectively. Although the frequency of M. pneumoniae among 5.9-year-old patients was higher, this difference did not reach statistical significance.

All 55 patients in the study groups (31 male, 24 female) had serologic tests. Forty-three patients had a throact swab for culture and PCR. Forty-nine patients were available for a clinical and serologic evaluation at 3-4 weeks. Nineteen patients (19/55, 34.5%) received diagnosis of M. pneumoniae pneumonia. The results of the serologic tests and PCR of these patients are summarized in Table I. None of the patients had M. pneumoniae positive culture in their throat swab. We could not isolated any other agents in culture medium. Two patients with positive IgG in non-Mycoplasma group (Cases 19 and 25) were considered to have past infection, because there were no increases in IgG level, and PCR was negative (Table II). Cases 29, 31 and 36 in the non-Mycoplasma pneumonia group were included in this group due to negative PCR test results according to inclusion criteria. We suggested that all cases

with positive PCR tests had increased IgG antibody levels in the convalescent period. The mean age of the control group was similar at 8.8±3.3 (1-13) years. Three children in this group had positive IgM (3/20,15%). However, they did not have any clinical findings of pneumonia and their PCR tests in throat swab were negative for M. pneumoniae.

Among patients who were diagnosed by acute and convalescent period serology according to increasing level of IgM and IgG positivity, 18 (90%) had positive PCR. This ratio increased to 100% after applying dot-blot hybridisation method to the PCR products. The diagnostic sensitivity of single sera IgM antibodies against M. pneumoniae pneumonia was 76%, and specificity was 97.1%. The positive predictive value of this test was 95%, and the negative predictive value was 84.6%. If positive sera against M. pneumoniae and PCR on throat swab were used together in diagnosis of M. pneumoniae pneumonia in the acute period, sensitivity, specificity, and positive and negative predictive values increased to 100%.

Table I. Properties of M. Pneumoniae-Positive Patients (n:19)

		1st Day		3rd-4th Week		1st Day
Case#	Age (year)	IgM	IgG	IgM	IgG	PCR
1	5.3	+	+	+	+	+
2	5.6	–	–	+	–	+
3	5.2	+	+	+	+	+
4	9	+	+	+	+	+
5	7.8	+	+	+	+	+
6	9	+	+	+	+	+
7	10	++	+	++	+	+
8	8	++	+	++	+	+
9	2	–	–	+	+	+
10	5.8	–	+	–	+	+
11	8.5	+	–	+	–	+
12	7	+	–	0	0	+
13	7	+	–	0	0	+
14	8	+	+	+	+	+
15	5	+	–	0	0	+
16	9	–	–	+	+	+
17	9	+	+	+	+	+
18	13	–	–	+	+	+
19	10	–	–	+	+	+

0 : Not analyzed.
PCR: polymerase chain reaction.

Table II. Properties of M. Pneumoniae-Negative Cases (n:36)

		1st Day		3rd-4th Week		1st Day
Case#	Age (year)	IgM	IgG	IgM	IgG	PCR
1	10	–	–	–	–	–
2	10	–	–	–	–	0
3	5.5	–	–	–	–	–
4	6.6	–	–	–	–	–
5	10	–	–	–	–	0
6	14	–	–	–	–	–
7	11	–	–	–	–	0
8	9	–	–	–	–	–
9	4.1	–	–	–	–	0
10	13	–	–	–	–	0
11	11	–	–	–	–	0
12	8	–	–	–	–	0
13	8	–	–	–	–	0
14	5.8	–	–	–	–	0
15	12	–	–	–	–	0
16	8.5	–	–	–	–	–
17	5.3	–	–	–	–	–
18	10.5	–	–	–	–	–
19	6	–	+	–	+	–
20	8	–	–	–	–	0
21	12	–	–	–	–	–
22	1.8	–	–	–	–	–
23	5.5	–	–	–	–	–
24	6	–	–	–	–	0
25	1.2	–	+	–	+	–
26	12	–	–	–	–	–
27	10.1	–	–	–	–	–
28	7	–	–	–	–	–
29	3.5	–	–	0	0	–
30	8	–	–	–	–	–
31	2.5	–	–	0	0	–
32	4	–	–	–	–	–
33	8	–	–	–	–	–
34	10	–	–	–	–	–
35	8	–	–	–	–	–
36	8	–	–	0	0	–

0: Not analyzed; PCR: polymerase chain reaction.

There were no differences between M. pneumoniae-positive and –negative patients with respect to mean age, the nature and duration of complaints, clinical findings, or epidemiological features (Table III). The most frequent radiological finding was unilateral or bilateral interstitial infiltration in both M. pneumoniae-positive (55.5%) and –negative (54%) patients.

Although the response rate to macrolide antibiotics was higher in Group 1 than Group 2 (94.4% vs. 80.6%), the difference was not statistically significant. A total of six patients in both groups did not respond to therapy (2 erythromycin, 3 azithromycin, and 1 clarithromycin).

Table III. Characteristics of Patients in Group 1 and 2 (n: 55)

	M. pneumoniae positive cases (n:19)	M. pneumoniae negative cases (n:36)	Significance
Age (mean±SD; year)	7.6±2.3	7.9±3.2	NS
Duration of complaints (mean±SD; day)	14.8±13.4	16.8±14.1	NS
Complaints n (%)			
Cough (dry)	14 (74)	21 (58)	NS
Cough (productive)	6 (32)	14 (39)	NS
Rhinorrhea	6 (32)	15 (42)	NS
Headache	9 (47)	10 (28)	NS
Chest pain	2 (11)	6 (17)	NS
Dyspnea	4 (21)	6 (17)	NS
Nausea/vomiting	7 (37)	12 (33)	NS
Anorexia	14 (74)	17 (47)	NS
Weight loss	3 (16)	2 (6)	NS
Fever	4 (21)	7 (19)	NS
Myalgias/arthralgias	4 (21)	10 (28)	NS
Dermatologic lesions	2 (11)	2 (6)	NS
Malaise	6 (32)	14 (39)	NS
Wheezing	2 (11)	2 (6)	NS
Clinical finding n (%)			
Pharyngeal erythema	13 (68)	26 (72)	NS
Cervical adenopathy	1 (5)	8 (22)	NS
Generalized adenopathy	2 (11)	3 (8)	NS
Rhonchi	14 (74)	28 (78)	NS
Wheezing	6 (32)	14 (39)	NS
Sinusitis	7 (37)	13 (36)	NS
Positive family history n (%)	11 (58)	19 (53)	NS
Admission in June to January n (%)	10 (53)	17 (47)	NS

NS: Not significant.

Discussion

The diagnosis of M. pneumoniae pneumonia is done by the isolation of the microorganism by culture from the respiratory secretions, the detection of antigen and nucleic acids, or the serologic tests mentioned above. Although culture used to be the 'gold standard' for diagnosis, it may take 1-3 weeks to grow the organism, it may yield 30% false negative results[11], and it may continue to be positive during convalescence[12]. Culture was not helpful in our investigation either. It was known that cold agglutinin could be positive in cases with atypical pneumonia[13]. But its diagnostic value is limited. We did not use cold agglutinin assay because ELISA has been the most powerful method to search for specific IgM in recent years[11,14,15]. The disadvantage of this method is that IgM cannot be detected for at least one week after the symptoms have started and some patients may be missed in the acute phase[14,16]. If single serum analysis of IgM level is used for diagnosis, healthy children who have had recent M. pneumoniae infection may get misdiagnosed. Although a second test during convalescence covers the gap, it may only give a retrospective diagnosis and does not contribute to the planning of therapy. In our study, we used immunocapture ELISA, which was reported to be sensitive[17-20], as well as PCR, which was proposed to be a rapid and reliable method[21,22]. On the other hand, the results of dot-blot hybridisation along with PCR are completely parallel to the serologic tests on double sera, and the diagnosis can be established rapidly and reliably. These data are similar to those of Waris et al.[9] who found that ELISA when used with PCR in the acute phase is the most rapid and reliable method of diagnosis for M. pneumoniae. Dorigo-Zetsma et al.[22] also proposed that a combination of PCR and complement-fixation test may cover diagnosis of all patients. Similar to our results, Kessler et al.[21] reported that the sensitivity of

PCR increases when used in combination with the hybridisation technique. The frequency of 34.5% among atypical pneumonia of childhood shows that M. pneumoniae is a common etiologic factor in community-based pneumonia[6,23,24]. Dereli et al.[25] researched IgM as a diagnostic tool for Chlamydial infections in infants. They demonstrated that IgM antibody was positive in 12 cases among 20 culture-positive cases. However, its frequency may be underestimated due to utilization of a single ELISA method on a single serum sample. It has been emphasized in the literature that M. pneumoniae pneumonia is being under-reported because of inadequate use of the diagnostic tools[5,26,27]. On the other hand, a cost-benefit analysis between ELISA and PCR test was done, and the ELISA test is cheaper than PCR. With two repeated analyses, cost of ELISA was only 30% of PCR cost.

Although M. pneumoniae infection is most frequently encountered between 5 to 9 years of age, occurrence in lower age groups has been reported[28]. The age range of our patients coincided with this trend, and only 5% were younger than 5 years of age. The lack of a significant difference between the M. pneumoniae-positive and –negative patients within a certain age range among those with atypical pneumonia implicates that M. pneumoniae may be a causative agent at younger ages as well. These data are also compatible with the literature[9,29]. Until recently, it has been thought that epidemiological features may be helpful in presumptive diagnosis[30]. However, recent studies show that not only epidemiological, but also clinical and radiological findings, are unhelpful in distinguishing M. pneumoniae pneumonia from other atypical pneumoniae or community-based pneumonia[7,26,31]. Our results support this view.

Although patients with M. pneumoniae pneumonia have responded better to macrolide antibiotics, the difference from other atypical pneumoniae is not significant[5,22,32-34]. Our results also reveal that macrolides may be good alternatives for patients over 2 years of age with pneumonia who can be treated as outpatients. We suggest that cases unresponsive to macrolides might be attributed to other agent, such as viruses.

In conclusion, M. pneumoniae was the etiologic agent in 34.5% of atypical pneumonia in childhood in our community. The epidemiological, clinical, and radiological findings failed to distinguish the etiology of the pneumonia. Consequently, patients with community-based pneumonia seem to be effectively treated with macrolides on an outpatient basis. However, severely ill patients who require admission need specific M. pneumoniae tests with ELISA and PCR in order to minimize the loss of time with beta lactam antibiotics, which are usually the first-line treatment in this group of patients.

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