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Comparison of PCR, Culture, and Serological Tests for Diagnosis of *Mycoplasma pneumoniae* Respiratory Tract Infection in Children

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For diagnosis of *Mycoplasma pneumoniae* infection we compared two rapid tests, PCR and the immunoglobulin M immunofluorescence assay (IgM IFA), with culture and the complement fixation test (CFT), in a prospective study among 92 children with respiratory tract infection and 74 controls. Based on positivity of culture and/or CFT as the diagnostic criterion, nine patients (10%) were diagnosed with *M. pneumoniae* infection. All patients positive by culture were also positive by PCR. In all controls cultures, PCRs, and serological assays were negative, except in one with a positive IgM IFA. The IgM IFA had a low positive predictive value of 50%. Only a combination of PCR (seven patients) and CFT (seven patients) allowed diagnosis of all cases.

Mycoplasma pneumoniae causes 15 to 20% of community-acquired pneumonia (6) in older children and adults and a variety of respiratory tract infections in younger children. Diagnosis of *M. pneumoniae* infection relies mainly on laboratory tests. Culturing *M. pneumoniae* from clinical specimens is laborious and may take up to 5 weeks. The sensitivity of culture is lower than that of serological assays (9). The complement fixation test (CFT) is the most widely used serological assay. The sensitivity of this assay depends on whether the first serum sample is collected early or late after the onset of illness and on the availability of paired sera collected with an interval of 2 to 3 weeks. In order to diagnose *M. pneumoniae* infection more rapidly, PCR and immunoglobulin M (IgM) assays which may allow diagnosis in 1 or 2 days have been developed. Although IgM assays are more sensitive than CFT, the IgM response may be nonspecific (12) or absent, particularly in adults (17). PCR does not have these disadvantages, but it carries the risk of detecting healthy carriers of *M. pneumoniae*.

In various studies PCR has been compared to serological diagnosis of *M. pneumoniae* infection. However, the serological diagnosis was based on the IgM response (1, 13) and/or on CFT results from a single serum sample (15, 19). In another study applying PCR for diagnosis of *M. pneumoniae* infection, serological assays were not used at all (8). None of the studies prospectively compared culture and CFT with rapid tests like PCR and IgM assays, including controls at the same time. We therefore designed a 15-month prospective study among children with respiratory tract infection and age-matched controls. We collected throat swab samples to detect the presence of *M. pneumoniae* by culture or its DNA by PCR, and we analyzed sera by CFT and an IgM immunofluorescence assay (IFA).

(This study was presented in part at the International Organization for Mycoplasmology Congress, 14 to 19 July 1996, Orlando, Fla. [5a].)

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MATERIALS AND METHODS

This study was approved by the Medical Ethical Committee of the investigating institute, the Academic Medical Center in Amsterdam (AMC). Written informed consent was obtained from patients and controls.

Patients and controls. For a 15-month period, June 1994 through October 1995, children with signs of community-acquired respiratory tract infection admitted to the Outpatient Department of Pediatrics at the AMC and Boven-IJ Hospital were eligible for inclusion. Entry criteria were (i) age between 0.5 and 18 years, (ii) a nonproductive cough associated with (a) signs of upper or lower respiratory tract infection for at least 3 days or (b) a sore throat or chest pain, and (iii) a body temperature of >37.5°C. (iv) Additionally, at least one of the following signs had to be present: malaise, muscle pain, or headache. Patients treated with non-β-lactam antibiotics in the preceding 14 days were excluded. At least one blood sample was collected for serological testing. A cotton-tipped throat swab was used to obtain material from the space between the palatine arches for PCR and culture of *M. pneumoniae*. The throat swab was placed into 2 ml of transport medium (PPLO broth [Difco, Detroit, Mich.], yeast extract [10%], unheated horse serum [20%], glucose [0.5%], phenol red [0.002%], and penicillin [1,000 U/ml]) and processed at the laboratory on the day of collection.

Controls were children admitted for diseases other than respiratory tract infection. Entry criteria for them were age matching (50% variation in age was allowed, with a maximum 5-year difference), matching regarding the time of entry into the study, and no antibiotic use in the 14 days before entry in the study, except β-lactams. From controls a throat swab was obtained and processed for PCR and culture. Blood was collected only if the disease justified the sampling of blood. Sera obtained at the time of the throat swab sampling were used for serological testing.

Serology for *M. pneumoniae*. An IFA detecting *M. pneumoniae* IgM antibodies (Zeus Inc., Raritan, N.J.) was performed on a 20-fold-diluted first serum sample from which IgG had been removed with rheumatoid factor absorbent (Behring, Marburg, Germany). Slides were interpreted according to the manufacturer's instructions. Sera were analyzed by CFT using a commercially available *M. pneumoniae* antigen (Virion, Rüschlikon, Switzerland). A fourfold titer rise for paired sera or a single titer of 1:≥128 was regarded as positive.

PCR and culture for *M. pneumoniae*. The throat swabs were twirled in the transport medium, and aliquots were used for culture and PCR. The remainder was stored at −70°C. For PCR, 0.1 ml of throat specimen was subjected to proteinase K lysis and processed for PCR with P1 gene-specific primers (8). An amplification control (AC) was added to test the amplifiability of *M. pneumoniae* DNA in the samples (18). When inhibition occurred, a 1/10 dilution of the lysate was retested. Positive samples were reanalyzed for confirmation by a nested PCR (5). For culture, 50 μl of throat specimen was plated on SP4 agar (16), supplemented with amphotericin B (5 μg/ml) and colistin (500 U/ml) but without

TABLE 1. Clinical signs and symptoms and laboratory findings of 16 patients who were positive for *M. pneumoniae* in any of the four diagnostic tests

Patient	Period of disease at first sampling (days)	Age	ESR[a]	Leukocyte count (10^9/liter)	Chest radiograph abnormalities	CFT titer[b] in sample:		Result of:				Diagnosis of *M. pneumoniae* infection[c]
						1	2	IgM IFA	PCR on throat specimens at: AMC (n = 92)	PCR on throat specimens at: SSI (n = 16)	Culture	
1	11	14	110	11.5	+	≥128	≥128	+	+	+	+	+
2	7	9	76	7.0	+	≥128	≥128	−	+	+	+	+
3	5	3	25	11.7	−	32	≥128	+	+	+	+	+
4	28	5	15	12.3	−	32	≥128	+	−	−	−	+
5	14	1	61	12.3	+	≥128	NA[d]	+	+	+	−	+
6	7	13	16	14	−	≥128	64	+	−	−	−	+
7	6	9	NT	6.7	+	32	32	+	+	+	+	+
8	7	9	43	7.2	+	≥128	≥128	+	+	+	+	+
9	5	16	24	7.5	−	64	64	−	+	+	+	+
10	17	3	11	6.8	−	16	8	+	−	−	−	−
11	7	7	4	8.5	−	32	32	+	−	−	−	−
12	14	6	43	11.3	+	32	32	+	−	−	−	−
13	18	2	34	7.9	+	32	16	+	−	−	−	−
14	4	7	5	9.1	NA	8	8	+	−	−	−	−
15	3	2	5	12.5	NA	64	64	+	−	−	−	−
16	3	3	32	37.8	NA	32	32	+	−	−	−	−

[a] ESR, erythrocyte sedimentation rate; NT, not tested.
[b] From 65 of the 92 included patients a second serum sample was collected 2 to 3 weeks after the first sample. CFT positivity was defined as ≥128 or a fourfold titer rise.
[c] Defined as positive culture and/or positive CFT.
[d] NA, not available.

thallium acetate (SP4-AC), and on Chanock and Herderschêe agar. Two hundred fifty microliters was cultured in 2 ml of SP4-AC broth and Chanock broth. Agars and broths were incubated at 36°C in 5% CO_2. Broths were subcultured on agar upon color change. The identity of putative *M. pneumoniae* colonies was verified by indirect immunofluorescence of unfixed colonies and by nested PCR (5).

Throat specimens from patients and controls positive by either serology, culture, or PCR were sent to the Statens Serum Institut (SSI, Copenhagen, Denmark) for PCR with primers amplifying a fragment corresponding to bp 178 to 331 of the P1 gene (10). An AC was included in each reaction. PCR-positive specimens were reanalyzed by PCR with primers amplifying a fragment corresponding to bp 114 to 513 of the P1 gene. A patient was designated PCR positive when confirmed PCRs at the AMC as well as at the SSI were positive. Throat specimens from patients who were *M. pneumoniae* positive by CFT or by PCR at either laboratory were also cultured at the SSI with Hayflick's, SP4, and soy peptone medium.

Interpretation. Patients with a positive culture and/or CFT were considered to have an *M. pneumoniae* infection.

Additional microbiological testing. On the request of the pediatrician, standard microbiological procedures were performed on sera and throat swabs.

Clinical data. Clinical data from patients were collected by the pediatrician using a questionnaire. Laboratory findings were extracted from the hospital information system.

RESULTS

Ninety-two patients and 74 controls were included in the study. From all patients and from 32 controls at least one serum sample was collected. The IgM IFA on the first serum sample was positive for 14 patients (15%) (Table 1) and for one control. From all subjects testing positive in the IgM IFA and from 51 IgM IFA-negative patients a second serum sample was collected 2 to 3 weeks after the first. CFT was positive for seven patients (8%). CFT on paired sera from the IgM IFA-positive control was negative.

PCR for *M. pneumoniae* was positive for seven patients (8%). PCR on follow-up throat samples obtained from four of them (patients 1, 3, 7, and 8 in Table 1) 4 to 12 weeks after the first sample was negative. Throat samples from the 74 controls were PCR negative. Inhibition of PCR was observed in 33 samples (20%), equally distributed among samples obtained from patients and controls. Diluting the lysates 10-fold before PCR resolved this inhibition in all cases.

M. pneumoniae was cultured from throat samples from six patients (7%). These samples were also PCR positive. All controls were culture negative. Seventeen throat samples (16 from patients and 1 from a control) were subjected to PCR for *M. pneumoniae* at the SSI. PCR results at the AMC and the SSI were concordant (Table 1).

Nine (10%) of the 92 patients met the diagnostic criteria for *M. pneumoniae* infection (patients 1 to 9 in Table 1). Four of them were diagnosed by culture and CFT, two were diagnosed by culture only, and three were diagnosed by CFT only. PCR was positive for all culture-positive patients and one culture-negative patient with a positive CFT. For seven additional patients the IgM IFA was positive whereas all other tests were negative.

Routine microbiological investigations revealed etiologic agents other than *M. pneumoniae* in 23 patients (25%), namely, *Haemophilus influenzae* (n = 4), *Moraxella* (*Branhamella*) *catarrhalis* (n = 1), *Bordetella pertussis*(n = 1), *Chlamydia* species (n = 3), respiratory syncytial virus (n = 4), coxsackie B virus (n = 3), adenovirus (n = 3), parainfluenza virus (n = 2), influenza B virus (n = 1), and Epstein-Barr virus (n = 1). In these patients, all of the four methods performed for diagnosis of *M. pneumoniae* infection were negative.

Clinical data were obtained from 89 patients (97%) (Table 2). Fifty-four patients (61%) met the criteria for lower respiratory tract infection (rales and wheezes with or without an abnormal chest radiograph). *M. pneumoniae* was significantly more often the cause of these severe infections (8 of 54 patients, 15%) than of upper respiratory tract infections (1 of 35 patients, 3%) ($P < 0.001$). Only the absence of coryza significantly correlated with *M. pneumoniae* infection ($P < 0.001$) (Fisher's exact test).

TABLE 2. Clinical features of 89 prospectively studied children with community-acquired respiratory tract infection

Clinical feature	No. of patients (%)		
		With feature and:	
	Investigated	*M. pneumoniae* infection (n = 9)	Other or unknown etiology (n = 80)
Age (yr)[a]	89	1–16 (9)	0–16 (5)
Cough	89	9 (100)	74 (93)
Coryza	89	0 (0)	59 (74)[b]
Malaise	88	6 (75)	59 (74)
Headache	72	2 (29)	17 (26)
Myalgia	72	1 (14)	8 (12)
Earache	89	0 (0)	13 (16)
Fever >38°C	89	4 (44)	30 (38)
Pharyngeal erythema	88	5 (56)	31 (39)
Cervical adenopathy	88	2 (25)	22 (28)
Rales, wheezes	89	8 (89)	46 (56)
Chest radiograph	69	5[c] (56)	27[c] (45)
Erythrocyte sedimentation rate[a]	88	15–110 (43)	1–115 (15)
Leukocyte count (10^9/liter)[a]	88	7–14 (11)	4–37 (11)

[a] Numbers represent range (median).
[b] $P < 0.001$ (Fisher's exact test).
[c] Number of abnormal chest radiographs.

DISCUSSION

Recently much emphasis has been placed on rapid diagnosis of *M. pneumoniae* infection by PCR (1, 8, 13, 15, 19) and IgM assays (2). We prospectively studied 92 children with community-acquired respiratory infections and 74 controls to compare diagnosis of *M. pneumoniae* infection by two rapid tests, PCR and IgM IFA, with diagnosis by the more traditional methods of culture and CFT. Our criteria for diagnosis of *M. pneumoniae* infection were a positive culture and/or positive CFT. According to these criteria, nine patients (10%) had an *M. pneumoniae* infection (Table 1).

Throat specimens from seven of the nine patients (78%) diagnosed with *M. pneumoniae* infection were PCR positive. Thus, the sensitivity of the PCR was 78%; the specificity and positive predictive value were 100%. The sensitivity of the PCR was relatively low because two CFT-positive patients were negative by PCR. This may have been caused by sampling errors. Another possibility is that the *M. pneumoniae* load was below the detection level of both PCRs or that *M. pneumoniae* already had disappeared from the throat at the time of sampling.

Conversely, PCR was positive for two other patients who had an *M. pneumoniae* infection based on a positive culture, whereas the CFT was negative. The negative CFT for these patients may be due to impaired immune responses (1, 13). All culture-positive patients were also positive by PCR. The strong positive predictive value indicates that PCR positivity can be added to the criteria for diagnosis of *M. pneumoniae* infection and can even replace culture positivity.

Sera analyzed by IgM IFA were positive in 14 patients (Table 1). The sensitivities of IgM IFA and of PCR were equal (78%). The specificity of IgM IFA was low (92%), and its positive predictive value was only 50%. One control was also positive by IgM IFA, whereas all other tests were negative. Even when single CFT titers of 64 were regarded as positive (14), IgM IFA still had a low positive predictive value (57%). We therefore will not add a positive IgM IFA to our criteria and conclude that the IgM IFA should not be used as the single assay to diagnose *M. pneumoniae* infection (4).

Although the number of *M. pneumoniae*-positive patients in our study was relatively small, PCR and CFT were complementary in four of the nine patients (44%) diagnosed. If either PCR or CFT had been used as the single test, diagnosis would not have been established in two patients (22%) with *M. pneumoniae* infection. We therefore use PCR for rapid diagnosis of *M. pneumoniae* infection. In case of a negative PCR, a CFT on paired sera is necessary to either confirm or reject the diagnosis of *M. pneumoniae* infection. Our findings are concurrent with those in the study of Abele-Horn et al. (1). However, for serological diagnosis they applied a microparticle agglutination test and added immunoblotting in unclear cases. As the microparticle agglutination assay exclusively detects IgM antibody (3) and immunoblotting is not widely available, we prefer the use of CFT on paired sera.

Several authors have reported carriage of *M. pneumoniae* after symptomatic infection (7) or after treatment (11). We did not find indications for carriership. Follow-up throat swabs from four PCR-positive patients who had been treated with antibiotics were PCR negative, suggesting eradication of *M. pneumoniae*. In addition, all controls were *M. pneumoniae* PCR negative.

Foy et al. (6) reported several clinical parameters which positively and negatively correlated with *M. pneumoniae* infection. In our study the absence of coryza was the only parameter that correlated with an *M. pneumoniae* infection ($P < 0.001$) (Table 2). This finding shows the difficulty in discriminating *M. pneumoniae* from other (viral) pathogens causing respiratory tract infection on clinical parameters only and emphasizes the need for laboratory confirmation.

In conclusion, the rapid IgM IFA has a low positive predictive value, and PCR on throat swab samples has a relatively low sensitivity. Therefore, PCR should be combined with CFT to allow both fast and reliable diagnosis of *M. pneumoniae* infection.

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