

Detection of *Mycoplasma pneumoniae* in Clinical Samples from Pediatric Patients by Polymerase Chain Reaction

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The polymerase chain reaction (PCR) technique was used to detect *Mycoplasma pneumoniae* DNA in clinical samples (nasopharyngeal aspirations or bronchoalveolar lavages) obtained from 100 children, 1 month to 16 years old. PCR allowed the detection of *M. pneumoniae* DNA from 20 out of the 100 patients studied. In 16 cases, PCR positivity was associated with acute respiratory symptomatology. For five PCR-positive patients, a positive culture or a serological response evidenced acute *M. pneumoniae* infections. A lack of antibody response was observed particularly with immunocompromised children and infants less than 12 months old. The amount of *M. pneumoniae* DNA in the PCR was estimated in a semiquantitative way by comparison of its hybridization signal with those obtained for 100, 10, and 1 color-changing unit (CCU) of the *M. pneumoniae* FH strain. Small amounts ($\leq 10^2$ CCU/ml) of *M. pneumoniae* were found in samples from asymptomatic patients, while larger amounts ($\geq 10^2$ to $\geq 10^4$ CCU/ml) were found for 8 out of 10 patients with acute pneumonia.

Mycoplasma pneumoniae is a common cause of a wide range of upper and lower respiratory tract infections, especially in children and in young adults. Routine laboratory diagnosis of *M. pneumoniae* infection is based mainly on serology and, to a lesser extent, on cultivation. The complement fixation test is widely used for *M. pneumoniae* antibody detection but lacks specificity because of its cross-reactivity with similar antigens, and it is poorly sensitive, especially with specimens from young children (8, 10). Enzyme-linked immunosorbent assay (3, 7, 15) and micro-particle agglutination (5) have been reported to be both more specific and more sensitive for antibody detection, but they usually require paired sera to demonstrate rises in antibody titer. Culture of *M. pneumoniae* is time-consuming, requires up to 4 weeks for results, and is relatively insensitive.

These drawbacks of serology and cultivation emphasize the need to develop rapid tests for the diagnosis of *M. pneumoniae* in order to allow the initiation of antibiotic treatment at the onset of infection. For this purpose, antigen detection (9) and molecular hybridization with DNA probes (6, 11, 14) have been proposed; despite good specificities, these tests do not allow the detection of low levels of *M. pneumoniae*. More recently, C. Bernet et al. (1) and J. Skov Jensen et al. (13) reported the use of polymerase chain reaction (PCR) for in vitro amplification of *M. pneumoniae* DNA. Using different sets of primers, these authors showed that PCR allowed the specific detection of *M. pneumoniae* DNA sequences, while DNA from other *Mycoplasma* species gave negative results. The sensitivity of the PCR assay was evaluated with simulated clinical samples (artificially seeded throat swabs or bronchoalveolar lavages). In these experiments, the detection level of *M. pneumoniae* was estimated in a range of 10 to 40 color-changing units (CCU). The specificity, the exquisite sensitivity, and the relative rapidity of PCR make this technique promising for the rapid

diagnosis of *M. pneumoniae* infections. However, the use of PCR as a diagnostic method still needs to be validated with clinical samples, and no report of such data has been published to date.

In this prospective study, we have evaluated the use of PCR to detect *M. pneumoniae* in clinical samples (nasopharyngeal aspirations or bronchoalveolar lavages) obtained from 100 children, 1 month to 16 years old. The results are compared with cultivation and serological data for *M. pneumoniae*.

MATERIALS AND METHODS

Patients. Clinical specimens were obtained from 100 children admitted to Trousseau Hospital between February and June 1991. Informed consent was obtained from the parents. The mean age was 7 ± 0.9 years (range, 1 month to 16 years). Children were divided into four groups according to their clinical status: group I ($n = 28$) included children with acute respiratory diseases (pneumonia, bronchitis, and attacks of asthma), group II ($n = 9$) included children with cystic fibrosis, group III ($n = 40$) included children with chronic pulmonary disease but without acute symptomatology (obstructive bronchopneumopathy and asthma and one patient with lipidic interstitial pneumopathy), and group IV ($n = 23$) included immunocompromised children. Clinical samples obtained for *M. pneumoniae* cultivation and DNA amplification were nasopharyngeal aspirations (NPA) ($n = 83$), bronchoalveolar lavages (BAL) ($n = 30$), or pleural fluid ($n = 1$). In this study, BAL were done for indications other than *M. pneumoniae* detection. Each time it was possible, paired sera were obtained for *M. pneumoniae* antibody detection. Acute-phase and convalescent-phase sera were taken 1 to 7 and 8 to 30 days, respectively, after the onset of disease.

DNA extraction and amplification. DNA was extracted from clinical samples by the method described by Boom et al. (2). Briefly, 100 μ l of a BAL or an NPA sample was incubated for 10 min at room temperature with 40 μ l of silica

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particles suspended in 900 μ l of lysis buffer (1 M guanidine isothiocyanate, 0.1 M Tris HCl [pH 6.4], 0.036 M EDTA, 1% Triton X-100). After being centrifuged (1 min, 12,000 \times g), the pellet was washed twice with 1 M guanidine isothiocyanate-0.1 M Tris HCl (pH 6.4), twice with ethanol (70%), and once with acetone. The dried pellet was then resuspended in 100 μ l of distilled water, heated for 10 min at 56°C, and centrifuged (2 min, 12,000 \times g), and the DNA-containing supernatant was stored at 4°C.

Prior to *M. pneumoniae* amplification, each DNA sample was tested for its ability to be amplified with the β -globin-specific primers KM38 and KM39 (12). A positive signal was defined by a 262-bp fragment visualized on an ethidium bromide-stained agarose gel. In our study, a positive signal was constantly detected with control dilutions of human DNA corresponding to 5 to 10 cells.

For *M. pneumoniae*-specific amplification, we used the primer set MP5-1 (5'GAAGCTTATGGTACAGGTTGG)-MP5-2 (5'ATTACCATCCCTTGTTGTAAGG) (purchased from Genset, Paris, France), described by C. Bernet et al. (1). A 10- μ l DNA sample was incubated in a 100- μ l reaction volume containing 0.1 μ M (each) primer, 200 μ M (each) deoxynucleoside triphosphate, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 1 U of *Taq* polymerase (AmpliTaq; Perkin-Elmer, Saint-Quentin, France). Amplification was carried out for 40 cycles (1 min at 92°C, 2 min at 55°C, and 1 min at 72°C) in a DNA thermal cycler (Perkin-Elmer). Positive controls (DNA from the *M. pneumoniae* FH strain) and negative controls (H₂O, human DNA, and DNA from *Escherichia coli*, *Mycoplasma orale* T519, *Mycoplasma salivarium* A889, and *Mycoplasma genitalium* G37c) were run in the same way. PCR products were analyzed on a 1.5% agarose gel, with a positive PCR yielding a 144-bp fragment. After Southern transfer on a GeneScreen Plus membrane (Du Pont de Nemours, Les Ulis, France), hybridization was performed with 5' ³²P-end-labeled oligonucleotide MP5-4 (5'CGTAAGCTATCAGCTACATGG AGG) (1) in order to confirm specific amplification. Filters were hybridized for 12 h at 42°C with 10⁶ cpm of labeled probe per ml of hybridization buffer (3 \times SSC [1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 5 \times Denhardt solution, 0.5% sodium dodecyl sulfate [SDS], 30% formamide, 0.1 mg of salmon sperm DNA per ml) and washed twice for 15 min at room temperature in 2 \times SSC-1% SDS and once for 10 min at 50°C in 1 \times SSC-0.1% SDS. Autoradiography was performed with Agfa-Curix RP-1 films for 3 and 24 h. All of the samples were tested in duplicate. A positive signal was defined by the detection of a reproducible hybridization signal in two independent amplification reactions and no signal with negative controls. The results of *M. pneumoniae* DNA amplification (MP-PCR) were considered only when DNA was found to be amplified with β -globin-specific primers. Thus, samples positive for β -globin and *M. pneumoniae* amplification were considered MP-PCR positive, samples positive for β -globin and negative for *M. pneumoniae* amplification were considered MP-PCR negative, and samples negative for β -globin and *M. pneumoniae* amplification were not taken into account and were classified as indeterminate.

***M. pneumoniae* cultivation.** Isolation of *M. pneumoniae* from clinical samples was performed in modified SP-4 medium with the *M. pneumoniae* culture kit Pneumofast (International Mycoplasma-DBV Production, Sanary, France) according to the manufacturer's recommendations. Color-changing unit titers of *M. pneumoniae* FH cultures were determined by serial 10-fold dilutions in liquid SP-4 medium.

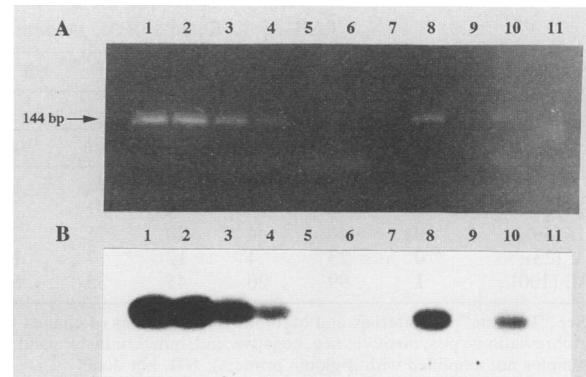


FIG. 1. Analysis of PCR products obtained from clinical specimens. (A) Ethidium bromide-stained 1.5% agarose gel. The positive controls were the PCR products obtained with DNA extracted from serial 10-fold dilutions of *M. pneumoniae* FH corresponding to 1,000 (lane 1), 100 (lane 2), 10 (lane 3), and 1 (lane 4) CCU. The negative controls were DNA from *M. orale* T519 (lane 5) and *M. salivarium* A889 (lane 6), H₂O with no DNA (lane 7), and human DNA from peripheral blood mononuclear cells (lane 11). Also shown are a negative BAL sample (lane 9), a positive BAL sample (lane 8), and a positive NPA sample (lane 10). (B) Southern transfer of the agarose gel shown in panel A and hybridization with the 5' ³²P-end-labeled oligonucleotide MP5-4. Hybridization signals of clinical samples were compared with signals corresponding to 100, 10, and 1 CCU of *M. pneumoniae* FH (lanes 2 to 4). The amount of *M. pneumoniae* DNA in the PCR (10 μ l of DNA) was estimated to range from >1 to \leq 10 CCU for the sample in lane 8 (i.e., 10² to 10³ CCU per ml of sample) and \leq 1 CCU for the sample in lane 10 (i.e., \leq 10² CCU per ml of sample).

One color-changing unit was estimated to be equivalent to 10 to 100 organisms (1).

Serology. Determination of *M. pneumoniae*-specific antibody was performed by using a commercial microparticle agglutination assay (Serodia Myco II; Fujirebio, Inc., Tokyo, Japan) according to the manufacturer's recommendations. An antibody titer of <40 was regarded as negative. A threefold rise in titers of paired sera and titers of \geq 160 were considered significant.

RESULTS

In order to appreciate the sensitivity of our PCR assay, we amplified DNA extracted from serial 10-fold dilutions of *M. pneumoniae* FH at a known concentration. One color-changing unit of *M. pneumoniae* per 0.1 ml of PCR sample (estimated to be equivalent to 10 to 100 organisms) was consistently detected, and 0.1 CCU was detected in only 25% of the reactions. The detection limit of the PCR assay was thus estimated to range from 10 to 100 CCU per ml of sample (i.e., 10² to 10³ organisms). In addition, a reproducible decrease in hybridization signal intensity was obtained for the dilutions corresponding to 100, 10, and 1 CCU. In further PCR experiments, these standard dilutions were systematically included, allowing semiquantitation of *M. pneumoniae* particles in clinical samples (Fig. 1). Amplification of human DNA and DNA from *M. orale* T519, *M. salivarium* A889, and *M. genitalium* G37c gave negative results, confirming the specificity of the primer set MP5-1-MP5-2 described by C. Bernet et al. (1).

When the PCR assay was applied to clinical samples, DNA extraction appeared to be a crucial step. In preliminary experiments, we previously compared three different tech-

TABLE 1. Results of *M. pneumoniae* DNA amplification, serology, and culture

Clinical group ^a (no. of patients)	No. of children with the following result ^b by:												
	Culture		PCR				Serology ^c			PCR-serology			
	Pos	Neg	Pos	Neg	Ind	Valid	Pos	Neg	ND	Pos-pos	Neg-neg	Pos-neg	Neg-pos
I (28)	1	27	9	9	10	18	4	13	1	3	7	6	1
II (9)	0	9	3	4	2	7	0	5	2	0	3	2	0
III (40)	0	40	4	20	16	24	1	23	0	1	20	3	0
IV (23)	0	23	4	12	7	16	0	12	4	0	8	4	0
All (100)	1	99	20	45	35	65	5	53	7	4	38	15	1

^a See "Patients" in Materials and Methods for definitions of clinical groups.

^b Abbreviations: pos, positive; neg, negative; ind, indeterminate; valid, total positive and negative PCR results, excluding indeterminate PCR results (i.e., those for samples not amplified with β -globin primers); ND, not done.

^c Results of *M. pneumoniae* serology are indicated for the 65 children with valid PCR tests (a threefold rise in titers of paired sera or titers of ≥ 160 were considered significant).

niques of DNA preparation from clinical samples (BAL and NPA): one included lysis with proteinase K followed by conventional phenol-chloroform extraction and ethanol precipitation, one was rapid lysis with proteinase K followed by heat inactivation, and the third was the guanidine isothiocyanate technique described in Materials and Methods. When the quality of the DNA used for amplification was tested with β -globin primers, the first two methods gave poor results (less than 10% positive amplification), while the third method allowed the amplification of about 80% of the samples. In addition, similar results were obtained when control BAL and NPA were artificially seeded with 100 CCU of *M. pneumoniae* and amplified with the MP5-1-MP5-2 primers. All samples negative for β -globin amplification were also negative for *M. pneumoniae* amplification, indicating that the inhibition of amplification was most likely due to the presence of trace contaminants. In contrast, *M. pneumoniae* DNA was detected by PCR in all samples positive for β -globin amplification. In these cases, the hybridization signal intensity was equivalent to that of the corresponding control standard (100 CCU of the FH strain) diluted in buffer. Thus, dilution of *M. pneumoniae* in samples positive for β -globin amplification seems to have little influence on PCR sensitivity.

During the study conducted between February and June 1991, 83 NPA, 30 BAL, and 1 sample of pleural fluid were collected from 100 children admitted to Trousseau Hospital. In order to detect possible inhibition of the PCR, DNA extracted from these samples was tested with the KM38 and KM39 β -globin-specific primers. A characteristic β -globin-amplified product was obtained for 75% of the samples, and 65 children had at least one β -globin-positive sample. The results of MP-PCR were considered only when DNA was found to be amplified with β -globin-specific primers. Thus, in order to eliminate possible false-negative results, only samples positive for β -globin and negative for MP-PCR were considered MP-PCR negative, while samples negative for β -globin and MP-PCR were not taken into account and were classified as indeterminate. Consequently, MP-PCR was considered indeterminate for 35 children whose samples were negative for β -globin amplification. Among the 65 remaining children, 20 were found positive and 45 were found negative for MP-PCR (Table 1). According to each clinical group, MP-PCR was more frequently positive in groups I (acute respiratory diseases, including pneumonia and asthma attacks, 9 of 18 tested found positive) and II (cystic fibrosis, 3 of 7 tested found positive) than in groups III (chronic pulmonary disease without acute symptoms, 4 of

24 tested found positive) and IV (immunocompromised children, 4 of 16 tested found positive).

A correlation of MP-PCR results with those of culture and serology is shown in Table 1. Among the 100 children tested, a positive culture of *M. pneumoniae* was found only once and was associated with a positive PCR. Serological data were available for 58 out of the 65 children with valid PCR tests (excluding indeterminate results). PCR was concordant with serology in 42 cases. Thirty-eight MP-PCR-negative children had no *M. pneumoniae* antibody, four MP-PCR-positive children had *M. pneumoniae* antibody titers of ≥ 160 , and one of them experienced seroconversion. Discrepancies were observed in 16 cases. One MP-PCR-negative child with acute pneumonia had an *M. pneumoniae* antibody titer of $\geq 20,480$. In contrast, serology was found negative for 15 MP-PCR-positive children; these last cases will be detailed below.

Detailed results for the 20 children (7 girls and 13 boys) with positive MP-PCR results are shown in Table 2. In 16 cases, PCR detection of *M. pneumoniae* was associated with acute respiratory symptomatology: acute pneumonia ($n = 10$), asthma attacks ($n = 2$), cystic fibrosis with pulmonary exacerbation ($n = 3$), and histiocytosis X with acute bronchitis ($n = 1$). No pathogenic bacterial agent other than *M. pneumoniae* could be detected, except with patient 3, for whom *Haemophilus influenzae* was isolated from sputum. *M. pneumoniae* DNA was detected in 13 NPA, 7 BAL, and 1 sample of pleural fluid and in both the NPA and BAL specimens from one child. For each specimen, the amount of *M. pneumoniae* DNA in the PCR was estimated in a semi-quantitative way by comparison of hybridization signals with those obtained for 100, 10, and 1 CCU of *M. pneumoniae* FH (Fig. 1). PCR values were then converted into the equivalent color-changing units per milliliter of clinical sample. *M. pneumoniae* PCR quantitation results ranged from low values ($\leq 10^2$ CCU/ml) to high values ($\geq 10^4$ CCU/ml). Low values were found in all asymptomatic (group III) patients and cystic fibrosis (group II) patients and in the two patients with acute asthma. In contrast, the amount of *M. pneumoniae* in clinical samples was higher for 8 out of 10 patients with acute pneumonia (groups I and IV). The single culture-positive sample (from patient 4) was quantified by PCR as $\geq 10^4$ CCU/ml. In addition, the low level found for patient 5 could possibly be due to high dilution of the microorganism in pleural fluid. For patient 20, the amount of *M. pneumoniae* in the BAL specimen was found to be 10-fold greater than that in the NPA specimen. With regard to *M. pneumoniae* serology, three points deserve to be noted. (i) In 4

TABLE 2. Results for children with positive *M. pneumoniae* PCRs^a

Clinical group ^b and patient no.	Sex/age	Clinical status	Clinical sample	PCR quantitation ^c	MP antibody titer	
					Acute-phase serum	Convalescent- phase serum
I						
1	F/5 yr	Atypical pneumonia	NPA	$n \geq 10^4$	<40	5,120
2	M/7 yr	Acute pneumonia	NPA	$10^3 < n < 10^4$	ND	5,120
3	F/16 mo	Acute pneumonia	NPA	$10^2 < n < 10^3$	ND	2,560
4 ^d	M/2 mo	Respiratory distress, pneumonia	BAL	$n \geq 10^4$	<40	<40
5	M/12 mo	Pneumonia, pleural effusion	PF	$n \leq 10^2$	ND	<40
6	F/2 yr	Acute bronchopneumonia	NPA	$10^3 < n < 10^4$	<40	ND
7	M/11 yr	Acute pneumonia	NPA	$n \leq 10^2$	<40	ND
8	M/11 yr	Asthma attack	NPA	$n \leq 10^2$	<40	ND
9	F/6 yr	Asthma attack	NPA	$n \leq 10^2$	<40	ND
II						
10	M/3 mo	Cystic fibrosis, pulmonary exacerbation	BAL	$n \leq 10^2$	ND	<40
11	M/6 yr	Cystic fibrosis, pulmonary exacerbation	BAL	$n \leq 10^2$	ND	<40
12	F/16 yr	Cystic fibrosis, pulmonary exacerbation	NPA	$n \leq 10^2$	ND	ND
III						
13	M/3 yr	Lipidic interstitial pneumopathy	BAL	$n \leq 10^2$	640	ND
14	F/5 yr	Asthma, asymptomatic	NPA	$n \leq 10^2$	<40	<40
15	M/8 yr	Chronic bronchopneumopathy	NPA	$n \leq 10^2$	<40	ND
16	M/12 yr	Asthma, asymptomatic	NPA	$n \leq 10^2$	40	40
IV						
17	M/3 yr	Histiocytosis X, bronchitis, fever	BAL	$10^3 < n < 10^4$	ND	<40
18	F/8 yr	Leukemia, pneumonia	NPA	$10^3 < n < 10^4$	<40	<40
19	M/12 yr	Leukemia, pneumonia	BAL	$10^2 < n < 10^3$	<40	<40
20	M/8 yr	Bruton syndrome, pneumonia	BAL	$10^2 < n < 10^3$	<40	<40
			NPA	$n \leq 10^2$		

^a Abbreviations: PF, pleural fluid; MP, *M. pneumoniae*; ND, not done.

^b See "Patients" in Materials and Methods for definitions of clinical groups.

^c Amounts (n) of *M. pneumoniae* are given as the equivalent color-changing units per milliliter of sample.

^d Patient no. 4 was the only one found positive for *M. pneumoniae* by culture.

cases, seroconversion or antibody titers of ≥ 160 in convalescent-phase serum were concordant with PCR results. (ii) In 10 cases, antibodies were not detected in paired sera or in convalescent-phase serum and seemed inconsistent with PCR results. However, this lack of antibody response was observed particularly for all immunocompromised, group IV patients ($n = 4$) and infants less than 12 months old (patients 4, 5, and 10), one of whom was positive for *M. pneumoniae* cultivation. (iii) In five cases, antibodies were not detected in acute-phase sera obtained 2 to 7 days after the onset of disease. Unfortunately, convalescent-phase sera were not available, and one cannot exclude a further increase in antibody titers.

DISCUSSION

The aim of this study was to evaluate the use of the PCR technique for the diagnosis of *M. pneumoniae* infections in children. One of the major drawbacks we encountered was obtaining DNA pure enough to be amplified. Inhibition of amplification was more likely due to the presence of trace contaminants. Such inhibition in other systems has been previously described as a source of false-negative PCR results (4). Therefore, the diagnosis of infectious agents by using PCR imperatively requires adaptation of the DNA preparation to the nature of the clinical samples, and the quality of the DNA to be amplified must be tested before PCR results are interpreted. In this study, even if the guanidine isothiocyanate DNA extraction method were the

most reliable, only 75% of the clinical samples could be amplified, and efforts to improve DNA quality are currently under way.

When applied to clinical samples, PCR detected *M. pneumoniae* far more frequently than culture. Limited data comparing PCR and culture methods are available. However, PCR was shown to be more sensitive and reproducible than culture for detecting *M. pneumoniae* in throat swabs from experimentally infected hamsters (1). Taking into account the semiquantitative PCR results, the culture-positive sample was one of those containing the largest amounts of *M. pneumoniae* ($>10^4$ CCU/ml). One can hypothesize that culture positivity may be partly related to the presence of a large number of organisms in the sample with the culture system we used.

Among PCR-positive patients, one experienced seroconversion and three already had high antibody titers when the sera were obtained. By the microparticle agglutination assay, antibody titers of ≥ 160 have been shown to be diagnostically indicative of an acute *M. pneumoniae* infection (5), and these three cases could be considered in agreement with the PCR results. In contrast, MP-PCR was found negative for one patient with acute pneumonia and an *M. pneumoniae* antibody titer of $\geq 20,480$. Different hypotheses could account for this possibly false-negative PCR result. First, *M. pneumoniae* may not have been detected because the number of microorganisms in the sample was less than the detection level of the PCR assay. Second, genetic diversity may exist among *M. pneumoniae* strains and amplification

failure may be due to genomic modifications in *M. pneumoniae* DNA target sequences that were no longer recognized by the primers. If this is the case, the use of more than one primer pair will be helpful in avoiding false negatives. A large number of *M. pneumoniae* clinical isolates will have to be tested before this hypothesis is ascertained.

The main point remains to interpret the significance of the positive PCR results associated with negative serological responses. These discrepancies may be partly explained by the timing of the serum samples. In five cases, a single serum sample was obtained 2 to 7 days after the onset of symptoms. This interval may have been too short for a serological response to develop. In 10 other cases, no serological evidence of acute *M. pneumoniae* infection was found by analyzing paired or convalescent-phase sera. These cases can account for false-positive PCR results. False positives due to the amplification of DNA sequences unrelated to *M. pneumoniae* seem unlikely, since the primers were shown to not amplify DNA from a large range of bacterial species (1; also this study). In addition, PCR products were analyzed by hybridization with an internal probe in order to confirm the reaction specificity. Contamination by the carryover of PCR products is one of the major drawbacks of the PCR technique which can lead to false positives. Guidelines for the general handling of the PCR procedure have been proposed in order to minimize the risk of contamination (4). Per these recommendations, PCR experiments were carried out with special care to avoid carryover, and negative controls were randomly included in each reaction set to monitor contamination. Moreover, a sample was considered PCR positive only when it was clearly reproducible in at least two independent experiments. For two seronegative children, the possibility of a false-positive PCR result seems very unlikely. One had a positive culture, and for the other, *M. pneumoniae* DNA was detected by PCR in both BAL and NPA samples which were independently processed. Such cases could rather then account for false-negative serological results. The presence of seronegative immunocompromised children and young infants with high PCR values could be even more relevant to this hypothesis. Consequently, PCR might be of great use in detecting *M. pneumoniae* infections in patients with immunological impairment. On the other hand, PCR may have detected *M. pneumoniae* in circumstances other than acute infection, such as persistence in the respiratory tract or asymptomatic infection. This might be the case for group II and III patients, for whom *M. pneumoniae* was found in amounts less than 10^2 CCU/ml. Similarly, in a recent study using a probe test (11), positive probe test results were found for control patients, but the values were lower than those for patients with confirmed *M. pneumoniae* infections. Thus, raising the PCR positivity limit to values greater than 10^2 CCU/ml as indicative of acute symptomatic infection deserves serious consideration. However, PCR detection of *M. pneumoniae* in asymptomatic patients may be of importance, as it could lead to a better understanding of the epidemiology and pathogenicity of *M. pneumoniae*. Analysis of sequential samples will be needed to estimate for how long *M. pneumoniae* can be detected by PCR after the onset of infection. In our experience, PCR did not detect *M. pneumoniae* in a sample obtained 2 months after the onset of infection in patient 20, who had received erythromycin and had experienced clinical improvement in the meantime.

Finally, with respect to the possibly false-positive PCR results, the clinical outcomes of the patients could be critical to assessing the reality of *M. pneumoniae* infection. Among

the 20 PCR-positive patients, 5 group I and 3 group IV patients with acute pneumonia had received specific therapy with erythromycin. In four cases, culture and serology were negative and *M. pneumoniae* was detected only by PCR. The treatment was effective, and all patients had complete clinical and radiological recoveries within their 2-month follow-up visits. In these cases, the clinical outcome was compatible with *M. pneumoniae* infection. Patients with mild symptomatology and asymptomatic patients were not treated. Cystic fibrosis patients received broad-spectrum-antibiotic therapy. For these patients, it seems difficult to assess the role of *M. pneumoniae* in the course of the disease.

The poor sensitivity of culture and the possibility of impaired serological responses make the PCR assay promising for the diagnosis of *M. pneumoniae* infections. In addition, PCR is fast enough to allow for the early application of therapy with a specific antibiotic. However, standardization of the PCR assay, including technical improvement of the DNA extraction step and PCR quantitation as well as interpretation of positive results, will be needed before this test can be proposed on a routine basis. For this purpose, the use of a nonisotopically labeled probe would make the test much more practical. Easier detection of *M. pneumoniae* by PCR may lead to an emphasis on its role in pulmonary or extrapulmonary infections. However, additional studies are still required in order to validate the PCR assay with a large number of clinical samples.

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