Analysis of mycoplasmal pleural effusion by the polymerase chain reaction

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Abstract

Ten pediatric patients with mycoplasmal pleuritis were tested for the presence of *Mycoplasma pneumoniae* in pleural fluid by the polymerase chain reaction (PCR). Three of the four PCR positive cases left a persistent consolidation. The remaining one was an infant who required mechanical ventilation. PCR may be useful in predicting delayed resolution of roentgenographic abnormality.

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Pleural effusion is a well recognised complication of mycoplasmal infection.¹ The purpose of this study was to investigate pleural fluid samples from patients with mycoplasmal infection for the presence of the chromosomal DNA of *Mycoplasma pneumoniae*.

Patients

Ten pediatric patients with serologically diagnosed mycoplasmal infection who were involved in this study are listed in table 1. Routine bacterial cultures from throat swabs, pleural fluid and/or blood, and serological examinations disclosed that no other bacterial or viral pathogens were present as far as tested. Since cases 4, 7, 8, and 9 had a severe or pro-

longed clinical course, these cases are briefly illustrated below.

CASE 4

A 2 year old previously healthy girl was referred to the hospital because of a high fever and severe cough that had lasted for a week. On admission, a chest radiograph revealed a massive pleural effusion in the left lung (fig 1A) and a thoracocentesis yielded a serous fluid (sample 1). Treatment was started with intravenous cefotiam and oral erythromycin. Despite repeated removal of the fluid, a chest radiograph taken on the fourth day revealed an entirely opaque left lung field with a deviation of the mediastinum to the right (fig 1B). A closed chest tube thoracostomy was performed which yielded a bloody fluid (sample 2). The treatment was changed to intravenous cefotaxime and intravenous clindamycin with the addition of oral clarithromycin. A chest radiograph taken one month after admission still showed a dense consolidation (fig 1C).

CASE 7

An 11 year old boy having neuronal ceroid lipofuscinosis was admitted to the hospital because of a fever and cough. A pleural effusion in the right lung was noticed five days after admission, and a thoracocentesis yielded a bloody pleural fluid without any noticeable mechanical injury. He subsequently required a left thoracocentesis on the next day. Although his condition improved with intravenous minocycline, a residual consolidation was still observed in the right lung three months after discharge.

CASE 8

An 11 month old boy with Down's syndrome was admitted to the hospital because of a fever and cough. He was intubated due to poor oxygenation and an arterial oxygen of 6.67-8.00 kPa was maintained under 100% oxygen supplement. A pleural effusion in the right lung became evident four days after admission. The patient gradually recovered with intravenous minocycline without showing any residual abnormalities on a chest radiograph.

CASE 9

A 5 year old previously healthy boy was referred to the hospital because of fever and a cough. Intravenous clindamycin was administered. A small pleural effusion was noticed in the left lung two days after admission and intravenous minocycline along with oral clarithromycin was added to the treatment. Despite these treatments, the fluid collection pro-

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Figure 1 Chest radiographs of case 4. Panel A, taken on admission; panel B, taken immediately before drainage (four days after admission); panel C, taken at the recovery stage (one month after admission).

Figure 2 Chest radiographs of case 9. Panel A, taken before drainage (10 days after admission), left decubital view. Arrowheads indicate the retention of fluid; panels B and C, taken at the recovery stage (two months after admission).

Table 1 Characteristics of the 10 cases, pleural fluid, and results of PCR

Case	1	2	3	4
Age (years)/sex	12/M	3/M	15/F	3/F
Affected side	Right	Left	Right	Left
Day of appearance*	<0 (0)	2 (2)	<0 (6)	<0 (0), (5)
Removal of effusions (volume in ml)	Drainage	Thoracocentesis (100)	Thoracocentesis (340)	Thoracocentesis (repeated), drainage
Antimycoplasmal antibody titre†				
Acute	<40	8	<40	<40
Highest observed	160	256	80	20480
Pleural fluid				(Sample 1)
Appearance	Serous	Serous	Serous	Serous
Protein (g/l)	49	26	46	44
Lactate dehydrogenase (U/l)	3236		611	11670
Cell count (/mm ³)	46			Numerous
Sample volume tested (ml)	4.0	25	3.4	10
Other involvement‡	Liver dysfunction	Liver dysfunction, skin rash	None	None
Resolution of chest roentgenograms§ (months)	0.7	0.5	0.5	12
PCR#	(-)	(±)	(-)	(+)

* The day of admission was denoted as day 0. The day of removal was shown in parentheses. <0 means that the effusion was already present on admission.

† Particle agglutination test was used for all cases except for case 2 in which complement fixation test was used.

‡ Liver dysfunction, maximum alanine aminotransferase value (U/l); 210 in case 1, 2004 in case 2, 478 in case 5, 342 in case 10.

gressed (fig 2A). Ten days after admission, a thoracocentesis was performed followed by administration of intravenous dexamethasone, which resulted in disappearance of the fever. A segmental consolidation remained at two months after admission in the absence of signs of active inflammation (fig 2B/C).

With the exception of case 8 the acute illness in these four cases was no more severe than in the other six cases. Apparently none of the 10 cases were subject to respiratory morbidity beyond the acute illness, although extensive investigations such as bronchography were not performed.

Methods

The characteristics of the pleural effusions are listed in table 1. The sample volume tested, which is shown in table 1, is an approximate

volume of the material that was actually used in one polymerase chain reaction (PCR) experiment, being calculated from the final volume of the DNA solution. Amplifications were carried out essentially by the same methodology as described elsewhere.^{2,3} General precautions were undertaken to avoid cross contamination. Eleven pleural fluid samples from 11 patients with pleuritis in which causes other than *M pneumoniae* infection were identified served as controls.

Results

The results of PCR amplification with the 12 pleural fluid samples from the 10 patients with mycoplasmal infection are shown in fig 3 and in table 1. Of these, two sequential samples from case 4, samples from cases 7, 8, and 9 yielded a strongly positive band of *M pneumoniae*, while negative results were obtained with the samples from cases 1, 3, 5, 6, and 10. A sample from case 2 showed a faintly positive band that was scarcely visible, whose result was considered as indeterminate. None of the 11 pleural fluid samples from the controls yielded a positive band.

Discussion

A remarkable finding in this study is the strongly positive bands of *M pneumoniae* DNA which were observed using the samples from cases 4, 7, 8, and 9. One major concern is that two of the samples with the positive PCR results were bloody. In case 4, sample 1 (apparently serous fluid) had already contained the genome of *M pneumoniae* before it became bloody, and in case 7, a serum sample which was obtained at the same time of thoracocentesis was performed did not yield a positive band. These facts suggest that the positive PCR results can be considered to represent a massive invasion of the pleural space by this organism rather than simple contamination with the organism from blood.

Three explanations can be offered for the differences concerning the PCR results. First, the PCR positive and negative cases arise from substantially different pathophysiological

Figure 3 Results of PCR using pleural fluid samples. Ethidium bromide stained agarose gels. Lane 1, positive control (1 pg of whole chromosomal DNA from a strain *M pneumoniae* IID 817); lane 2, negative control (a buffer control); lane 3, negative control (a sham operation with water); lane 4, pleural fluid sample from case 1; lane 5, case 2; lane 6, case 3; lane 7, case 4 (sample 1); lane 8, case 4 (sample 2); lanes M, a size marker (pUC 19 digested with *Hinf* I); lane 9, case 5; lane 10, case 6; lane 11, case 7 (right lung); lane 12, case 7 (left lung); lane 13, case 8; lane 14, case 9; lane 15, case 10. Numbers at the right are base pairs. Number 108 at the left indicates the size of the expected band. Closed squares on lanes 5 and 12 indicate the amplified bands which are only faintly visible.

Table 1 Continued

	5	6	7	8	9	10
	10/M Left 4 (4) Thoracocentesis (120)	7/M Right <0 (0) Thoracocentesis (>100)	11/M Bilateral 5 (5), 6 (6) Thoracocentesis, drainage	0.9/M Right 4 (8) Thoracocentesis (85)	5/M Left 2 (10) Thoracocentesis (190)	4/F Bilateral <0 (1) Thoracocentesis (130)
	<40 1280	320 640	<40 160 (Right)	2560 2560	<40 1280	80 640 (Right)
(Sample 2) Bloody	Serous 29 2650	Serous	Bloody 26 914 900	(Left) Serous 23	Serosanguinous 46 2387 1047	Serous 26 1680
8	8.5 Liver dysfunction, skin rash, SIADH	17 None	10 None	10 None	3.6 None	3.6 Liver dysfunction
(+)	0.5 (-)	0.7 (-)	>3 (+)	1 (+)	>2 (+)	0.5 (-)

§ Time interval from the admission to the normalisation of the chest radiographs.

(+) = positive; (±) = indeterminate; (-) = negative.

SIADH = syndrome of inappropriate secretion of antidiuretic hormone.

mechanisms, that is, the former being caused by the direct invasion by this organism, and the latter by the indirect mechanisms. Second, these are within a continuum of the same disease, the clinical severity being simply proportional to the amounts of the organism in the fluid as represented by the PCR positivity. Third, an unrecognised superinfection such as by adenovirus type 7⁴ predisposed these patients to have severe lung damage secondarily resulting in an active state of *M pneumoniae* replication, or vice versa.

The exact nature of the persistent consolidation could not be clarified in this study. Due to the fundamentally benign nature of the infection, pathological features of the affected lung have been described only in a few articles.⁵⁻⁷ Within this experience, severe and fatal cases were associated with interstitial fibrosis.^{6,7} We believe that the persistent consolidation that was observed in our study may represent a rather milder presentation of localised fibrotic changes.

In conclusion, the positive PCR results using pleural fluid samples in mycoplasmal pleuritis were strongly associated with residual radiographic abnormality. In this regard, PCR may

provide a predictive result with a single acute phase specimen.

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