Clinical Utility of Polymerase Chain Reaction for Diagnosis of Smear-negative Pleural Tuberculosis

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Background: Polymerase chain reaction (PCR) is a molecular biology technique which can detect *Mycobacterium tuberculosis* (TB) genome in pleural fluid; however, the results are variable.

Methods: Two hundred and twelve pleural fluid specimens suspected to be possibly associated with tuberculosis with negative acid-fast smears were sent to our laboratory to test for the presence of *M. tuberculosis* DNA using nested PCR, the target for the amplification being a segment of IS6110 in the genome of *M. tuberculosis*. The final diagnosis of TB pleurisy was based on combining clinical judgment with radiologic findings, microbiologic tests, and the histopathologic findings. Forty-nine patients were excluded due to incomplete or inconsistent clinical information.

Results: Of 163 patients enrolled, PCR was positive in 23 (43.4%) of 53 patients with TB pleurisy and 5 (4.5%) of 110 patients with non-TB pleurisy, with a sensitivity and specificity of 43.4% and 95.5%, respectively. Positive culture of pleural fluid was found in 15 (28.3%) of the TB pleurisy group and none in the non-TB group. Fifteen (55.6%) of 27 with pleural biopsy demonstrated chronic granulomatous inflammation with or without caseous necrosis. Of these 27 patients, PCR was positive in 12 (44.4%). A higher proportion (70.4%) of patients with TB pleurisy was diagnosed when PCR was combined with biopsy results.

Conclusion: These data indicate that PCR alone has limited value in diagnosis of TB pleurisy with negative smear. However, when used in combination with pleural biopsy, it can be used to increase early detection of TB pleurisy in such patients. [*J Chin Med Assoc* 2007;70(4):148–151]

Key Words: PCR, pleural effusion, tuberculosis

Introduction

Tuberculosis (TB) pleurisy is the main extrapulmonary presentation of TB secondary to TB lymphadenitis.[1] If TB pleurisy is left untreated, the pleural effusion will usually resolve spontaneously, however, pulmonary or extrapulmonary TB within the subsequent 5 years will develop in approximately 65% of patients.[2] Diagnosis of TB pleurisy is usually accomplished with radiologic and clinical findings, pathology of pleural tissue from biopsy, and several laboratory methods. Conventional methods include direct examination of pleural fluid with Ziehl-Neelsen staining of acid-fast bacilli and culture. Ziehl-Neelsen staining is rapid and inexpensive but requires bacillar concentration of 10,000/mL and has a low sensitivity of approximately 0–1%.[3,4]

Culture is more sensitive (11–50%);[5,6] only 10–100 bacilli yield to the diagnosis, but require 2–6 weeks to grow *Mycobacterium tuberculosis*. Pleural biopsy has high sensitivity (70–80%),[3,4,7] but the procedure is not free of risk. Polymerase chain reaction (PCR) is a molecular biology technique that can detect *M. tuberculosis* genome in pleural fluid or tissue specimens. The sensitivity (31.3–81%) and specificity (96.6–100%) are variable.[8–10] A recent study suggests that PCR has a potential role in confirmation of TB pleurisy but may

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not be useful in excluding the disease.[11] We report our experience with an in-house IS6110-based nested PCR assay for diagnosis of TB pleurisy.

Methods

Patients

The study comprised 212 patients with negative acid-fast pleural effusion smears who were suspected to have TB pleurisy and who had undergone TB PCR study in Taipei Veterans General Hospital between January 2002 and November 2003. TB pleurisy was defined as when at least 1 of the following criteria were met: (1) chronic granulomatous inflammation with or without caseous necrosis is found on pleural biopsy; (2) *M. tuberculosi*s is cultured from pleural effusion; (3) compatible clinical symptoms and signs with good anti-TB treatment response are found. Forty-nine patients with undetermined etiology were excluded, and a total of 163 patients including 53 with TB pleurisy and 110 with non-TB pleurisy were enrolled in the study.

Pleural effusion samples

For each subject, at least 30 mL of pleural fluid was collected in a syringe during thoracentesis. The resuspended pellets were submitted for acid-fast staining, and innoculated (0.1 mL) on Lowenstein–Jensen medium at 37°C and assessed weekly for mycobacterial growth for a total of 8 weeks. The remainder of pellets was tested by an in-house nested PCR assay. TB was identified by a positive niacin test result.

Nested PCR assay

The suspended pellets were washed twice with 0.5–1 mL of TEX (Tris-EDTA-Triton X-100) lysis buffer consisting of 1% Triton X-100 in 10 mM Tris-EDTA (10 mM Tris/HCl, pH 8.0, and 1 mM EDTA) and was sedimented by centrifugation at 12,500*g* for 5 minutes. Each sediment was suspended in 100 μL of TEX lysis buffer, and the mixture was incubated at 95°C for 30 minutes to break down the bacterial cell walls and to release the DNA for subsequent nested PCR amplification. Ten microliters of clinical sample was amplified by the in-house nested PCR assay with IS6110-specific primers. The outer primers were INS1 and INS2, as described previously.[12] The inner primers were the sense primer (5′-AGATGCACCGTCGAACGG-3′) and the antisense primer (5′-GCCACGTAGGCGAACCCTG-3′), corresponding to the base pairs 652–668 and 836–854, respectively. Briefly, using the outer primers, the DNA samples were added to 15 μL of the master reaction mixture containing 1.5 mM $MgCl_2$, 10 mM Tris/HCL, 0.1% Triton X-100, 50 mM KCl, 200 μM of each deoxyribonucleotide triphosphate, 0.5 μM of each outer primer, and 0.5 U of Taq polymerase (Ampli Taq; Perkin Elmer, Cetus, Norwalk, CT, USA). After denaturation at 94°C for 5 minutes, the mixture was subjected to 30 cycles of denaturation at 94°C for 1 minute, annealing at 65°C for 2 minutes, and primer extension at 72°C for 5 minutes. For the nested PCR, 75 μL of the same master reaction mixture containing 0.5 μM each of inner primer and 2.5 U of Taq polymerase were added to the previous PCR product in a final volume of 100 μL. The nested PCR was carried out for 30 cycles under the same amplification conditions, with the omission of the terminal primer extension. Amplification was done in the GeneAmp PCR system 9,600 thermal cycles (Perkin Elmer Applied Biosystems). The PCR products (203 bp) were analyzed by 2% NuSieve 3:1 agarose (FMC, Rockland, MD, USA) gel electrophoresis.

Statistical analysis

We compared demographic categorical data using χ^2 or Fisher's exact test, and continuous data using the Mann-Whitney *U* test. We assumed statistical significance at $p < 0.05$. The sensitivity, specificity, positive predictive value, and negative predictive value of PCR were calculated to predict TB pleurisy occurrence.

Results

We studied 163 patients with negative acid-fast pleural effusion smears; 53 patients were diagnosed with TB pleurisy. Among these 53 cases, 14 had chronic granulomatous inflammation with good anti-TB treatment response, 1 had chronic granulomatous inflammation with caseous necrosis, 15 had *M. tuberculosi*s cultured from pleural effusion, and 26 had compatible clinical symptoms and signs with good anti-TB treatment response. One-hundred and ten patients did not have TB, including 42 para-pneumonic effusions, 1 virus-related pleural effusion, 28 malignant pleural effusions, 14 congestive heart failure-related effusions, 4 chylous effusions, 3 SLE-related effusions, 1 collagen vascular disease-related effusion, 15 other transudate pleural effusions, and 1 hypothyroidism-related effusion (Table 1). PCR for *M. tuberculosis* DNA, done on the pleural effusions, was positive in 23 (43.4%) of 53 TB pleurisy patients, and in 5 (4.5%) of 110 non-TB pleurisy patients. In the 53 TB pleurisy cases, culture of pleural effusion was positive in 15 (28.3%), and it was negative for all 110 non-TB pleurisy cases. The nested PCR for *M. tuberculosis* DNA was 43.4% sensitive and

Table 1. Demographic data of 163 patients

	TB pleurisy ($n=53$)	Non-TB pleurisy ($n=110$)	p
Sex, *n* (%)			0.087
Male	44 (83)	80 (72.7)	
Female	9 (17)	30 (27.3)	
Median age, yr	76	78	0.708
Pleural effusion samples, *n* (%)			
Positive PCR	23 (43.4)*	5 (4.5)	<0.001
Positive Ziehl-Neelsen stain	0	0	
Positive TB culture	15 (28.3)	0 (0)	<0.001

**Seven in 15 positive TB culture, 16 in 38 negative TB culture.*

Table 2. Diagnostic methods in 27 patients who received pleural biopsy in the TB pleurisy group

Diagnostic method	*n*	Sensitivity (%)
Effusion culture	3	11.10
Effusion PCR	12	44.40
Pleural biopsy	15	55.50
Effusion PCR + effusion culture	14	51.90
Effusion PCR + pleural biopsy	19	70.30

95.5% specific, having a positive predictive value of 82% and negative predictive value of 77.7%.

Among the 53 TB pleurisy patients, 27 received pleural biopsy; chronic granulomatous inflammation with or without caseous necrosis was found in 15 (55.6%) of the 27 patients with pleural biopsy. Of these 27 patients, PCR and culture of pleural fluid was positive in 12 (44.4%) and 3 (11.1%) patients, respectively. Among 12 of 27 TB pleurisy patients without granulomatous inflammation, PCR was positive in 4 patients. A higher proportion (19 patients, 70.4%) of patients with TB pleurisy was diagnosed when PCR was combined with biopsy results (Table 2).

Discussion

TB pleurisy is the main extrapulmonary TB presentation secondary to TB lymphadenitis.[1] If TB pleurisy is left untreated, pleural effusion will usually resolve spontaneously; however, pulmonary or extrapulmonary TB within the subsequent 5 years will develop in approximately 65% of cases.[2] As in other high-incidence areas of TB, TB pleurisy is the most common etiology of pleural effusion (25%), other than malignancy, para-pneumonic effusion, and congestive heart failure.[13] Taiwan is also an area with high TB incidence. Thus, TB pleurisy should be ruled out in patients with undiagnosed exudative pleural effusions. Considerable advances have been made in improving the speed of detection of mycobacterium using PCR techniques. We have shown PCR amplification to be sensitive and specific for the rapid detection of TB bacilli DNA in respiratory specimens, and demonstrated a high sensitivity and specificity of 91.7% and 98.6%, respectively.[14] However, TB pleurisy remains a diagnostic challenge. Pleural fluid staining for acid-fast bacilli and culture of *M. tuberculosis* has a poor yield. We currently perform this assay for the detection of pleural TB with negative acid-fast smears. In this study, based on the final diagnosis, we found that PCR assay was more sensitive than microbiologic tests and cultures of pleural fluid in the diagnosis of smear-negative TB pleurisy, where almost half of the cases of treatment began without substantiation of diagnosis. However, the limitation of relying on PCR alone is the low sensitivity (43.4%) yield due to the low number of TB bacilli in the pleural fluid, which was demonstrated by the low culture (28.3%) yield from pleural fluid. Others have reported experiences of PCR amplification with pleural fluid and found them only positive in 17.1% of TB pleurisy, and positive culture in 24.6% of patients with TB pleurisy.[15] Previous reports of PCR specificity ranged from 95.4 to 100%.[16–18] The specificity of our PCR result was 95.5%, with 5 of 110 non-TB pleurisy cases demonstrating positive PCR examination. However, only 1 of these 5 patients received pleural biopsy, and 1 had previous TB history. Diagnosis of TB pleurisy in these 5 patients may be underestimated.

Of 27 patients with pleural biopsy, PCR and culture of pleural fluid was positive in 12 (44.4%) and 3 (11.1%) patients, respectively. Among 12 of 27 TB pleurisy patients without granulomatous inflammation,

PCR was positive in 4 patients. A higher proportion (70.4%) of patients with TB pleurisy was diagnosed when PCR was combined with biopsy results.

In conclusion, although PCR used in this study had a higher sensitivity than other diagnostic options, using PCR alone is not sensitive enough for rapid diagnosis of TB pleurisy. However, for patients with a typical clinical presentation for TB pleurisy, it can still play an important role in early detection of nondiagnostic exudative pleural effusion with negative acid-fast smear, especially when combined with pleural biopsy presenting chronic granulomatous inflammation with or without caseous necrosis.

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