The Role of Mycobacterium Tuberculosis PCR in the Early Diagnosis of Tuberculosis among Patients with Massive Pericardial Effusion

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Background --- Tuberculosis is one of the most common causes of pericardial effusion in the Philippines. The early diagnosis of tuberculosis among patients with pericardial effusion remains elusive to this date. Polymerase Chain reaction, a technique for amplifying small amounts of DNA when only small amount of cells are available, can amplify "fingerprint" strains of M. tuberculosis DNA in pericardial fluid with excellent specificity. This study was conducted to describe the role of Mycobacterium tuberculosis polymerase chain reaction (PCR) in the early diagnosis of TB in patients with moderate to massive pericardial effusion by comparing it to existing diagnostic standards

Methods and Results --- Twenty-three subjects with moderate to massive pericardial effusion were included in this study. Majority were males with an average age of 40.2 ± 15.2 yrs. The average widest diameter of pericardial fluid by 2DED was 3.7 ± 1.3 cm before pericardiocentesis/ pericardiostomy, with 77% of the subjects had RA collapse and 64% had RV collapse on presentation. All pericardial fluid specimen tested were exudative based on Light's criteria. Cytological analyses were done on 19 subjects with 42% had findings characteristic of both acute and chronic inflammation. Five subjects had findings suggesting malignancy. Nine subjects had documented PPD results and two tested positive. All AFB smears done at the Philippine Heart center and Lung center of the Philippines showed negative results. Four out of 22 subjects had positive MTB cultures. Two of the subjects had a positive pericardial biopsy result for tuberculosis. Ten out of 23 subjects had their PCR done with a positive result in only one of the subjects. All of the specimens that tested negative for PCR also had negative results on MTB culture and pericardial biopsy.

Conclusion --- AFB smear appears to have a limited use in pericardial fluid analysis. The role of PCR-TB in the early diagnosis of PTB cannot be fully assessed in this study. The lack of subjects with positive PCR result prevented us from giving any definite conclusion. PCR-TB seemed to correlate well with MTB culture and pericardial biopsy. All of the subjects who had a negative PCR-TB also had negative TB results on their culture and biopsy. TB culture and pericardial biopsy appear to complement each other, with the latter having the advantage of an earlier result and the potential to show other diagnosis aside from TB. The routine use of AFB smear and routine determination of serum and pericardial LDH and protein should be looked into. ***Phil Heart Center J 2007; 13(2):109-112.***

Key Words: Pericardial effusion ■ Tuberculosis ■ Mycobacterium tuberculosis ■ Pericardiocentesis ■ Pericardiostomy ■ Polymerase Chain Reaction ■ Acid Fast Bacilli (AFB) smear ■ TB culture, diagnosis

Tuberculosis remains as one of most common di agnosis among patients with pericardial effusion in the Philippines. Nadurata et al reviewed 438 cases of pericardial effusion admitted at the Philippine heart center from 1985–1999 and reported that 25.1% (n=110) of the cases were attributed to tuberculosis.[1] The early diagnosis of tuberculosis among patients with pericardial effusion remains elusive to this date. Tuberculous pericarditis can either present in a transudative or exudative state depending on its stage.[2] AFB smears are frequently negative. AFB cultures, the current gold standard against which all other methods are measured, lack sensitivity and may take up to 8 weeks to obtain final results. Pericardial biopsies are frequently non-specific. A positive PPD tests supports the diagnosis but does not confirm it. Not infrequently, a trial of anti-Koch's regimen is started when tests are inconclusive.

Polymerase Chain reaction is a technique for amplifying small amounts of DNA when only small amount of cells are available. The technique utilizes oligodeoxyribonucleotides that are complimentary to the DNA strands of interest. The oligodeoxyribonucleotides are then annealed to the ends of the DNA and serve as primers for the in vitro copying of each DNA strand using a heat-stable DNA polymerase. The chemically synthesized primers are in excess so that when the reaction mixture is heated, the DNA strands separate and reanneal with more primer on cooling. This process of heating, cooling, and synthesis can be repeated many times, and in the process the DNA fragment of interest is greatly amplified.[3] Polymerase chain reaction (PCR) amplifies mycobacterial DNA to "fingerprint" strains of

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M. tuberculosis in pericardial fluid with excellent specificity.[4,5] TB PCR has been shown to have a sensitivity and a specificity of 66.7% and 99.6%, respectively, for the diagnosis of pulmonary TB from respiratory samples.[6] TB AMPLICOR was found to be more sensitive than the combination of Ziehl-Neelsen staining of smears and radiometric culture for M. tuberculosis and was a rapid and highly specific diagnostic test for TB meningitis.[7] An early diagnosis of TB pericarditis facilitates definitive treatment. Strang et al noted a decrease in mortality and a decreased need for repeat pericardiocentesis when anti-TB regimen was given with prednisolone for the first 11 weeks.[8] This study was conducted to describe the role of Mycobacterium tuberculosis polymerase chain reaction (PCR) in the early diagnosis of TB in patients with massive pericardial effusion by comparing it to existing diagnostic standards, such as AFB smear, AFB culture, pericardial biopsy, and biochemical and cytologic analysis.

Methods

Inclusion/ exclusion criteria

Patients aged 18 years old and above admitted at the Philippine Heart Center from January 01, 2005 to August 30, 2006 with echocardiographic evidence of pericardial effusion requiring pericardiocentesis and/ or pericardiostomy tube insertion, for diagnostic or therapeutic purposes, were included in the study. Patients with known metastatic disease were not included. Patients who had open heart surgery for the past 3 months were also not included in the study. Clinical data from each patient was collected on a standardized form by the investigator. It included the patients age, sex, hospital number, echocardiographic findings relevant to pericardial effusion, and surgical procedures done if any.

Subjects

Twenty-three subjects were included in the study with an average age of 40.2 ± 15.2 yrs. Fifteen subjects (65%) were male. Patients had an average of 3.7 ± 1.3 cm as their widest diameter in 2DED before pericardiocentesis/ pericardiostomy. Seventy-seven percent of the subjects had RA collapse while 64% had RV collapse on presentation.

Samples

All Pericardial fluid analyses were done at the Philippine Heart Center unless otherwise specified. Portions were sent for cell count, differential count and cytology, biochemistry (LDH, total protein), and for Ziehl-Neelsen staining, gram-stain and culture. Some specimens were sent to the Lung Center of the Philippines for TB culture and sensitivity. Finally, pericardial fluid specimens were sent to San Lazaro hospital (SACTL) or UP PGH for TB PCR (AMPLICOR MTB Test. Roche diagnostic systems, Inc; Branchburg NJ). Pericardial samples were sent for biopsy, if possible. PCR TB. AMPLICOR MTB amplifies a 585-bp region of the 16S rRNA gene sequence common to all mycobacteria. Carryover contamination is prevented by incorporation of dUTP in place of dTTP in the amplification reaction and utilization of uracil-N-glycosylase (AmpErase) to enzymatically cleave any contaminating amplicon carried over from previous reactions. AmpErase is subsequently inactivated at temperatures used for thermal cycling. For amplification, 50 µl of neutralized specimen is added to 50 µl of master mix. The tray containing specimens and controls is then placed in a TC-9600 thermal cycler and amplified according to the following program: hold at 50ºC for 2 min; 2 cycles of 98ºC for 20 s, 62ºC for 20 s, and 72º for 45 s; hold at 72ºC for 5 min; and hold at 72ºC indefinitely. Detection on M. tuberculosis complex organism is accomplished by hybridization of the amplified product to a DNA probe specific for organisms of the M. tuberculosis complex. Following amplification, 100µl of denaturation solution is added to all tubes; this is followed by a 10-min room temperature incubation to allow complete denaturation of the double-stranded products.100µl of hybridization buffer is added to a microwell plate coated with a DNA probe specific for members of the M. tuberculosis complex. Twenty-five microliters of denatured amplicon is then added, and hybridization is carried out at 37ºC for 90 min. Detection of hybridized duplex is accomplished with an avidin-horseradish peroxidase conjugate- tetramethylbenzidine substrate system. The reaction is stopped by addition of dilute hydrosulfuric acid, and the results are read at 450nm. A result is considered positive if the absorbance is greater than or equal to 0.35.5

Results

Table 1. Diagnostic examination done on the pericardial fluid specimens of the patients included in this study

Diagnostic Test	Number of tests done	Number of specimen w/ positive results
PCR	10	1
AFB smear	23	0
AFB culture	22	4
Biopsy	19	2

Table 1 depicts the diagnostic examinations done on the pericardial fluid specimen of the 23 subjects included in this study. Ten out of 23 subjects had their PCR done with a positive result in only one of the subjects. All AFB smears done at the Philippine Heart Center and Lung center of the Philippines showed negative results. Four out of 22 (18%) had positive MTB cultures.

A pericardial biopsy specimen is considered positive for TB when it shows chronic granulation with caseous necrosis. Two out of 19 subjects who had pericardial

biopsy done, had this finding, while one had chronic granulation with caseous necrosis. Two out of 19 subjects who had pericardial biopsy done, had this finding, while one had chronic granulation without caseous necrosis. It is interesting to note that 4 out of 19 subjects eventually had malignant pericardial biopsy findings.

Table 2. Results of Pericardial Fluid Exam (PCR, AFB smear, TB culture, Pericardial Biopsy) compared to PPD,

Diagnostic Exam		Biochemical (Light's criteria)		Cytology				PPD	
		Transudate	Exudate	Acute	Chronic	both	Malignant	(+)	(-)
PCR	+	0	1	0	0	0	1	0	0
	-	0	7	0	1	2	2	1	4
AFB	+	0	0	0	0	0	0	0	0
smear	-	0	21	1	3	8	5	2	7
TB	+	0	4	0	0	3	0	1	0
culture	-	0	16	1	3	5	4	1	7
Biopsy	+	0	2	0	0	0	0	0	0
	-	0	16	1	2	5	4	2	6

Table 2 shows the biochemical and cytologic analysis of the specimen. All the specimens tested were exudative. The pericardial fluids were categorized into transudate or exudate based on Light's criteria, which includes the determination of pericardial fluid and serum LDH and protein. A pericardial fluid/ serum LDH ratio of more than 0.6, a pericardial fluid/ serum protein ratio of more than 0.5, or an LDH greater than 200 u/L were considered exudative. Cytolological analysis was done on the pericardial fluid of 19 subjects, primarily used to detect malignancy. Eight out of 10 (42%) had findings characteristic of both acute and chronic inflammation. Five subjects had findings suggesting malignancy. All subjects that showed malignant changes in their pericardial biopsy also had findings suggestive of malignancy in their cytological analysis. Nine subjects had documented PPD results and two tested positive. Both of them had negative biopsy results, although one of them had a positive AFB culture.

Table 3. Comparison of Results of Pericardial Fluid

		PCR		AFB smear		TB culture		Biopsy	
		(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
PCR	(+)	1	0	0	1	0	0	0	0
	(-)	0	9	0	9	0	9	0	8
AFB	(+)	0	0	0	0	0	0	0	0
smear	(-)	1	9	0	23	4	18	2	17
TB	(+)	0	0	0	4	4	0	2	2
culture	(-)	0	9	0	18	0	18	0	15
Biopsy	(+)	0	0	0	2	2	0	2	0
	(-)	0	9	0	17	2	15	0	17

PCR-TB seemed to have a good negative predictive value. All of the specimens that tested negative for PCR (9 subjects) also had negative results on MTB culture and pericardial biopsy. Only one out of ten subjects tested positive for PCR. Whether this is due to lack of sensitivity, still needs to be investigated. Factors that may decrease the yield of PCR-TB include a bloody sample which is quite common in pericardial fluid samples. AFB smear appears to have a limited use in pericardial fluid analysis. All subjects who tested positive for PCR-TB, MTB culture and pericardial biopsy showed negative AFB smear. Four specimens that eventually grew MTB on culture had negative initial AFB smears. Two out of four subjects who had a positive MTB culture had biopsy findings showing chronic granulation with caseous necrosis. One out of four had chronic granulation only without caseous necrosis. Two patients who had a normal pericardial biopsy result were eventually diagnosed to have malignancy.

Conclusion

The role of PCR-TB in the early diagnosis of PTB cannot be fully assessed in this study. The lack of positive results and the scarcity of subjects who underwent this test prevented us from giving any definite conclusion. PCR-TB seemed to correlate well with MTB culture and pericardial biopsy. All of the subjects who had a negative PCR-TB had negative TB results on their culture and biopsy. It was unfortunate that none of our subjects who grew MTB on culture had PCR analysis. This study was primarily limited by the cost of the PCR procedure. With more subjects, PCR-TB may be able to show its real potential. TB culture and pericardial biopsy appeared to complement each other. Though TB culture seems to be more sensitive than pericardial biopsy in this study, the latter has the advantage of an earlier result and the potential to show other diagnosis aside from TB. Pericardial biopsy was able to give a definite diagnosis in 6 out of 19 subjects in this study. The routine use of AFB smear and routine determination of serum and pericardial LDH and protein should be investigated. All specimens tested for AFB smear yielded negative result. AFB smear was so insensitive in this study that all subjects who had definite TB on culture and pericardial biopsy had negative AFB smears. With regards to the routine determination of LDH and protein, all of the subjects were eventually categorized as exudative based on the Light's criteria. The clinical significance of this practice still confuses the author. Massive pericardial effusion is a condition that demands prompt medical attention. In this study, 5 out of 23 patients had already expired while another 5 of them are suffering from metastatic disease. The early diagnosis and management of these cases cannot be overemphasized. Tuberculosis, being the leading cause of massive pericardial effusion in this country must be

diagnosed and excluded early to facilitate definitive management.

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