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Comparison of PCR, Culture, and Histopathology for Diagnosis of Tuberculous Pericarditis

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Nucleic acid amplification techniques for the diagnosis of tuberculosis (TB) are rapidly being developed. Scant work, however, has focused on pericardial TB. Using cryopreserved specimens from a prior study of pericarditis, we compared PCR to culture and histopathology for the diagnosis of tuberculous pericarditis in 36 specimens of pericardial fluid and 19 specimens of pericardial tissue from 20 patients. Fluid and tissue were cultured on Lowenstein-Jensen and Middlebrook solid media and in BACTEC radiometric broth. Tissue specimens were stained with hematoxylin-eosin, Ziehl-Neelsen, auramine O, and Kinyoun stains and were examined for granuloma formation and acid-fast bacilli. PCR was performed with both fluid and tissue with IS*6110*-based primers specific for the *Mycobacterium tuberculosis* complex by published methods. Sixteen of the 20 patients had tuberculous pericarditis and 4 patients had other diagnoses. TB was correctly diagnosed by culture in 15 (93%) patients, by PCR in 13 (81%) patients, and by histology in 13 of 15 (87%) patients. PCR gave one false-positive result for a patient with *Staphylococcus aureus* pericarditis. Considering the individual specimens as the unit of analysis, *M. tuberculosis* was identified by culture in 30 of 43 specimens (70%) from patients with tuberculous pericarditis and by PCR in 14 of 28 specimens (50%) from patients with tuberculous pericarditis ($P > 0.15$). The sensitivity of PCR was higher with tissue specimens (12 of 15; 80%) than with fluid specimens (2 of 13; 15%; $P = 0.002$). In conclusion, the overall accuracy of PCR approached the results of conventional methods, although PCR was much faster. Therefore, PCR merits further development in this regard. The sensitivity of PCR with pericardial fluid was poor, and false-positive results with PCR remain a concern.

Nucleic acid amplification techniques have the potential to revolutionize investigative microbiology and have already begun to affect clinical diagnostic microbiology. Because most mycobacterial cultures require 10 days to 8 weeks to become positive, diagnostic assays based on nucleic acid amplification methods such as PCR, transcription-mediated amplification, ligase chain reaction, and Q-beta replicase amplification promise to dramatically decrease the time required to identify organisms in clinical specimens (for a review, see reference 8).

These methods have been extensively tested for the detection of *Mycobacterium tuberculosis* in sputum specimens (3, 8, 15, 16, 20, 25). The first two nucleic acid amplification-based kits for the laboratory identification of *M. tuberculosis* in sputum have recently been licensed for commercial use (14, 18). With the exceptions of cerebrospinal fluid (17) and pleural fluid (12), comparatively little work has focused on the utility of diagnostic PCR with extrapulmonary specimens (9, 15).

In 1990, we reported a rapid rise in the incidence of human immunodeficiency virus-associated pericarditis at the Muhimbili Medical Center in Dar es Salaam, Tanzania (1), and then demonstrated in a subsequent study that this was due to tuberculosis (TB) (2). Establishing the diagnosis of tuberculous pericarditis in most of these patients required many weeks and extensive cultivation by multiple methods. We took advantage of archived specimens of pericardial fluid and tissue to assess the utility of PCR to diagnose tuberculous pericarditis by comparing it to conventional culture and histopathology.

MATERIALS AND METHODS

Patients. Twenty-two Tanzanian patients (ages, 10 to 42 years; 19 males and 3 females) with large pericardial effusions documented by echocardiography underwent invasive diagnostic or therapeutic procedures as described previously (2). For 20 of these patients, frozen pericardial specimens were available for PCR analysis, and the present report is restricted to the results for these 20 patients. A final diagnosis of tuberculous pericarditis was defined as a positive culture for *M. tuberculosis* or typical granuloma formation in biopsy tissue, supported by all laboratory, radiographic, and clinical data, including response to therapy, with 6 months of follow-up and no alternate diagnosis.

Microbiology. Thirty-six pericardial fluid specimens and 19 pericardial tissue specimens were obtained from these 20 patients by either pericardiocentesis or subxiphoid pericardiostomy as described previously (2). Samples of pericardial fluid were considered to be distinct specimens if they were obtained from different procedures performed on different days. Tissue specimens were aseptically divided into pieces of approximately 1 g. At Tanzania's National Tuberculosis Reference Laboratory, the tissue specimens were aseptically homogenized. Aliquots of tissue and fluid specimens were cultured on both pyruvate- and glycerol-based Lowenstein-Jensen (LJ) media. Cultures were held for 8 weeks before they were discarded. Smears were stained with auramine O and were examined by fluorescence microscopy.

The remainder of each specimen was cryopreserved in liquid nitrogen, transported to Duke University Medical Center (Durham, N.C.), and stored at −70°C for approximately 1 year. After they were thawed, the tissue specimens were aseptically homogenized. Fluid and tissue specimens were inoculated onto Middlebrook 7H10 and 7H11 solid media and into BACTEC 12B radiometric

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broth both with and without PANTA antibiotic supplement. Cultures were held for 8 weeks before they were discarded. Cultures for bacteria, viruses, fungi, and mycoplasmas were performed by standard methods as described previously (2).

Histopathology. One specimen of pericardial tissue was available from each patient. One piece of each fresh tissue specimen was placed in formalin, fixed, embedded in paraffin, sectioned, stained with hematoxylin-eosin and Ziehl-Neelsen stains, and examined microscopically by pathologists at Muhimbili Medical Center. At the Duke University Medical Center, cryopreserved specimens were thawed and then similarly fixed, embedded, sectioned, and stained with Kinyoun stain for acid-fast bacilli and with hematoxylin-eosin, periodic acid-Schiff, Gomori methenamine silver, Masson trichrome, and Gram stains. Tuberculous pericarditis was diagnosed when typical granuloma formation, composed of epithelioid histiocytes and mononuclear inflammatory cells, was present in the absence of other diagnoses.

Sample preparation for PCR. Tissue samples were thawed, ground in saline and 0.2% bovine serum albumin in a Dounce homogenizer, and stored at −70°C. Aliquots of 0.5 ml of ground tissue or pericardial fluid were placed in 1.5-ml screw-top vials and were centrifuged at 12,000 rpm in an Eppendorf Microfuge (model 5415) centrifuge for 10 min. The supernatant was discarded, and 100 μl of lysis buffer (1.0% Triton X-100 and 10% Chelex) (24) was added to the pellets. The tissue samples were incubated at 55°C for 2 h in the presence of 100 μg of protease K per ml. All samples were sonicated for 15 min, heated to 95°C for 10 min, and then frozen at −20°C until assayed.

PCR. Aliquots of 1 to 10 μl of the prepared samples were used directly in the PCR. PCR was performed with the primers and under the conditions described by Eisenach et al. (6), with the following modifications. Cycle times were reduced from 2 to 1 min, and reaction products were analyzed on 3% Nusieve (FMC Corporation, Philadelphia, Pa.) and 1% agarose gels and were stained with ethidium bromide. An internal control for the amplification reaction was included in each tube (7). All manipulations were performed by using dedicated pipettors and plugged tips to prevent PCR product carryover. Following electrophoresis, the reaction products were transferred to a Nytran membrane (Schleicher & Schuell Inc., Keene, N.H.) by standard Southern blotting (21). The 123-base PCR product, corresponding to a 123-bp sequence located within IS*6110*, was generated from *M. tuberculosis* and was identified by hybridization with a ^{32}P-labeled 123-bp sequence generated from a previous PCR with DNA known to be that of *M. tuberculosis* (7). Filters were placed against X-OMAT film (Kodak, Rochester, N.Y.) for 20 h, and the film was developed in an automatic film processor.

Analysis. The results of culture, histopathology, and PCR were compared to a final diagnosis of TB separately for individual patients as the unit of analysis and separately for individual specimens as the unit of analysis. Statistical significance was determined with the chi-square test with Yates' correction or Fisher's exact test for tables with an expected cell size of <5.

RESULTS

Of 20 patients with large pericardial effusions who required invasive procedures, 16 had a final diagnosis of tuberculous pericarditis on the basis of culture or histology supported by all available information. Two had staphylococcal pericarditis. Two remained idiopathic, despite extensive testing for infectious, malignant, autoimmune, and other causes (2).

Tuberculous pericarditis was established by culture in 15 of the 16 patients and by histology in 13 of 15 patients (biopsy was not performed for 1 patient) (*P* was not significant). Therefore, TB was diagnosed by culture in two patients missed by histology and by histology in one patient missed by culture (Table 1). Overall, the sensitivity and specificity of culture were 94 and 100%, respectively. For histology, the sensitivity and specificity were 87 and 100%, respectively. In the one culture-negative patient, the pericardium itself showed nonspecific chronic inflammation, but the adjacent subxiphoid lymph node showed typical caseating granulomas. This patient was treated with anti-TB drugs, and follow-up at 6 weeks revealed marked clinical improvement with no effusion by echocardiography.

Culture of pericardial tissue had a slightly higher diagnostic yield than culture of pericardial fluid. *M. tuberculosis* was cultured from the pericardial tissue of 14 of the 15 patients from whom tissue specimens were obtained. For two patients these were the only samples positive by culture. Pericardial fluid was culture positive for 13 of 16 patients (*P* was not significant). Smears of specimens from seven patients (six smears of pericardial tissue and one smear of pericardial fluid) were positive ($P = 0.04$). Liquid (BACTEC) culture medium gave positive results for 11 of 16 patients, while solid media (Middlebrook and LJ) were positive for 15 of 16 patients (*P* was not significant). Middlebrook agars yielded positive cultures for 13 of 16 patients (7H10, 13 of 16 patients; 7H11, 11 of 16 patients), and LJ slants yielded positive cultures for 9 of 16 patients (*P* was not significant).

TABLE 1. Results of culture, histology, and PCR analysis for 20 patients with large pericardial effusions

Method	No. of the following patients positive/no. of patients tested (%):	
	Patients with tuberculous pericarditis	Patients without tuberculous pericarditis
Culture	15/16 (94)	0/4
Histology	13/15 (87)	0/4
PCR	13/16 (81)	1/4 (25)

PCR analysis was carried out with specimens from all 20 patients, including pericardial fluid from 16 patients and pericardial tissue from 19 patients (Table 1). PCR was positive for TB for 14 patients and negative for 6 patients. For 13 of 14 PCR-positive patients, cultures were positive for *M. tuberculosis*. The 14th PCR-positive patient had frankly purulent pericardial fluid showing many gram-positive cocci in clusters and growing *Staphylococcus aureus* from cultures of both pericardial fluid and pericardial tissue. *S. aureus* was also isolated from blood and from a septic ankle. This patient had no other evidence of TB (including treatment and follow-up), and this patient may be considered to have a false-positive PCR result. Of six PCR-negative patients, three had tuberculous pericarditis, two had idiopathic pericarditis, and one had *S. aureus* pericarditis. Thus, the PCR result was false negative for three patients. For one of these three patients cultures were also negative and TB was diagnosed histologically, i.e., both PCR and culture results were false negative for this patient. Regarding all 20 patients, PCR had a sensitivity of 81% and a specificity of 75% for the diagnosis of tuberculous pericarditis.

From a laboratory perspective, considering individual specimens as the unit of analysis, 43 pericardial specimens (28 fluid and 15 tissue specimens) were obtained from patients with tuberculous pericarditis (Table 2). Twelve specimens (eight fluid and four tissue specimens) were obtained from patients without TB. Overall, 30 of the 43 specimens (70%) grew *M. tuberculosis*. Again, culture of tissue (14 of 15 specimens) provided a slightly higher yield than culture of fluid (16 of 28 specimens; $P = 0.02$). It is important to point out that for two patients the specimen of pericardial fluid obtained first was culture negative, while specimens from subsequent procedures were positive. Overall, cultures in liquid (BACTEC) media were positive for 23 specimens (53%), while cultures on solid media were positive for 27 of the 43 specimens (63%; *P* was not significant). For the solid media, Middlebrook agars were positive for 24 specimens (56%) (7H10, 20 of 43 specimens; 7H11, 21 of 43 specimens), and LJ slants were positive for 17 specimens (40%) (*P* was not significant).

Thirty-five specimens from all 20 patients were analyzed by PCR, including 19 specimens of pericardial tissue and 16 specimens of pericardial fluid (Table 2). Of 15 pericardial tissue specimens from patients with TB, 12 were positive for *M. tuberculosis* by PCR and 3 were repeatedly false negative. Of four pericardial tissue specimens from patients without TB, one was repeatedly false positive by PCR (as noted above) and three were correctly negative. Thus, compared to a final diagnosis of pericardial TB based on the combined conventional

TABLE 2. Results of culture, histology, and PCR analysis for specimens of pericardial fluid and pericardial tissue

Method	No. of positive specimens from the following patients/no. of specimens tested (%):	
	Patients with tuberculous pericarditis	Patients without tuberculous pericarditis
Culture		
Overall	30/43 (70)	0/12
Pericardial fluid[a]	16/28 (57)	0/8
Liquid media	15/28 (54)	0/4
Solid media	13/28 (46)	0/4
Pericardial tissue[a]	14/15 (93)	0/4
Liquid media	8/15 (53)	0/4
Solid media	14/15 (93)	0/4
Histopathology	13/15 (87)	0/4
PCR		
Overall	14/28 (50)	1/7 (14)
Pericardial fluid[b]	2/13 (15)	0/3
Pericardial tissue[b]	12/15 (80)	1/4 (25)

[a] $P = 0.02$ for pericardial fluid versus pericardial tissue.
[b] $P = 0.002$ for pericardial fluid versus pericardial tissue.

methods of analysis of pericardial tissue, the sensitivity of PCR was 80% and its specificity was 75%. In contrast, only 2 of 13 specimens of pericardial fluid were positive for *M. tuberculosis* by PCR ($P = 0.002$ compared to tissue). Nine specimens were false negative with positive controls and two specimens were false negative with negative controls, indicating the presence of internal inhibitors that blocked the PCR. All three specimens of pericardial fluid from patients without TB were PCR negative. Therefore, for the diagnosis of TB with pericardial fluid, PCR had a sensitivity of only 15% but a specificity of 100%.

DISCUSSION

Rapid diagnosis and treatment are crucial to reducing mortality and morbidity from pericardial disease. Because the differential diagnosis of effusive pericarditis is broad, the ability to diagnose tuberculous pericarditis promptly would greatly facilitate the management of many patients with pericarditis. Smears for acid-fast bacilli are fast, but they are insensitive and do not distinguish between different mycobacteria. Diagnosis by culture, the criterion standard, is specific and relatively sensitive but slow. Histopathology may be less sensitive and specific in the context of human immunodeficiency virus infection, in which the tissue reaction to TB includes a spectrum of changes which may not involve the presence of granulomas (10). Thus, PCR technology has the potential to become an important method of diagnosing pericardial disease.

Diagnosis by PCR took about 1 to 2 days in our study. The use of PCR for the diagnosis of TB from pericardial fluid has been reported for an isolated patient (19). PCR for the diagnosis of TB was also assessed with 294 extrabronchial specimens, including 6 pericardial fluid specimens, but the results were reported only in aggregate; the pericardial specimens were not distinguished from other body fluids and aspirates (5). Pfyffer et al. (15) included 14 culture-negative pericardial fluid specimens in their evaluation of the Amplified *Mycobacterium tuberculosis* Direct Test; 1 specimen was positive by this method. Although the sample size is small, the present report is the first systematic evaluation of PCR for the diagnosis of tuberculous pericarditis and the first report of the use of PCR for diagnosis of TB with pericardial tissue.

The sensitivity and specificity of PCR have reportedly been comparable to those of culture in studies of sputum (4) and cerebrospinal fluid (13). In our study, the sensitivity and specificity of PCR approached those of culture pericardial tissue, but the sensitivity of PCR with pericardial fluid was poor. This result is similar to that of Vlaspolder et al. (23), who reported only 20% sensitivity (but 96% specificity) with pleural exudates. Six false-negative PCR results occurred for patients for whom culture results indicated a low burden of *M. tuberculosis* organisms. These indicators included low colony counts on solid media, BACTEC positivity alone with no growth on solid media, or only one of several specimens yielding positive cultures. Two of our false-negative results were due to the presence of inhibitors. Many possible reasons for low sensitivity have been proposed in previous studies of PCR. Among these are specimen source; methods of specimen preparation; the presence of inhibitors such as heparin, hemoglobin, phenol, and sodium dodecyl sulfate; and the numbers of bacteria or their DNA in the specimens (11). As with other methods of testing for microorganisms, the sensitivity of PCR may be improved by testing several samples from each patient. In addition, purification of the specimen through a 50% sucrose solution has been shown to improve the ability to detect DNA (22).

The false-positive PCR result may have been due to crossover contamination, again highlighting this crucial operational issue in the commercial application of PCR technology. The presence of nonviable mycobacterial DNA from past infection may cause false-positive results with sputum samples, but it is not a probable cause in pericarditis.

We conclude that the advantages of rapid diagnosis of tuberculous pericarditis by PCR support continued efforts to improve the sensitivity of the assay. We anticipate that technical advances in sample preparation and that the elimination of inhibitors will make the PCR assay a valuable tool in the diagnosis of tuberculous pericarditis.

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