Xpert MTB/RIF Assay for Diagnosis of Pleural Tuberculosis[▽]

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We prospectively investigated the diagnostic utility of the Xpert MTB/RIF (*Mycobacterium tuberculosis*/rifampin [RIF] resistance) assay in 20 cases with confirmed tuberculous pleural effusion. The sensitivity and specificity of the Xpert assay in pleural fluid were 25% and 100%, respectively. All cases positive by the Xpert assay were also positive by pleural fluid culture.

The detection of *Mycobacterium tuberculosis* in sputum samples from suspected pulmonary tuberculosis (TB) patients has been significantly improved by the Xpert MTB/RIF (*Mycobacterium tuberculosis*/rifampin [RIF] resistance) assay (Xpert; Cepheid, Sunnyvale, CA). This automated system employs real-time PCR and molecular beacon probes to determine the presence of *M. tuberculosis* complex DNA, as well as *rpoB* gene mutations conferring rifampin resistance, rapidly and with high accuracy (1, 2, 7). The assay seems predestined for use with other specimens than sputum, such as cerebrospinal fluid or effusions from various sites, but data are still scarce. Pleural TB is the second most frequent form of extrapulmonary TB and the most frequent cause of exudative pleural effusions in areas with a high prevalence of HIV infection. Patients present at all ages with uni- or bilateral pleural effusion and acute to subacute onset of chest pain, fever, weight loss, breathlessness, and cough. The diagnostic workup includes pleural fluid aspiration and pleural biopsy, as pleural fluid smear and culture are often negative due to the paucibacillary nature of pleural TB (9). We investigated the utility of the novel Xpert MTB/RIF assay for diagnosing pleural TB from pleural fluid.

Our prospective series included 25 consecutive patients referred to Tygerberg Hospital, a tertiary medical institution in Cape Town, South Africa, with an undiagnosed pleural effusion and high clinical suspicion of pleural TB for sputum collection, pleural biopsy, and fluid aspiration. Sputum was collected from patients able to produce samples spontaneously and processed with routine methods for smear and culture. Abrams needle biopsy specimens were conducted and the specimens analyzed as published previously (4); specimens were considered positive when granulomatous inflammation with or without necrosis and/or acid-fast bacilli (AFB) were found by Ziehl-Neelsen (ZN) microscopy or when *M. tuberculosis* was cultured from them. A minimum of 160 ml pleural fluid was collected from each patient and split for two mycobacterial cultures and one Xpert measurement. The samples for culture were centrifuged for 20 min at 3,000 × g and 4°C,

and the pellets were decontaminated for 20 min with an equal volume of 2.5% sodium hydroxide-*N*-acetyl-L-cysteine (NaOH-NALC; Merck, Cape Town, South Africa) and neutralized with sterile phosphate-buffered saline (PBS, pH 6.8) added to a total volume of 40 ml. A second centrifugation step followed (3,000 × g at 4°C for 20 min). The remaining pellets were resuspended in PBS, the bacterial concentrations were titrated to 0.5 McFarland standard, and 0.5 ml of this mixture was inoculated into mycobacteria growth indicator tubes (MGIT; Becton Dickinson, Sparks, MD) supplemented with 0.8 ml oleic acid-albumin-dextrose-catalase with the PANTA antibiotic mixture (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) and incubated in a Bactec MGIT 960 machine (Becton Dickinson). Growth was monitored hourly, and time to positivity (TTP) recorded. The presence of AFB was confirmed in positive tubes by ZN staining and microscopy. For Xpert analysis, 50 ml of pleural fluid was centrifuged as described above and the pellet resuspended in 2 ml PBS. One milliliter of this was processed for Xpert analysis, and the quantitative cycle threshold (*C_T*) value of *rpoB* probe B detection recorded according to published protocols (1, 5). The activity of adenosine deaminase (ADA) was determined as previously described. An ADA value of ≥50 U/liter was considered positive (6).

The diagnosis of TB was confirmed in 20 patients (80%), of whom 19 had a culture positive for *M. tuberculosis* from any specimen. One patient was confirmed based on typical clinical presentation, high activity of ADA with a predominantly lymphocytic effusion, and a good response to antituberculosis

TABLE 1. Diagnostic accuracy of assays used on samples from 20 TB and 5 non-TB patients

Method	No. of samples that were:			Sensitivity (%)	Specificity (%)
	Positive	Negative	Not done		
Pleural fluid ADA	22	3	0	100	60.0
Pleural biopsy specimen histology/ZN microscopy/liquid culture	18	5	2	94.7	100
Sputum smear microscopy	6	7	12	54.5	100
Pleural fluid liquid culture	9	16	0	45.0	100
Pleural fluid Xpert assay	5	20	0	25.0	100

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FIG. 1. Effect of frozen sample storage on C_T value of probe B. The storage of sputum pellet samples at -80°C for 30 days in PBS (pH 6.8) without glycerol increased the calculated mean C_T value of probe B in samples determined to be *M. tuberculosis* positive by the Xpert assay ($\Delta C_T = 2.11$, $n = 11$). This difference is statistically significant (*, $P = 0.0021$, paired two-tailed Student's t test).

treatment. Diagnoses for the other 5 patients were echinococcosis ($n = 1$), liver abscess with pleural effusion ($n = 1$), bacterial infection ($n = 2$), and renal failure with bacterial infection ($n = 1$).

The sensitivities of mycobacterial culture and the Xpert assay were 45% and 25%, respectively (Table 1). All cases with positive Xpert results were also pleural fluid culture positive, and the average TTP (403 h) tended to be shorter in Xpert-positive fluids (358 h) than in Xpert-negative fluids (458 h), suggesting a quantitative relationship of viable bacteria with a positive Xpert result. One Xpert result also indicated RIF resistance, which was confirmed on MGIT testing. The best diagnostic accuracy was found with pleural biopsy specimens, followed by pleural fluid ADA values of ≥ 50 U/liter, sputum smear microscopy, pleural fluid culture, and Xpert analysis (Table 1). The relatively low sensitivity of the Xpert assay contrasts with recent reports from regions with a low TB prevalence. Hillemann et al. (8) found 3 positive Xpert results out of 113 pleural fluid samples (2.9%) which were all negative on mycobacterial culture, and Causse et al. (3) found 4 positive Xpert results out of 34 tested fluids (11.8%), all confirmed on mycobacterial culture.

The close relationship of the viable bacterial load with a positive Xpert result in pleural fluid is an interesting observation. It confirms previous findings in sputum and spiked spu-

tum that liquid culture TTP and Xpert C_T value are quantitatively correlated (1, 5). Pleural TB is a paucibacillary form of the disease, and accordingly, positive Xpert cases were all close to the negative threshold of >37 C_T s (mean $\pm$ standard deviation, 29.6 ± 4.4 , and range, 23.6 to 35.3). This indicates that sample quality could be a crucial factor for using the Xpert assay on pleural fluid. For instance, we recently observed a mean increase of the C_T of 2.1 when comparing frozen and fresh sputum pellets (Fig. 1). This alone would have made one of our positive samples negative. For this pioneering study, we empirically concentrated the fluid by a factor of 25 but did not further explore options to increase the number of bacteria in the sample. There might be considerable potential to increase the sensitivity of the Xpert assay by optimizing sample collection, transport, or preparation. Larger studies are needed to show whether the association of positive Xpert results with positive culture indicates a low risk of false-positive Xpert readings in pleural fluid.

In conclusion, we found that use of the Xpert assay on pleural fluid samples is feasible but has low sensitivity and is linked to a positive pleural fluid culture. There is an indication for high specificity, which must be verified with larger studies, including more patients with a pleural effusion due to other causes than TB. Before this is attempted, the methods for collection, storage, and preparation of pleural fluid samples need to be optimized in order to increase the sensitivity of the Xpert assay on pleural fluid.

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