

Comparison Studies of GENXPRT versus MGIT Culture of Bronchial Lavage in Sputum Smear Negative Suspected Pulmonary Tuberculosis

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Abstract Introduction: Xpert MTB/RIF (Cepheid, USA) is an automated real-time PCR system that simultaneously detects TB and resistance to rifampicin. The test has excellent accuracy when performed on sputum and is endorsed by the World Health Organisation (WHO) and the USA Federal Drug Administration. **Aims:** To measure the diagnostic yield of bronchoalveolar lavage gene Xpert and compare it with traditional mycobacterial cultures in smear-negative and sputum-scarce pulmonary tuberculosis. **Methods:** A prospective observational study at a tertiary care hospital in India was conducted. A total of 200 patients who were 18 years or older under evaluation for clinico-radiological features of PTB with a history of a cough >2 weeks and a chest X-ray with pulmonary parenchymal involvement were recruited after ensuring two consecutive sputum negative smears or inability to produce adequate sputum. A volume of one ml of BAL sample was used to detect mycobacterium tuberculosis by the G4 version of Xpert[®] MTB/RIF (Cepheid, USA) and by traditional mycobacterial cultures, cartridges without initial decontamination or centrifugation. Sensitivity, specificity and predictive values were calculated with 95% class intervals (CIs). McNemar's test was used for comparison of sensitivities. **Result:** The results showed that the AFB smear test had 76.83% (95% CI: 66.20-85.44) sensitivity, 100.0% (95% CI: 96.92-100.0) specificity with 100.% PPV and 86.13% (95%CI: 80.72-90.21) NPV compared to culture report. The Gene Xpert test had better sensitivity 84.15% (95% CI: 74.42-91.28) and good specificity 97.52% (95% CI: 92.75-99.47) with 97.52% (95% CI: 92.75-99.47) PPV and 89.84% (95% CI: 84.30-93.58) NPV compared to AFB smear test. The Gene Xpert test to detect resistance against culture report had 89.47% (95% CI: 66.86-98.70) sensitivity, 90.61% (95% CI: 85.39-94.43) specificity with 50.0% (95% CI: 38.27-61.73) PPV and 98.90% (95% CI: 95.67-99.67) NPV. **Conclusion:** Xpert MTB/RIF has high sensitivity and specificity for diagnosis of both smear positive and smear negative PTB cases with high rates of detection of RIF resistance and greater concordance with gene sequencing for RIF resistance when compared with culture.

Keywords: bronchoalveolar lavage tuberculosis

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1. Introduction

Tuberculosis (TB) remains one of the major global health threats leading to massive morbidity and mortality. [1,2] One in three persons across the world representing 2-3 billion individuals are known to be infected with *Mycobacterium Tuberculosis* (*M. Tuberculosis*) of which 5-15% are likely to develop active TB disease during their lifetime. [3] The 1990 World Health Organization (WHO) report on the Global Burden of Disease ranked TB as the seventh most most morbidity-causing disease in the world and expected it to continue in the same position up to 2020. In 2014, an estimated 9.6 million people fell ill due to TB, around 1.5 million people died from the disease

including 1.1 million HIV-negative persons and 400,000 HIV patients. While TB is present in every country majority of TB sufferers live in low income and middle income countries especially in regions such as Sub-Saharan Africa and South East Asia. [2,3]

Tuberculosis (TB) remains a common public health hazard in the underdeveloped world like India causing major public health burden. With a population of about 1250 million, India is the largest country in the Region. It is ranking first among the high burden countries and contributed one-fourth of estimated global incident TB cases in 2011. As per WHO estimates, prevalence and incidence rates of all forms of tuberculosis were respectively 249 and 181 per 1,00,000 population in 2011. [4]

TB deaths are unacceptable because the widely available anti-tubercular drugs have cure rates of more

than 90%. The main challenge with TB is obtaining a rapid and accurate diagnosis to initiate early treatment. Only about 20 - 40% of pulmonary TB patients are smear-positive, while rest of the patients had either smear-negative or sputum-scarce disease. [5,6] The key to this challenge is having a more accurate and rapid diagnostic modality and obtaining a high-quality biological specimen. Bronchoscopy with Bronchoalveolar Lavage (BAL) is routinely performed for these set of patients in a suspected case of pulmonary tuberculosis. [7,8]

Xpert MTB/RIF (Cepheid, USA) is an automated real-time PCR system that simultaneously detects TB and resistance to rifampicin. The test has excellent accuracy when performed on sputum and is endorsed by the World Health Organisation (WHO) [9,10] and the USA Federal Drug Administration [11] for this purpose. In addition to containing PCR reagents and TB-specific primers, each MTB/RIF cartridge contains a set quantity of *Bacillus globigii* spores and a primer pair specific for the DNA in these spores. [12] If the amplification of this internal positive control fails, or occurs after 38 cycles, the test result is designated invalid. [13]

Fiber-optic bronchoscopy is helpful in early detection and confirmation of sputum smear-negative and sputum-scarce PTB by providing high-quality biological samples like bronchoalveolar lavage (BAL) fluid. [14] There are no formal recommendations on utilizing Xpert[®] MTB/RIF on BAL fluid due to limited literature.

The aim of this study was to measure the diagnostic yield of bronchoalveolar lavage gene Xpert and compare it with traditional mycobacterial cultures in smear-negative and sputum-scarce pulmonary tuberculosis.

2. Methodology

A prospective observational study at a tertiary care hospital in India was conducted. Any patient 18 years or older under evaluation for clinico-radiological features of PTB with a history of a cough >2 weeks and a chest X-ray with pulmonary parenchymal involvement was recruited after ensuring two consecutive sputum negative smears or inability to produce adequate sputum. Prior to enrolment patients had undergone evaluation for alternate diagnosis, further imaging studies, had received non-TB antibiotics at the discretion of the treating physician. Patients who had received more than 2 weeks of anti-tubercular therapy (ATT) in the past 90 days, unfit or unwilling for bronchoscopy, unwilling for follow-up were excluded from the study.

The study was approved by the Institutional Ethics Committee. At the time of enrolment written informed consent was obtained from each participant. Research information sheet was provided in local language to all the participating subjects. Each patient was evaluated by baseline demographic, clinical, radiological, and laboratory information were recorded in standardised proformas. HIV testing was done after providing pretest counselling.

Bronchoscopy was done for each patient. Flexible fiber-optic bronchoscopy which was performed using a bronchoscope of 6.2 mm insertion tube diameter (Pentax model EB-1970K). The visible part of the bronchial tree

was inspected, and bronchoalveolar lavage was done from a single or multiple segments of the lung based on the radiographic findings and the discretion of the investigators. After instilling aliquots of 50 ml each BAL samples were collected in a trap bottle 3 times and then transferred and to a Falcon tube.

A volume of one ml of BAL sample was transferred to the G4 version of Xpert[®] MTB/RIF (Cepheid, USA) cartridges without initial decontamination or centrifugation. The remaining BAL fluid was processed by the standard decontamination protocol, using NALC-NaOH method and centrifuged. AFB smear was done according to the standard protocol for Ziehl-Neelson staining. [15] The centrifuged sample after decontamination was inoculated for liquid culture in BACTEC mycobacterium growth indicator tube (MGIT) 960 system (BD Diagnostics, USA). Isolates were identified as MTB by immunochromatographic test kit (SD MPT64TB Ag kit). Any diagnostic sample that was detected as non-tuberculous mycobacterium (NTM) by culture method was considered as “non-TB.”

A final diagnosis of PTB was based on composite reference standard (CRS) which included two criteria-Culture confirmed PTB and Probable PTB. “Culture confirmed PTB” were cases with MTB culture positive on MGIT. “Probable PTB” were cases without MTB on culture or alternate diagnosis, showing resolution in the clinical and radiological features of PTB to ATT. The response to ATT was monitored during follow up of patients every 2 months for a total of 6 months. Rest of the cases either with an alternate diagnosis or showing no improvement with ATT were considered “Non-TB”

Data collection was completed with Microsoft excel 2010. Statistical analysis was performed using Statistiscal Package for Social Sciences (SPSS) for MAC, version 17.0 (IBM, Chicago, USA). Descriptive statistics were performed. Sensitivity, specificity and predictive values were calculated with 95% class intervals (CIs). McNemar’s test was used for comparison of sensitivities. A two-tailed $P < 0.05$ was considered statistically significant.

3. Results

A total of 200 subjects fulfilling the inclusion criteria were included in the study. The mean age of the was 39.49 ± 13.43. There were 121 (60.5%) males and 79 (39.5%) females (Table 1).

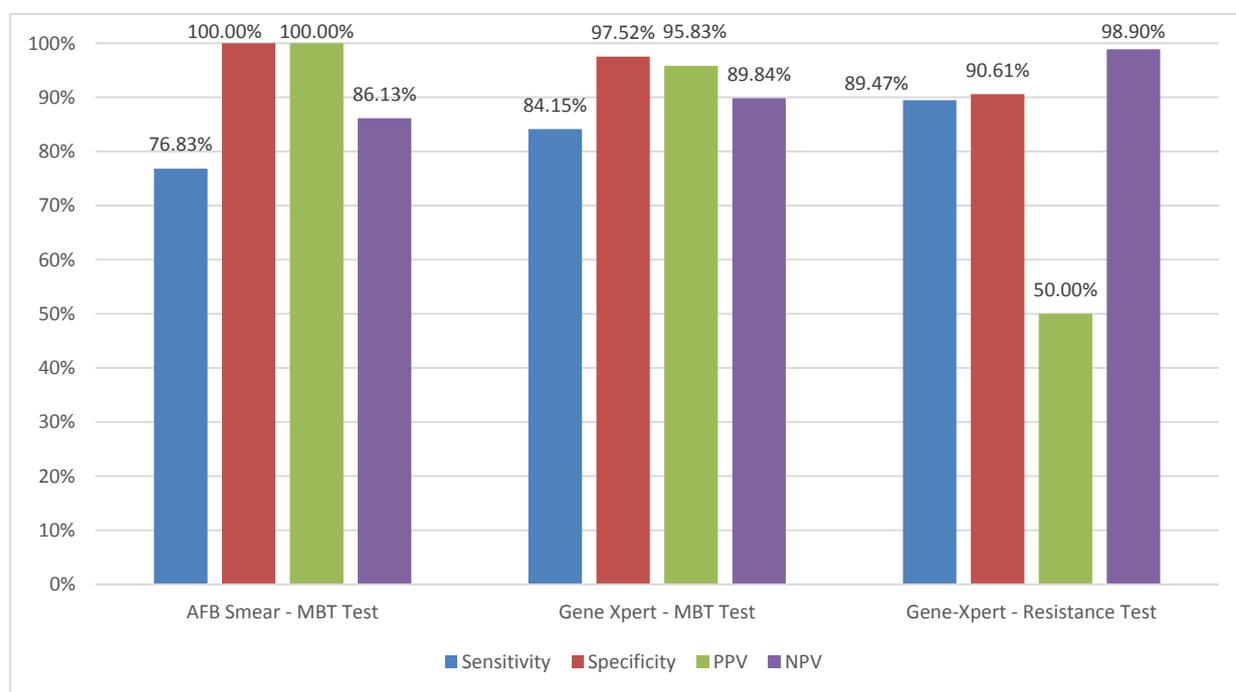
The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the AFB smear test and Gene Xpert was evaluated for MTB and rifampicin resistance (Table 2). The results showed that the AFB smear test had 76.83% (95% CI: 66.20-85.44) sensitivity, 100.0% (95% CI: 96.92-100.0) specificity with 100.% PPV and 86.13% (95%CI: 80.72-90.21) NPV compared to culture report. The Gene Xpert test had better sensitivity 84.15% (95% CI: 74.42-91.28) and good specificity 97.52% (95% CI: 92.75-99.47) with 97.52% (95% CI: 92.75-99.47) PPV and 89.84% (95% CI: 84.30-93.58) NPV compared to AFB smear test. The Gene Xpert test to detect resistance against culture report had 89.47% (95% CI: 66.86-98.70) sensitivity, 90.61% (95% CI: 85.39-94.43) specificity with 50.0% (95% CI: 38.27-61.73) PPV and 98.90% (95% CI: 95.67-99.67) NPV (Figure 1)

Table 1. Demographic details of the study population (n=200)

| Variables | Mean | S.D. | Range |
|-----------|-------|-------|-------|
| Age | 39.49 | 13.43 | 14-70 |
| Sex | N | % | |
| Male | 121 | 60.5 | |
| Female | 79 | 39.5 | |

Table 2. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) AFB smear and Gene Xpert compared to culture (n=200)

| Variables | Percentage (95% CI) | | | |
|-------------------|---------------------|---------------------|---------------------|---------------------|
| | Sensitivity | Specificity | PPV | NPV |
| MTB Test | | | | |
| AFB Smear | 76.83 (66.20-85.44) | 100.0 (96.92-100.0) | 100.0 (-) | 86.13 (80.72-90.21) |
| Xpert | 84.15 (74.42-91.28) | 97.52 (92.75-99.47) | 95.83 (88.23-98.60) | 89.84 (84.30-93.58) |
| Resistance | | | | |
| Xpert | 89.47 (66.86-98.70) | 90.61 (85.39-94.43) | 50.0 (38.27-61.73) | 98.90 (95.67-99.67) |

**Figure 1.** Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) AFB smear and Gene Xpert compared to culture (n=200)

4. Discussion

To date, sputum smear microscopy remains the first microbial analysis for both the diagnosis of TB and the assessment of patient infectiousness, which guides airborne isolation measures. [16] However, the limited sensitivity of this method hinders its widespread application for TB diagnosis. In addition, smear microscopy shows limited specificity, because AFB staining cannot distinguish between MTB and non-tuberculosis mycobacteria (NTM). Although culture is the 'gold standard' for TB diagnosis, it is slow and may take up to 2–8 weeks to yield results. The Xpert MTB/RIF assay is a rapid, automated molecular test with good sensitivity for PTB on sputum samples. However, with regard to its utility on BALF samples, especially in smear-negative cases and in patients who find it difficult to cough up sputum, only a few studies have been conducted so far.

In this study, the Xpert MTB/RIF assay was found to have a sensitivity of 84.15% (95% CI: 74.42-91.28), which was higher than the sensitivity of smear microscopy (76.83%; 95% CI: 66.20-85.44). Furthermore, the PPV for the assay was 97.52% (95% CI: 92.75-99.47) and the NPV was 89.84% (95% CI: 84.30-93.58). This is an important finding especially in a high TB burden country like India as this test will help in rapid diagnosis of smear-negative TB cases which were earlier a challenge for the TB control programmes. The results of our study are comparable to those a recent meta-analysis which reported the pooled sensitivity of Xpert in smear positive-culture positive PTB as 98%, and a sensitivity was 67% and specificity 99% for smear negative TB. [17] The results for the sensitivity of the Xpert MTB/RIF assay are also consistent with previous studies conducted in neighbouring countries; a study conducted in Pakistan by Ullah et al. (2017), [18] who reported a sensitivity of 80.0%; in Korea by Lee et al. (2013), [19] who reported a

value of 81.6%. These results further strengthen the evidence indicating the superiority of Xpert MTB/RIF over smear microscopy for the detection of TB when using BALF samples.

In our study, the sensitivity of Xpert to detect RIF resistance in pulmonary samples positive for PTB was 89.47% (95% CI: 66.86-98.70) sensitivity, 90.61% (95% CI: 85.39-94.43) specificity with 50.0% (95% CI: 38.27-61.73) PPV and 98.90% (95% CI: 95.67-99.67) NPV. These results are consistent with previously reported data for RIF resistance. [20,21,22] The Xpert MTB/RIF assay simultaneously identifies MTB and detects rifampicin resistance with molecular beacons to detect five overlapping 81-bp regions in the *rpoB* gene known as the rifampicin resistance determining region. [23] It has been shown that 95% of all rifampicin-resistant MTB strains contain mutations in the region of the *rpoB* gene. [24] Given the importance of the prompt and accurate identification of rifampicin resistance, Xpert MTB/RIF has been recommended for confirming rifampicin resistance in all TB cases in the USA. [25] According to a national survey of drug-resistant TB in China performed in 2007, 6.7% of new TB cases and 29.4% of previously treated cases were resistant to rifampicin. [26]

While Xpert MTB/RIF may be the foremost choice amongst all molecular diagnostic tests, it has its own limitations. Resistance to RIF is taken as a surrogate marker for MDR-TB, but certain strains may exhibit only mono-resistance to RIF that may not warrant full line MDR therapy, thus, leading to over-estimation of the MDR-TB cases. Likewise, a study from Mumbai, India demonstrated how specimens with rifampicin results reported as sensitive by GeneXpert could be resistant to isoniazid. [27] Other drawbacks of Xpert MTB/RIF are requirement of stable electrical power supply, temperature control and annual calibration of instrument. Regardless of all these limitations, addition of Xpert MTB/RIF assay to the present set of diagnostic modalities for TB on account of its unambiguous, rapid results, and high sensitivity and specificity will facilitate early diagnosis.

To conclude, our study highlights that Xpert MTB/RIF has high sensitivity and specificity for diagnosis of both smear positive and smear negative PTB cases with high rates of detection of RIF resistance and greater concordance with gene sequencing for RIF resistance when compared with culture. Our findings are similar to those reported by studies previously done in other countries. In resource-limited settings and less accessible areas where establishing a sophisticated laboratory for culture and DST conforming to the prescribed biosafety levels is difficult, Xpert MTB/RIF provides a viable option. Widespread application of this assay can increase the case detection rates of both drug sensitive and MDR-TB, thereby facilitating early treatment decisions and curbing transmission.

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