

# Diagnostic Yield of Bronchoalveolar Lavage Gene Xpert in Smear-Negative and Sputum-Scarce Pulmonary Tuberculosis

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## ABSTRACT

**Objective:** To measure the diagnostic yield of Bronchoalveolar Lavage (BAL) gene Xpert (Xpert MTB/RIF assay), to detect *Mycobacterium tuberculosis* (MTB) and rifampicin resistance and compare it with that of mycobacterial cultures in a suspected case of pulmonary tuberculosis.

**Study Design:** An analytical study.

**Place and Duration of Study:** Department of Pulmonology, Fauji Foundation Hospital (FFH), Rawalpindi, from December 2012 to August 2013.

**Methodology:** BAL specimens of 93 patients with suspected pulmonary tuberculosis with smear-negative or sputum-scarce disease, who presented to the Department of Pulmonology, FFH, Rawalpindi were inducted. A smear-negative case was one in whom three consecutive early morning sputum samples did not reveal acid fast bacilli when examined by microscopy with Zeihl Nelson (ZN) stain. Patients who had sputum amount less than 1 ml were defined to have sputum-scarce disease. The same was evaluated with ZN stain, gene Xpert and mycobacterial cultures. Sensitivity analysis was carried out using culture as the gold standard.

**Results:** The frequency of positive mycobacterial cultures was 85 (91.4%). The sensitivity, specificity, positive predictive value and negative predictive values of BAL gene Xpert to detect *Mycobacterium tuberculosis* were 91.86%, 71.42%, 97.53% and 41.66% respectively. Xpert MTB/RIF assay had a sensitivity and specificity of 83.33% and 100% to detect rifampicin resistance.

**Conclusion:** Bronchoalveolar lavage gene Xpert had a superior diagnostic yield in patients with either smear-negative or sputum-scarce pulmonary tuberculosis. Hence a positive Xpert MTB/RIF assay may be a useful adjunct to diagnosis and detection of MDR-TB in bronchoalveolar lavage specimens.

**Key Words:** Tuberculosis pulmonary. Sputum. Bronchoalveolar lavage. *Mycobacterium tuberculosis*. Cultures.

## INTRODUCTION

Tuberculosis (TB) remains a common public health hazard in the underdeveloped world like Pakistan. Only about 20 - 40% of pulmonary TB patients are smear-positive, while rest of the patients had either smear-negative or sputum-scarce disease.<sup>1,2</sup> Bronchoscopy with Bronchoalveolar Lavage (BAL) is routinely performed for these set of patients in a suspected case of pulmonary tuberculosis.<sup>3,4</sup>

Bronchoalveolar lavage is sent for Acid Fast Bacilli (AFB) smear by ZN stain) and mycobacterial cultures. The sensitivity of ZN stain remains low (41%). Mycobacterial cultures, considered as the gold standard (with 86% sensitivity) but they are expensive and results take 6 - 8 weeks for diagnosis.<sup>5</sup>

Traditional nucleic acid amplification tests do not have a role in routine diagnosis again because of their poor

sensitivity and complexity.<sup>6</sup> Recently a single-tube, single-use sample-processing cartridge system with multicolor real-time PCR capacity for the detection of *Mycobacterium tuberculosis* (MTB), commonly known as Gene-Xpert, has been developed with the additional feature of detecting mutations in the 81-bp Rifampicin Resistance-Determining Region (RRDR) of the *rpoB* gene, that occurs in 95 - 98% of all rifampin-resistant strains.<sup>6</sup> This assay is able to detect MTB and rifampicin resistance within 2 hours. The advantages of this test include high sensitivity and specificity, low complexity, low cost, wide availability and less manpower involved. This test is found useful in diagnosis and management of suspected cases of pulmonary tuberculosis.<sup>7</sup>

As smear-negative patients form the bulk of cases and delay in diagnosis in this subset often leads to increased morbidity and mortality, so if found superior to mycobacterial cultures that are the gold standard, BAL gene Xpert can rapidly detect the mycobacteria and rule out rifampicin resistance on the same day, helping in the diagnosis and management of these patients.

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.

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## METHODOLOGY

This was an analytical study, carried out in the Department of Pulmonology, Fauji Foundation Hospital, Rawalpindi, on 93 patients with suspected pulmonary tuberculosis from December 2012 to August 2013. Permission was taken from the Hospital Ethical Committee. The financial expenses were borne by the Hospital.

Sample size was calculated using the WHO sample size calculator, using the sensitivity of the Xpert assay being bronchial washing or Bronchoalveolar Lavage (BAL) fluid for the diagnosis of PTB as 81.6%,<sup>8</sup> and mycobacterial cultures having sensitivity of 86%.<sup>5</sup> By using the sensitivity calculator and an absolute precision of 0.08, the sample size was determined as 93.

The diagnostic yield was defined and measured in terms of frequency and validity by calculating sensitivity, specificity, positive and negative predictive values. A patient was considered a tuberculosis-suspect, on the basis of clinical and radiological features compatible with a diagnosis of pulmonary tuberculosis.<sup>9</sup>

A smear-negative case was one in whom three consecutive early morning sputum samples did not reveal acid fast bacilli when examined by microscopy with Zeihl Nelson stain.<sup>10</sup> Patients that had sputum amount less than 1 ml were defined to have sputum-scarce disease.<sup>11</sup>

A confirmed case of pulmonary tuberculosis was one in whom *Mycobacterium tuberculosis* (MTB) grew on BAL mycobacterial cultures by LJ medium, that was taken as the gold standard.

Patients of either gender aged above 12 years of age, that had suspected pulmonary tuberculosis on clinical or radiological grounds and had taken anti-TB medications for less than 1 week, were included in the study. Smear-positive cases, those with disseminated or extra pulmonary tuberculosis, HIV positive and immunocompromised patients were excluded from the study. Following written consent for bronchoscopy, demographic and clinical data was collected.

Bronchoscopy was performed by transnasal route and bronchoscope was wedged into the subsegmental bronchus of interest and about 100 ml of Bronchoalveolar Lavage (BAL) was obtained in two aliquots by instillation of sterile normal saline.<sup>10</sup> It was sent for ZN stain and mycobacterial cultures by Lowenstein Jenson (LJ) medium to the Hospital Laboratory and to WHO sponsored National Institute of Health (NIH) Laboratories, Islamabad, for gene Xpert to detect *Mycobacterium tuberculosis* (MTB) and rifampicin resistance.

BAL specimens were processed by NIH laboratory using standardized protocols and quality assurance procedures for the Xpert MTB/RIF assay.<sup>11</sup> The results

of all tests were read by a trained technologist and reported for detection of MTB and presence or absence of rifampicin resistance.

BAL specimens for smear microscopy and mycobacterial cultures were evaluated at Fauji Foundation Hospital Laboratory and results were available after 3 days and 6 weeks respectively.

SPSS version 16 was used for statistical analysis. Demographic features including age, gender, clinical and radiological features, past history of tuberculosis were recorded. Frequency of AFB smear, gene Xpert assay and mycobacterial cultures was calculated by percentages. Validity of these tests was calculated in terms of sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Values (NPV).

## RESULTS

The mean age of the patients was  $38.56 \pm 19.045$  years. About 36 patients (38.7%) were males while the rest were female patients. The common presenting complaints included cough with or without sputum (44.1%), fever (33.3%) and hemoptysis (22.6%).

Radiological features compatible with the diagnosis of tuberculosis included cavity (32.3%), consolidation (34.4%), nodulo-striate opacities (20.4%), miliary shadows (2.2%) and others (10.8%). Around 28 patients (30.1%) were treated for tuberculosis while the rest of the patients (69.9%) were never treated. The demographic features of these patients are presented in Table I.

Out of 93 patients who were tuberculosis suspect, 85 (91.4%) cases were confirmed as pulmonary tuberculosis on mycobacterial cultures. The frequency, sensitivity, specificity, positive predictive value and negative predictive values for BAL ZN stain and gene Xpert are presented in Table II.

**Table I:** Demographic features of the cases.

Demographic characteristics	
Age	Mean $38.56 \pm 19.045$ years
Gender	
Male	36 (38.7%)
Female	57 (61.3%)
Presenting complaints	
Cough (sputum)	41 (44.1%)
Fever	31 (33.3%)
Hemoptysis	21 (22.6%)
Radiological findings	
Cavitation	30 (32.3%)
Consolidation	32 (34.4%)
Nodulo-striate opacities	19 (20.4%)
Miliary shadows	2 (2.2%)
Others	10 (10.8%)
Previous treatment	
Treated TB	28 (30.1%)
Never treated	65 (69.9%)

**Table II:** Frequency and validity of BAL AFB smear and gene Xpert as compared to mycobacterial cultures.

Test on BAL	Frequency and percentage n (%)	Sensitivity (%) a/a+c	Specificity (%) d/d+b	PPV (%) a/a+b	NPV (%) d/c+d
ZN stain for AFB					
BAL +ve	34 (36.6%)	39.53%	100%	100%	11.86%
BAL -ve	59 (63.4%)				
Gene Xpert for MTB					
MTB detected	81 (87.09%)	91.86%	71.42%	97.53%	41.66%
MTB not detected	12 (12.9%)				

Six patients (6.45%) had rifampicin resistance (and also isoniazid resistance i.e MDR-TB) detected on mycobacterial cultures, while the rest had susceptible strains. Five patients (5.37%) had rifampicin resistance detected on BAL gene Xpert, there was only one patient with rifampicin resistance detected on mycobacterial cultures that was not detected on gene Xpert, hence BAL gene Xpert was able to detect rifampicin resistance in 83.33% of cases in this study with a specificity of 100%.

## DISCUSSION

In this study, the authors evaluated the diagnostic yield of BAL gene Xpert to detect MTB and rifampicin resistance in smear-negative and sputum-scarce pulmonary TB and compared it with that of mycobacterial cultures which were taken as the gold standard.

Mycobacterial cultures for detection of *Mycobacterium tuberculosis* use either solid (Lowenstein Jensen media) or liquid broth system (MGIT 960). LJ medium is highly specific but is expensive, laborious, requires trained personnel, not widely available and takes 6 - 8 weeks to give the results. Though results by MGIT 960 medium come earlier as compared to LJ medium, delay in diagnosis in smear-negative especially drug resistant strains can have serious consequences for the patient as well as the community.<sup>15,16</sup> The Xpert MTB/RIF assay is, however, a simple assay that can be performed with minimal training. The results are available within a couple of hours.<sup>6</sup> At present, costs for the Gene Xpert system are similar to those required to set up an automated liquid culture system for tuberculosis but as this test is offered at WHO centres for TB control so they are available free of charge to the patient. Although it is routinely performed for identification of pulmonary tuberculosis by using frozen sputum or BAL specimens, but research has shown that it may be a valuable aid in identification of mycobacteria in other body fluids like Cerebrospinal Fluid (CSF), pleural and ascetic fluid and will have wider applicability in future.<sup>12</sup>

Numerous studies have demonstrated the utility of Xpert MTB/RIF assay in diagnosis of pulmonary tuberculosis. In a multicentre implementation study by Boehme and colleagues, MTB/RIF test sensitivity was 76.9% in smear-negative, culture-positive patients with 99.0%

specificity while its sensitivity for rifampicin resistance was 94.4% and specificity was 98.3%.<sup>13</sup> This study showed a sensitivity of 91.86% but a specificity of 71.42% that is lower than in the international studies. However, the sensitivity and specificity of MTB/RIF assay to detect rifampicin resistance in our study was 83.33% and 100% which is higher than the international studies.

There were certain limitations of the study. First, mycobacterial culture was performed by using the solid culture medium (LJ medium). Studies have shown the automated liquid culture medium (MGIT 960) to be superior to the LJ medium because of its higher sensitivity and shorter time for detection of mycobacteria.<sup>14</sup> Further studies are required in this respect. In this study, the yield of mycobacterial cultures on BAL specimens was higher than the international studies (91.4% vs. 55 - 88%), this may be due to a selection bias as patients who had a strong suspicion of active TB clinically and radiologically were enrolled in this study by non-probability consecutive sampling.<sup>17,18</sup>

Secondly, the sensitivity and positive predictive value of Xpert MTB/RIF assay to detect MTB was very high (91.86% and 97.53% respectively), while the specificity and negative predictive value in this study was low (71.42% and 41.66%) as compared to the International studies (100% and 92.1% respectively).<sup>8,19</sup> Possible explanations include false positive tests in the presence of a negative culture, use of solid culture medium as the gold standard or laboratory error. Well designed future studies eliminating these errors are required. The study was performed in smear-negative and sputum-scarce pulmonary tuberculosis that excluded many of the MDR-TB patients. This may be the reason for the low frequency of drug-resistant TB in this study (6.45%).

## CONCLUSION

Bronchoalveolar lavage gene Xpert had a superior diagnostic yield (with high sensitivity and positive predictive value) to detect *Mycobacterium tuberculosis* and rifampicin resistance (with high sensitivity and specificity), in those cases of pulmonary tuberculosis who have either smear-negative or sputum-scarce disease. This test has the advantages of being inexpensive, requires less manpower and gives results on the same day. Hence, a positive Xpert MTB/RIF

assay may be a useful adjunct to diagnosis and detection of MDR-TB in bronchoalveolar lavage specimens.

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