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IMPROVING DIAGNOSIS OF PULMONARY TUBERCULOSIS IN SMEAR-NEGATIVE AND SPUTUM-SCARCE PATIENTS USING BRONCHOALVEOLAR LAVAGE GENE XPERT AS A DIAGNOSTIC TOOL

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ABSTRACT

***Objective**: To assess the diagnostic accuracy of Bronchoalveolar Lavage (BAL) gene xpert (Xpert MTB/RIF assay) in smear-negative and sputum-scarce patients to detect mycobacterium tuberculosis using mycobacterial culture as the gold standard.*

***Study Design:** Cross-sectional validation study*

***Place and Duration of Study:** Department of Pulmonology, Pakistan Institute of Medical Sciences (PIMS), Islamabad, from July 2024 to December 2024.*

***Methodology**: A total of 215 patients with suspected pulmonary tuberculosis who were either smear-negative or sputum-scarce were included. Smear-negative cases were defined as those with three sputum specimens negative for Acid-Fast Bacilli (AFB) on microscopy using the Ziehl-Neelsen (ZN) staining technique[8]. Sputum-scarce cases were those unable to produce sputum or provide sputum samples of less than 1 mL[8]. The diagnostic performance of BAL GeneXpert, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy, was calculated against the BAL culture results.*

***Results**: Frequency of BAL culture was 195 out of 215(90.6%).BAL GeneXpert demonstrated a sensitivity of 93.33%, specificity of 85%, PPV of 98.38%, NPV of 56.67% and overall accuracy of 92.56%. Tuberculosis was identified in 185 out of 215 cases using BAL gene xpert.*

***Conclusion**: BAL GeneXpert exhibits superior diagnostic accuracy in detecting tuberculosis among smear-negative and sputum-scarce patients, making it a reliable diagnostic tool in such cases.*

Keywords: *Tuberculosis, Bronchoalveolar Lavage, GeneXpert, Pulmonary TB, Mycobacterial Culture.*

INTRODUCTION

Tuberculosis (TB) is still a major killer disease throughout the world; it is the leading cause of death due to an infectious disease. About 10 million people developed TB in 2019, while an estimated 1.2 million people died from the disease globally [1]. The national TB prevalence rate is 5%, and Pakistan has ranked 5th among these 30 highest-burdened countries out of 118 countries globally, contributing 87% of the global total TB cases [2]. Sputum microscopy for AFB is equally inexpensive and could be done on a sputum sample; however, the yield is low only 44% of cases are smear-positive even at an advanced stage [3]. This limitation is especially observed in smear-negative TB patients, who form a considerable proportion of TB patients. For example, as per data from the European Centre for Disease Prevention and Control for Italy, smear-negative TB was as high as 68.1% of all TB incidences in 2014 [3]. The use of smear microscopy for TB diagnosis was associated with a longer time to treatment initiation [4] Median diagnostic delay was longer in those with smear-negative pulmonary tuberculosis. [5] Delaying the diagnosis worsens illness severity, prolongs patient suffering, increases the risk of patient death, and enables the transmission within the community[6] Bronchoscopy, particularly bronchoalveolar lavage, turns out to be an essential tool in such situations to obtain respiratory samples in cases where sputum is suboptimal or smear findings are negative [7,8].In addition to these, poor quality of sputum and microscopic observation can also contribute to smear-negative results, which ultimately reduce the sensitivity of AFB smears in the diagnosis of TB. On the other hand, despite being considered the gold standard for TB detection, culture does not provide a prompt result and requires 2–8 weeks for final determination. [9]Gene xpert technology has brought about a marvellous change in the diagnostic process of TB due to its ability to detect both M. tuberculosis and rifampicin resistance in one day [9,10]. When implemented on BAL samples, gene Xpert has high diagnostic efficacy in smear-negative or sputum-scarce pulmonary TB patients, making therapy initiation more efficient[11]. Gene Xpert having high sensitivity (88%) and specificity (99%) in the detection of smear-negative TB has raised hopes of increased case detection in low- and middle-income countries.[11] Due to the high frequency of smear-negative TB and poor performance of traditional diagnostic tests, the present study will compare the diagnostic accuracy of BAL gene Xpert with that of mycobacterial culture in smear-negative and sputum-scarce suspected pulmonary TB patients attending the Pulmonology Department of PIMS Hospital, Islamabad

Methodology

This cross-sectional validation research was carried out at the Pulmonology Department of Pakistan Institute of Medical Sciences (PIMS), Islamabad, over a six-month duration from July 2024 to December 2024 after seeking permission from the ethical review board and written informed consent from the patient. Financial expenses were borne by the hospital. The sample size was calculated using the WHO sample size calculator. With a significance level of 0.05, a statistical power of 95%, a Type I error rate of 5%, an expected sensitivity of the BAL gene xpert test at 91.8%[8], a projected specificity of 71.4%[8], and an estimated M. tuberculosis prevalence of 91.4%[8], the sample size for the study was calculated as 215. A smear-negative case was one in whom three consecutive sputum samples did not reveal acid-fast bacilli when examined by microscopy with Zeihl Nelson stain.[8].Patients who had sputum amounts less than 1 ml were defined to have sputum-scarce disease[8]. GeneXpert MTB/RIF is a molecular real-time polymerase chain reaction (PCR) test that detects Mycobacterium tuberculosis DNA and the most common mutations in the rpoB gene causing resistance to rifampin. The sample test is faster than reference culture methods and can produce results in only 2 hours [12]. Bronchoscopy is a safe procedure and complications ranged from <0.1 to 11%, with mortality reported between 0 and 0.1%. [13]

Patients of either gender above 15 years of age who were smear-negative or who were sputum-scarce with suspected pulmonary TB and having stable vitals (more than 92 % oxygen saturation at room air with a blood pressure of 120/80 to 140/90) were included in this study. Smear-positive cases, those with extrapulmonary tuberculosis, those who were not giving consent and those who were having

contraindications for bronchoscopy (uremia, coagulopathy, thrombocytopenia, coagulopathy and severe pulmonary hypertension) were excluded from the study. The procedure involved the insertion of a flexible bronchoscope through the vocal cords and into the lumen of the trachea and bronchi of interest. Direct visualization is provided by fiberoptic video imaging. [14].After the bronchial tree had been inspected, BAL was performed by instilling sterile saline (0.9%) in serial 20-mL aliquots (up to a maximum of 200 mL). At least 50% of the total volume of the aspirate was returned [15]Samples were sent to the WHO-supported National Institute of Health (NIH) Laboratories for Gene xpert testing and mycobacterial cultures using the Lowenstein-Jensen (LJ) medium to detect Mycobacterium tuberculosis (MTB).SPSS version 22 was used for statistical analysis. Continuous data, including age, were described using mean and standard deviation, while categorical variables, including gender, comorbidities, BAL gene xpert, and mycobacterial culture, were represented in frequencies and percentages. A 2x2 contingency table was used to identify the true positives, true negatives, false positives and false negatives. Diagnostic tests metrics, including sensitivity, specificity, positive predictive values, negative predictive values, and overall accuracy were computed using the sample data formulas.

Results

Of 215 patients who were included in the study, the participants' mean age was 50.46 years (SD = ± 18.99). Men were(144) 67 % and women were (71)33%. The most common symptoms at the time of presentation were chronic cough in (184)85% of the patients, fever in (161)75% of patients, weight loss in(147)68%, night sweats in(115) 53% and breathlessness in only (94)43% of patients. Coexisting conditions were Diabetes Mellitus type(75) (35%), Hypertension (54)25%, and CKD(23) 10%.

Demographic features are present in Table 1

Out of 215 patients who were tuberculosis suspects 195 cases were confirmed as pulmonary tuberculosis on mycobacterial culture.

Sensitivity, specificity, positive predictive value and negative predictive value for gene xpert are presented in Table 2

Table 1: Demographic and Clinical Characteristics of Study Participants

Characteristic	N (%)
Age (years)	50.46 ±18.99
Gender	
Male	144(67%)
Female	71 (33%)
Comorbidities	
Diabetes Mellitus	75 (35%)
Hypertension	54 (25%)
Chronic Kidney Disease	23 (10%)
Symptoms	
Cough	184(85%)
Fever	161 (75%)
Weight Loss	147 (68%)
Night Sweats	115 (53%)
Shortness of Breath	94(43%)

Diagnostic Performance of BAL GeneXpert

The BAL Gene Xpert test detected Mycobacterium tuberculosis in 185 out of 215 patients, while mycobacterial culture confirmed TB in 195 patients.

Table 2: Diagnostic Performance of BAL Gene Xpert Compared to Mycobacterial Culture

	Mycobacterial culturere	Mycobacterial cultureulture	Total

	Positive	**Negative**	
BAL Gene Xpert Positive	182	3	185
BAL Gene Xpert Negative	13	17	30
Total	195	20	215

Calculation of Diagnostic Accuracy Metrics

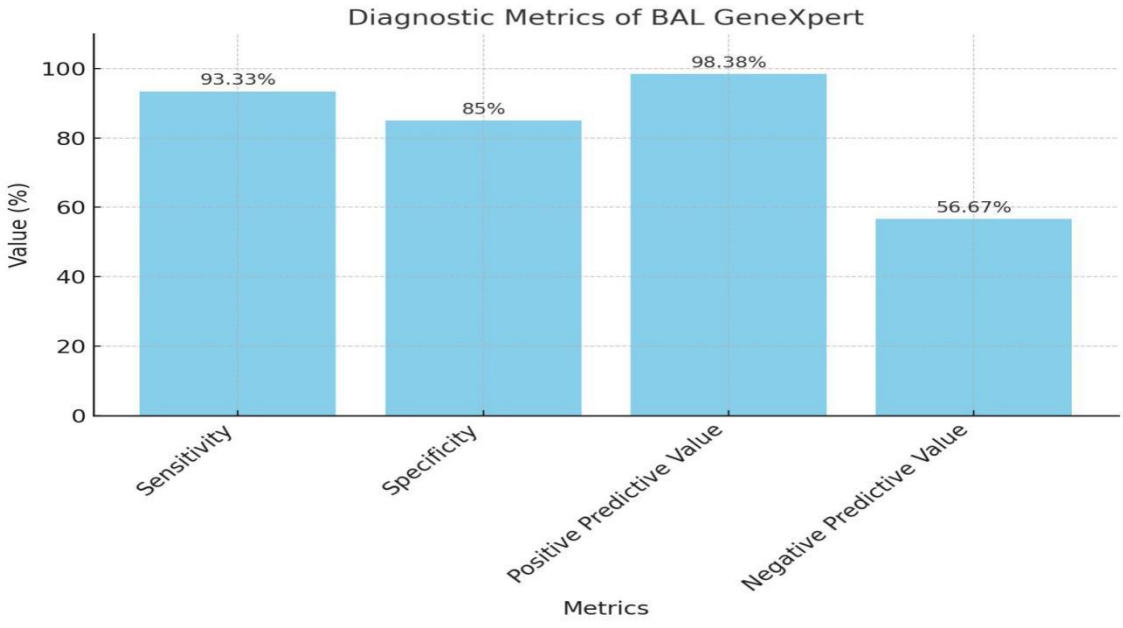
- **Sensitivity:** (True Positives / (True Positives + False Negatives)) × 100
  = (182 / (182 + 13)) × 100
  = 93.33%
- **Specificity:** (True Negatives / (True Negatives + False Positives)) × 100
  = (17/17 + 3)) × 100
  = 85%
- **Positive Predictive Value (PPV):** (True Positives / (True Positives + False Positives)) × 100
  = (182 / (182 + 3)) × 100
  = 98.38%
- **Negative Predictive Value (NPV):** (True Negatives / (True Negatives + False Negatives)) × 100
  = (17/ (17 + 13)) × 100
  = 56.67%
- **Diagnostic accuracy** = {TP + TN}/{TP + FP + FN + TN} ×100
  =92.56%
- ***Table 3: Diagnostic Accuracy Metrics of BAL GeneXpert***

Metric	**Value (%)**
Sensitivity	93.33%
Specificity	85%
Positive Predictive Value	98.38%
Negative Predictive Value	56.67%
Dianostic accuracy	92.56%

Figure 4 : sensitivity, specificity, positive predictive value (PPV) and negative predictive (NPV) of BAL Gene Xpert

Discussion

This study assessed the diagnostic accuracy of BAL gene xpert in a population of smear-negative and sputum-scarce pulmonary tuberculosis (PTB) patients, using mycobacterial culture as the gold standard. The results demonstrate that BAL gene xpert exhibits high sensitivity (93.33%) and specificity (85%), with an



overall accuracy of 92.56%, positive predictive value (PPV) of 98.38% and negative predictive value of 56.67. These metrics underscore the potential of BAL gene xpert as a reliable diagnostic tool in this challenging subset of PTB patients, aligning with recent studies which highlight the assay's robust performance across diverse clinical settings. Our results exactly match with a study done by Yasin M et all [16].

According to yasin M et all .,2022 [16] compared to culture as gold standard the sensitivity of gene xpert was 93.24% while its specificity was 84.62% with positive predicted value (PPV) of 97.18%, negative predicted value (NPV) of 68.75% and accuracy of 91.95%.

The sensitivity and specificity of BAL gene xpert in our study are almost equivalent to the sensitivity and specificity of Raja K., et all [17]. According to Raja S K et all, the sensitivity and specificity of BAL gene xpert compared to culture were 94.74% and 80.43% respectively while in our study sensitivity and specificity were 93.33 and 85% respectively, which is almost equivalent. High sensitivity suggests that GeneXpert is particularly useful in settings where traditional sputum-based diagnostics fail to detect mycobacteria due to low bacterial load.

The high PPV of 98.38% observed supports the utility of gene xpert in accurately confirming tuberculosis cases among those who test positive. This is particularly vital in reducing the risk of overtreatment and ensuring appropriate management of resources within healthcare settings (Aror D et al, 2020)[18].

This study also underscores the limitations of conventional diagnostic methods, such as smear microscopy, which has been shown to have a low diagnostic yield in smear-negative patients. The rapid and accurate detection capabilities of gene xpert for Mycobacterium tuberculosis and rifampicin resistance signify a considerable advancement in the diagnosis of tuberculosis, facilitating early and appropriate therapeutic interventions. (Rimal R et al 2022)[19]

The sensitiviy, specificty and PPV of BAL gene xpert by Satish Chandra Kilaru et all.,2019 [20] to be reported as 90%, 52% and 29% respectively while in our study it was 93.33,85% and 56.67% respectively.

Additionally, the use of bronchoscopy and BAL to obtain diagnostic samples from patients who cannot produce adequate sputum is validated by our findings and others, underscoring the value of this approach in resource-limited settings where sputum collection may be challenging (Kim YW et al 2019[21].

The negative predictive value was 56.67% which was low compared to other studies.

Despite these promising results, our study is not without limitations. The single-centre design may restrict the generalizability of the findings and the high prevalence of TB in this study could influence the diagnostic metrics, especially the NPV, which are known to be prevalence-dependent. If the prevalence of a disease is high, the negative predictive value can be low even if sensitivity and specificity are very high. (Akobeng AK 2007) [22]

Conclusion

BAL gene xpert demonstrates excellent diagnostic performance for detecting pulmonary TB in smear-negative and sputum-scarce patients, making it a valuable addition to the diagnostic arsenal in high-burden TB settings. Future multi-centre studies are warranted to validate these findings and explore strategies to further improve the NPV of gene xpert in these patient populations

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