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Diagnostic Performance of Xpert MTB/RIF in Bronchoalveolar Lavage of Sputum-Scarce Recurrent Pulmonary Tuberculosis Cases

Pratibha Sharma[1] Abhishek Kumar[2] Mamatha S[3] Ranganath T. Ganga[4]

[1]Department of Microbiology, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India
[2]Department of Pulmonary Medicine, Teerthanker Mahaveer Medical College and Research Center, Moradabad, Uttar Pradesh, India
[3]Department of Respiratory Medicine, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru, Karnataka, India
[4]Department of Pulmonary Medicine, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India

Address for correspondence Ranganath T. Ganga, MD, Department of Pulmonary Medicine, All India Institute of Medical Sciences, D Block, 1st Floor, Gate No 4, Tatibandh, GE Road, Raipur 492099, Chhattisgarh, India (e-mail: ranganathtg@aiimsraipur.edu.in).

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Abstract

Introduction Xpert MTB/RIF has greater sensitivity and specificity than smear microscopy. Bronchoalveolar lavage (BAL) is safe and valuable tool in sputum-scarce and sputum-negative tuberculosis (TB) patients. Our study evaluated the performance of Xpert in BAL specimen of sputum-scarce recurrent TB cases exclusively.

Materials and Methods Sputum-scarce recurrent TB patients who underwent BAL between July 2018 and July 2019 were included. The diagnostic performance of Xpert and acid-fast bacilli (AFB) smear examination in BAL specimen was compared with liquid culture Mycobacterium TB (MTB) and composite reference standard.

Results A total of 126 patients were included in the study. MTB culture was positive in 70 cases and nontuberculous mycobacteria were seen in five cases. Xpert was positive was in 63 patients. Sensitivity of Xpert and AFB smear was 84.29% (95% confidence interval [CI]: 73.62–91.89) and 18.57% (95% CI: 10.28–29.66), respectively, with $p < 0.001$ proving the superiority of Xpert. Xpert had a specificity of 97.96 (89.15–99.95), positive predictive value of 93.65% (95% CI 85.19–97.42), and negative predictive value of 80.36% (95% CI: 70.26–87.63). Smear had a specificity of 91.84% (95% CI: 80.21–97.58) against 97.96 (89.15–99.95) of Xpert, and smear was positive in nontuberculous mycobacterium cases as well. Xpert showed no cross-reactivity between mycobacterial species. Rifampicin resistance was seen in 8 (12.69%) cases, and 21 patients had other diagnoses.

Conclusion Xpert has greater sensitivity in comparison to AFB smear in BAL specimen. Sputum-scarce recurrent TB cases have a similar chance of rifampicin resistance as sputum smear-positive cases should undergo BAL for Xpert analysis routinely.

Keywords
- bronchoalveolar lavage
- pulmonary tuberculosis
- recurrent tuberculosis
- sputum-scarce pulmonary tuberculosis
- tuberculosis
- Xpert MTB/RIF

Introduction

Tuberculosis (TB) continues to challenge countries worldwide and the End TB strategy of the World Health Organization (WHO) and is still the leading cause of death among the infectious disease patients despite the availability of effective medication.[1] India leads the countries with a high TB burden.[2] In 2017, there were 28,00,000 cases in our country accounting for 25% of all the TB cases worldwide, and there were 4,23,000 deaths due to TB. To combat this global epidemic, the Government of India embarked upon the National Strategic Plan (NSP) 2020–2025 for TB elimination. The NSP aims at 80% reduction in TB, 90% reduction in TB mortality,

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and 0% patient having catastrophic expenditure due to TB by 2025. Early diagnosis and treatment initiating are essential for breaking the chain of transmission and achieving the aims of NSP. Confirmation of TB is a limiting factor for treatment initiation in sputum-scarce cases. Bronchoalveolar lavage (BAL) is a safe and valuable tool for TB in sputum-scarce patients.[3] Xpert MTB/RIF is a WHO approved molecular test for TB diagnosis with high sensitivity than sputum smear examination.[4] Data regarding the use of Xpert in daily practice in BAL samples is limited by small sample size and non-uniform sampling techniques. In this study, we evaluated the performance of Xpert in BAL in recurrent TB cases exclusively who run a high chance of harboring drug-resistant TB.[2]

Objective

This study aimed to assess the performance of Xpert in BAL in the sputum-scarce recurrent pulmonary TB (PTB) cases.

Materials and Methods

Study Setting

This retrospective study was conducted in July 2019 at a 500-bedded tertiary care teaching hospital in north India. The study was permitted by the Institutional Ethics Committee.

Study Population

Data of patients who had undergone bronchoscopy between July 2018 and July 2019 were obtained from medical case records. For study purpose, patients were suspected to be having recurrent PTB if they had taken at least 6 months of antitubercular treatment (ATT) in the past and had a history of cough, fever, hemoptysis, and loss of appetite for more than 15 days. Only sputum-scarce patients were included for analysis.

Bronchoscopy Procedure

Bronchoscopy was performed in a dedicated suite with the EB-1970TK Video Bronchoscope (PENTAX Medical, Montvale, New Jersey, United States). Approximately 20 to 40 mL of 0.9N saline in 20-ml aliquots was used to obtain washings.

Mycobacteriology

BAL samples were decontaminated with 4% sodium hydroxide and centrifuged at 3,000 g for 20 minutes. Decontaminated samples were inoculated in MGIT culture system (Becton Dickinson, Sparks, Maryland, United States) and incubated for 6 weeks, and positive cultures were identified as Mycobacterium TB (MTB).

Xpert was performed on 2.5 mL of unprocessed sample as per the manufacturer's instruction, and results were read on a computer as either positive for MTB and rifampicin-sensitive/resistant or MTB negative.

Final Diagnosis

Final diagnosis was based on a composite reference standard (CRS). CRS included confirmed PTB cases who were MTB culture-positive and probable TB cases who improved on ATT at the end of 2 months and continued further ATT till 6 months.

Statistical Analysis

Data collection was completed using Microsoft Excel 2015. Data validation and descriptive statistical analysis was performed using SPSS, Version 16 (IBM Corp., Armonk, New York, United States). Sensitivity, specificity, positive predictive value, and negative predictive value with 95% confidence intervals were calculated. A two-tailed p-value <0.05 was considered to be statistically significant.

Results

Patient Profile

Records of 179 patients of suspected recurrent PTB were available and 53 were excluded. A total of 126 patients had undergone bronchoscopy during the study period. Two samples were contaminated, and 124 samples were finally included in the study. ►**Fig. 1** shows the flow of patients in the study. The clinicodemographic and radiological profiles are shown in ►**Table 1**.

Computed tomography (CT) of the thorax was performed in 51 (72%) of culture-positive TB cases: 20 (39.2%) had patchy consolidation, 16 (37.2%) had random nodularity, and 15 (29.4%) had multiple cavities as their predominant lesions. Majority of the lesions were seen in the upper lobes followed by the middle lobes, with both sides being equally affected.

Performance of Diagnostic Tests

Using culture as the reference standard, Xpert was positive in 63 cases and acid-fast bacilli (AFB) smear was positive in 14 PTB cases. The sensitivity of Xpert was 84.29 (95% confidence interval [CI]: 73.62–91.89) and that of AFB smear was 18.57 (95% CI: 10.28–29.66). Xpert performed significantly better ($p < 0.001$).

Using CRS, sensitivity of Xpert and AFB smear was 77.78 (95% CI: 67.17–86.27) and 16.05 (95% CI: 8.83–25.88), respectively, and the difference was statistically significant ($p = 0.001$). ►**Table 2** shows the sensitivity, specificity, positive predictive value, and negative predictive value of Xpert and AFB smear in comparison to culture and composite reference.

Rifampicin-resistant cases were diagnosed by Xpert: among the 63 Xpert-positive cases, rifampicin resistance was seen in 8 (12.6%) and 2 (3.1%) cases were rifampicin intermediate. ►**Table 3** shows drug susceptibility pattern in Xpert-positive cases.

Final Diagnosis

Recurrent PTB was diagnosed in 81 (65.3%) cases: 70 culture confirmed and 11 probable TB based on clinical response to ATT. Non-TB diagnosis was made in 26 (21%). No specific diagnosis was made in 17 (13.7%) cases. Diabetes was seen in seven (10%) culture-confirmed TB cases ($p = 0.351$), and one diabetic patient had rifampicin resistance on Xpert. HIV

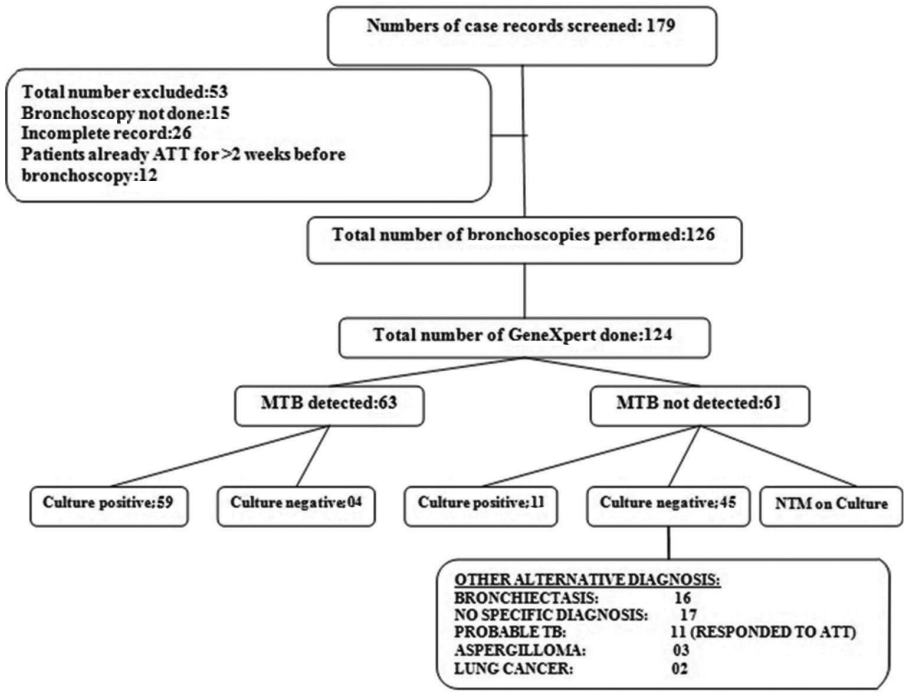

Fig. 1 Flow of patients in the study. ATT, antitubercular treatment; MTB, *Mycobacterium* tuberculosis; NTM, nontuberculous mycobacterium.

Table 1 Clinicodemographic and radiological profile of the study population (n = 124)

Characteristics	*n* (%)
Age (years)[a]	49.9 (17–90)
Gender	
Male	87 (70.2)
Female	37 (29.8)
Smoking status	
Smoker[b]	79 (63.7)
Nonsmoker	45 (36.3)
Diabetic status	
Diabetic	12 (9.7)
Nondiabetic	112 (90.3)
HIV positive	03 (2.4)
Residence	
Urban	90 (72.6)
Rural	34 (27.4)
Clinical features	
Cough	113 (91.1)
Fever	104 (83.8)
Weight loss	68 (54.8)
Breathlessness	42 (33.8)
hemoptysis	21 (16.9)
Thorax CT findings (n = 70)	
Patchy consolidation	23 (18.5)
Cavitary lesions	24 (19.4)
Random nodules	17 (13.7)
Bronchiectasis	06 (4.8)

Abbreviations: HIV, human immunodeficiency virus; CT, computed tomography.
[a]Age is mentioned as mean with minimum and maximum age. [b]Smoker includes both current and ex-smokers.

infection was seen in three (2.4%) cases, and all had culture and Xpert-confirmed TB. ►**Table 4** summarizes all the diagnoses established in the study population.

Discussion

This study was conducted in a TB high burden setting. Although many studies have been conducted in sputum-scarce patients, our study was performed exclusively on recurrent TB cases. BAL samples were obtained in 124 patients. All samples were processed for Xpert and liquid culture MTB.

The study clearly showed that Xpert has very high sensitivity and specificity in the diagnosis of PTB in sputum-scarce cases. In our study, BAL Xpert in comparison with MTB culture showed a sensitivity of 84.29% and specificity of 97.96%, similar to other recent studies.[4-8] In all these studies, sensitivity was 57 to 92% and specificity was 94 to 100%. Positive predictive value and negative predictive value in our study was 93.65 (95% CI: 85.19–97.42) and 80.36 (95% CI: 70.26–87.63), respectively. Comparing both BAL AFB with BAL Xpert, Xpert outperformed smear (sensitivity of 84.29 vs. 18.57%), similar to previous data.

Rifampicin resistance on Xpert was seen in 12.89% (8), 3.17% (2) had intermediate resistance to rifampicin, and rest 84.12% (53) were rifampicin sensitive similar to drug-resistance pattern seen in the first National Anti-tubercular Drug Resistance Survey by Mishra and Mulani.[9] The added advantage of Xpert was that rifampicin-resistant patients were diagnosed and put on appropriate treatment without loss of time.

Table 2 Performance of diagnostic tests

Variable	Percentage (95% confidence intervals)			
	Sensitivity	Specificity	PPV	NPV
Compared with gold standard				
AFBS	18.57 (10.28 to 29.66)	91.84 (80.21 to 97.58)	92.86 (63.74 to 98.97)	45.71 (42.78 to 48.68)
XPERT	84.29 (73.62 to 91.89)	97.96 (89.15 to 99.95)	93.65 (85.19 to 97.42)	80.36 (70.26 to 87.63)
Compared with composite reference standard				
AFBS	16.05 (8.83 to 25.88)	90.70 (77.86 to 97.41)	76.47 (53.02 to 90.35)	34.45 (33.38 to 39.63)
XPERT	77.78 (67.17 to 86.27)	100.00 (91.78 to 100.00)	100.00 (94.88 to 100.00)	70.49 (61.38 to 78.22)
MTBC	86.90 (77.78 to 93.28)	100.00 (91.78 to 100.00)	100.00 (92.14 to 100.00)	79.63 (69.26 to 87.15)

Abbreviations: AFBS, acid-fast bacilli smear; MTBC, *Mycobacterium* tuberculosis culture; NPV negative predictive value; PPV, positive predictive value.
Notes: Comparing sensitivity of AFBS and XPERT with positive culture, $p < 0.001$. Comparing sensitivity of AFBS and XPERT with CRS, $p = 0.001$. Comparing sensitivity of XPERT and MTBC, $p < 0.001$.

Table 3 Rifampicin sensitivity pattern among Xpert positive cases

Rifampicin sensitivity pattern (*n* = 63)			
	Sensitive	Resistant	Intermediate
n	53	08	02
%	84.12	12.69	3.17

Table 4 Final diagnosis of all the study population (*n* = 124)

Final diagnosis	*n* (%)
Pulmonary tuberculosis	
Culture confirmed	70 (56.4)
Clinically diagnosed	11 (8.8)
Other than tuberculosis	
No specific diagnosis	17 (13.7)
Bronchiectasis	16 (12.9)
Nontuberculous mycobacteria	5 (4.0)
Aspergilloma	3 (2.4)
Lung cancer	2 (1.6)

Xpert-positive and culture-negative results were seen in four cases. This was possible due to including patients on ATT for less than 2 weeks or due to patients receiving β-lactam antibiotics with antitubercular activity during the initial period.[10,11] Xpert being a polymerase chain reaction test amplifies any MTB DNA found in the sample and is read as positive, but it does not differentiate between dead and live bacilli; hence, results need to interpreted with caution,[12] and Xpert cannot be relied upon to assess immediate treatment response.[13]

The rate of nontuberculous mycobacterium (NTM) isolation varies widely from 0.5 to 8.6% across India,[14] 1 to 3.5% in Mumbai,[14] 3.9% in all clinical specimens in Vellore,[15] 9.9% in Delhi.[16] In our study, 6.6% (five) samples were identified as NTM, and all these were negative on Xpert and positive on AFB smear. There was no cross-reactivity between MTB and NTM isolates, and no false-positive results were observed with Xpert, as in other studies.[17,18]

The available molecular tests for TB diagnosis in BAL fluid include multiplex polymerase chain reaction with a sensitivity of 92.1% and a specificity of 98%.[19] The other approved molecular diagnostic test is GenXpert Ultra, which has a sensitivity of 90.28%.[20]

The strengths of our study were sufficiently large sample size and uniform cases, and all our patients were suspected of recurrent TB, which is a unique and uniform sampling technique. The limitations of the study were that solid culture was not used and CT of the thorax was not performed in all the cases, which could have added more information.

Conclusion

BAL is a safe and useful tool in recurrent TB cases. BAL Xpert has higher sensitivity and specificity for TB confirmation. Immediate availability of rifampicin sensitivity is an advantage. Sputum-scarce recurrent TB patients have similar rifampicin resistance as sputum-positive cases. Xpert-positive and culture-negative cases need to be interpreted keeping the clinical background in mind.

Conflict of Interest
None declared.

Acknowledgment
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