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Original Research Article

A PROSPECTIVE STUDY FOR EVALUATION AND COMPARISON OF BAL CBNAAT, BAL AFB SMEAR, AND RADIOLOGICAL METHODS (CT CHEST) FOR DETECTION OF TUBERCULOSIS IN SPUTUM SMEAR-NEGATIVE OR NON-SPUTUM PRODUCING CLINICALLY SUSPECTED PULMONARY TUBERCULOSIS

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ABSTRACT

Background: Sputum smear-negative or non-sputum producing Pulmonary tuberculosis (PTB) patients present a diagnostic dilemma, leading to delayed treatment and continued transmission The objective is to evaluate and compare the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of bronchoalveolar lavage cartridge-based nucleic acid amplification test (BAL CBNAAT), BAL acid-fast bacilli (AFB) smear, and computed tomography of the chest (CT chest) in sputum smear-negative or non-sputum producing clinically suspected PTB patients, taking liquid AFB culture as the gold standard.

Materials and Methods: A prospective observational study was conducted on 150 clinically suspected PTB patients who were sputum smear-negative or non-sputum producing at Bundelkhand Medical College, Sagar, MP. All patients underwent CT chest followed by flexible fiberoptic bronchoscopy to collect BAL fluid. BAL specimens were subjected to AFB smear, CBNAAT, and liquid AFB culture. Statistical analysis was performed using standard diagnostic performance metrics with P-values calculated via Chi-square test.

Results: Out of 150 patients (mean age 49.05 ± 17.13 years; 60% male), 37 cases (24.7%) were confirmed as active PTB by liquid culture. BAL CBNAAT demonstrated the highest sensitivity (97.3%), specificity (92.9%), PPV (81.8%), NPV (99.1%), and diagnostic accuracy (94.0%). CT chest showed a sensitivity of 89.2%, specificity of 85.8%, PPV of 67.3%, NPV of 96.0%, and accuracy of 86.7%. BAL AFB smear demonstrated a sensitivity of 64.9%, specificity of 92.9%, PPV of 75.0%, NPV of 89.0%, and accuracy of 86.0%. Concordance between BAL CBNAAT and BAL AFB smear was high, but CBNAAT identified 13 additional cases missed by smear microscopy.

Conclusion: BAL CBNAAT is a highly sensitive and accurate rapid diagnostic tool for smear-negative or sputum-scarce PTB patients.

Keywords: Tuberculosis, Pulmonary, Bronchoalveolar Lavage, GeneXpert MTB-RIF, Sputum Smear Negative, X-Ray Computed Tomography.

INTRODUCTION

Tuberculosis (TB), caused by the acid-fast bacillus Mycobacterium tuberculosis, continues to pose an immense public health challenge worldwide, particularly in low- and middle-income countries.[1] Pulmonary tuberculosis (PTB) accounts for more than 80% of all TB cases and represents the primary

reservoir for airborne transmission.[2] In the immunocompetent host, the lungs are involved in approximately 79% to 87% of TB cases.[3] A single untreated individual with active pulmonary TB can infect ten to fifteen close contacts annually, underscoring the vital imperative for rapid diagnostic identification and early therapeutic intervention.[1,4]

Despite the availability of highly effective short-course anti-tubercular therapy (ATT), tuberculosis remains a leading cause of mortality among infectious diseases globally. In strategic frameworks such as the World Health Organization (WHO) End TB Strategy and India's National TB Elimination Program (NTEP), early case detection is emphasized as a foundational pillar.[5] Conventional diagnostic algorithms rely heavily on direct sputum smear microscopy for acid-fast bacilli (AFB). However, sputum smear microscopy suffers from low sensitivity (ranging between 30% and 60%) and requires a high bacillary threshold of 5,000 to 10,000 bacilli/mL for visual detection.[6] Consequently, a substantial proportion of active PTB cases are paucibacillary, presenting as sputum smear-negative or unable to produce adequate sputum samples.[7,8]

Patients with sputum smear-negative PTB remain capable of disease transmission and experience significant diagnostic delays, often receiving empirical broad-spectrum antibiotics or delayed ATT.[9,10] While mycobacterial culture remains the gold standard for diagnosis, its clinical utility is constrained by a lengthy turnaround time of two to six weeks. [1[1] High-Resolution Computed Tomography (HRCT) of the chest provides detailed cross-sectional visualization of parenchymal abnormalities such as tree-in-bud opacities, fibrocavitary lesions, and centrilobular nodules; nevertheless, radiological features alone lack absolute microbiological specificity.[12,13]

Flexible fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) offers a direct, targeted sampling mechanism to retrieve cellular and microbiological specimens from affected subsegmental airways.[14] The integration of Cartridge-Based Nucleic Acid Amplification Testing (CBNAAT / GeneXpert MTB/RIF) on BAL fluid allows rapid automated real-time polymerase chain reaction (PCR) detection of M. tuberculosis DNA along with simultaneous screening for rifampicin resistance within two hours.[15,16] Therefore, this prospective study was undertaken to evaluate and compare the diagnostic performance—specifically sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy—of BAL CBNAAT, BAL AFB smear, and CT chest in sputum smear-negative or non-sputum producing clinically suspected PTB patients.

MATERIALS AND METHODS

Study Design and Setting: This prospective observational comparative study was conducted in the Department of General Medicine in collaboration with the Department of Respiratory Medicine at Bundelkhand Medical College and Hospital, Sagar, Madhya Pradesh, India, over a 12-month period following institutional ethical approval.

Ethical Considerations: Ethical approval was granted by the Institutional Ethics Committee of Bundelkhand Medical College, Sagar (Approval Letter Ref No. BMC/IEC/2022/147). Written informed consent was obtained from all participating patients or their legally acceptable representatives prior to enrollment. Patient confidentiality and anonymity were strictly maintained in accordance with the Declaration of Helsinki (revised 2000).

Sample Size Calculation: The sample size was calculated using the standard formula for diagnostic accuracy studies: $N = [Z^2 * p * (1 - p)] / M^2$, where $Z = 2.3$ (for 98% confidence level), $p = 0.47$ (estimated prevalence of smear-negative PTB based on pooled literature), and $M = 0.10$ (margin of error). The initial calculated sample size was 131. Accounting for a 10% non-response or procedure intolerance rate, the final sample size was set at 150 patients.

Inclusion Criteria:

1. Patients aged ≥ 18 years presenting with clinical features suggestive of pulmonary tuberculosis (e.g., persistent cough ≥ 2 weeks, fever, night sweats, weight loss > 1.5 kg/month, anorexia, hemoptysis, chest pain).
2. Patients who were either sputum smear-negative on two consecutive morning samples or unable to produce sputum spontaneously.
3. Patients providing written informed consent for fiberoptic bronchoscopy.

Exclusion Criteria

1. Sputum smear-positive pulmonary tuberculosis cases.
2. Isolated extra-pulmonary tuberculosis without lung parenchymal involvement.
3. Human Immunodeficiency Virus (HIV) positive individuals.
4. Patients medically unfit for bronchoscopy (e.g., refractory hypoxemia PaO2/FiO2 < 200, uncorrected coagulopathy, acute cardiovascular instability, recent myocardial infarction, severe hypercapnia, status asthmaticus).

Study Protocol and Bronchoscopy Procedure: Enrolled patients underwent detailed history taking, physical examination, routine hematological investigations, chest radiograph (PA view), and non-contrast CT chest. CT chest scans were evaluated by experienced radiologists for signs suggestive of TB (consolidation, fibrocavitary lesions, thick-walled cavities, centrilobular nodules, interstitial shadows, bronchiectasis). Bronchoscopy was performed in a dedicated suite under local anesthesia (topical 2% lidocaine spray) and conscious sedation using short-

acting benzodiazepines. A flexible fiberoptic bronchoscope was inserted via the transnasal route. After visual inspection of the tracheobronchial tree, the bronchoscope tip was wedged into the involved subsegmental bronchus corresponding to the radiological lesion. Serial instillation of 50 to 120 mL of sterile normal saline was performed, followed by gentle manual suctioning to harvest BAL fluid. BAL fluid was divided into aliquots and immediately dispatched for: (a) Ziehl-Neelsen (ZN) staining for AFB smear microscopy, (b) Automated Cartridge-Based Nucleic Acid Amplification Testing (CBNAAT / GeneXpert MTB/RIF assay), and (c) Liquid AFB culture using the Mycobacterial Growth Indicator Tube (MGIT 960) system, which served as the gold standard reference.

Statistical Analysis

Data were compiled into Microsoft Excel and analyzed using SPSS Version 25.0. Categorical variables were presented as numbers and percentages. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy were computed for BAL CBNAAT, BAL AFB smear, and CT chest using liquid AFB culture as the reference standard. Associations between categorical variables and final TB diagnosis were evaluated using the Chi-square test or Fisher's exact test. A P-value < 0.05 (italicized P) was considered statistically significant.

RESULTS

Demographic Profile and Baseline Characteristics: The study evaluated 150 clinically suspected PTB patients. The mean age of the study population was 49.05 ± 17.13 years (range: 22 to 80 years; median: 47.5 years). The highest proportion of cases was observed in the 26–35 years (19.3%) and 46–55 years (19.3%) age groups. Males comprised 60.0% (n=90) of the cohort, while females accounted for 40.0% (n=60). Farmers (28.0%), housewives (26.7%), and labourers (16.7%) constituted the majority of occupational categories. A higher proportion of patients resided in rural areas (59.3%) compared to urban areas (40.7%). Furthermore, 56.7% belonged to low socioeconomic status (SES), 35.3% to middle SES, and 8.0% to high SES. [Table 1]

Table 1: Baseline Socio-Demographic Characteristics of Suspected PTB Patients (N = 150)

Parameter	Category	Number of Patients (%)
Age Group	<= 25 years	13 (8.7%)
	26 - 35 years	29 (19.3%)
	36 - 45 years	21 (14.0%)
	46 - 55 years	29 (19.3%)
	56 - 65 years	24 (16.0%)
	66 - 75 years	28 (18.7%)
	> 75 years	6 (4.0%)
Gender	Male	90 (60.0%)
	Female	60 (40.0%)
Residence	Rural	89 (59.3%)
	Urban	61 (40.7%)
Socioeconomic Status	Low	85 (56.7%)
	Middle / High	65 (43.3%)

Clinical Symptoms and Comorbidities: Cough and weight loss were the most predominant presenting symptoms, each recorded in 55.3% (n=83) of cases. Breathlessness was present in 53.3% (n=80), fever in 52.7% (n=79), hemoptysis in 47.3% (n=71), chest pain in 46.0% (n=69), and loss of appetite in 42.7% (n=64). Chronic Obstructive Pulmonary Disease (COPD) was the most frequent comorbidity, observed in 50.0% (n=75) of cases, followed by hypertension in 17.3% (n=26) and diabetes mellitus in 13.3% (n=20).

Radiological and Bronchoscopic Findings: In CT chest evaluations, fibrocavitary lesions (48.6% vs 19.5%, P = 0.000) and consolidation (45.9% vs 19.5%, P = 0.001) were significantly associated with a final diagnosis of TB. Other radiological features strongly correlated with confirmed TB included bronchiectasis (27.0% vs 1.8%, P = 0.000), interstitial shadows (24.3% vs 2.7%, P = 0.000), thick-walled cavities (18.9% vs 1.8%, P = 0.000), and other non-specific opacities (32.4% vs 1.8%, P = 0.000). On bronchoscopy, mucoid secretions (70.3% vs 31.9%, P = 0.000), endobronchial masses (29.7% vs 5.3%, P = 0.000), and bronchiectatic involvement (35.1% vs 2.7%, P = 0.000) were significantly more frequent among TB cases. [Table 2]

Table 2: Association of Radiological (CT Chest) and Bronchoscopic Findings with Final TB Diagnosis

Diagnostic Feature	Confirmed TB (n=37)	Non-TB (n=113)	P-value
CT: Consolidation	17 (45.9%)	22 (19.5%)	0.001
CT: Fibrocavitary Lesions	18 (48.6%)	22 (19.5%)	0.000
CT: Thick Wall Cavity	7 (18.9%)	2 (1.8%)	0.000
CT: Interstitial Shadows	9 (24.3%)	3 (2.7%)	0.000
CT: Bronchiectasis	10 (27.0%)	2 (1.8%)	0.000
CT: Mass Lesions	5 (13.5%)	4 (3.5%)	0.027
CT: Nodules	2 (5.4%)	8 (7.1%)	0.723

Bronchoscopy: Mucoid Secretions	26 (70.3%)	36 (31.9%)	0.000
Bronchoscopy: Endobronchial Mass	11 (29.7%)	6 (5.3%)	0.000
Bronchoscopy: Bronchiectasis	13 (35.1%)	3 (2.7%)	0.000

Diagnostic Performance of Modalities: Out of 150 patients, liquid AFB culture confirmed active TB in 37 cases (24.7%). BAL CBNAAT tested positive in 44 cases (29.3%), BAL AFB smear was positive in 32 cases (21.3%), and CT chest was classified as suggestive of TB in 49 cases (32.7%). [Table 3]

Table 3: Diagnostic Findings across Evaluated Modalities (N = 150)

Diagnostic Test	Positive / Suggestive	Negative / Not Suggestive
BAL CBNAAT Result	44 (29.3%)	106 (70.7%)
BAL AFB Smear Result	32 (21.3%)	118 (78.7%)
CT Chest Diagnosis	49 (32.7%)	101 (67.3%)
Final Diagnosis (Liquid Culture)	37 (24.7%)	113 (75.3%)

Cross-Tabulation and Head-to-Head Comparison: When comparing BAL CBNAAT with BAL AFB smear, 23 cases were positive on both modalities. Importantly, 13 cases were positive on BAL CBNAAT but negative on BAL AFB smear, highlighting the incremental diagnostic yield of CBNAAT in smear-negative samples. Only 1 case was smear-positive but CBNAAT-negative. Furthermore, among 75 patients whose CT chest was not suggestive of TB, all 75 (100%) were negative on BAL CBNAAT and BAL AFB smear. Among 75 patients with CT findings suggestive of TB, BAL CBNAAT was positive in 36 cases (48.0%) and BAL AFB smear was positive in 24 cases (32.0%).

Comparative Diagnostic Metrics: BAL CBNAAT exhibited superior sensitivity (97.3%), specificity (92.9%), PPV (81.8%), NPV (99.1%), and diagnostic accuracy (94.0%). CT chest demonstrated high sensitivity (89.2%) and NPV (96.0%), but lower PPV (67.3%) due to false positives. BAL AFB smear demonstrated a sensitivity of 64.9%, specificity of 92.9%, PPV of 75.0%, NPV of 89.0%, and accuracy of 86.0%. [Table 4]

Table 4: Comparative Diagnostic Performance Metrics against Reference Standard (Liquid AFB Culture)

Diagnostic Modality	Sensitivity	Specificity	PPV	NPV	Accuracy
BAL CBNAAT	97.3%	92.9%	81.8%	99.1%	94.0%
CT Chest	89.2%	85.8%	67.3%	96.0%	86.7%
BAL AFB Smear	64.9%	92.9%	75.0%	89.0%	86.0%

DISCUSSION

Prompt and accurate diagnosis of pulmonary tuberculosis remains a paramount objective in clinical medicine. Sputum smear-negative PTB presents a major clinical challenge, as these patients frequently experience delayed diagnosis, undergo empirical antimicrobial trials, and continue to transmit infection within the community.[1,7] This study comprehensively evaluated BAL CBNAAT, BAL AFB smear, and CT chest in a cohort of 150 sputum smear-negative or non-sputum producing clinically suspected PTB patients.

In our study, 60.0% of patients were male, and the highest age distribution occurred in the economically productive age bracket (26–55 years). This male predominance and age distribution align with published literature by Agrawal et al,[17] (65.9% male) and Das & Panda,[18] (66.9% male), reflecting higher environmental and occupational exposures among adult males. Farmers (28.0%) and labourers (16.7%) constituted a major portion of our cohort, further corroborating the association between low socioeconomic status, occupational exposure, and TB vulnerability.[19]

COPD was identified in 50.0% of our study participants, representing the most common comorbidity. Chronic structural lung alteration in COPD severely impairs local mucociliary clearance and macrophage activity, predisposing patients to mycobacterial infection.[20] Similar findings were reported by Das & Panda,[18] (30.2% COPD prevalence). Furthermore, symptoms such as chronic cough, weight loss, and dyspnea overlap extensively between COPD and PTB, underscoring the critical need for advanced diagnostic modalities in this subgroup.

Radiological imaging via CT chest demonstrated high diagnostic sensitivity (89.2%) and NPV (96.0%). Fibrocavitary lesions (48.6%), consolidation (45.9%), bronchiectasis (27.0%), and thick-walled cavities (18.9%) showed statistically significant correlations with confirmed TB ($P < 0.05$). These results closely parallel findings by Avashia et al,[16] and Ghanshyam et al,[21] who observed consolidation and cavitary patterns in 33.3% and 41.0% of smear-negative cases, respectively. However, the PPV of CT chest was relatively modest (67.3%), as fungal infections, non-tuberculous mycobacteria, and chronic inflammatory states can mimic tuberculous lesions.

Fiberoptic bronchoscopy provided significant direct visual clues, with mucoid secretions (70.3%) and endobronchial masses (29.7%) strongly associated with active TB. More importantly, BAL fluid harvesting bypasses upper airway contamination and yields high-grade microbiological specimens directly from affected subsegments.[14,22]

The central finding of this study is the outstanding diagnostic performance of BAL CBNAAT, which achieved a sensitivity of 97.3%, specificity of 92.9%, PPV of 81.8%, NPV of 99.1%, and an overall accuracy of 94.0%. In direct comparison, BAL AFB smear yielded a sensitivity of only 64.9%. CBNAAT successfully identified 13 active TB cases that were missed by smear microscopy. These findings are strongly supported by Dwari et al,[15] who demonstrated a statistically significant 10.7% yield increase with BAL CBNAAT over sputum testing, and Bhatia et al,[23] who reported superior sensitivity and NPV for CBNAAT in BAL specimens.

CONCLUSION

The remarkable diagnostic accuracy and high NPV (99.1%) of BAL CBNAAT indicate that a negative BAL CBNAAT result, in conjunction with non-suggestive CT imaging, can effectively rule out active pulmonary tuberculosis, thereby avoiding unnecessary empirical anti-tubercular therapy. Integrating fiberoptic bronchoscopy and BAL CBNAAT into standard diagnostic algorithms for smear-negative or sputum-scarce suspected PTB patients significantly accelerates definitive decision-making and optimizes clinical outcomes.

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