

Diagnostic Yield of Induced Sputum and Bronchial Aspirate in Sputum Smear Negative Pulmonary Tuberculosis Patients

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Abstract

Introduction: Tuberculosis is one of the most important public health problem worldwide, detecting patients with active pulmonary tuberculosis is an important disease control measure. Diagnosing sputum smear negative pulmonary tuberculosis is challenging and in this study, an attempt is made to compare the diagnostic yield of smear and CBNAAT with culture in smear negative PTB patients.

Aims and Objectives: 1. To determine the diagnostic yield of CBNAAT, AFB smear, AFB Culture on induced sputum and bronchial aspirate 2. To evaluate the sensitivity, specificity, PPV, NPV of CBNAAT in sputum negative respiratory samples as compared with AFB culture.

Methods: This is a prospective observational study done in the department of Pulmonary Medicine, Goa Medical College, from October 2016 to August 2018. All patients of suspected Tuberculosis based on clinical and radiological diagnosis underwent sputum induction and flexible video-bronchoscopy as per standard protocol. Our study had a different and unique approach where smear negative PTB patients were subjected to sputum induction procedure initially, those which were positive on induced-sputum CBNAAT were diagnosed as PTB and started on treatment. However cases which were induced-sputum CBNAAT negative were subjected to fiberoptic bronchoscopy. The bronchial aspirate from the involved segment was collected and sent for smear, CBNAAT and Culture.

Results: The total number of patients included were 202 cases. Males- 64.9%, females- 35.1%. The most common symptom was cough with expectoration in 99% individuals. Multiple heterogenous opacities on chest radiography seen in 65.3% of cases. The overall diagnostic yield of AFB smear, CBNAAT and AFB Culture were 41/202(20.29%), 134/202(66.33%) and 144/202(71.28%) respectively. The sensitivity, specificity, PPV and NPV of induced sputum smear was 28.5%, 98.7%, 96.7% and 76.7% and that of Induced sputum CBNAAT was 84.2%, 98.0%, 94.1% and 94.9% respectively. The sensitivity, specificity, PPV and NPV of bronchial aspirate smear was 26.31%, 100%, 100% and 69.2% and that of BA CBNAAT was 84.6%, 98.4%, 97% and 91.1% respectively. In this study around 17 patients were culture positive and CBNAAT negative, among 17 cases 3 patients(17.64%) were diagnosed to have Non tuberculous mycobacterial pulmonary disease on AFB culture.

Conclusions: Our study shows that CBNAAT has high sensitivity and specificity in diagnosing smear negative pulmonary tuberculosis than AFB smear microscopy as comparable to AFB culture which is gold standard. Hence all smear negative patients should be evaluated with CBNAAT and also helps in diagnosing RIFAMPICIN resistant Tuberculosis. Use of procedures like induced sputum further increases yield of diagnosis and is a powerful tool in diagnosis of smear negative PTB suspects reserving invasive procedure like Fiberoptic bronchoscopy for undiagnosed cases.

Keywords: Fiberoptic Bronchoscopy; Induced sputum; Bronchial aspirate; CBNAAT; Pulmonary tuberculosis.

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INTRODUCTION

Tuberculosis is one of the most important public health problems worldwide and is declared as “global emergency” by the World Health Organization¹. Detecting patients with active Pulmonary Tuberculosis (PTB) disease is an important component of tuberculosis control programs. Early detection and appropriate treatment renders these patients non-infectious and interrupts the chain of transmission of tuberculosis.

The most widely used method for diagnosis is Acid Fast Bacilli (AFB) staining by fluorescent microscopy, but it is seen that in up to 50% of active pulmonary tuberculosis cases smear microscopy is negative². Various other methods to collect specimen for diagnosis in smear negative cases are Induced Sputum (IS), Bronchial aspirate(BA)/washing (BW), Broncho-Alveolar Lavage (BAL) and Gastric Washings (GW) samples. Each of these methods have its own advantages and disadvantages and diagnostic yield varies accordingly. Hence in this study effort was made to diagnose smear negative pulmonary tuberculosis using induced sputum. Invasive procedure like fibreoptic bronchoscopy was reserved for cases which were not diagnosed by using induced sputum samples.

In this study, induced sputum and bronchial aspirate samples collected from suspected smear negative pulmonary tuberculosis patients were sent for AFB smear, Cartridge based nucleic acid amplification test (CBNAAT) and AFB culture. CBNAAT in smear negative pulmonary tuberculosis patients was not being done in this institution so far, hence an attempt was made in this study to compare its diagnostic yield with culture in smear negative PTB.

AIMS AND OBJECTIVES

1. To diagnose Pulmonary Tuberculosis in sputum smear negative patients.
2. To determine the diagnostic yield of induced sputum and bronchial aspirate in diagnosing the sputum smear negative pulmonary tuberculosis.
3. To evaluate the sensitivity, specificity, PPV, NPV of CBNAAT in sputum negative respiratory samples as compared with AFB culture.
4. To detect Rifampicin resistance pulmonary tuberculosis in sputum smear negative pulmonary tuberculosis patients.

METHODS

This is a prospective observational study done in the department of Pulmonary Medicine, Goa Medical College, from October 2016 to August 2018 after obtaining

permission from the institution ethical committee. All patients of suspected Tuberculosis according to Revised national tuberculosis control Programme algorithm were included in this study and after obtaining consent for the study patients underwent sputum induction and flexible video-bronchoscopy as per standard protocol.

A total of 202 patients were included in the study, all patients included in the study were Pulmonary Tuberculosis suspect had presented with complaints of cough with expectoration >2 weeks and constitutional symptoms of PTB like loss of weight, loss of appetite, Fever and hemoptysis and with Radiological features suggestive of Pulmonary Tuberculosis like infiltrative lesions, cavities, consolidation, nodules, mediastinal lymphadenopathy, CT scan showing centrilobular nodules with tree in bud appearance.

All patients with AFB smear microscopy on 2 Sputum samples reporting negative were subjected to Sputum induction with nebulization of 3% hypertonic saline under precautionary measures. 3 samples were collected in 3 different falcon tubes and sent to IRL lab for AFB smear, CBNAAT and AFB solid Culture. Those patients with Induced sputum AFB smear and CBNAAT negative cases were further evaluated with Fibreoptic Bronchoscopy.

Fibreoptic Bronchoscopy was performed under local anaesthesia with mild sedation with midazolam(1-3mg)³. Fibreoptic bronchoscopy was performed in cases who were negative for AFB Smear and CBNAAT on Induced sputum samples. With the help of CT Thorax after localizing the infected part of the lung, bronchial washings were collected in mucus traps by instillation of 20-40 cc of normal saline via the working channel of the bronchoscope followed by application of suction. Three samples of Bronchial aspirate were collected in 3 different falcon tubes. All the samples collected were subjected to concentration by modified Petroffs method in IRL lab before subjecting for AFB smear, CBNAAT and AFB solid Culture.

Inclusion Criteria

Patients with clinical and radiological features suggestive of tuberculosis and sputum AFB smear reporting negative as per RNTCP algorithm. Patients consenting for Sputum Induction and Fibreoptic Bronchoscopy

Age above 18 years.

Exclusion Criteria

Patients who are smear positive for microscopy

Patients with active hemoptysis

Patients who are having HBV, HCV, HIV coinfection

STATISTICAL ANALYSIS

Data collection was done in Epidata Manager V4.2.0.0 and Data entry client V4.2.0.0 2014. Statistical analysis was performed using SPSS Software. Descriptive statistics were performed. Sensitivity, specificity and predictive values were calculated with MedCalc for windows version 17.0. 95% confidence intervals(CIs) were calculated using Extract Clopper Pearson test. Chi square test was used and the p value <0.05 was statistically significant.

RESULTS

A total of 202 patients are included in study who were suspected pulmonary tuberculosis as per RNTCP algorithm. The baseline demographic, clinical and radiological characteristics of patients included in study are shown in Table 1. The total number of patients studied in our study were 202 cases. Males were most commonly affected than females i.e 64.9% and females were 35.1%.

The most common symptom was cough with expectoration seen in 99% individuals. Multiple heterogenous opacities were noted on chest radiography in majority of patients i.e 65.3% of cases as details provided in Table 1.

The overall diagnostic yield of AFB smear, CBNAAT and AFB Culture were 41/202(20.29%), 134/202(66.33%) and 144/202(71.28%) respectively as shown in Table 2. The diagnostic yield of AFB smear on induced sputum was 15.34%(31/202) and yield increased by 9.90%(10/102) after subjecting patients to fiberoptic bronchoscopy procedure. The diagnostic yield of CBNAAT on Induced sputum was 46.53%(100/202) and yield increased by 33.33%(34/102) on diagnosing by fiberoptic bronchoscopy using bronchial aspirate CBNAAT samples in negative Induced sputum CBNAAT patients. The diagnostic yield of AFB Culture on Induced sputum and BA were 51.98%(105/202) and 38.23%(39/102) respectively.

Table 1. Baseline characteristics of Patients evaluated for Sputum smear negative Pulmonary Tuberculosis.

Gender	No. of patients	Percentage
Female	131	64.9%
Male	71	35.1%
Age(years)	No. of Patients	Percent
10 – 24	35	17.3%
25 – 39	54	26.7%
40 – 54	63	31.2%
55 – 69	41	20.3%
70+	9	4.5%
Symptoms	No. of patients	Percentage
Cough with expectoration	200	99%
Fever	121	59.9%
Loss of appetite	188	93%
Loss of weight	165	81.6%
Hemoptysis	25	12.3%
Radiological features	No. of patients	Percentage
Consolidation	102	50.4%
Cavitation	56	27.7%
Mediastinal lymphadenopathy	53	26.2%
Multiple opacities	132	65.3%
Centrilobular nodules	129	63.8%
Military nodules	4	1.9%
H/O Old PTB	63	31.18%
Diabetes Mellitus	64	31.68%

Table 2. Diagnostic yield of induced sputum and bronchial aspirate using AFB smear, CBNAAT and AFB culture

Diagnostic yield	Induced Sputum	Bronchial Aspirate
AFB Smear	(31/202) 15.34%	(10/102) 9.90%
CBNAAT	(100/202) 46.53%	(34/102) 33.33%
AFB Culture	(105/202) 51.98%	(39/102) 38.23%

The sensitivity and specificity of CBNAAT on induced sputum and bronchial aspirate when compared against AFB Culture was 84.2% and 84.6% and 98.0% and 98.4% on BA respectively, whereas sensitivity and specificity of AFB smear on induced sputum when compared against

AFB Culture was 28.5% and 98.97% and on BA samples were 26.31% and 100% as shown in Table 3 with 95% confidence interval. All the results analysed were statistically significant with p value <0.005.

Table 3. Diagnostic performance of AFB smear and CBNAAT on induced sputum and bronchial aspirate when compared against induced sputum and bronchial aspirate AFB Culture with 95% Confidence Interval:

Samples	Induced sputum AFB smear	Induced sputum CBNAAT	BA-AFB smear	BA-CBNAAT
Sensitivity	28.57% (20.18 % to 38.21%)	89.52% (82.03% to 94.65%)	26.31% (13.40% to 43.10%)	84.6% (69.47% to 94.14%)
Specificity	98.97% (94.39 % to 99.97%)	93.81% (87.02% to 97.70%)	100% (94.31% to 100%)	98.4% (91.47% to 99.96%)
PPV	96.77% (80.66 % to 99.54%)	94.00% (87.80% to 97.15%)	100% -	97% (82.46% to 99.57%)
NPV	56.14% (53.10 % to 59.13%)	89.22% (82.51% to 93.55%)	69.2% (65.04% to 73.12%)	91.1% (83.18% to 95.57%)

PPV-Positive predictive value, NPV- Negative predictive value

Among 202 cases 100(49.5%) samples of induced sputum CBNAAT revealed MTB detected and among them 98 cases were RIFAMPICIN sensitive and 2 cases were RIFAMPICIN resistant. Remaining 102 cases underwent fibreoptic

bronchoscopy and bronchial aspirate CBNAAT revealed MTB detected in 34 cases(33.33%) and among them 32 were RIFAMPICIN sensitive and 2 were RIFAMPICIN resistant as shown in Figure 1.

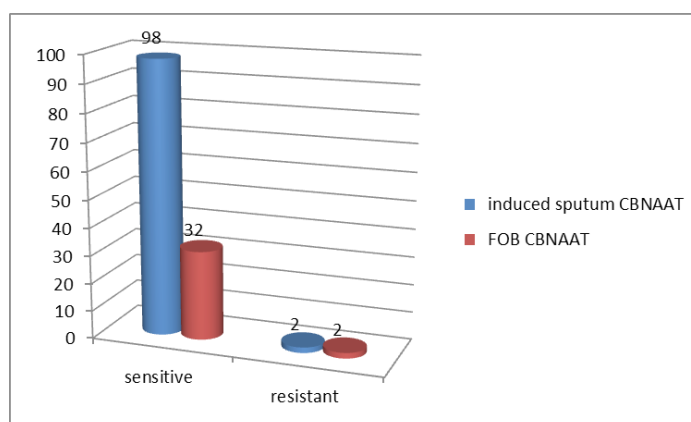


Figure 1. Rifampicin status in CBNAAT positive cases using Induced sputum and Bronchial aspirate

In this study around 17 patients were culture positive and CBNAAT negative, among 17 cases 3 patients(17.64%) were diagnosed to have Non tuberculous mycobacterial pulmonary disease on AFB culture.

DISCUSSION

In present study males were commonly affected than

females and are compared with other studies as shown in Table 4. Cough with expectoration was the most common and predominant symptom seen in 99% of cases, which was comparable with nageswar et al and Nikhil et al. however in Khalil et al loss of weight was the predominant symptom seen in 57% of cases as shown in Table 5.

Table 4. Sex Distribution

Gender	Khalil et al ⁹	Theoren et al ¹⁰	Nikhil et al ¹¹	Present study
Male	38.7%	54%	60%	64.9%
Female	61.3%	46%	40%	34.1%

Table 5. Symptoms Of Pulmonary Tuberculosis

Symptoms	Khalil et al ⁹	Nageswar et al ⁴	Nikhil et al ¹¹	Present study
Cough with expectoration	44.1%	95%	100%	99%
Fever	33.3%	75%	78.3%	59.9%
Loss of appetite	46.1%	75%	58.2%	81.7%
Loss of weight	57%	79%	46.7%	92%
Hemoptysis	22.6%	15%	11.7%	12.8%

Present study induced sputum AFB smear against Induced sputum AFB culture showed sensitivity, specificity, PPV, and NPV of 28.5%, 98.7%, 96.7% and 56.17% respectively which was comparable with scoch et al¹² which showed 47%, 100%, 100% and 89.0% respectively. The results in

nageswar rao were showing sensitivity, specificity, PPV, and NPV 72.7%, 100%, 100% and 76.7% respectively. Our study had lower sensitivity compared to other studies as shown in Table 6.

Table 6. Induced sputum AFB smear compared to Induced sputum Culture

Ind. Sp. Smear	Nageswar rao et al ⁴	Scoch et al ¹²	Present study
Sensitivity	72.7%	47%	28.5%
Specificity	100%	100%	98.7%
PPV	100%	100%	96.7%
NPV	76.7%	89.0%	56.17%

In present study induced sputum CBNAAT against induced sputum AFB culture showed sensitivity, specificity, PPV, and NPV of 84.2%, 98%, 94.1% and 94.2% respectively which was comparable with sharma et al⁸ where sensitivity, specificity, PPV, and NPV of 84.2%, 98%, 94.1% and

94.9% respectively. However in mohanty et al¹³ sensitivity, specificity, PPV, and NPV of 61.9%, 96.5%, 96.3% and 63.6% respectively which had low sensitivity and NPV compared to our study as they had compared induced sputum CBNAAT against BAL culture as shown in Table 7.

Table 7. Induced sputum CBNAAT compared to Induced sputum Culture

Ind. Sp. CBNAAT	Mohanty et al ¹³	Sharma et al ⁸	Present study
Sensitivity	61.9%	84.2	84.2%
Specificity	96.5%	98.0	98%
PPV	96.3%	94.1	94.1%
NPV	63.6%	94.9	94.2%

Present study BA AFB Smear against BA Culture showed sensitivity, specificity, PPV, and NPV of 26.31%, 100%, 100% and 69.2% respectively which was comparable with

nageswar rao et al, Bernard et al and perrie et al. above mentioned studies also showed similar sensitivity and specificity compared to our study as shown in Table 8 .

Table 8. BA AFB Smear compared to BA Culture

BA smear	Nageswar rao et al ⁴	Barnard et al ¹⁴	Theoren et al ¹⁰	Perrie et al ¹⁵	Present study
Sensitivity	22.2%	41%	58.4%	25.5%	26.31%
Specificity	100%	98.6%	100%	95.8%	100%
PPV	100%	94.1%	100%	90.1%	100%
NPV	85.3%	75.8%	93.6%	45.5%	69.2%

Present study showed BA CBNAAT against BA Culture sensitivity, specificity, PPV, and NPV of 84.6%, 98.4%, 97% and 91.1% respectively and these results were comparable

with studies done by mohanty et al, nargeswar et al and Bernard et al as shown in Table 9.

Table 9. BA CBNAAT compared to BA Culture

BA CBNAAT	Bernard et al ¹⁴	Nageswar et al ⁴	Theoren et al ¹⁰	Mohanty et al ¹³	Present study
Sensitivity	92.3%	81.4%	100%	88.09%	84.6%
Specificity	87.7%	93.6%	95.9%	96.5%	98.4%
PPV	80.0%	73.3%	62.5%	97.36%	97%
NPV	95.5%	95.7%	100%	84.84%	91.1%

Our study had a different and unique approach where smear negative pulmonary tuberculosis patients were included in the study where all patients are subjected to sputum induction procedure initially. In our study 202 cases were subjected to sputum induction and among them 100 cases came positive on CBNAAT and were diagnosed as pulmonary tuberculosis and started on treatment according to RNTCP treatment protocol. However 102 cases which were CBNAAT negative were subjected to fiberoptic bronchoscopy, bronchial aspirate, from the involved segment was collected and sent for BA smear, BA CBNAAT and BA Culture. Among 102 cases BA CBNAAT was positive in 34 cases and BAL Smear was positive in only 10 cases. BA CBNAAT negative cases were kept under close follow up for BA culture. BA Culture was positive in 39 cases.

LIMITATIONS OF THIS STUDY

The study had no clinical follow-up of the patients which does not give a clinical reference for the samples tested by Xpert MTB/RIF and culture. However, our study fulfills the objective which was to evaluate the diagnostic utility of Xpert MTB/RIF assay in smear negative PTB samples using culture as the reference standard.

Comparison of procedures of induced sputum and bronchoscopy was not done in our study as all patients were not subjected to bronchoscopy, only those cases who were undiagnosed on IS i.e. only when IS CBNAAT was negative were subjected to bronchoscopy, reserving bronchoscopy for evaluation of undiagnosed cases.

In this study only spot induced samples were tested for AFB smear and CBNAAT, studies have shown that subsequent samples increase the diagnostic yield, which was not studied in our subjects

CONCLUSION

Our study shows that CBNAAT has high sensitivity and specificity for diagnosis of smear negative pulmonary tuberculosis comparable to AFB culture. CBNAAT detected more number of cases of PTB compared to AFB smear microscopy. Hence all patients should be evaluated with CBNAAT as per guidelines which has increased diagnostic yield and also helps in diagnosing RIFAMPICIN resistant Tuberculosis. Using procedures like induced sputum further increases yield of diagnosis and is a powerful tool in diagnosis of smear negative PTB suspects reserving invasive procedure like bronchoscopy (BA-CBNAAT) for undiagnosed cases.

Acknowledgement

The authors would like to thank all the patients and their

relatives for providing consent and participation in the study.

Conflict of Interest

There are No conflicts of interest.

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Cite this article: Ashwini Chawhan, Sanjivani Keny, Durga Lawande, et al. *Diagnostic Yield of Induced Sputum and Bronchial Aspirate in Sputum Smear Negative Pulmonary Tuberculosis Patients. International Journal of Research in Medical and Clinical Sciences*. 2025; 3(2): 84-90.

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