# TO STUDY THE USEFULNESS OF CBNAAT (CARTRIDGE BASED NUCLEAR ACID AMPLIFICATION TEST) IN BAL (BRONCHOALVEOLAR LAVAGE) SAMPLES IN THE DIAGNOSIS OF SMEAR-NEGATIVE/NON SPUTUM PRODUCING PATIENTS WITH SUSPECTED TUBERCULOSIS

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**ABSTRACT**

**BACKGROUND**

Sputum smear negative pulmonary tuberculosis remains a significant burden with a definite role in disease transmission too. They sometimes pose a diagnostic challenge to the treating physician. CBNAAT, a newly endorsed WHO technique, which not only detects the tubercle bacilli but also tells about the resistance to rifampicin, may have a role in sputum smear negative patients if bronchoalveolar lavage fluid is made available.

**MATERIAL AND METHODS**

Clinico-radiologically suspected patients of pulmonary tuberculosis who were either sputum negative or not bringing out adequate sputum sample were included in the study. Included patients who do not have contraindications to bronchoscopy were subjected to the procedure and lavage fluid was obtained. Smear and CBNAAT examination of the fluid were done. The data recorded was then analysed statistically.

**RESULT**

In our study out of total 72 cases, 56.9% were male while 43.1% population were of female. Majority of patients belonged to Urban (86.1%) as compared to rural area (13.9%). The most common lesions detected by chest imaging were consolidation (33.3%) followed by fibrocavitary (11.1%). Otherwise not specified opacities constituted about 27.8%. Out of 37 bacteriologically confirmed cases 3 were positive in BAL smear microscopy, while 34 were positive by CBNAAT. Out of 34 CBNAAT positive samples, 3 were resistant to Rifampicin.

**CONCLUSION**

CBNAAT done on broncho-alveolar lavage fluid obtained via bronchoscopy can be an important adjunct to bacteriological confirmation of suspected cases who were otherwise sputum negative or not bringing adequate sputum sample. Moreover resistance to rifampicin can be detected prior to the treatment.

**KEYWORDS**

CBNAAT, Tuberculosis, BAL, Gene Xpert.

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## INTRODUCTION

Tuberculosis is one of the oldest diseases known to human being, still causing a large number of mortality and morbidity throughout the developing world. Many patients presenting with active Pulmonary Tuberculosis (PTB) may, however, exhibit negative sputum Acid-Fast Bacilli (AFB) smears. In low Tuberculosis (TB) burden country like France 73% of all TB cases were pulmonary tuberculosis. Among them about 50% were sputum smear negative.[1] In our institute in 2012 out of 519 of PTB case 167 were sputum negative, 130 cases were sputum negative out of 399 PTB cases in 2013 whereas in 2014 out of 439 of all PTB cases 227 were diagnosed as sputum negative PTB.

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Thus about more than half of the cases were sputum negative in the last three years.

A substantive number of pulmonary tuberculosis patients remain undiagnosed by conventional sputum microscopy. These cases also play an important role in the disease transmission. Moreover on the basis of Chest radiography only as the diagnostic tool, many patients are wrongly started on Anti-Tubercular Treatment (ATT). In the above two situations, Cartridge Based Nuclear Acid Amplification Test (CBNAAT) of BAL (Bronchoalveolar Lavage) looks convincing as a good diagnostic method for the purpose of diagnosing or ruling out pulmonary tuberculosis. By detecting active pulmonary TB early, an appropriate treatment can be initiated, lung damage can be prevented and disease transmission can be pre-emptively blocked. Fiberoptic bronchoscopy is considered a good option for these cases that pose a diagnostic challenge.[2] although smear microscopy is still exhibiting low sensitivity on fiberoptic bronchoscopy samples with 5-35% on Bronchial Aspirates (BA) and 10-30% on Bronchoalveolar Lavages (BAL).[3,4]

Furthermore, while mycobacterial culture remains the gold standard for laboratory diagnosis of TB, it requires 2–6 weeks to confirm a diagnosis and there are fewer accredited labs. This results in delay in initiating appropriate treatment while waiting for the confirmation, except for the cases where there is strong clinical suspicion to initiate a presumptive anti-TB therapy. Several Polymerase Chain Reaction - (PCR) based molecular methods have recently been developed for early TB diagnosis and rapid detection of drug resistance from clinical specimens.[5,6] The CBNAAT (Cartridge Based Nuclear Acid Amplification Test) is one of these methods and consists of a hemi-nested real-time PCR test that simultaneously identifies mycobacterium tuberculosis and detects rifampicin resistance as a surrogate of Multidrug Resistance (MDR), directly from clinical specimens. Since December 2010, WHO has recommended the CBNAAT as a bona fide test due to its high-quality performance as compared to microscopy, especially in cases of smear-negative cases.[7]

## MATERIAL AND METHODS

This study was to evaluate the diagnostic value of the CBNAAT on BAL samples obtained through fiber-optic bronchoscopy for an early diagnosis of pulmonary TB in patients with either negative sputum smear for AFB or who could not produce an expectorated sputum sample.

### Inclusion Criteria

Patients with clinical suspicion of PTB based on symptoms (e.g., cough more than two weeks, hemoptysis, fever, asthenia, loss of weight and night sweats) or radiological features (e.g., nodule, consolidation, cavation and other opacities) who either have a negative sputum AFB smear microscopy or were unable to produce sputum were included in the study.

### Exclusion Criteria

Sputum positive cases, isolated extra-pulmonary tuberculosis, HIV positive patients and patients not fit for bronchoscopy procedure, e.g. those having refractive hypoxemia, bleeding disorders, cardiovascular instability, status asthmaticus and marked hypercapnia were excluded from the study.

### Selection of Cases

Patients having clinical or radiological suspicion of pulmonary tuberculosis who met both inclusion and exclusion criteria were then planned for a diagnostic fiberoptic bronchoscopy procedure at MY Hospital, Indore. In this group, we included those cases whose symptoms did not resolved completely after appropriate antibiotic treatment.

### Bronchoscopic Procedure

Pre-operative procedure included overnight fasting, lignocaine sensitivity test, Inj. ondansetron administration, intravenous drip, intramuscular atropine administration, oropharyngeal local 2% lignocaine spray, intra-nasal application of 5% lignocaine jelly.

After taking informed consent, under all aseptic precautions bronchoscopy was performed via transnasal route using a flexible fiberoptic bronchoscope by a trained chest specialist to collect BAL specimens. Lignocaine 2% was used as local anaesthesia to anaesthetize vocal cords and bronchial tree. The lung section samples were chosen based on chest X-ray or CT-scan abnormalities. BAL samples were obtained after instillation of 100-200mL isotonic saline in serial aliquots using 10ml disposable syringe with bronchoscope wedged in a segmental or sub-segmental bronchus and then aspirated. BAL samples thus obtained were then analyzed by microscopic examination and by CBNAAT.

### Microscopic Examination

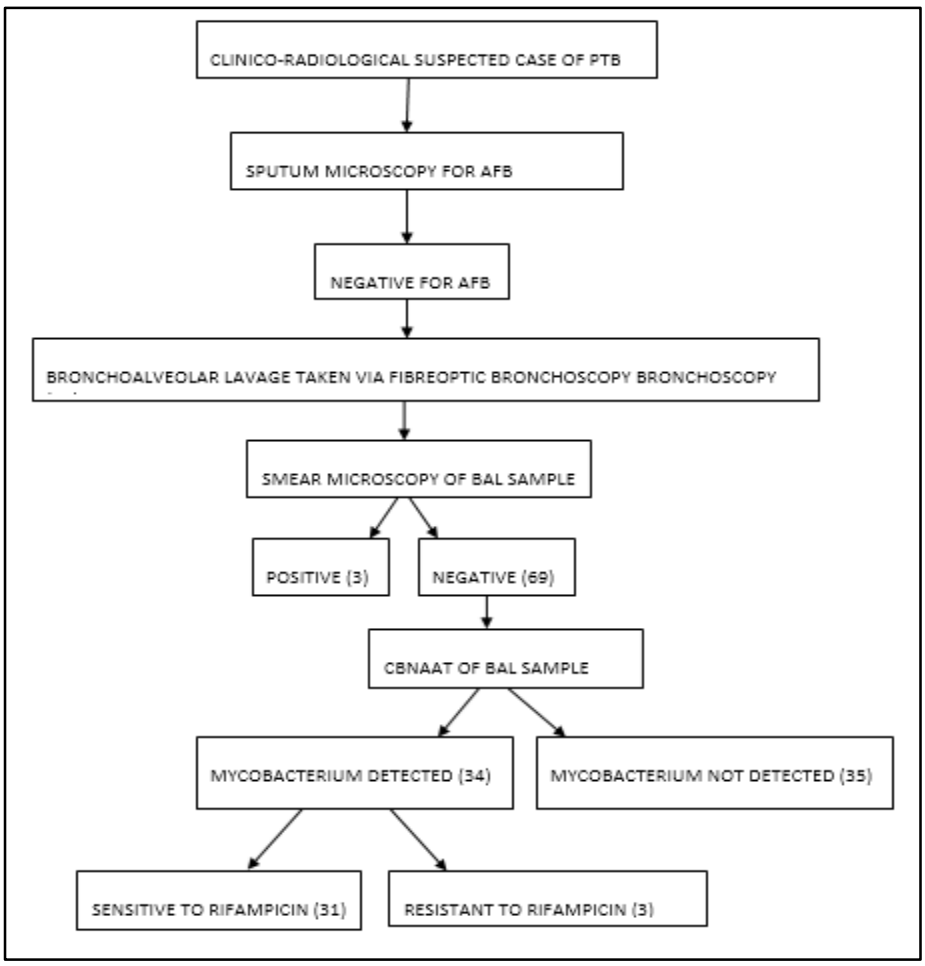
For the smear examination, fixed preparations were stained with ZN staining and visualized under an oil field microscope (At 400x magnification). Each slide was observed for 5-10min, corresponding to 100 fields examined. Samples which were negative for AFB microscopy underwent CBNAAT.

### CBNAAT

For the CBNAAT, a 500µL BAL sample was poured into a single-use disposable cartridge that is placed into the Xpert Dx module with the results produced in less than 2 hours. Each PCR run comprised an internal control for sample processing (DNA extraction) and PCR validity (Presence of inhibitors) with positive and negative controls tested everyday. The system automatically interpreted all results from measured fluorescent signals, with embedded calculation algorithms, into the following categories: invalid, if PCR inhibitors are detected with amplification failure; negative or positive. If positive, the strain was categorized as susceptible or resistant to rifampicin.

## RESULTS

In our study among all 72 sputum negative suspected PTB patients underwent bronchoscopy and BAL samples were collected. All the 72 BAL samples underwent smear microscopy and 3 smear samples came positive of AFB, remaining 69 BAL samples which were negative for smear microscopy underwent CBNAAT. Among these 69 BAL samples, mycobacterium tuberculosis was detected in 34 BAL samples. Among these 34 BAL samples, 31 BAL samples were sensitive to rifampicin while 3 were resistance. Rest 35 BAL CBNAAT negative patients were kept in follow-up on antibiotic therapy, on the basis of BAL culture and sensitivity report and majority of them were improved.




| Demographic Characters | Frequency | Percentage |
|---|---|---|
| Male | 41 | 56.9 |
| Female | 31 | 43.1 |
| Urban | 62 | 86.1 |
| Rural | 10 | 13.9 |

***Table 1: Demographic Characteristics***

| Symptoms | Frequency | Percentage |
|---|---|---|
| Cough | 52 | 72.2 |
| Hemoptysis | 7 | 9.7 |
| Loss of appetite | 43 | 59.7 |
| Weight loss | 36 | 50 |
| Fever | 50 | 69.4 |
| Night sweats | 14 | 19.4 |

***Table 2: Presenting Symptoms***

| Radiological Characters | Frequency | Percentage |
|---|---|---|
| Consolidation | 24 | 33.3 |
| Fibrocavitary lesions | 08 | 11.1 |
| Nodules | 02 | 2.8 |
| Mass | 02 | 2.8 |
| Interstitial shadows | 02 | 2.8 |
| Thick wall cavity | 02 | 2.8 |
| Bronchiectasis changes | 05 | 6.7 |
| Other opacities | 20 | 27.8 |
| Normal | 07 | 9.7 |

***Table 3: Radiological Features***

| Results | Frequency | Percentage |
|---|---|---|
| Total cases | 72 | 100 |
| Bacteriologically confirmed PTB | 37 | 51.4 |
| BAL Smear positives | 03 | 4.2 |
| BAL Smear negative, but CBNAAT positive | 34 | 47.2 |
| Rifampicin resistant | 03 | 4.2 |
| Rifampicin sensitive | 31 | 43.1 |

***Table 4: Results of Tests***

| Total clinic-radiological PTB suspects (n= a+b+c) | Sputum Smear positive | BAL smear positive (a) | CBNAAT positive (b) | Total Bacteriological confirmed cases (a+b) | Neither BAL smear nor CBNAAT positive (c) |
|---|---|---|---|---|---|
| 72 | 0 | 3 | 34 | 37 | 35 |

***Table 5: Comparison of CBNAAT with smear microscopy***

**Discussion**

Sputum negative pulmonary tuberculosis constitutes about 50% of all new cases of pulmonary tuberculosis. Although the relative transmission rate of smear negative tuberculosis is lower than that of smear positive cases, it is still responsible for 17% of tuberculosis transmission.[8]

Conventional laboratory techniques like direct microscopy are less sensitive and going for culture is a time consuming process for the diagnosis of tuberculosis.

Therefore it is the need of time to develop new techniques for rapid identification of the Mycobacterium tuberculosis in pauci-bacillary samples. Recently, attention has been devoted to latest nucleic acid amplification diagnostic processes due to their speed and accuracy.

| Techniques | Advantages | Disadvantages |
|---|---|---|
| Ziehl-Neelsen Microscopy | Cheap | At least 10,000 bacili per ml of sputum is required Low sensitivity in paucibacillary samples |
| Fluorescent Microscopy | •More samples can be examined than ZN microscopy •Good for high load settings (>25 smear per day) •Require less time than ZN microscopy | •Costly compared to ZN Microscopy |
| Solid Culture (LJ) | •Sensitivity 80-85% & Specificity 98% •Ideal for diagnosis and follow-up •Applicable for MDR-TB & XDR-TB | •Growth takes 6-8 weeks •Lengthy time for certification • Required trained manpower |
| Liquid Culture (MGIT) | •More sensitive & can be positive even when bacterial load is low(10-100 bacilli/ml) •Rapid detection (4-21 days) and DST (15-28 days) •Applicable for diagnosis and follow-up •Applicable for MDR-TB & XDR-TB | •BSL-III facility essential •Continuous power supply •Trained Manpower •Higher contamination |
| Molecular DST(LPA) | •Rapid turnaround time (TAT), within 5days •Highly sensitive and specific (99% RIF &80%INH) •High Out-put laboratory with GT-BLOT (40 test per day) | •Trained manpower •Only applicable for smear positive TB patients •Labour Intensive •Detects only for first line Drug (H&R) •Only for diagnosis |
| CBNAAT | •Rapid turnaround time within 2 hours •require bio-safety conditions similar to the conventional sputum smear microscopy sample •Minimal Training for LT •Inbuilt quality control for process | •Stable electricity supply •Require ambient operating temperatures max. 30C •Only detects Rifampicin resistance •Annual Calibration •Only for diagnosis |

***Table 6.[9]: Comparison of various method for PTB diagnosis***

A 2013 Cochrane systematic review showed that this test is highly accurate.[10] when compared to culture, Xpert has about 88% sensitivity and 98% specificity for pulmonary TB in adults. In smear-negative patients with TB, Xpert had a sensitivity of 67%. For rapid detection of rifampicin resistance, the sensitivity is 94% and specificity is 98%. As per Panayotis Ioannidis et al.[11] positive and negative predictive values of GeneXpert MTB/RIF assay for the pulmonary are 93.5% and 91.7% and for the extrapulmonary samples, they are 50% and 100%, respectively. For microscopically negative specimens, the respective values are 79% and 95.6%.

In our study out of total 72 cases, 56.9% were male while 43.1% population were of female. Majority of patients belonged to Urban (86.1%) as compared to rural area (13.9%). Most common symptom in our study was cough (72.2%) followed by fever (69.4 %). In a similar study from France Le Palud et al. in 2014 found cough (51.9%) as the main symptom followed by general symptoms (45.1%).[12] In majority of patients in our study more than two symptoms were present. The most common lesions detected by chest imaging were consolidation (33.3%) and fibrocavitary diseases (11.1%). Otherwise, not specified opacities constituted about 27.8%. Chest imaging was normal in 9.7% cases, whereas in study by Le Palud et al. nodules (53.7) were the most common radiological finding followed by Pneumonia (27.1%).[12]

Out of 72 clinico-radiologically suspected patients, only 3 were BAL smear positive while another 34 were further detected by CBNAAT giving a total bacteriological figure of 37. Out of 34 CBNAAT positive samples, 3 were resistant to Rifampicin. Raquel Moure et al. in their research in 2012 concluded that out of 108 smear-negative extrapulmonary samples 58.3% were positive with the Xpert MTB/RIF assay (GX) for Mycobacterium tuberculosis.[13] In a similar study by Vadwai in 2011, the sensitivity of the Xpert assay was 64% for smear-negative cases.[14] Our results are little lower (47.2%) than these two study as we had not performed CBNAAT on BAL smear AFB positive samples and our sample size was much less as compared to other studies.

Lee et al. recruited 132 patients in a single South-Korean centre and reported sensitivity and specificity values (relative to the culture) of 81.6% and 100.0% for the Xpert® MTB/RIF assay, compared to 13.2% and 98.8% respectively for smear microscopy.[15] Theron et al. In their study of 154 patients in a South-African single-centre study analysed the BAL samples in which sensitivity and specificity values compared to the culture were 92.6% and 96.0% for the Xpert® MTB/RIF assay, and 57.7% and 99.3% for SM, respectively.[16] Le Palud et al. concluded that as compared to culture, sensitivity and specificity values were 80.0% and 98.6% for the Xpert® MTB/RIF assay.[12]

**CONCLUSION**

In summary, our study confirmed the usefulness of the Xpert® MTB/RIF assay (CBNAAT), compared to Smear Microscopy, for the early diagnosis of suspected pulmonary TB requiring fibre-optic bronchoscopy, performed on per procedure samples. Gene Xpert MTB/RIF assay is efficient and reliable technique for the rapid smear negative cases. Its simplicity, sensitivity, speed and automation, make this technique a very attractive tool for diagnosis of Mycobacterium tuberculosis from smear negative cases of TB suspects. Meanwhile it has an added advantage of detection of multi-drug resistant cases.

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