

# To Study the Usefulness of CBNAAT(Cartridge based Nuclear Acid Amplification Test) in BAL (Bronchoalveolar Lavage) Samples for the Diagnosis of Smear-Negative / Non Sputum Producing Patients with Suspected Tuberculosis

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**Abstract :** ***Background:** sputum smear-negative and sputum scarce pulmonary tuberculosis is a diagnostic challenge. .CBNAAT provide a early diagnosis on BAL specimen obtained by bronchoscopy. **Methodology:** It is a prospective observational study. Conducted in 140 Patients of suspected pulmonary TB diagnosed on the basis of clinical radiological with either negative sputum smear for AFB or who could not produce an expectorated sputum in the department of pulmonary medicine, Kamala Nehru Chest Hospital, Jodhpur to evaluate the diagnostic role of the CBNAAT in BAL sample. **Results:** Out of 140 patients 83 patient had MTB detected in BAL by CBNAAT and 10 AFB smear microscopy positive .among 83 CBNAAT positive samples, 9 (10.84%)were resistant to Rifampicin. **Conclusion:** Our study demonstrated that BAL for CBNAAT has a superior diagnostic value than BAL AFB smear microscopy and also help in early diagnosis of smear negative pulmonary tuberculosis and help on differentiation of other disorder where clinical picture mimic tuberculosis.*

**Keywords:** Cartridge based nuclear acid amplification test, bronchoalveolar lavage, flexible fiberoptic bronchoscopy

## 1. Introduction

Tuberculosis remains a major global health problem causing significant morbidity and mortality in 2017, there have been 10.0 million people developed TB disease and 558 000 people developed TB that was resistant to rifampicin (RR-TB), and 1.3 million deaths among HIV-negative people and there were an additional 300 000 deaths from TB among HIV-positive people<sup>1</sup>

At present Sputum smear microscopy for Acid Fast Bacilli (AFB) has been the mainstay for the diagnosis of pulmonary tuberculosis (PTB) and chest X-Ray. Sputum smear for acid-fast bacilli (AFB) is the most reliable tool for diagnosis of PTB. It has a very poor sensitivity (30–70%) despite high specificity (98–99%).<sup>5</sup> From the available published evidence, it has been observed that the number of bacilli in the sputum correlates well with sputum smear microscopy results when the smears are prepared properly and examined. Sputum microscopy is a highly specific test, a low-cost, appropriate technology but sputum smear microscopy alone, although inexpensive, misses many patients and detects only those with relatively advanced disease, having a bacillary load

of at least 10,000 bacilli per ml of sputum. Culture for AFB in Lowenstein-Jensen medium is considered as the gold standard test for detection of pulmonary tuberculosis, but it is time consuming and takes about 6 to 8 weeks. Also it requires 100 AFB bacilli per ml of sputum to be culture positive.<sup>2</sup>

Only about 20 - 40% of pulmonary TB patients are smear positive, while rest of the patients had either smear negative or sputum-scarce disease.<sup>3</sup>

A patient with symptoms suggestive of TB whose two sputum smear examination is negative for AFB, with evidence of pulmonary TB by Chest X ray or microbiological method (approved molecular methods or culture positive) is classified as smear negative pulmonary tuberculosis. Lack of sputum production, low bacterial load and improper technique can be the factors for SSN-TB<sup>4</sup>

Bronchoalveolar lavage is sent for Acid Fast Bacilli (AFB smear by ZN stain) and mycobacterial cultures. The sensitivity of ZN stain remains low (41%). Mycobacterial cultures, considered as the gold standard (with 86%

sensitivity) but they are expensive and results take 6 – 8 weeks for diagnosis.<sup>5</sup>

Recently introduced Cartridge based nuclear acid amplification test (CBNAAT) was endorsed by WHO in December 2010 which can detect Mycobacterium tuberculosis complex and RIF resistance directly from clinical samples has been shown to be rapid, yielding results in less than 2 hours. The assay based on nested real-time PCR and molecular beacon technology is not prone to cross-contamination, requires minimal biosafety facilities, can be performed by technicians with little training; and has high sensitivity in smear-negative pulmonary TB.<sup>6</sup>

In our study we used the BAL Sample CBNAAT taken by fibre optic bronchoscopy as primary tool for diagnosis of smear negative pulmonary tuberculosis and early treatment renders those patients non infectious, interrupts the transmission of TB and reduce the incidence of MDR-TB in those patient.

## 2. Material and Methods

The study will be conducted in Kamla Nehru Chest Hospital, Dr S N Medical college Jodhpur, a tertiary care center for respiratory diseases in western part of Rajasthan, India.

This will be a prospective observational study.

140 Patients of suspected pulmonary TB diagnosed on the basis of clinic radiological with either negative sputum smear for AFB or who could not produce an expectorated sputum sample admitted in department of pulmonary medicine of our hospital, who are willing to participate in the study will be enrolled after proper counseling. The protocol will be explained to the patient/care provider before enrolment and informed consent will be taken from each patient.

The study was approved by the ethical committee.

### Patient evaluation

Baseline demographic, clinical radiological and laboratory information were recorded

### Bronchoscopy and Bronchoalveolar lavage sample

After taking informed consent, under all aseptic precautions bronchoscopy was performed via trans-nasal route using a flexible fibre-optic 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 10-50 ML isotonic saline using 10 ml 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.

## 3. Results

**Table 1:** Baseline characteristics of study population

Characteristics	N%
Age in year	38+/_5
Sex male	106
female	34
Past history of ATT taken	64

**Table 2:** Distribution of symptoms among study population

Symptoms	No. of patients	Percentage
Cough	133	95
Expectorant	66	47.14
Chest pain	51	36.43
Fever	71	50.71
Hemoptysis	39	27.86
Weight Loss	40	28.57
SOB	36	25.71

In our study 133 (95%) patients has cough, and with expectorant 66(47.14%), fever 71 (50.71%),chest pain 51 (36.43), wight loss 40(28.57%),hemoptysis 39(27.86%) ,sob 36(25.71%)

**Table 3:** Chest x-ray abnormality in study population

Abnormality	Total	
	N	%
Consolidation	48	34.28
other opacity	38	27.14
Fibrocavity	18	12.85
Pleural effusion with collapse	12	8.57
Infiltrates	6	4.21
mass	4	2.85
reticular nodule	4	2.85
collapse	2	0.71
Normal chest xray	8	5.71

In our study out 140 subjects most common chest xray finding consolidation n=48 (34.28%) following other opacity n=38 (27.14%), fibrocavity 18 (12.85%),pleural effusion with collapse n=12(8.57%),infiltrates n=6(4.21%), mass 4 (2.85%) collapse n=2(0.71%) while normal chest x ray in 8(5.71%) subjects.

**Table 4:** Chest x-ray laterality finding in study population

Detect	Total	
	N	%
Right	83	59.29
Left	35	25
B/L	14	10
NAD	8	5.71
Total	140	100

In our study most common side involvement were right n=83(59.29%) While left sided involvement were n=35(25%) and bilateral were 14(10%)

**Table 5:** Chest x-ray zone involvement in study population

Zone	Total	
	N	%
Upper Zone	45	32.14
Mid Zone	28	20
Lower Zone	28	20
Hilar	20	14.29
Mid Zone and Lower Zone	9	6.43
Upeer Zone and Mid Zone	2	1.42
Normal	8	5.71
Total	140	100

In our study out of 140 subjects most of subjects were upper zone involvement n=45(32.14%). lower zone and mid zone involvement equally n=28(20%) and hilar involvement observed in 20 (14.29%).

**Table 6:** Chest x-ray lesion in study population

Chest X-ray Classification	Total	
	N	%
Normal 0	9	6.42
Minimal 1	72	51.42
Moderate 2	35	25
Advanced 3	24	17.14
Total	140	100

In our study most commonly subjects were mild lesion n=72(51.42%) following moderate n=35(25%), advanced 24(17.14%)

**Table 7:** FOB finding in BAL specimen MTB detected patients

S. No	Fob finding	No.	Percentage
1	Hyperemia and congestion mucosa	28	33.73
2	Pus or abnormal secretion	18	21.68
3	Ulceration	4	4.81
4	Loss of normal architectural of tracheobronchial tree	2	2.4
5	Narrowing of bronchus	2	2.4
6	White cheese like material	4	4.81
7	normal mucosa	25	30.12
total		83	

In our study 83 BAL specimen MTB detected subjects most common abnormal FOB finding were hyperemia and congestion n=28(33.73%), following pus or abnormal secretion n=18(21.68%), ulceration and white cheese like material in n=4(4.81%), loss of normal architectural of tracheobronchial tree and narrowing of bronchus n=2(2.4%) observed normal mucosa were seen in n=25(30.12%) subjects.

**Table 8:** Distribution of CBNAAT result in study population (N=140)

CBNAAT	No. of patients	Percentage
Detect	83	59.29
Not detect	57	40.71
Total	140	100.00

In our study out of 140 subjects 83 (59.29%) were BAL specimen CBNAAT MTB detected

**Table 9:** CBNAAT MTB detected result in relation to Rifampicin resistance (n=140)

CBNAAT	No. of patients	Percentage
Detect	83	59.29
Not detect	57	40.71
Total	140	100.00

**P value 0.010 (S)**

In our study out of 83(BAL specimen CBNAAT positive) patients 9 (10.84%) were rifampicin resistance

**Table 10:** Test result in comparison to BAL AFB smear and BAL CBNAAT

Results	Frequency	Percentage
Total case	140	100
confirmed PTB	83	59.28
BAL AFB Smear Positives	10	7.14
BAL CBNAAT Positive	83	59.28
Rifampicin resistant	9	10.84
Rifampicin sensitive	74	89.15

In our study out of 140 subjects confirmed PTB were 83 (59.28%). out of 83 subjects n=10(7.14%) were BAL for AFB smear positive and 83 (59.29%) subjects were cbnaat detected

#### 4. Discussion

The present study was conducted at Kamla Nehru chest hospital (Department of Tuberculosis & Respiratory Medicine), Dr.S.N medical college JODHPUR which included 140 patients with sputum smear negative and clinico-radiological suspected tuberculosis. All patients were subjected to Flexible fiberoptic Bronchoscopy. Out of 140 patients, 83 (59.29%) were diagnosed with pulmonary tuberculosis. Majority of the patients were males and were in the (31-40) age group. 94 (67.14%) were smoker and 64(46%) history of ATT.

In our study out of 140 patients, among the abnormal radiological findings most common finding was consolidation 48 (34.28%), followed by opacity 38 (27.14%), fibro cavity 18 (12.85%), pleural effusion with collapse 12(8.57%), infiltrates 6(4.21%), mass 4 (2.85%), collapse n=2(.71%) and while normal chest x ray in 8(5.71%) subjects respectively. Similar study by **Sanajy avashial et al**<sup>7</sup> reported on 74 patients the most common chest x ray finding was consolidation 24(33.3%), fibrocavitary lesion 08(11.1%), nodules 02(2.8%), mass 02 (2.8%) interstitial shadows 02(2.8%), thick walled cavity 2 (2.8%) bronchiectatic changes 05 (6.7%) other opacity 20 (27.8%) normal chest xray 7(9.7%) respectively. **Study conducted by, k.f khalil et al**<sup>8</sup>, **B.Hazarika et al**<sup>9</sup>, **D barnard**<sup>10</sup> et al also found that most common chest radiology abnormality was consolidation and fibrocavity lesion ranging 10-12% of patients.

Also our study concluded that right lung involvement was maximum i.e 83(59.29%), followed by left sided involvement 35(25%) and bilateral involvement 14(10%). Similar study by **Chodhary et al**<sup>11</sup> on 108 patients found that the lesion most commonly was on right sided 58 (53.70%) followed by left sided 29 (26.85%) and bilateral in 4 (3.84%) patients respectively.

Also our study concluded that out of 140 patients chest radiography, maximum were showing upper zone involvement 45(32.14%) followed by equal involvement of lower zone and mid zone 28(20%) and hilar involvement observed in 20 (14.29%). Pulmonary tuberculosis typically involves the apical and posterior segments of the upper lobes of patients. And the anterior segment of the upper lobe and anterior segment of the lower lobe being less involved as the mycobacterium bacilli is aerobic in nature.

Our study also concluded that most patients chest xray were having mild lesion 72(51.42%) followed by moderate lesion 35(25%) and advanced lesion 24(17.14%) respectively . Similar study by **Chodhary et al<sup>12</sup>** on 108 patients had chest Xray lesion mild, moderate and advanced lesion 53(49.07%),23(21.29%) and 9 (8.33%) respectively. Hence Radiologic findings for pulmonary TB are not diagnostic but rather suggestive of PTB. The limitation of our study was not that we could not Xray findings with CT(computed tomography)

On Endobrochial examination by FOB 83 out of 140 BAL specimens in whom MTB was detected the most common abnormal FOB finding were hyperemia and congestion 28(33.73%),following pus and increased secretion ,18(21.68%) had ulceration 4 (4.81%),white cheese like material 4 (4.81%), loss of normal architecture of tracheobronchial tree 2(2.4%) and narrowing of bronchus 2(2.4%) was observed and normal mucosa was seen in 25(30.12%) subjects Hyperemia and congested mucosa with mucopurulent secretions was noted in majority of patients .

Similar study done by **Chodhary et al<sup>13</sup>** observed that bronchoscopic findings were: Congestion and hyperemia in majority 41 (37.96%) patients, 21 (19.44%) patients had Granulomatous and Tubercles with Caseous Material,19 (17.59%) patients had narrowing of segmental opening, Ulceration and erosion was seen in 16(14.81%) patients ,bleeding from the bronchus in 7(6.48%) patients , 6 (5.56%)patients with Necrotic red Patches with Sloughed Mucosa and endobronchial growth was seen in 5(4.63%) patients. A study by **Altaf Bachh, A et al<sup>14</sup>** 70.6% of cases showed congestion with hyperemia of bronchial mucosa on bronchoscopy, 28% of patients had erosion, ulceration and granulation on bronchoscopy, all of these were diagnosed as active pulmonary tuberculosis by demonstration of acid fast bacilli in bronchial washing smear or culture or postbronchoscopy sputum .**Kulpati et al<sup>15</sup>**, observed the coating of mucosa of involved segments with yellowish white secretions in almost all patients and also revealed mild to moderate hyperemia after bronchial wash. Segmental bronchus was narrowed in 20% patients, and ulceration was seen in 20% patients. **Purohit et al<sup>16</sup>**, reported ulceration in 64% of patients; 60% had frothy secretion from the bronchus. A moderate hyperemia of bronchial mucosa was observed in all the patients. Similar observations were made by **Panda et al<sup>17</sup>**, according to their study, 44% had normal bronchial mucosa, 21% had unhealthy mucosa with granulations, 35% had discharge of mucous from bronchus, five per cent had growth, three per cent had external compression and three per cent had bleeding from bronchus and some cases had multiple findings.

To conclude FOB finding is non specific by appearance alone but is helpful in diagnosis by showing different stages of EPTB and hence Bronchoscopic morphological features have prognostic significance.

Out of 140 patients 83 patients had MTB detected in BAL by CBNAAT and 10 AFB smear microscopy positive . Among the 83 CBNAAT positive samples, 9 (10.84%) were resistant to Rifampicin.Similar observational study was seen by **Sanjay et al<sup>18</sup>** observed that total 69 out of 34

(49.27%)patients were positive for BAL specimen CBNAAT MTB detected and 3 (8.82%)were in rifampicin resistance. **Thitta Mohant et al<sup>19</sup>** Observed that Sputum induction smear positive was 29 out of 100. Sputum induction CBNAAT was positive among 23 (32%), and BAL CBNAAT yield was 38/71 (54%). Study conducted by **B Hazarika et al<sup>20</sup>**concluded that Out of 162 patients 74 (45.67%) were BAL CBNAAT positive cases and the number of rifampicin sensitive cases were 65(87.84%) and rifampicin resistant cases were 9 (12.16%) **s.c.kiluru et al<sup>21</sup>** study reported that out of 51 patients 31 (60.78%)patients were BAL Gene xpert mtb detected and 3 (9.6%) were rif resistance

## 5. Conclusion

BAL for CBNAAT has a superior diagnostic value than BAL AFB smear microscopy and also helps in early diagnosis of smear negative pulmonary tuberculosis and help on differentiation of other disorders where clinical picture mimics tuberculosis.

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the institutional ethics committee

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