A Dissertation On

"DIAGNOSTIC YIELD OF BRONCHIAL WASH IN A SUSPECTED CASE OF SMEAR- NEGATIVE AND SPUTUM-SCARCE PULMONARY TUBERCULOSIS BY CBNAAT AND LIQUID MYCOBACTERIAL CULTURES (MGIT) IN RGGGH"

Dissertation submitted to The Tamil Nadu Dr.M.G.R. Medical University in partial fulfilment of the

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BONAFIDE CERTIFICATE

This is to certify that the dissertation titled **"DIAGNOSTIC YIELD OF BRONCHIAL WASH IN A SUSPECTED CASE OF SMEAR- NEGATIVE AND SPUTUM-SCARCE PULMONARY TUBERCULOSIS BY CBNAAT AND LIQUID MYCOBACTERIAL CULTURES (MGIT) IN RGGGH"** is the bonafide work done by **Dr. A.PANDIYAN** during his **M.D (Tuberculosis and Respiratory Diseases)** course in the academic years 2017-2020, at the Institute of Thoracic Medicine and Rajiv Gandhi Government General Hospital – Madras Medical College, Chennai. This work has not previously formed the basis for the award of any degree.

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CONTENTS

INTRODUCTION

Tuberculosis is an ancient and highly infectious disease that can potentially involve any organ or system in the body. Tuberculosis remains the major health problem globally.

According to WHO 2018 global tuberculosis report , Worldwide, TB is one of the top ten causes of death and the leading cause from a single infectious agent(5) .

In 2017, TB caused an estimated 1.3 million deaths among HIV-negative people and an additional 300 000 deaths from TB among HIV-positive people(5)

Globally, 10 million people developed TB disease in 2017 among which 5.8 million men, 3.2 million women and 1.0 million children. There were cases in all countries and age groups, but overall 90% were adults (aged ≥ 15 years), 9% were people living with HIV (72% in Africa) and two thirds were in eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%)(5).

Drug-resistant TB continues to be a public health crisis. Worldwide in 2017, 558,000 people developed TB that was resistant to rifampicin , the most effective firstline drug, and of these, 82% had multidrug-resistant TB (MDR-TB).(5)

Three countries accounted for almost half of the world's cases of MDR/RR-TB: India (24%), China (13%) and the Russian Federation (10%).

Among cases of MDR-TB in 2017, 8.5% were estimated to have extensively drug-resistant TB (XDR-TB).(5)

About 1.7 billion people, 23% of the world's population, are estimated to have a latent TB infection, and are thus at risk of developing active TB disease during their lifetime.(5)

The disease burden caused by TB is falling globally, in all WHO regions, and in most countries, but not fast enough to reach the first milestones of the End TB Strategy.(5)

By 2020, the TB incidence rate needs to be falling at 4–5% per year, and the proportion of people with TB who die from the disease (case fatality ratio, CFR) needs to fall to 10%. In 2017, the proportion of people with TB who died from the disease was 16%, down from 23% in 2000.(5)

The TB mortality rate is falling at about 3% per year, and the overall reduction in the period 2000–2017 was 42%.

Early diagnosis and successful treatment of people with TB prevents millions of deaths each year , but there are still large and persistent gaps in detection and treatment. Worldwide in 2017, 6.4 million new cases of TB were officially notified to WHO. This has been increasing since 2013, following 4 years (2009–2012) in which 5.7–5.8 million new cases were reported annually, mainly due to increased reporting of detected cases by the private sector in India and, in 2017, an upturn in notifications in Indonesia.(5)

The 6.4 million cases reported represented 64% of the estimated 10 million new cases that occurred in 2017. Ten countries accounted for 80% of the 3.6 million global gap, the top three being India (26%), Indonesia (11%) and Nigeria (9%)

Globally, 160 684 cases of MDR/RR-TB were detected and notified in 2017. Of these, a total of 139 114 people (87%) were enrolled on treatment with a second-line regimen, up from 129 689 in 2016 but still only 25% of the estimated 558 000 people who developed MDR/RR-TB in 2017. China and India alone accounted for 40% of the global gap, but Treatment success remains low, at 55% globally.(5)

The main health-care interventions to prevent new infections of Mycobacterium tuberculosisand their progression to TB disease are treatment of latent TB infection and vaccination of children with the BCG vaccine.

TB preventive treatment for a latent TB infection is expanding, but most of those for whom it is strongly recommended are not yet accessing care, whereas coverage of BCG vaccination is high.(5)

WHO has strongly recommended treatment for latent TB infection in two priority groups: people living with HIV, and children aged under 5 years who are household contacts of someone who has bacteriologically confirmed pulmonary TB.(5)

The End TB Strategy milestones for 2020 and 2025 can only be achieved if TB diagnosis, treatment and prevention services are provided within the context of progress towards universal health coverage , and if there is multisectoral action to address the social and economic factors that drive TB epidemics.(5)

TB incidence needs to be falling at 10% per year by 2025, and the proportion of people with TB who die from the disease needs to fall to 6.5% by 2025. Such levels have only been achieved in the context of UHC, combined with social and economic development that reduces known risk factors for TB infection and disease

Early diagnosis is imperative for early patient management and successful patient outcomes. False-negative results and misdiagnosis of TB suspects are common in developing nations, as most TB control programmes use Ziehl-Neelsen (ZN) smear microscopy, which has poor sensitivity and multiple visits are required that leads to higher default. Mycobacterial culture, although considered as the gold standard but is slow and usually takes 2-6 weeks time to yield a final result and requires proper infrastructure and technical expertise .

The mean time for the detection of culture of Mycobacterium tuberculosis on LJ medium is 31±9 days and so it is obligatory to device a rapid & reliable diagnostic culturemethod in detection of mycobacterium tuberculosis. The MGIT system Mycobacterial Growth Indicator Tube, which is a broth based liquid culture medium , has considerably improved the mean time of detection by 18±14 days. This method is, noninvasive, and non-radiometric, not labour intensive and very easy to use, can be executed in any laboratory

There are number of Nucleic Acid Amplification methods that have been developed for rapid detection and identification of Mycobacterium tuberculosis (MTB) in clinical specimens of pulmonary and extra-pulmonary tuberculosis cases . These techniques not only provide the advantage of rapidity of diagnosis but also detect even low MTB genomic copies in various specimens.

More recently, the WHO endorsed the CBNAAT for the diagnosis of TB . The CBNAAT utilizes a DNA PCR technique for simultaneous detection of Mycobacterium tuberculosis and Rifampicin resistance related mutations. It is the first fully automated bench top cartridge based nucleic acid amplification (CB-NAAT) assay for TB detection that includes all necessary steps of DNA PCR. It gives results

within 2 hours. Diagnostic accuracy of CBNAAT for pulmonary TB has been reported high . Patients with high risk of tuberculosis like presumptive HIV-associated TB patients and pediatric presumptive including extra pulmonary cases in whom AFB smear examination is usually negative, are the most likely to be benefited from CBNAAT

A smear-negative case was one in whom two samples, one spot sample and one early morning sample did not reveal acid fast bacilli when examined by microscopy with Zeihl Nelson stain and fluorescent microscopy. Patients that had sputum amount less than 1 ml were defined to have sputum scarce disease

REVIEW OF LITERATURE

Tuberculosis (TB) is a severe and contagious disease caused by Mycobacteria tuberculosis complex (MTBC). Most often involving the lungs, TB is transmitted by cough, with an infectious dose of less than 10 bacilli.

Mycobacterium belongs to the family Mycobacteriaceae, order Actinomycetales.

Properties of genus Mycobacterium :

• Acid fastness: They resist decolorization by dilute mineral acids.

Acid fastness is due to-

(l) presence of high content of mycolic acids in the cell wall, and

(2) integrity of the cell wall.

• Guanine plus cytosine (G+C) content of DNA of Mycobacterium is 61-71 mol %, the only exception being M. leprae with G+C content of 54 to57 mol %.(29)

Mycobacteria are non,motile, non-sporing, non encapsulated, weakly gram-positive, straight or slightly curved rod-shaped bacteria, averaging 3 by 0.3 μ in size and visible by light microscopy which are obligate aerobes (or microaerophilic). they sometimes show branching filamen1ous forms resembling fungal mycelium (myces meaning fungus, reflecting the mould-like pellicle formation on liquid media).(29)

Traditionally, mycobacteria are grown on solid, enriched media where rough, pigmented colonies generally appear 4 to 6 weeks after inoculation. MTBC can also be cultivated in specialized liquid media where characteristic “cords” visible by light microscopy are formed. Rapid liquid culture systems (e.g., BACTEC) have been adapted for use with mycobacteria and shorten time to detection of growth to a little as 9 to 16 days, depending on the initial concentration of the bacteria in the specimen tested(29)

ANTIGENIC STRUCTURE

Antigens of M. tuberculosis are mainly of two types:

1. Cell wall (insoluble) antigens:
2. Cytoplasmic (soluble) antigens(29)

The cell wall consists of several distinct layers:

- Peptidoglycan layer: It maintains the shape and rigidity oflhe cell.
- Arabinogalactan layer: It facilitates the survival of M. tuberculosis within the macrophages.
- Mycolic acid layer: lt is responsible for acid fas1ness and also reduces the entry of most antibiotics.
- Outermost layer: It consists of lipids (mycocerosates and acylglycerols), glycolipids and mycosides (phenolic glycolipids).
- Proteins (e.g. porins, transport proteins):they are found throughout the various layers.
- Plasma membrane: This layer is present beneath the cell wall, into which various proteiris, phosphatidyli11ositol mannosides, and lipoarabinomannan are inserted.

2. Cytoplasmic (soluble) antigens: These include antigen 5, antigen 6, antigen 60; and are used in serodiagnosis of tuberculosis(29)

RISK FACTORS FOR TB TRANSMISSION

Characteristics of source case:

Sputum-smear positivity for AFB

Cough strength and frequency

Presence of lung cavitation Humidity

Effective treatment

Delayed diagnosis

Laryngeal tuberculosis(31)

Environmental factors:

Air circulation, ventilation

Room volume

UV light

Proximity to the source case

Duration of contact with infected air (31)

Forceful expiratory efforts such as coughing and sneezing, discharge minute respiratory droplets of sputum containing viable bacilli that, when increasingly reduced in size by evaporation, become infectious droplet nuclei, each measuring less than 5 μ.

Due to small size, droplet nuclei have an extremely slow settling rate in air (0.5 mm/s or less). This permits their transport by air currents for significant distances. Larger respiratory droplets, on the other hand, settle out of the air quickly, and travel only a few feet from the source case. The microbes suspended within droplet nuclei are highly susceptible to germicidal levels of ultraviolet light. The small size of droplet nuclei also facilitates penetration of the bronchial defenses allowing access to terminal alveolar macrophages

The dominant predictors of infectiousness in source cases are the presence of cough, lung cavitation on chest radiograph, and acid-fast bacilli visible in sputum by smear microscopy. Sputum-smear microscopy positive cases excrete about 108 bacilli per mL of sputum compared to less than 103 bacilli per mL of sputum in smear-negative cases

PATHOLOGY:

Deposition of tubercle bacilli in the alveoli of the lungs is followed by vasodilation and an influx of polymorphonuclear leucocytes and macrophages to the area. (26) After several weeks, the polymorph numbers diminish and macrophages predominate. The macrophages develop pale foamy cytoplasm rich in lipid and crowd together as epithelioid cells to form the tubercle or unit lesion of tuberculosis . Some mononuclear cells fuse to form the multinucleated or Langhans' giant cell (26). Lymphocytes surround the outer margin of the tubercle and in the centre of the lesion a zone of caseous necrosis may appear that may subsequently calcify. (9)

Primary infection is usually evident as a subpleural tubercle (the primary or Ghon focus), which may be in any lung zone and which drains via lymphatics to hilar lymph nodes to form the primary complex. (26)

Most primary infections heal with or without calcification of the primary complex, although haematogenous spread probably occurs via the lymphatics in the majority of infected subjects, resulting in the seeding of tubercle bacilli to other parts of the lung as well as other organs(26)

The primary lesion sometimes progresses and the pathological changes are then similar to those seen in reactivation tuberculosis.

Reactivated pulmonary tuberculosis is most often seen in the upper lung zones and is limited in extent, most frequently to the posterior segment of the upper lobe or the apex of the lower lobe.

The high ventilation–perfusion ratios, with alveolar PO2 elevated relative to other zones, is believed to predispose to reactivation at these sites . Proliferation of tubercle bacilli in the caseouscentres is followed by softening and liquefaction of the caseous material, which may discharge into a bronchus with resultant cavity formation. 104 bacilli per gram are found in caseous tissue, up to 109 organisms may be harboured within a single cavitary lesion, Fibrous tissue forms around the periphery of such tuberculous lesions but is usually incapable of limiting extension of the tuberculous process.

Haemorrhage may result from extension of the caseous process into vessels within the cavity walls. Spread of caseous and liquefied material through the bronchial tree may disseminate the infection to other lung zones with or without the development of a vigorous inflammatory exudate or tuberculous pneumonia.

Rupture of a caseous pulmonary focus into a blood vessel may result in miliary (milia, a seed) tuberculosis, with the formation of multiple 0.5–2mm tuberculous foci in the lung and in other organs .

Encroachment on bronchi of pulmonary or lymph node caseous material may give rise to tuberculous bronchitis. Rupture of caseous glands into trachea or major bronchi causes collapse of lung or even sudden death by suffocation in young children.

PRIMARY TUBERCULOSIS

From the site of implantation, the organisms disseminate via the lymphatics to the regional lymph nodes. The lesion at the primary site of involvement, draining lymphatics and the inflamed regional lymph node constitute the primary complex. When the primary site of implantation is in the lung, it is called **Ghon's focus**. The draining lymphatics and the involved lymph nodes together with Ghon's focus constitute the primary complex [**Ghon complex**]. In children, the lymph node component may be much larger than the Ghon's focus(26)

The tubercle bacilli then disseminate via the haematogenous route to other parts of the lung and many organs of the body. Thus, primary TB is a widely disseminated infection. Most of these metastatic foci heal.

However, some of these metastatic foci may remain dormant and may reactivate at a later date when the host immunity decreases.

In most of the patients, the primary complex resolves without becoming clinically apparent. This occurs when the immune status of the host is good, and healing occurs by fibrosis and calcification.

In a minority of patients, progressive primary TB due to the extension of the inflammatory process at the site of the primary focus can occur. In the lung, this can present as an area of consolidation TB pneumonia(26)

Caseation necrosis at the Ghon's focus may lead to liquefaction. Expectoration of the liquefied material can leave a cavity with shaggy margins in the pulmonary parenchyma which may be apparent on the chest radiograph.

Mediastinal and tracheobronchial lymph nodes may produce compression of the adjacent bronchus. If this obstruction is complete, the lung distal to the site of bronchial obstruction becomes atelectatic [epituberculosis](24) . If the obstruction is incomplete, it may act as a "ball-valve" and results in obstructive emphysema.

The inflamed caseous lymph nodes may erode through the walls of the bronchus and result in bronchogenic dissemination. Bronchial mucosal involvement may result in TB bronchitis. In a patient with overwhelming infection, large number of Mycobacterium tuberculosis may gain access to the circulation and result in miliary and meningeal TB.

In majority of the patients, the initial focus of infection subsides. Cicatrization, scar formation and often calcification develop. Repeated episodes of extension of infection followed by healing and fibrosis may result in the formation of “onion skin” or “coin lesion”(23)

Post-primary Tuberculosis

Rarely, the primary lesion may progress directly to the post-primary form, characterized by extensive caseation necrosis and cavitation.

Reactivation or reinfection TB may occur due to old age, malnutrition, malignant disease, HIV infection and acquired immunodeficiency syndrome [AIDS], use of immunosuppressive drugs and intercurrent infections. While reactivation can occur at any site, postprimary TB classically involves the apical and posterior segments of the upper lobes,

or, the superior segment of the lower lobes in more than 95 per cent of the cases.(25)

Post-primary lesions are different from primary lesions in that, local progression and central caseation necrosis are much more marked in post-primary TB as compared to primary TB. Tuberculosis cavities are abundant sites for the growth of Mycobacterium tuberculosis as the temperature in them is optimal, there is abundance of oxygen and various nutrients derived from the cell wall are readily available.

The bacilli in the wall of the cavity gain free access into the sputum and are expectorated. Such patients are said to have “open tuberculosis” and are infectious to the community. If these bacilli are aspirated from the cavity to other parts of the lung via the bronchi, many secondary pulmonary lesions develop. Early in the illness, TB cavities are moderately thick walled, usually have a smooth inner surface, lack an air-fluid level and are surrounded by an area of consolidation.

Later, in the chronic phase of the disease, the wall may become thin, and the cavities may appear spherical.

SYMPTOMS

Patients with pulmonary TB present with constitutional and respiratory symptoms.

Constitutional symptoms include tiredness, headache, weight loss, fever, night sweats and loss of appetite. (24)

The classic symptoms and signs of TB Cough, fever , weight loss, night sweats, sputum production ,and haemoptysis. (24)

TYPICAL CHEST RADIOGRAPH PATTERNS

Four radiographic features suggest active TB:

(1) Nodular opacities located in the apical-posterior segments of the upper lobes or superior segment of the lower lobes,

(2) associated volume loss and fibrosis,

(3) lung cavitation, and

(4) endobronchial spread.(30)

Endobronchial spread to dependent lung segments fills lung acinar units, resulting in 4- to 5-mm size nodular opacities on plain film (acinar shadows) and a “tree-and-bud” pattern on computed tomography(7)

ATYPICAL CHEST RADIOGRAPH PATTERNS

Atypical features include lower lung zone infiltrates without cavitation, unilateral pleural effusion , and ipsilateral hilar or mediastinal adenopathy and are seen in children, the elderly, the immunocompromised persons.(6)(30).

Intrathoracic TB lymphadenitis is often better appreciated on CT where lymph node enlargement, rim enhancement, and low central attenuation are characteristic features.

Miliary TB is rare but, produces a distinctive, readily recognizable radiographic pattern: Innumerable, interstitial nodules uniformly distributed throughout all lung fields, without reduction in lung volumes(6)(7)(30)

TESTING FOR LATENT TB INFECTION

There are two main tests for the identification of LTBI:

(1) Tuberculin skin test (TST) and

(2) IFN-γ release assays (IGRAs).

Both are indirect and evaluate the presence of host cell–mediated immunity rather than detect actual mycobacterial organisms or antigens(8)

Since there is no gold standard for LTBI, sensitivity and specificity are typically estimated using surrogate reference standards.

Sensitivity is estimated among culture-confirmed TB cases, while specificity is estimated among low-risk individuals with no known TB exposure in low-incidence settings(10).

SMEAR MICROSCOPY:

Two types of acid-fast stains are commonly used :

1. Fluorochrome stain: Auramine O, with or without a second fluorochrome, Rhodamine

2. Carbolfuchsin stains: a mixture of fuchsin with phenol (carbolic acid)

a. Ziehl–Neelsen (hot stain)

b. Kinyoun (cold stain)(12)

ZIEHL-NEELSEN STAINING PROCEDURE:

- A smear is made from yellow coloured portion of the sputum using broom stick. A good smear is evenly spread, neither too thick nor too thin. (12)
- Allowed to dry for 15–30 mins.
- Then slide is fixed by passing it over a flame 3–4 times for 3–5 seconds each time.
- 1% carbolfuchsin is poured to cover the slide.
- The slide is gently heated with carbolfuchsin , until vapours rise andDo not boil.
- Carbolfuchsin is left on the slide for 5 mins.
- The slide is gently washed with tap water until all free carbolfuchsin stain is washed away.
- 25% Sulphuric acid is poured and allowed to stand for 2–4 mins.
- 0.1% methylene blue is poured over the slide and left for 30 secs. Then slide is rinsed with tap water and allowed to dry.
- Mycobacteria are stained red and the background is light blue(12)

GRADING OF SMEAR

EXAMINATION FINDING	NO OF FIELDS EXAMINED	GRADING
NO AFB IN 100 OIF	100	0
1-9 AFB IN 100 OIF	100	SCANTY
10-99 AFB IN 100 OIF	100	1+
1-10 AFB PER OIF	50	2+
MORE THAN 10 AFB PER OIF	20	3+

OIF – Oil Immersion Field

FLUORESCENCE STAINING PROCEDURE

SMEAR PREPARATION-

- Prepare smear in oval shape in the centre of the slide for good spreading of sputum firmly press the stick perpendicular to the slide and move in small concentric circles
- It should be neither too thick nor too thin. Allow smear to air dry at room temperature
- Heat fix by passing the slide over flame 2-3 times for about 2-3 seconds each time

STAINING

- 0.1% Auramine solution Poured over the slide (12)
- Keep the staining reagent for at least 20 min; make sure that the smear area
- Rinse with water and drain
- Apply 0.5% acid alcohol for 3 minutes
- Gently rinse with water
- To add 0.5% potassium permanganate solution for 1 minute. Gently rinse with water and drain
- Mycobacteria are stained yellow-orange against a dark background. (12)

FLUORESCENT MICROSCOPY GRADING :

200-250x Magnification	400x Magnification	GRADING
NO AFB per 1 length	NO AFB per 1 length	0
1-29 AFB per 1 length	1-19 AFB per 1 length	SCANTY
30-299 AFB per 1 length	20-199 AFB per 1 length	1+
10-100 AFB Per 1 field on average	5-50 AFB per 1 field on average	2+
More than 100 AFB Per 1 field on average	More than 50 AFB per 1 field on average	3+

200-250x Magnification: 1 length =30 fields = 300 HPF

400x Magnification: 1 length =40 fields = 400 HPF

KINYOUN STAINING PROCEDURE

- Cover a heat-fixed, dried smear with a small rectangle of filter paper.
- Add 5–7 drops of carbolfuchsin stain to moisten filter paper. Allow to stand for 5 mins.
- Remove paper with forceps, rinse slide with water, and drain.
- Decolorize with acid–alcohol
- Counterstain with methylene blue (1–2 minutes).
- Rinse, drain and dry .
- Mycobacteria are stained red and the background is light blue(12)

BRONCHOSCOPY

Bronchoscopy is a valuable diagnostic and therapeutic tool in Pulmonary Medicine. It plays an important role in the diagnosis of smear negative pulmonary tuberculosis.

HISTORY:

Gustavkillian is considered as the father of modern bronchoscopy.

Chevalier Jackson developed a rigid esophagoscope . Derivatives of this device are now called rigid bronchoscopes. (13)

ShigetoIkeda , a thoracic surgeon, developed flexible fiberoptic bronchoscope in the year 1968, which is now known as the year of "second revolution" in bronchoscopy.(13)

Andersen first performed bronchoscopictransbronchial lung biopsy (TBLB) with the rigid bronchoscope in 1965.

In 1958, Eduardo Schieppati originally proposed transbronchial needle aspiration biopsy (TBNA) .

Ko-Pen Wang (1978) done successfulbronchoscopic Needle aspiration biopsy of paratracheal node through a flexible Bronchoscope. (13)

BEFORE BRONCHOSCOPY:

- Consent- Verbal and informed consent should be obtained
- DIET - Solid diet may be allowed upto 4 hours prior to procedure

  -Liquid diet intake upto 2 hours prior to procedure
- Prophylactic antibiotics before bronchoscopy to patients with asplenia, prosthetic heartvalve, or previous history of endocarditis. should be given
- FOB should be avoided for atleast 6 weeks following MI.
- Oral anticoagulants stopped at least 3 days before. Or their effect be reversed with vitamin K injection.(14)

DURING BRONCHOSCOPY:

- Patients vitals should be monitored .
- SpO2 should be maintained > 90% . Supplemental oxygen administered if necessary.
- The maximal total dose of lignocaine for topical anaesthesia should be limited to 8.2 mg/kg in adults
- Lignocaine gel(2%) is better compared to nasal spray

- Equipments for emergency resuscitation should be kept ready.(14)

AFTER BRONCHOSCOPY:

- Oxygen supplementation may be required in patients with impaired lung function
- A chest radiograph should be taken at least one hour after transbronchial biopsy to exclude air leak.(14)

CLEANING AND DISINFECTION:

- Immersion time of 20 minutes is recommended for bronchoscopes at the

  beginning and end of a session and in between patients.
- Longer immersion time of 60 minutes is recommended for known / suspected atypical mycobacterial infections and in HIV positive individuals with respiratory complaints. MAIC and other atypical mycobacteria are more resistant to glutaraldehyde.
- Patients with suspected tuberculosis should undergo FOB at the end of the list.(13)

PROCEDURE:

Flexible bronchoscopy and diagnostic techniques were performed under monitored anesthesia care as recommended by the American Thoracic Society and the British Thoracic Society. Bronchial washing was performed at the site of the lesion. After a full inspection of all the visible segmental and subsegmental bronchi, the flexible bronchoscope was wedged into a segmental bronchus , 10 mL of normal saline was repeatedly introduced until 20 mL of the aspirate was collected in the trap bottle.

NUCLEIC ACID AMPLIFICATION METHODS

In late 1990s, two commercially available nucleic acid amplification assays approved by the FDA.

(1)The AmplicorM. tuberculosis PCR assay

(2)The Amplified Mycobacterium tuberculosis Direct test (AMTD),

Both been approved for use with smear-positive specimens, But only AMTD was been approved for use on smear-negative respiratory specimens.(15)

Nucleic acid amplification assays perform well on smear-positive specimens, but sensitivity is lower with smear negative respiratory specimens, when compared with culture.

AMTD was highly sensitive and specific for detecting MTBC organisms within a few hours.

In 2012, a third nucleic acid amplification assay—the CBNAAT was approved by the FDA .

The assay has two components:

(1) a plastic cartridge that contains liquid sample processing and PCR buffers and lyophilized real-time PCR reagents

(2) the CBNAAT instrument, which performs sample processing and real-time PCR in a hands-free manner.(15)

This assay simultaneously detects M. tuberculosis and rifampin resistance by PCR amplification of the 81-bp fragment of the M. tuberculosis rpoBgene and subsequent probing for mutations associated with rifampin resistance. The internal control is a hemi-nested molecular beacon assay to detect BacillusglobigiiDNA.

Total hands-on time is less than 5 minutes per specimen, and results are available in less than 2 hours. (15)

The sensitivity versus culture when testing sputum specimens was 98% to 100% for AFB smear positive samples and 69% to 72% for smear-negative samples. Specificity of the assay in these two studies was 100%.

CBNAAT is a semi-quantitative nested real-time PCR in-vitro diagnostic test for:

(1) the detection of MTB DNA in sputum samples

(2) the detection of rifampicin resistance associated mutations of the rpoB gene.(16)

The CBNAAT system integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences. The system requires the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. The CBNAAT cartridge also contains a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers in the CBNAAT assay amplify a portion of the rpoB gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with rifampicin resistance(16)

QUALITY CONTROLS

Each CBNAAT cartridge includes a Sample processing control (SPC) and Probe Check control (PCC). Print out of the test result indicates the validation of controls.

Sample Processing Control (SPC): Ensures the sample was correctly processed. The SPC contains non-infectious spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of MTB. SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result will be "Invalid" if the SPC is not detected in a negative test.

Probe Check Control (PCC): Before the start of the PCR reaction, the CBNAAT system measures the fluorescence signal from the probes to monitor bead hydration, reaction-tube filling, probe integrity and dye stability. Probe Check passes if it meets the assigned acceptance criteria.(16)

INTERPRETATION OF RESULTS

The results are interpreted by the CBNAAT system from measured fluorescent signals and embedded calculation algorithms and will be displayed in the **"View Results"** window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template.

MTB DETECTED

If MTB target DNA is detected- the MTB result will be displayed at High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sputum sample.

MTB result	**Ct range**
High	<16
Medium	16-22
Low	22-28
Very Low	>28

RIFAMPICIN RESISTANCE RESULT TYPES:

- Rifampicin resistance DETECTED: a mutation in the rpoB gene has been detected that falls within the valid delta Ct setting.
- Rifampicin resistance NOT DETECTED: no mutation in the rpoB gene has been detected.
- Rifampicin resistance INDETERMINATE: the MTB concentration was very low and resistance could not be detected.(16)

LIMITATIONS OF CBNAAT

- Perform the test and validate results as per this SOP and details of test package insert
- Reliable results depend on proper specimen collection, handling, and storage.
- A positive test result does not necessarily indicate the presence of viable organisms. It is however, presumptive for the presence of MTB and rifampicin resistance.
- The results might be affected by antecedent or concurrent anti-TB drug therapy

CULTURE:

Culturing Mycobacterium tuberculosis is the gold standard investigating modality. Can be cultured in both solid media and liquid media

SOLID MEDIUMS

Egg based –

Lowenstein-Jensen(LJ),

Petragnani,

American Thoracic Society medium,

Agar-Based –

a)Middlebrook 7H10 &Middlebrook 7H10 selective.

b)Middlebrook 7H11 &Middlebrook 7H11 selective(Mitchisons)

c)Middlebrookbiplate(7H10/7H11S agar).

OTHER SOLID MEDIA

• Blood-based (Tarshis medium)

• Serum-based (Loeffler medium)

• Potato-based (Pawlowsky medium)

LIQUID MEDIUMS

- BACTEC 12B medium.
- Middlebrook 7H9 broth.
- Septichek AFB.
- Mycobacterial Growth Indicator Tube.

OTHER LIQUID MEDIA:

Dubos, Proskauer Sula, and Sauton media

For prime recovery of mycobacteria, a minimum of two one liquid medium and another solid medium is recommended.

MYCOBACTERIA GROWTH INDICATOR TUBE SYSTEMS:

- The Mycobacteria Growth Indicator Tube (MGIT) (BD Diagnostic) is a round-bottom glass tube that contains modified Middlebrook 7H9 broth base. (18)
- A fluorescent compound is embedded in silicone on the bottom of the tube. The fluorescent compound is sensitive to dissolved oxygen in the broth, the presence of oxygen in the uninoculated medium serves to quench the emission of fluorescent light. (18)
- As the actively growing mycobacteria consume the dissolved oxygen, the fluorescence is unmasked and can be detected manually by observing the tube under long-wave UV light (Wood's lamp). (18)
- Growth may also be detected by observing a nonhomogeneous turbidity or small grains or flakes in the culture medium.
- The MGIT system can be used to culture all specimen types except blood and urine.
- For optimal performance, OADC (oleic acid, albumin, dextrose, and catalase) growth supplement and an antibiotic mixture (PANTA: polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) are added to the broth. (18)

- The antibiotic mixture inhibits the growth of contaminating bacteria.
- The OADC supplement provides oleic acid, an important metabolic stimulant for mycobacteria; albumin, to bind toxic free fatty acids; dextrose, as an energy source; and catalase, to destroy toxic peroxides that may be present in the medium. (18)
- After adding the Supplement/PANTA mixture, the tube is inoculated with 0.5 mL of specimen or specimen concentrate; adding more than 0.5 mL of specimen may adversely affect the performance of the tube. The cap is replaced, and the ingredients are mixed by inverting the tube several times.
- For manual reading, tubes are placed in a 37°C incubator, and are read every other day starting on the second day after inoculation. Tubes are read with a Wood's lamp, placing the tube with the test mixture between a positive (sodium sulfite solution) and a negative (uninoculated MGIT tube) control.
- UV protective goggles are worn while looking for a bright orange color in the bottom of the positive tubes; an orange reflection is also seen at the meniscus.
- Positive tubes are stained for AFB, preferably using a carbolfuchsin method.

- Negative tubes are returned to the incubation rack and again observed at regular intervals for up to 6 weeks.(18)

Advantage:

(1) Detect growth faster (2- 3 weeks),

(2) Automated and continuous monitoring of the growth,.

(3) Lesscontamination issues**.**

Disadvantage:

They are expensive**.**

Principleused :

MGIT Uses an oxygen sensitive fluorescent compound, dissolved in the broth. Initially, the large amount of dissolved oxygen quenches emissions from the compound and little fluorescence can be detected. Later, actively respiring microorganisms consume the oxygen and the absence of oxygen allows the fluorescence to be detected .(18)

AIMS AND OBJECTIVES

AIMS :

To measure the diagnostic yield of Bronchial wash CBNAAT to detect Mycobacterium tuberculosis and compare it with liquid culture (MGIT) in a suspected case of smear negative and sputum scarce pulmonary tuberculosis

OBJECTIVES

- Measure the diagnostic yield of Bronchial wash CBNAAT, to detect Mycobacterium tuberculosis(MTB) and rifampicin resistance and compare it with liquid mycobacterial cultures (MGIT) (gold standard) in a suspected case of smear negative and sputum scarce pulmonary tuberculosis
- The diagnostic yield was measured in terms of frequency and validity by calculating sensitivity, specificity, positive and negative predictive values

MATERIALS AND METHODS

A patient of tuberculosis-suspect, on the basis of clinical and radiological features compatible with a diagnosis of pulmonary tuberculosis. A smear-negative case was one in whom two consecutive early morning sputum samples did not reveal acid fast bacilli when examined by microscopy with ZeihlNeelson stain. Patients that had sputum amount less than 1 ml were defined to have sputum- scarce disease. A confirmed case of pulmonary tuberculosis was one in whom Mycobacterium tuberculosis (MTB) grew on mycobacterial cultures by solid or liquid culture medium, that was taken as the gold standard. Patients of either gender aged above 12 years of age, that had suspected pulmonary tuberculosis on clinical or radiological grounds , were included in the study. Smear- positive cases, those with disseminated or extra pulmonary tuberculosis patients were excluded from the study. Following written consent for bronchoscopy, demo- graphic and clinical data was collected. Bronchoscopy was performed by transnasal route and bronchoscope was wedged into the subsegmental bronchus of interest and Bronchial wash was obtained by instillation of sterile normal saline. It was sent for ZN stain and mycobacterial liquid culture (gold standard), for CBNAAT to detect Mycobacterium tuberculosis(MTB) and rifampicin resistance.

SUBJECT SELECTION:

INCLUSION CRITERIA:

1. Patients with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, haemoptysis and loss of appetite with sputum smear negative for PTB

2. Patients with radiological features suggestive of pulmonary tuberculosis with smear negative for PTB

EXCLUSION CRITERIA:

1. Sputum positive for pulmonary tuberculosis
2. Patients unfit for FOB

LABORATORY METHODS

- Following written consent for bronchoscopy, Bronchoscopy was performed by transnasal route and bronchoscope was wedged into the subsegmental bronchus of interest and Bronchial wash was obtained by instillation of sterile normal saline. It was sent for ZN stain and mycobacterial liquid culture, for CBNAAT to detect Mycobacterium tuberculosis(MTB) and rifampicin resistance.
- Each bronchial wash sample received in the lab from the centers as per the collection and transportation policy of the laboratory, were divided into three parts;
- one part was immediately tested using CBNAAT, second part used for ZN smear microscopy and third part for MGIT BACTEC 320 liquid culture and performed on same day.
- For Liquid culture as much as sample was taken after sending for CBNAAT and ZN stain but it should be checked that volume remaining should not be less than 2 ml for processing.
- CBNAAT testing was performed according to the manufacturer's instructions . Sample reagent was added to bronchial wash fluid at a ratio of 2:1, manually agitated and kept for 10 min at room temperature, then shaken again and kept for 5 min; 2 ml of the inactivated material was transferred to the test cartridge and

inserted into the test platform. Only electronic results were used for comparison.

- Direct Smear microscopy was performed to investigate presence of acid fast bacilli with the second part of the specimen using conventional ZN staining method. Slides showing red coloured acid fast bacilli were taken as positive and negative slides were those without any acid fast bacilli .

Third part was processed using the N-acetyl-L cysteine- sodium hydroxide method (NALC-NaOH) as per the manufacturer's instructions, cultured on MGIT media and incubated in MGIT BACTEC 320 liquid culture system . Sodium hydroxide (NaOH) is a decontaminating agent and also acts as emulsifier and NALC acts as a mucolytic agent and also reduces the concentration of NaOHrequired . When the tubes were flagged positive by the system, ZN staining and culture on 5% sheep blood agar were performed from the tube directly to see any contamination as per the manufacturer's instructions. All tubes were checked for positivity till 42 days. Positive tubes are stained for AFB, preferably using a carbolfuchsin method. Negative tubes are returned to the incubation rack and again observed at regular intervals for up to 6 weeks.

STUDY CENTRE:

Rajiv Gandhi Government General Hospital, Chennai

DURATION OF THE STUDY:

12 months (APRIL 2018- MARCH 2019)

STUDY DESIGN:

Comparative study

SAMPLING FRAME:

Patients attending opd of department of thoracic medicine and admitted in wards in RAJIV GANDHI GOVT GENERAL HOSPITAL.

SAMPLE SIZE:

Sample size: 100

ETHICAL CLEARANCE: Obtained (Copy enclosed)

CONSENT : Informed consent will be obtained from all patients.

STUDY PROTOCOL:

From patients attending the thoracic medicine OPD, and patients admitted in wards, selected for clinical study as per inclusion/exclusion criteria the following data are collected:

-Demographic data

-Medical history,

-H/o prior ATT

-Complete blood counts,LFT, RFT, Microbiology, CXR, diagnostic and therapeutic procedures, response to therapy.

-patients with clinical suspicion of pulmonary tuberculosis with symptoms of cough with or without expectoration for >2 weeks, Fever, weight loss, fatigue, haemoptysis and loss of appetite will be subjected to investigations and xray chest if required HRCT/CT chest

Patients satisfying the inclusion criteria will be included in the study.

They will be given counselling and informed consent will be obtained for investigations.

SPONSORSHIP (YES/ NO): No

CONFLICT OF INTEREST : None

RESULTS

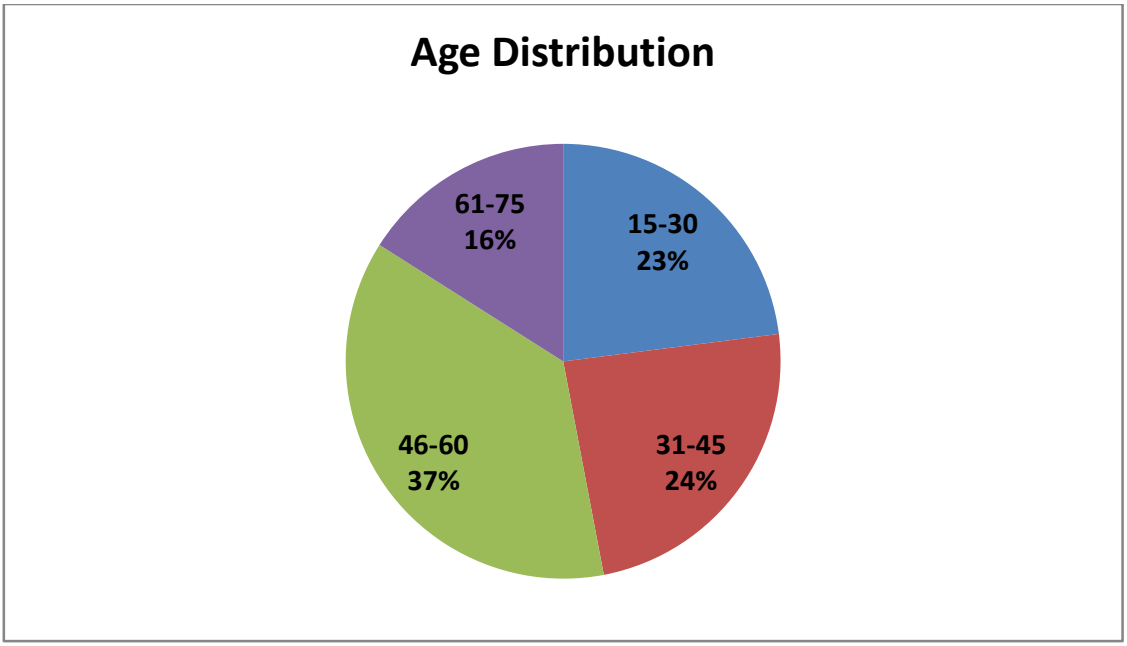
A total of 100 patients who satisfied the inclusion and exclusion criteria were included in the study . All the patients were undergone bronchoscopy

and the samples were sent for AFB, CBNAAT and MGIT.

AGE DISTRIBUTION:

Among the 100 patients, the commonest age group was 46 to 60 years, and 2nd commonest group 31 to 45.

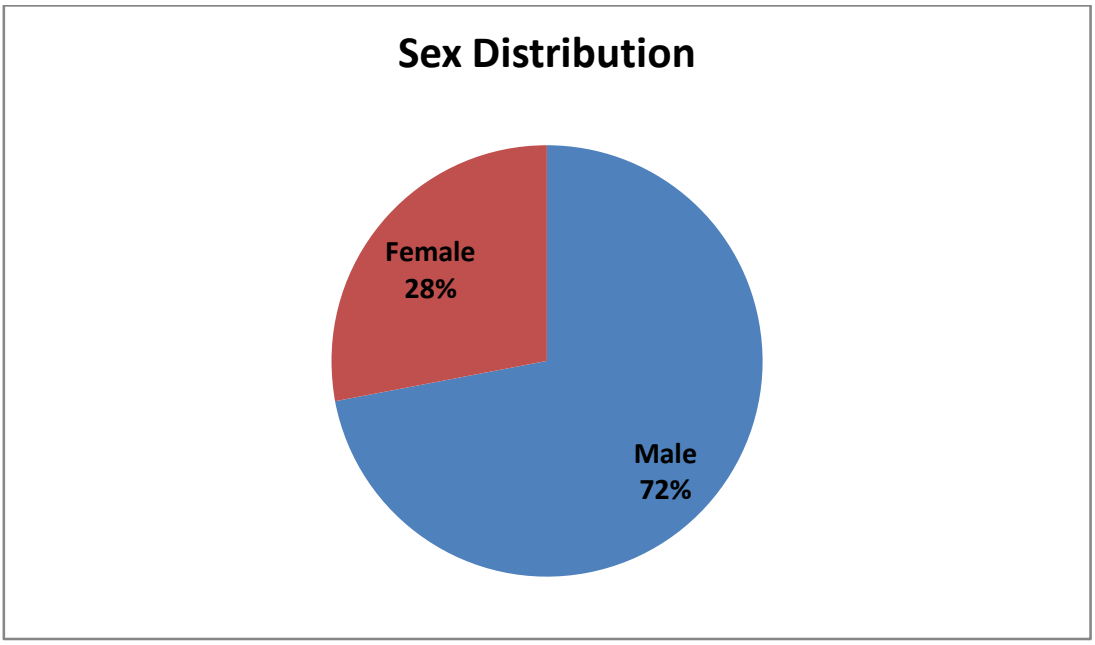
Diagram showing age distribution:



GENDER DISTRIBUTION:

In this study out of 100 patients 72 were male and 28 were females.

Diagram showing gender distribution:



DIABETIC STATUS:

In this study, out of 100 patients 19 were known diabetic and on regular treatment.

Diagram showing distribution of diabetic status:

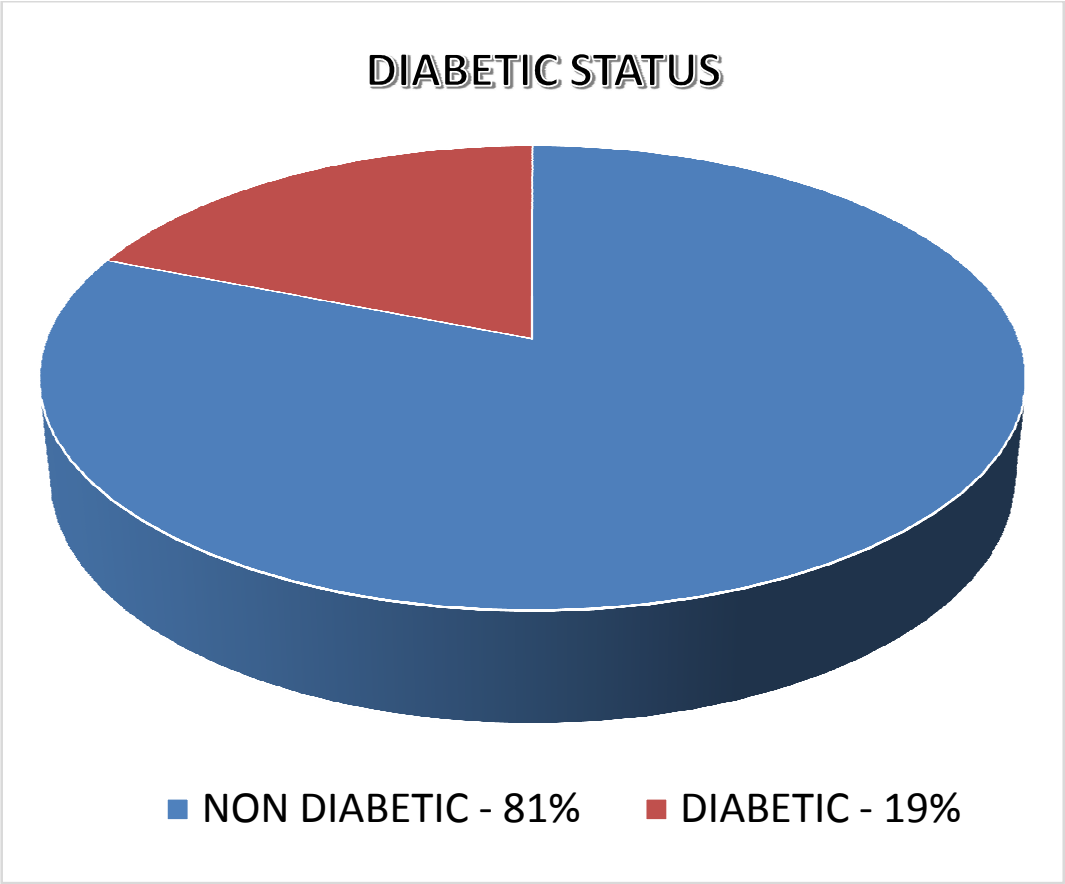


Table showing comparison of CBNAAT in diabetic and non diabetic patients:

	CBNAAT		TOTAL
	POSITIVE	NEGATIVE	
DIABETIC	13	6	19
NON-DIABETIC	29	52	81
TOTAL	42	58	100

Table showing comparison of MGIT in diabetic and non diabetic patients

	MGIT		TOTAL
	POSITIVE	NEGATIVE	
DIABETIC	11	8	19
NON-DIABETIC	27	54	81
TOTAL	38	62	100

Among the 100 patients 19 patients were known diabetic , out of this 19 patients CBNAAT was positive in 13 patients, MGIT positive in 11 patients

PRESENTING COMPLAINTS:

In this study , most common presenting complaint was cough with expectoration in 47 patients, followed by fever, loss of weight – loss of apetite and hemoptysis .

Diagram showing distribution of clinical features:

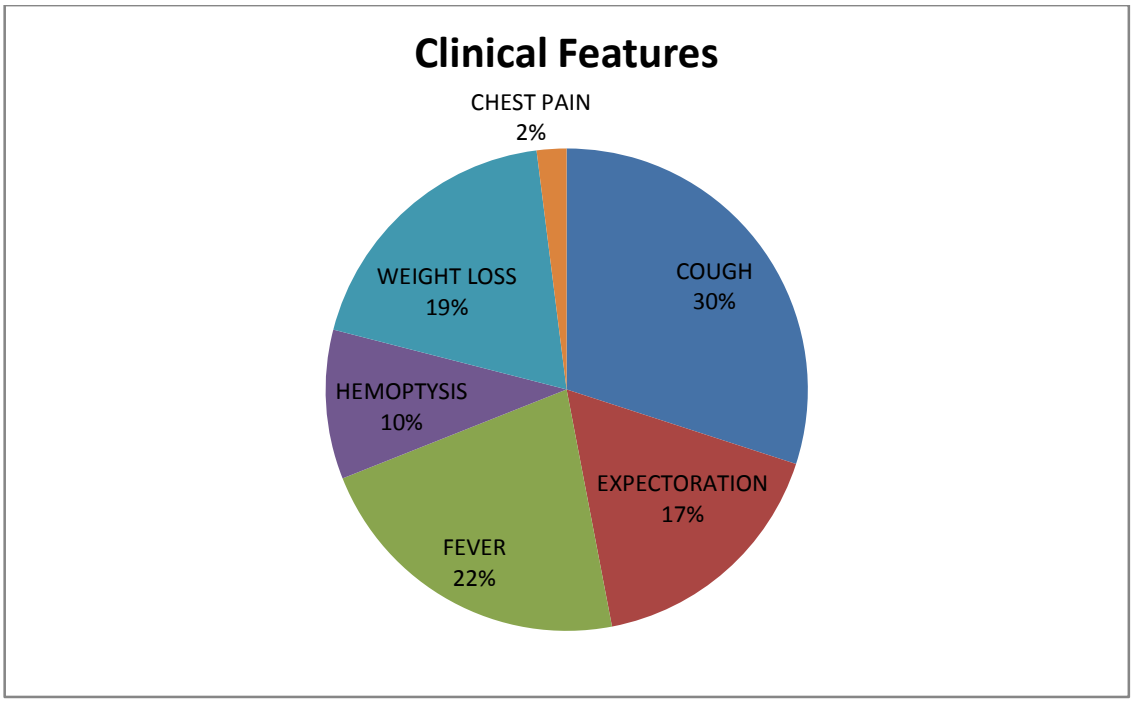


Table showing distribution of clinical features:

CLINICAL FEATURES	n
COUGH	30
EXPECTORATION	17
FEVER	22
HEMOPTYSIS	10
WEIGHT LOSS	19
CHEST PAIN	2

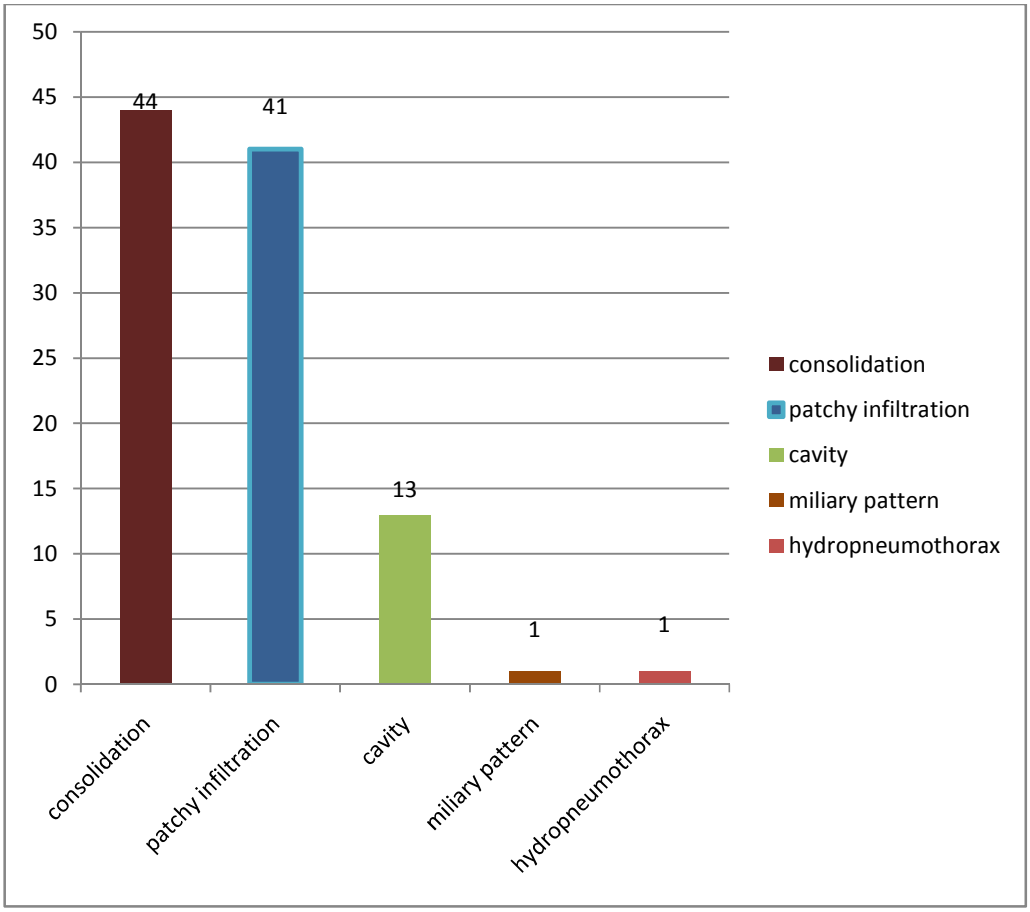
RADIOLOGICAL FINDINGS:

In our study M/C radiologic lesion was consolidation in 44% patients.

Table showing distribution of radiological features:

RADIOLOGICAL FINDINGS	n
Consolidation	44
Cavity	13
Patchy Infiltration	41
Miliary pattern	01
Hydropneumothorax	01

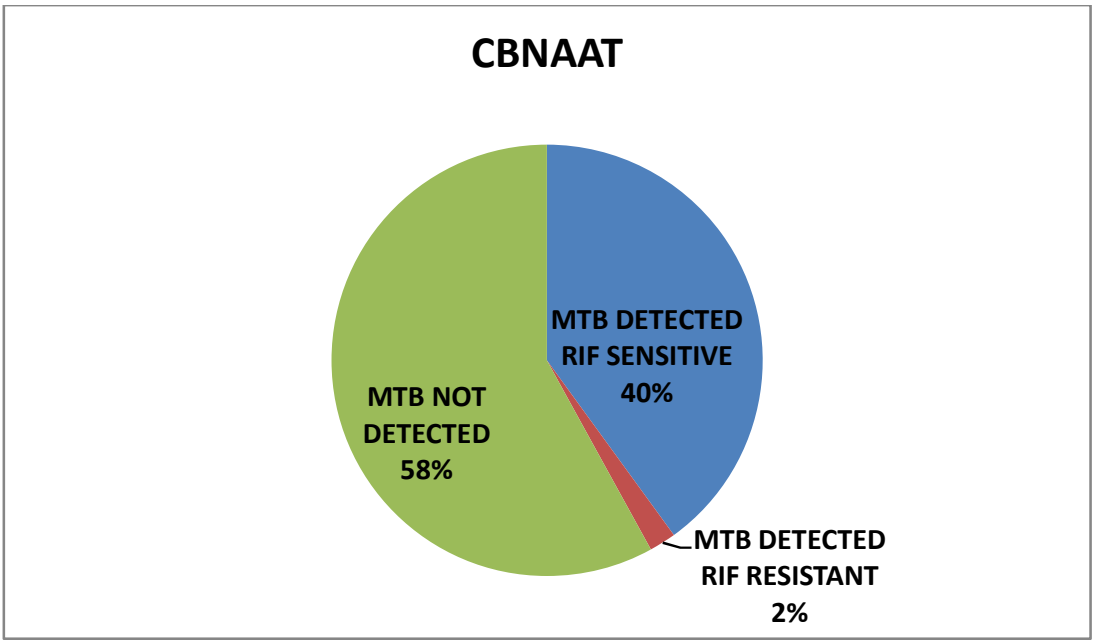
Diagram showing distribution of radiological features:



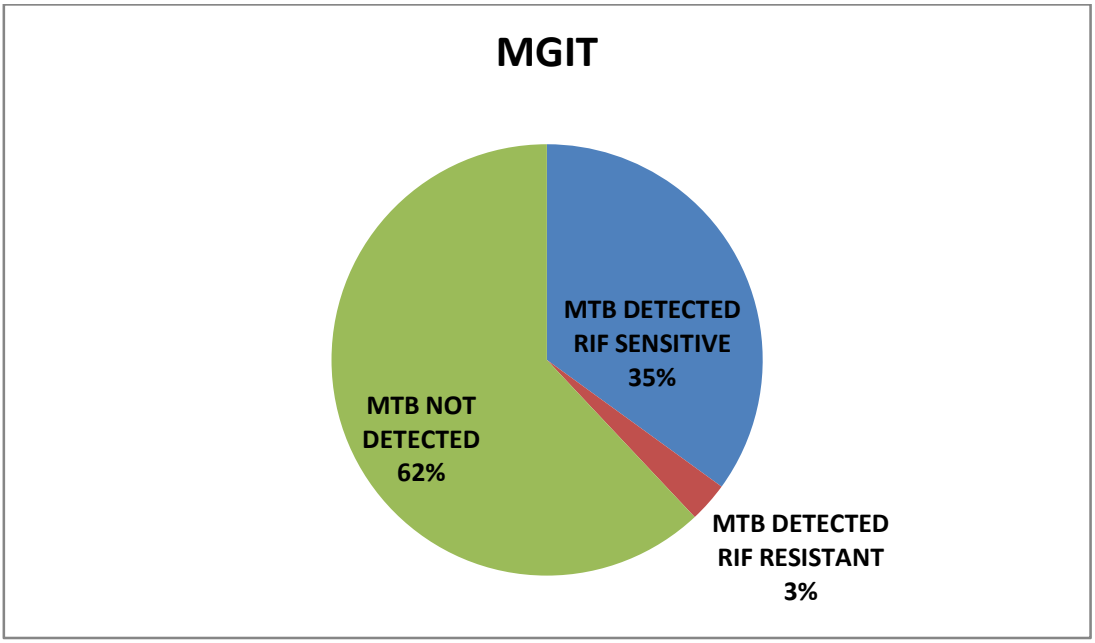
BRONCHIAL WASH CBNAAT Vs MGIT:

Following FOB, bronchial wash sample sent for CBNAAT and it was positive in 42 patients and among the 42 positive patients , 40 were rifampicin sensitive, 2 were rifampicin resistant

CBNAAT report:



MGIT REPORT:



In our study , 42 patients were positive in CBNAAT and in those 42 patients MGIT was positive in 35 patients. Among the 58 CBNAAT negative patients, 3 patients were positive in MGIT

Diagram showing diagnostic yield of CBNAAT:

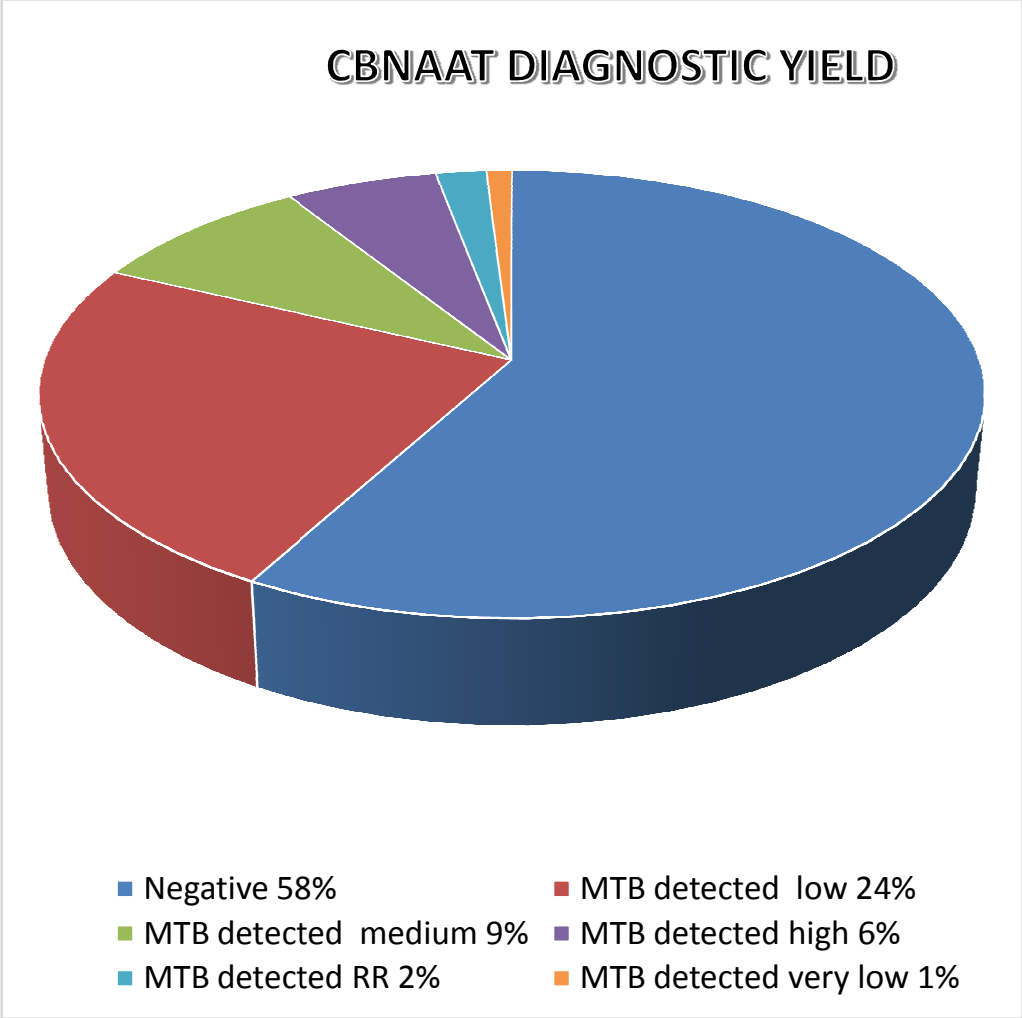


Diagram showing comparison of CBNAAT Vs MGIT (diagnosis)

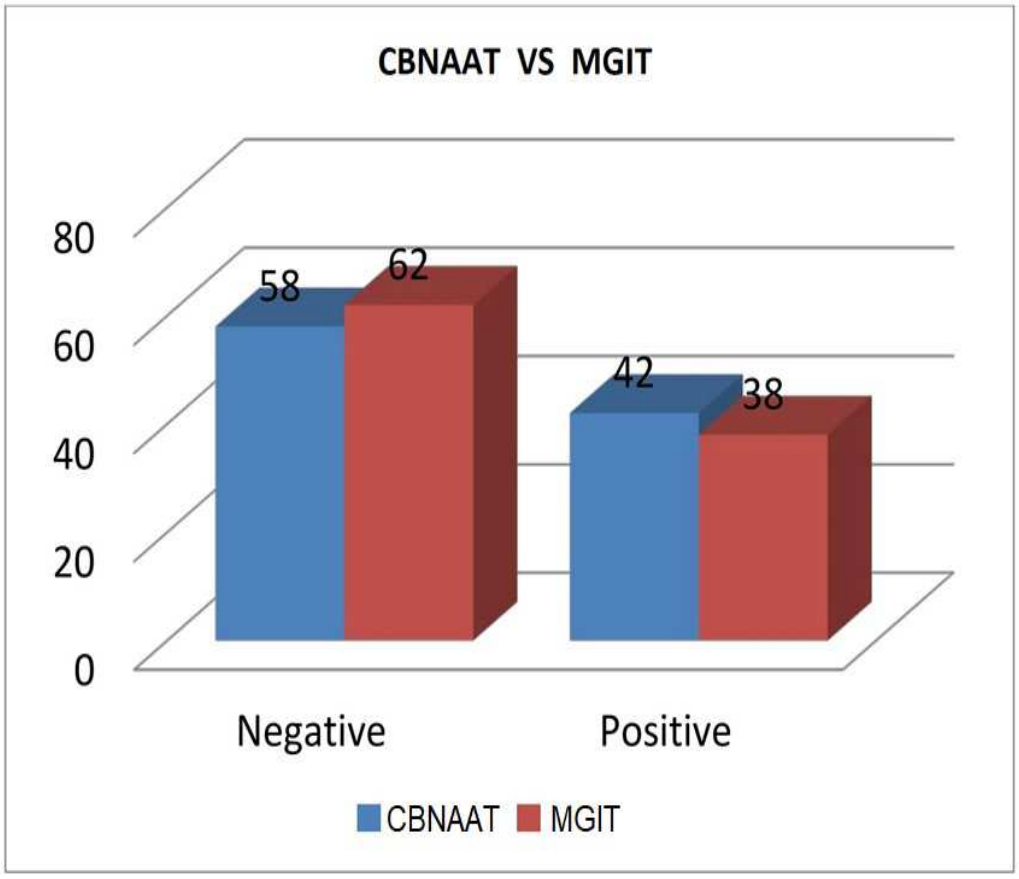
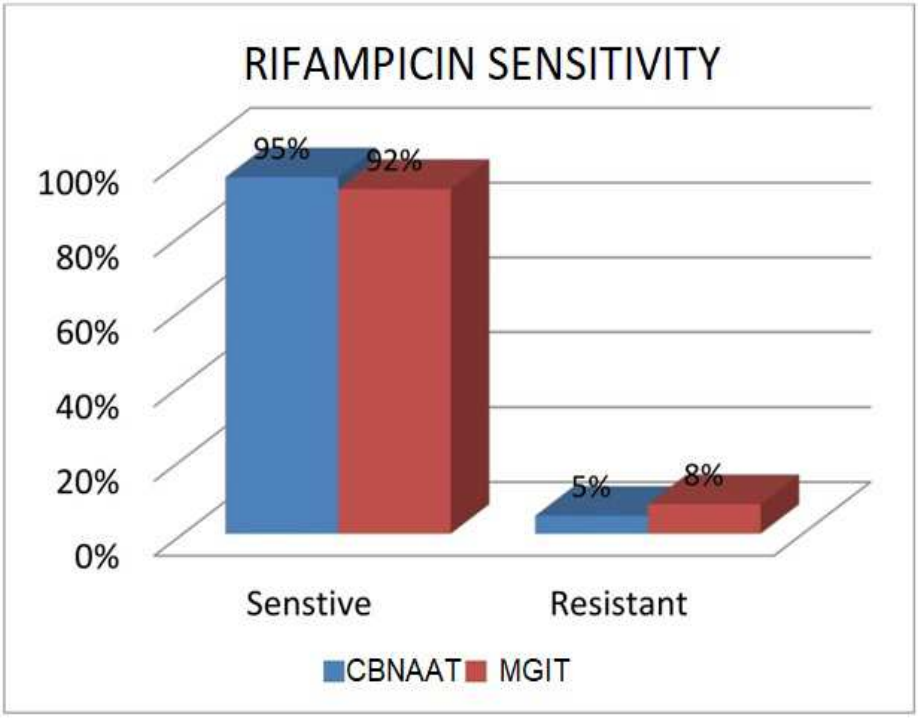


Diagram showing comparison of CBNAAT Vs MGIT (RIF sensitivity)



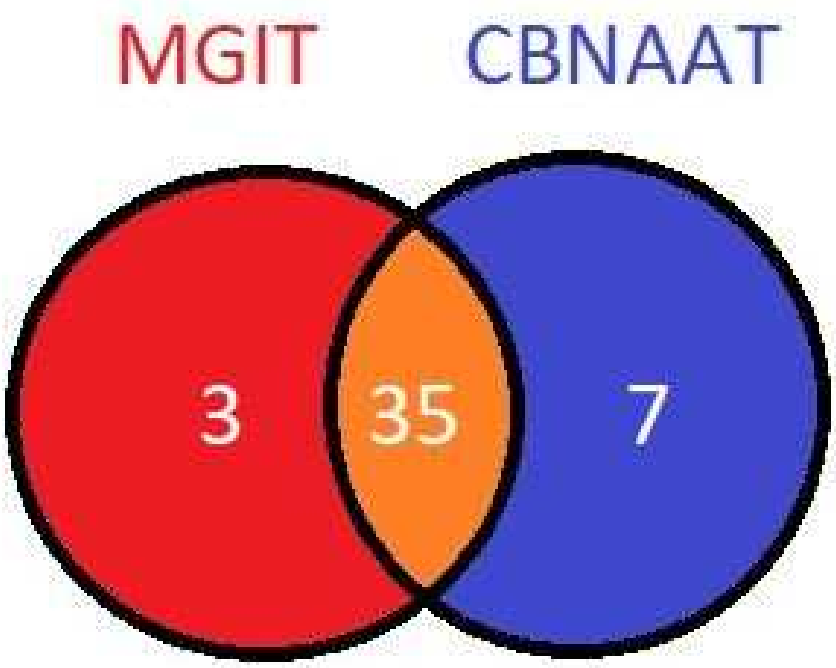
Among the 42 CBNAAT positive patients, 7 patients were negative in

MGIT.

Among the 42 CBNAAT positive patients, 40 patients were rifampicin sensitive & 2 patients were rifampicin resistant.

Among the 38 MGIT positive patients, 35 patients were rifampicin sensitive & 3 patients were rifampicin resistant

Venn diagram showing comparison of CBNAAT Vs MGIT (PTB diagnosis)



Both CBNAAT and MGIT are positive in 35 patients, 3 patients were MGIT positive and CBNAAT negative and 7 patients were CBNAAT positive and MGIT negative

Diagnostic yield of CBNAAT (diagnosis):

COMPARISON OF CBNAAT AND MGIT

		MGIT		
		Positive	Negative	Total
CBNAAT	Positive	35	7	42
	Negative	3	55	58
Total		38	62	100
Sensitivity (95% CI)		92.1 (78.6 to 98.3)		
Specificity (95% CI)		88.7 (78.1 to 95.3)		
PPV (95% CI)		83.3 (71.2 to 91.0)		
NPV (95% CI)		94.8 (86.1 to 98.2)		
Accuracy (95% CI)		90.0 (82.4 to 95.1)		

Diagnostiv yield of CBNAAT (RIF Sensitivity):

RIFAMPICIN SENSITIVITY				
		MGIT		
		Positive	Negative	Total
CBNAAT	Positive	35	7	42
	Negative	4	54	58
	Total	39	61	100
SENSITIVITY(95% CI)		89.74%(75.7 to 97.1)		
SPECIFICITY(95% CI)		88.52%(77.7 to 95.2)		
PPV (95% CI)		83.33%(71.1 to 91.0)		
NPV (95% CI)		93.10%(84.1 to 97.1)		
ACCURACY(95% CI)		89.00%(81.1 to 94.3)		

Table showing comparison of our results with various other studies:

STUDY	SENSITIVITY	SPECIFICITY	PPV	NPV
Our Study	92.1	88.7	83.3	94.8
Boehme et al	76.9	99	-	-
Theron G et al	93	77-98	-	-
Boehme CC et al (2010)	72.5	99.2	-	-
Lee HY et al	81.6	100	100	92.1
Kwak N et al	79.5	100	100	94
Palud P L et al	60	100	-	-
Kirwan DE et al	72.5-98.2	99.2	-	-
Steingart et al	67	99	-	-
Marlowe EM et al	72	98	-	-
Armand S et al	79	84	-	-
Moure R et al	75.3	100	-	-
Teo J et al	90.9	89	-	-
Saglam, L et al	86	57	99	22
Tortoli E et al	81.3	99.8	-	-

In our study, on comparing with MGIT in detecting MTB , CBNAAT diagnostic yield has been measured and it has the sensitivity of 92.1% , specificity was 88.7%, positive predictive value was 83.3% and negative predictive value was 94.8%

In diagnosing the rifampicin sensitivity , on comparing with MGIT, CBNAAT has the sensitivity of 89.7% , specificity was 88.5%, positive predictive value was 83.3% and negative predictive value was 93.1%.

DISCUSSION

In this study, the diagnostic yield of Bronchial wash (CB NAAT) to detect MTB and rifampicin resistance in smear-negative pulmonary TB was evaluated and compared it with that of mycobacterial cultures which were taken as the gold standard.

AGE DISTRIBUTION:

Among the tuberculosis suspected patients, the commonest age group was 46 to 60 years, and the mean age of this study was 44.7 years which correlates with the study conducted by Salome Phelamei et al[49] in which the age group affected was 35 to 54 yrs.

GENDER DISTRIBUTION:

Among the tuberculosis suspected patients 72% were men and 28% were women. Among the 72 males CBNAAT was positive in 29 males, MGIT was positive in 26 males. This correlates with study of Mohamed35 et al where 78% of men and 22% of women affected [48].

Among the 28 females CBNAAT was positive in 13 females, MGIT was positive in 12 females.

PRESENTING COMPLAINTS:

The commonest symptom was cough in 30% of the patients. The second commonest complaint in this study group was fever 22% of patients & followed by loss of weight & loss of apetite, Haemoptysis, expectoration are most common complaints in pulmonary Tuberculosis suspected patients. This correlates with the study of S.K Sharma et al[50].

DIABETIC STATUS:

Patients with diabetic mellitus are at higher risk for development of tuberculosis. Risk is highest with type 1 DM (24%).This correlates with study conducted by SchurmannR,Retamal G, et al. TB occurs predominantly lower lobes with cavitary lesions in diabetics.[45]

Among the 100 patients 19 patients were known diabetic , out of this 19 patients CBNAAT was positive in 13 patients, MGIT positive in 11 patients.

RADIOLOGICAL FEATURES:

In this study most common radiological feature was consolidation 44% [45], 2nd most common was patchy infiltration (reticulostriate nodules) 41% , followed by cavity 13% are most common presentations. This correlates with the study of Mc Adams HP, Erasmus J et al and l,ister et al[47]

Other lesions like miliary pattern was present in one patient and hydropneumothorax was present in one patient.

Yield was higher in cavitatory lung lesions, and yield will be near 100% if cavity is surrounded by consolidation.

BRONCHIAL WASH CBNAAT Vs MGIT IN SMEAR NEGATIVE PTB SUSPECTS:

In this study out of 100 patients , CBNAAT positive in 42 patients and in 2 patients rifampicin resistant. Among the 42 CBNAAT positive patients , no growth in 7 patients (MGIT negative). One patient was CBNAAT negative but bronchial wash AFB positive, a case of suspected Non Tuberculous Mycobacteria.

In our study, on comparing with MGIT in detecting MTB , CBNAAT diagnostic yield has been measured and it has the sensitivity of 92.1% , specificity was 88.7%, positive predictive value was 83.3% and negative predictive value was 94.8%.

The study conducted by Theron G et al (20) has sensitivity of 93% and specificity of 77- 98 and this correlates with our study which also has sensitivity 92.1%.

The study conducted by Teo J et al (20) in which speciicity was 89% this correlates with our study which has a specificity of 88.7%.

The Boehme et al (51) conducted a study with total of 1033 patients, in which specificity was 99%, because of lower sample size our study has lower specificity compared to Boehme study(51)

The steingart et al (52) conducted a multicentre study with total participants of 9557, because of much larger population comparing to our study population our study has lower specificity than study conducted by steingart et al (52)

In diagnosing the rifampicin sensitivity , on comparing with MGIT, CBNAAT has the sensitivity of 89.7% , specificity was 88.5%, positive predictive value was 83.3% and negative predictive value was 93.1%.

Compared to other study , in detecting rifampicin sensitivity, our study had a sensitivity of 89.74 and specificity of 88.52. it was little lesser than other study condutced by Boehme et al (51) in which study was conducted with larger number [1033] patients and sensitivity was higher than study conducted by kwak et al.(20)

CONCLUSION

Bronchial wash CBNAAT had a superior diagnostic yield (with high sensitivity and positive predictive value) to detect Mycobacterium tuberculosis and rifampicin resistance (with high sensitivity and specificity), in those cases of suspected pulmonary tuberculosis who have either smear-negative or sputum-scarce disease.

Bronchial wash CBNAAT has the advantages of being inexpensive, requires less manpower and gives results on the same day.

Using culture as the reference standard, CBNAAT on Bronchial wash showed a high sensitivity (92.1%). Furthermore, our study confirmed the advantage of early diagnosis of sputum smear-negative and sputum-scarce PTB by CBNAAT detecting more number of cases compared to smear microscopy.

The higher sensitivity is explained by the limit of detection of CBNAAT being 131 bacilli/ml compared to the limit of detection of AFB smear which is 10,000 bacilli/ml.

CBNAAT showed specificity of 88.7% .This is due to the higher CBNAAT positive culture negative cases observed asfalse positive cases when liquid culture (MGIT) is used as a gold standard. CBNAAT amplifies DNA from dead bacilli and reports a positive while cultures are negative

LIMITATIONS OF THE STUDY

- The study was conducted in a single centre with relatively less number of cases so the statistical power may be insufficient.
- Specificity in diagnaosis of Mycobaterium tuberculosis by CBNAAT was less than other studies conducted with large sample size so it needs further studies in future with large number of patients.
- In our study by using CBNAAT, Detection of Rifampicin resistance also has little less sensitivity and specificity, so it needs further evaluation studies with large number of patients with multi centre trial.

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ABBREVIATIONS

PTB	–	Pulmonary uberculosis
MTB	–	Mycobacterium Tuberculosis
MMC	–	Madras MedicalCollege
CBNAAT	–	Cartridge Based Nucleic Acid Amplification Test
MGIT	–	Mycobacterial Growth indicator tube
RGGGH	–	Rajiv Gandhi Government General Hospital
LJ	–	Lowenstein Jensen media

URKUND PLAGIARISM SCREEN SHOT

URKUND

Urkund Analysis Result

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PLAGIARISM CERTIFICATE

This to certify that this dissertation work titled **"DIAGNOSTIC YIELD OF BRONCHIAL WASH IN A SUSPECTED CASE OF SMEAR- NEGATIVE AND SPUTUM-SCARCE PULMONARY TUBERCULOSIS BY CBNAAT AND LIQUID MYCOBACTERIAL CULTURES (MGIT) IN RGGGH"** of the candidate **DR.A.PANDIYAN** with registration number **201727001** for the award of **MD** in the branch of **Tuberculosis & Respiratory Diseases.** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and the result shows **13 percentage** of plagiarism in the dissertation.

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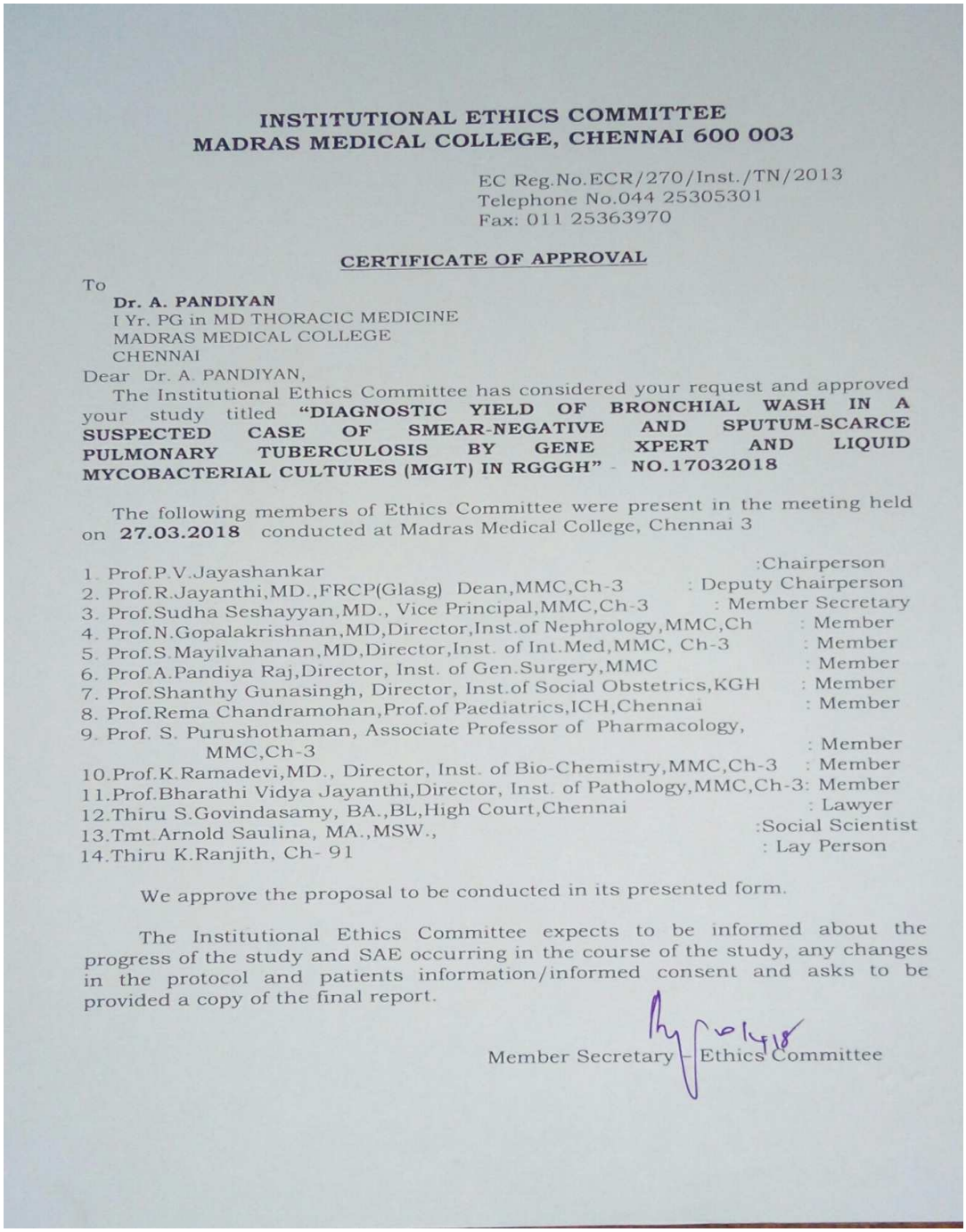

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

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CERTIFICATE OF APPROVAL

To
Dr. A. PANDIYAN
I Yr. PG in MD THORACIC MEDICINE
MADRAS MEDICAL COLLEGE
CHENNAI

Dear Dr. A. PANDIYAN,

The Institutional Ethics Committee has considered your request and approved your study titled **"DIAGNOSTIC YIELD OF BRONCHIAL WASH IN A SUSPECTED CASE OF SMEAR-NEGATIVE AND SPUTUM-SCARCE PULMONARY TUBERCULOSIS BY GENE XPERT AND LIQUID MYCOBACTERIAL CULTURES (MGIT) IN RGGGH" - NO.17032018**

The following members of Ethics Committee were present in the meeting held on **27.03.2018** conducted at Madras Medical College, Chennai 3

1. Prof.P.V.Jayashankar :Chairperson
2. Prof.R.Jayanthi,MD.,FRCP(Glasg) Dean,MMC,Ch-3 : Deputy Chairperson
3. Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3 : Member Secretary
4. Prof.N.Gopalakrishnan,MD,Director,Inst.of Nephrology,MMC,Ch : Member
5. Prof.S.Mayilvahanan,MD,Director,Inst. of Int.Med,MMC, Ch-3 : Member
6. Prof.A.Pandiya Raj,Director, Inst. of Gen.Surgery,MMC : Member
7. Prof.Shanthy Gunasingh, Director, Inst.of Social Obstetrics,KGH : Member
8. Prof.Rema Chandramohan,Prof.of Paediatrics,ICH,Chennai : Member
9. Prof. S. Purushothaman, Associate Professor of Pharmacology, MMC,Ch-3 : Member
10. Prof.K.Ramadevi,MD., Director, Inst. of Bio-Chemistry,MMC,Ch-3 : Member
11. Prof.Bharathi Vidya Jayanthi,Director, Inst. of Pathology,MMC,Ch-3: Member
12. Thiru S.Govindasamy, BA.,BL,High Court,Chennai : Lawyer
13. Tmt.Arnold Saulina, MA.,MSW., :Social Scientist
14. Thiru K.Ranjith, Ch- 91 : Lay Person

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary - Ethics Committee

PATIENT CONSENT FORM

Study Detail : "**Diagnostic yield of Bronchial Wash in a suspected case of Smear- Negative and Sputum-Scarce Pulmonary Tuberculosis by Gene Xpert and liquid mycobacterial cultures (MGIT) in RGGGH**"

Study Centre : Department of Thoracic Medicine, Department of Microbiology. Rajiv Gandhi Government General Hospital, Chennai.

Patient's Name :

Patient's Age :

Identification Number :

Patient may check (☑) these boxes

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ❑

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected. ❑

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ❑

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any ❑

unexpected or unusual symptoms.

I hereby consent to participate in this study. ❑

I hereby give permission to undergo complete clinical examination and necessary investigations. ❑

Signature of Investigator Signature/thumb impression

Study Investigator's Name: Patient's Name and Address:

Dr.A.PANDIYAN

நோயர்ஒப்புதல்படிவம்

ஆராய்ச்சிவிவரம்:Diagnostic yield of Bronchial Wash in a suspected case of Smear- Negative and Sputum-Scarce Pulmonary Tuberculosis by CBNAAT and liquid mycobacterial cultures (MGIT) in RGGGH.

ஆராய்ச்சிமையம்:நெஞ்சகமருத்துவத்துறை, நெஞ்சகமருத்துவநிலையம், சென்னைமருத்துவக்கல்லூரி, சென்னை.

நோயாளியின்பெயர்:

நோயாளியின்வயது:

நோயாளியின்எண்:

நோயாளிகள்இந்தக்கட்டங்களில் (☑) குறியிடவும் :

மேலேகுறிப்பிட்டஆராய்ச்சிநடைமுறையின்நோக்கம்எனக்குப்புரிந்ததுஎன்றுஉறுதி கூறுகிறேன். எனக்குக்கேள்விகேட்பதற்கானவாய்ப்பும், எனது சந்தேகங்களுக்கு மனநிறைவான பதிலும்கிடைத்தன.	☐
இந்தஆராய்ச்சியில்என்னுடையபங்கேற்புதன்னார்வமானதுஎன்றும்எந்தநேரத்திலும் , காரணமின்றியும், எனதுசட்டபூர்வ உரிமைகள் பாதிக்காத வகையிலும் இதில் இருந்துவிலகிக்கொள்ளஎனக்குத்தடையில்லைஎன்றும்உணர்ந்துகொண்டுள்ளேன்.	☐
இந்தஆராய்ச்சியில்இருந்துநான்விலகினாலும்நிகழ்கிறஆராய்ச்சியிலும், மேற்கொள்ளப்படும்சார்புடையகூடுதல்ஆய்வுகளிலும், இந்த மருத்துவப்பயிற்சி ஆய்வின் ஆதரவாளருக்கும், ஆதரவாளருக்காக வேலை செய்பவருக்கும், நெறிசார்குழுவுக்கும், ஒழுங்குமுறை அதிகாரிகளுக்கும் என்னுடைய உடல்நிலை அறிக்கைகளைதேர்ந்துகாணஎனதுஅனுமதிதேவையில்லைஎன்பதைஉணர்ந்துசம்ம திக்கிறேன்.	☐
மேற்குறிப்பிட்டஆய்வில்பங்கேற்கவும், ஆய்வின்போதான நெறிமுறைகளுக்கு உடன்படவும், ஆய்வுக்குழுவுடன் உளமாரஒத்துழைக்கவும் சம்மதிப்பதோடு ஏதேனும்உடல்நலக்குறைவால்அவதியுற்றாலோ, எதிர்பாராதவகையில் உடல் நிலையில்வழக்கத்துக்குமாறானஅறிகுறிகளைஉணர்ந்தாலோஉடனடியாகஆய்வுப்ப ணியாளரிடம்தெரிவிக்கவும்சம்மதிக்கிறேன்.	☐
இதன்மூலம்இந்தஆராய்ச்சியில்பங்குபெறஒப்புதல்அளிக்கிறேன்.	☐
இதன்மூலம்மருத்துவஆய்வுக்குக்கும்இன்றியமையாதசோதனைகளுக்கும்உட்படசம் மதிக்கிறேன்.	☐

ஆய்வாளரின்கையொப்பம் நோயாளியின்கையொப்பம் / கைரேகை

ஆய்வாளரின்பெயர்: மருத்துவர்.அ. பாண்டியன்நோயாளியின்பெயர்மற்றும்முகவரி:

PROFORMA

NAME OF THE PATIENT :

AGE / SEX :

OP/ NUMBER :

OCCUPATION :

ADDRESS :

CONTACT NUMBER :

COMPLAINTS :

PRESENT HISTORY :

PAST HISTORY :

FAMILY HISTORY :

GENERAL EXAMINATION :

Pallor: Icterus: Cyanosis: Clubbing:

Lymphadenopathy: Odema:

VITALS

Pulse Rate: BP: Respiratory rate: Temperature:

SYSTEMIC EXAMINATION

CARDIOVASCULAR SYSTEM:

RESPIRATORY SYSTEM :

ABDOMEN :

CENTRAL NERVOUS SYSTEM

BASIC BLOOD INVESTIGATIONS:

RBC TC DC HB PLATELETS ESR

RFT

SR. ELECTROLYTES

LFT

CXR:

CT-CHEST:

SPUTUM MICROSCOPY:

GENE XPERT

CULTURE REPORT

BRONCHOSCOPY FINDINGS AND REPORTS:

S.NO	NAME	AGE	Clinical features	SEX	ICTC	LFT/RFT	CXR	SP AFB	BW CBNAAT	BW AFB	MGIT
1	X1	29	Cough	M	neg	NDM	Rt U/L cavitatory consolidatior	neg	MTB det high - RS	neg	detected RS
2	X2	23	Cough	M	neg	NDM	B/L U/L Consolidation	neg	MTB det low - RS	neg	detected RS
3	X3	53	Hemoptysis	M	neg	NDM	Rt U/L ,M/L consolidation	neg	neg	neg	neg
4	X4	63	Cough	M	neg	DM	Lt U/L consolidation	neg	MTB det low - RS	neg	negative
5	X5	68	Hemoptysis	M	neg	NDM	Rt U/L cavitatory cons	neg	neg	neg	neg
6	X6	65	Cough	M	neg	DM	Rt M/L consolidation	neg	neg	neg	neg
7	X7	37	Expectoration	F	neg	NDM	Rt L/L consolidation	neg	neg	neg	neg
8	X8	68	Expectoration	M	neg	NDM	B/L U/L het opacity	neg	neg	neg	neg
9	X9	29	Cough	M	neg	NDM	Rt L/L consolidation	neg	neg	neg	neg
10	X10	45	Cough	M	neg	DM	B/L U/L Consolidation	neg	MTB det high - RS	neg	detected RS
11	X11	21	Hemoptysis	M	neg	NDM	Rt U/L consolidation	neg	MTB det low - RS	neg	detected RS
12	X12	44	Fever	M	neg	NDM	Rt U/L consolidation	neg	neg	neg	neg
13	X13	55	Weight loss	F	neg	NDM	Lt L/L consolidation	neg	neg	neg	neg
14	X14	28	Hemoptysis	M	neg	NDM	Lt U/L consolidation	neg	MTB det low - RS	neg	detected RS
15	X15	32	Cough	M	neg	NDM	B/L L/L Consolidation	neg	neg	neg	neg
16	X16	63	Cough	M	neg	DM	Rt L/L consolidation	neg	MTB det low - RS	neg	detected RS
17	X17	43	Fever	M	neg	NDM	Rt U/L patchy infiltration	neg	neg	neg	neg
18	X18	60	Fever	M	neg	DM	Lingula patchy infiltration	neg	neg	neg	neg
19	X19	37	Weight loss	F	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	neg
20	X20	16	Fever	F	neg	NDM	Multi lobar consolidation	neg	MTB detected - RR	neg	detected RR
21	X21	58	Cough	M	neg	DM	Rt U/L consolidation	neg	MTB det low - RS	neg	negative
22	X22	22	Fever	M	neg	NDM	Rt U/L cavitatory consolidatior	neg	Mtb det med - RS	neg	detected RS
23	X23	45	Fever	F	neg	DM	B/L L/L Consolidation	neg	MTB det low - RS	neg	detected RS
24	X24	23	Cough	F	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	neg
25	X25	55	Expectoration	F	neg	NDM	Lingular patchy infiltration	neg	neg	neg	neg
26	X26	55	Weight loss	M	neg	DM	Lt U/L consolidation	neg	MTB det Med - RS	neg	detected RS
27	X27	70	Chest pain	M	neg	NDM	Rt L/L consolidation	neg	MTB det low - RS	neg	negative
28	X28	48	Fever	M	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	neg
29	X29	20	Cough	M	neg	NDM	Rt U/L patchy infiltration	neg	neg	neg	detected RS
30	X30	60	Weight loss	M	neg	NDM	B/L U/L patchy infiltration	neg	neg	neg	neg
31	X31	50	Fever	M	neg	DM	Rt L/L consolidation	neg	neg	neg	neg
32	X32	31	Cough	M	neg	NDM	Rt L/L patchy infiltration	neg	neg	neg	neg
33	X33	38	Weight loss	M	neg	NDM	Rt U/L cavity	neg	MTB det low - RS	neg	detected RS
34	X34	20	Hemoptysis	M	neg	NDM	B/L U/L Cavity	neg	MTB det high - RS	neg	detected RS

35	XX35	46	Expectoration	M	neg	NDM	Rt L/L patchy infiltration	neg	neg	neg	neg
36	X36	30	Expectoration	M	neg	NDM	Lingular patchy infiltration	neg	neg	neg	neg
37	X37	61	Cough	F	neg	NDM	Lt U/L cavity	neg	neg	neg	neg
38	X38	36	Weight loss	F	neg	NDM	Rt M/L consolidation	neg	MTB det med - RS	neg	detected RS
39	X39	36	Cough	F	neg	NDM	Lt U/L cavity	neg	neg	positive	?NTM
40	X40	21	Fever	F	neg	NDM	Rt U/L consolidation	neg	MTB det low - RS	neg	detected RS
41	X41	57	Expectoration	M	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	neg
42	X42	65	Expectoration	M	neg	DM	Lt L/L cavitatory cons	neg	neg	neg	neg
43	X43	37	Expectoration	M	neg	NDM	B/L U/L patchy infiltration	neg	neg	neg	neg
44	X44	51	Expectoration	M	neg	NDM	Rt U/L patchy infiltration	neg	neg	neg	neg
45	X45	21	Hemoptysis	M	neg	NDM	B/L U/L Cavity	neg	MTB det low - RS	neg	negative
46	X46	56	Expectoration	M	neg	NDM	Rt L/L cavity	neg	neg	neg	neg
47	X47	59	Weight loss	M	neg	NDM	Rt U/L patchy infiltration	neg	neg	neg	neg
48	X48	47	Expectoration	F	neg	NDM	Rt U/L cavity	neg	neg	neg	neg
49	X49	60	Cough	F	neg	DM	B/L L/L Consolidation	neg	MTB det low - RS	neg	detected RS
50	X50	53	Weight loss	M	neg	DM	Lingular consolidation	neg	MTB det high - RS	neg	detected RS
51	X51	53	Weight loss	M	neg	NDM	Rt L/L patchy infiltration	neg	neg	neg	neg
52	X52	50	Hemoptysis	M	neg	DM	Rt U/L cavitatory cons	neg	MTB det low - RS	neg	detected RS
53	X53	15	Fever	F	neg	NDM	Lt U/L cavity	neg	MTB det low - RS	neg	detected RS
54	X54	72	Expectoration	F	neg	NDM	Rt U/L patchy infiltration	neg	neg	neg	neg
55	X55	37	Fever	F	neg	NDM	Rt M/L Consolidation	neg	neg	neg	neg
56	X56	63	Cough	F	neg	NDM	Lingular patchy infiltration	neg	neg	neg	neg
57	X57	18	Weight loss	M	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	neg
58	X58	44	Hemoptysis	M	neg	DM	Rt U/L consolidation	neg	MTB det low - RS	neg	detected RS
59	X59	60	Cough	F	neg	NDM	B/L L/L Consolidation	neg	neg	neg	neg
60	X60	25	Fever	M	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	detected RS
61	X61	53	Hemoptysis	M	neg	NDM	B/L L/L Consolidation	neg	neg	neg	neg
62	X62	24	Cough	M	neg	NDM	Rt U/L cavitatory consolidatior	neg	MTB det med - RS	neg	detected RS
63	X63	47	Weight loss	F	neg	NDM	Rt L/L consolidation	neg	neg	neg	neg
64	X64	69	Fever	M	neg	NDM	Rt U/L consolidation	neg	neg	neg	neg
65	X65	48	Weight loss	M	neg	DM	B/L L/L consolidation	neg	MTB det med - RS	neg	detected RS
66	X66	55	Cough	M	neg	NDM	Rt L/L patchy infiltration	neg	neg	neg	neg
67	X67	41	Fever	M	neg	NDM	Rt U/L patchy infiltration	neg	MTB det low - RS	neg	negative
68	X68	26	Fever	F	neg	NDM	Lt U/L consolidation	neg	MTB det high - RS	neg	detected RS
69	X69	39	Weight loss	M	neg	NDM	**Rt U/L cavity**	neg	MTB det med - RS	neg	detected RS

70	X70	34	Weight loss	M	neg	NDM	Lt U/L patchy infiltration	neg	MTB det low - RS	neg	negative
71	X71	27	Fever	F	neg	NDM	Lt U/L cavitatory consolidatior	neg	MTB det med - RS	neg	detected RS
72	X72	53	Expectoration	M	neg	DM	**Rt U/L consolidation**	neg	MTB det low - RS	neg	detected RS
73	X73	40	Weight loss	F	neg	NDM	Lt L/L consolidation	neg	MTB det low - RS	neg	detected RS
74	X74	50	Weight loss	F	neg	NDM	B/L L/L patchy infiltration	neg	MTB det v. low - RS	neg	negative
75	X75	58	Hemoptysis	M	neg	DM	Rt L/L, Lingula patchy infiltrati	neg	neg	neg	neg
76	X76	61	Cough	M	neg	NDM	B/L U/L patchy infiltration(silic	neg	neg	neg	detected RS
77	X77	20	Cough	M	neg	NDM	Lt L/L patchy infiltration	neg	neg	neg	neg
78	X78	52	Cough	F	neg	NDM	Rt L/L consolidation	neg	MTB det low - RS	neg	detected RS
79	X79	53	Weight loss	M	neg	NDM	Lt U/L cavity Cons	neg	MTB detected - RR	neg	detected RR
80	X80	52	Cough	M	neg	NDM	Rt U/L patchy infiltration	neg	neg	neg	neg
81	X81	54	Fever	M	neg	NDM	B/L U/L lingula consolidation	neg	MTB det low - RS	neg	detected RR
82	X82	55	Fever	F	neg	NDM	Rt M/L, L/L patchy infiltration	neg	neg	neg	neg
83	X83	45	Cough	M	neg	NDM	Lt L/L patchy infiltration	neg	neg	neg	neg
84	X84	62	Fever	M	neg	DM	Multi lobar consolidation	neg	MTB det low - RS	neg	detected RS
85	X85	22	Cough	M	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	neg
86	X86	37	Weight loss	M	neg	NDM	B/L U/L, Rt L/L patchy infiltrati	neg	neg	neg	neg
87	X87	18	Fever	M	neg	NDM	Rt U/L cavity	neg	MTB det med - RS	neg	detected RS
88	X88	19	Fever	F	neg	NDM	Multi lobar consolidation	neg	MTB det low - RS	neg	detected RS
89	X89	72	Cough	M	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	neg
90	X90	48	Cough	F	neg	NDM	Miliary patern	neg	MTB det low - RS	neg	detected RS
91	X91	54	Chest pain	M	neg	NDM	Lt Hydro pneumothorax	neg	MTB det high - RS	neg	detected RS
92	X92	45	Cough	M	neg	NDM	Lt L/L Cavity	neg	neg	neg	neg
93	X93	51	Expectoration	M	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	neg
94	X94	43	Expectoration	F	neg	NDM	Rt U/L patchy infiltration	neg	neg	neg	neg
95	X95	67	Expectoration	M	neg	DM	Lt U/L patchy infiltration	neg	neg	neg	neg
96	X96	62	Cough	M	neg	NDM	Rt L/L patchy infiltration	neg	neg	neg	neg
97	X97	39	Weight loss	M	neg	NDM	Lt U/L Consolidation	neg	MTB det med - RS	neg	detected RS
98	X98	56	Expectoration	M	neg	NDM	B/L U/L patchy infiltration	neg	neg	neg	neg
99	X99	32	Cough	M	neg	NDM	Lt U/L patchy infiltration	neg	neg	neg	neg
100	X100	46	Fever	M	neg	NDM	Lt L/L patchy infiltration	neg	neg	neg	neg