“Evaluating the diagnostic utility of sputum and bronchoalveolar Aspirate CBNAAT For Mycobacterium tuberculosis in diagnosis of sputum negative pulmonary tuberculosis-One-year tertiary hospital based cross-sectional study.”

By

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in

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EVALUATING THE DIAGNOSTIC UTILITY OF SPUTUM AND BRONCHOALVEOLAR ASPIRATE CBNAAT FOR MYCOBACTERIUM TUBERCULOSIS IN DIAGNOSIS OF SPUTUM NEGATIVE PULMONARY TUBERCULOSIS-ONE YEAR TERTIARY HOSPITAL BASED CROSS SECTIONAL STUDY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee.

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ABBREVIATIONS

AFB - ACID FAST BACILLI

BAL - BRONCHOALVEOLAR LAVAGE

BALF - BRONCHOALVEOLAR ALVEOLAR LAVAGE FLUID

BC - BEFORE CHRIST

BCE - BEFORE COMMON ERA

CBNAAT- CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST

CD - CLUSTER OF DIFFERENTIATION

CDC - CENTER FOR DISEASE CONTROL AND PREVENTION

CE - COMMON ERA

CFU - COLONY FORMING UNIT

CMI - CELL MEDIATED IMMUNITY

CRS - COMPOSITE REFERENCE STANDARD

CSF - CEREBROSPINAL FLUID

TYPE 2 DM - TYPE 2 DIABETES MELLITUS

DNA - DEOXYRIBO NUCLEIC ACID

DOTS - DIRECTLY OBSERVED TREATMENT SHORT COURSE THERAPY

DTH - DELAYED TYPE OF HYPERSENSITIVITY

EPTB - EXTRARESPIRATORY TUBERCULOSIS

FOB - FIBRE OPTIC BRONCHOSCOPE

H/O - HISTORY OF

HCW - HEALTH CARE WOKERS

HIV - HUMAN IMMUNODEFICIENCY VIRUS

IGRA - INTERFERON GAMMA RELEASE ASSAY

IL - INTERLEUKIN

IMID - IMMUNE MEDIATED INFLAMMATORY DISORDERS

INF - INTERFERON GAMMA

INH - ISONIAZID

LAM - LIPOARABINOMANNAN

LJ - LOWENSTEIN-JENSEN

LPA - LINE PROBE ASSAY

LTBI - LATENT TUBERCULAR BACILLI INFECTION

MDR - MULTIDRUG RESISTANT TUBERCULOSIS

MGIT - MYCOBACTERIAL GROWTH INDICATOR TUBE

MRC- MEDICAL RESEARCH CENTER

MTB - MYCOBACTERIUM TUBERCULOSIS

NPV - NEGATIVE PREDICTIVE VALUE

NTEP - NATIONAL TB ELIMINATION PROGRAMME

NTM - NON-TUBERCULAR MYCOBACTERIUM

PAS - PARA-AMINO SALYSILIC ACID

PCR - POLYMERASE CHAIN REACTION

PNA - PEPTIDE NUCLEIC ACID

PPV - POSITIVE PREDICTIVE VALUE

PTB - PULMONARY TUBERCULOSIS

RIF - RIFAMPICIN

RNA - RIBOXY NUCLEIC ACID

RNTCP - REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME

rRNA - RIBOSOMAL RIBOXY NUCLEIC ACID

SM - STREPTOMYCIN

TB - TUBERCULOSIS

TH - T HELPER CELLS

TNF - TUMOR NECROSIS FACTOR

WHO - WORLD HEALTH ORGANISATION

WRD - WHO RECOMMENDED RAPID DIAGNOSTIC

XDR - XTENSIVE DRUG RESISTANT TUBERCULOSIS

ZN - ZIEHL NEELSEN

ABSTRACT

BACKGROUND:

TB is a major global health issue, affecting 10.6 million people worldwide in 2022 alone which manifests as PTB constituting around 85% of total cases. Prompt and precise diagnosis is essential for efficient administration and regulation of TB, as delayed or overlooked diagnosis can lead to heightened transmission, disease advancement, and unfavorable outcomes. The traditional method for diagnosing pulmonary tuberculosis heavily relies on sputum smear microscopy and culture, which have drawbacks in terms of sensitivity and the time required to obtain results.

CBNAAT has significantly transformed the diagnosis of TB by offering quick and precise outcomes and it can test clinical samples directly for both the Mycobacterium tuberculosis complex and rifampicin resistance

This study was designed to assess the role of CBNAAT in diagnosing sputum smear-negative pulmonary TB in a tertiary care hospital. The diagnostic utility of CBNAAT was evaluated by analyzing sputum and bronchoalveolar aspirate samples. The study sought to gain insights into the most effective diagnostic approach for this challenging subset of tuberculosis cases by comparing the performance of CBNAAT on these two sample types.

OBJECTIVES:

1. To study and compare the sensitivity and specificity of sputum and bronchoalveolar CBNAAT for Mycobacterium tuberculosis in the diagnosis of sputum smear-negative pulmonary tuberculosis.

2. To study and compare bronchoalveolar lavage CBNAAT positivity and MTB culture positivity.

METHODS:

After obtaining approval and clearance from the institutional ethics committee, individuals fulfilling the inclusion criteria were enrolled for the study after obtaining informed consent. Demographic data was collected using a questionnaire. Patients provided early morning sputum samples for CBNAAT for MTB. If sputum CBNAAT for MTB was negative, patients underwent bronchoscopy to obtain bronchoalveolar lavage (BAL) samples, which were then subjected to CBNAAT for MTB and MGIT culture

RESULTS:

The study included 152 sputum smear-negative but suspected PTB patients.

The sensitivity and specificity of sputum CBNAAT for MTB was 18.52%, and 93.15% respectively. The sensitivity & specificity of BAL CBNAAT was 97.78% and 66.18% respectively. PPV of BAL CBNAAT was 65.67% and NPV of BAL CBNAAT was 97.83%.

CONCLUSION:

Bronchoscopic aspirate for CBNAAT is more sensitive and specific than sputum CBNAAT for MTB in negative sputum smear AFB samples for PTB diagnosis. Bronchoscopic aspirate is better for obtaining respiratory samples in patients who cannot produce sputum. Patients with cavity as radiological finding and sputum smear AFB negative should be subjected to bronchoscopy. However, interpreting positive

sputum CBNAAT samples with caution due to high chances of detection of dead bacilli and slow growth of mycobacterial growth.

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INTRODUCTION

Tuberculosis (TB) is a global health issue having infected 10.6 million people globally in 2022, out of which 5.8 million were men, 3.5 million were women, and 1.3 million were children.[1] Pulmonary tuberculosis (PTB) is predominant manifestation of this disease, constituting around 85% of total cases. [2] Prompt and precise diagnosis is essential for efficient administration and regulation of TB, as delayed or overlooked diagnosis can cause heightened transmission, disease advancement, and unfavorable results.

The traditional method for diagnosing pulmonary tuberculosis heavily relies on sputum smear microscopy & culture, which has drawbacks in terms of sensitivity and the time it takes to obtain results.[3] Sputum smear microscopy exhibits limited sensitivity, especially in instances of paucibacillary disease or in patients who struggle to generate sputum samples. [4] Culture techniques, although more accurate, can require several weeks to produce outcomes, resulting in a delay in the commencement of suitable treatment.[5]

Diagnosing pulmonary TB without detectable sputum is a difficult task. Approximately 22-61% of cases of pulmonary TB do not show any signs of the disease in sputum samples. These patients frequently exhibit non-specific symptoms and may have negative results in sputum smears or cultures, which can cause delays in diagnosis and increase the chances of disease transmission.[6] In such situations, it may be essential to perform invasive procedures such as bronchoscopy and bronchoalveolar lavage (BAL) to acquire respiratory samples for analysis.

The introduction of molecular diagnostic tests has improved the diagnosis of TB by offering quick & precise outcomes.[7] CBNAAT, is a PCR test that can test clinical samples directly for both the MTB complex and rifampicin resistance.[8]

Multiple studies have assessed the diagnostic accuracy of CBNAAT on different respiratory samples, such as sputum, bronchial washings, and bronchoalveolar lavage (BAL) fluid.[9,109,10] Although the effectiveness of CBNAAT on sputum samples is widely recognized, its ability to diagnose sputum-negative PTB cases using BAL samples is still being studied.

Present study is aims to assess the role of CBNAAT and bronchoscopy in diagnosis of sputum smear-negative PTB. The diagnostic power of CBNAAT will be evaluated by analyzing sputum and bronchoalveolar aspirate samples. The study seeks to gain insights into the most effective diagnostic approach for this challenging subset of tuberculosis cases by comparing the performance of CBNAAT on these two sample types.

AIMS AND OBJECTIVES

OBJECTIVES:

- Study and compare sensitivity and specificity of sputum and bronchoalveolar CBNAAT for Mycobacterium tuberculosis in diagnosis of sputum smear negative pulmonary tuberculosis.
- Compare BAL CBNAAT with MTB culture positivity

REVIEW OF LITERATURE

HISTORICAL LANDMARKS IN TUBERCULOSIS (TB) [11]

The Age of Discovery

Tuberculosis is caused by Mycobacterium tuberculosis complex (MTBC) and consists of 9 lineages some of which can cause infection in humans and some can infect few animals. MTBC consists of following species of Mycobacterium- tuberculosis, bovis, microti, canettii, caprae, africanum, and pinnipedii.[12,13] MTB is transmitted by humans whereas M. bovis is spread by cattle, bison, and deer; and M. avium is spread by poultry. Wild mammals and birds can contract M. bovis and M. avium infections, respectively, and these animals can occasionally spread their infections to domestic animals.[14]

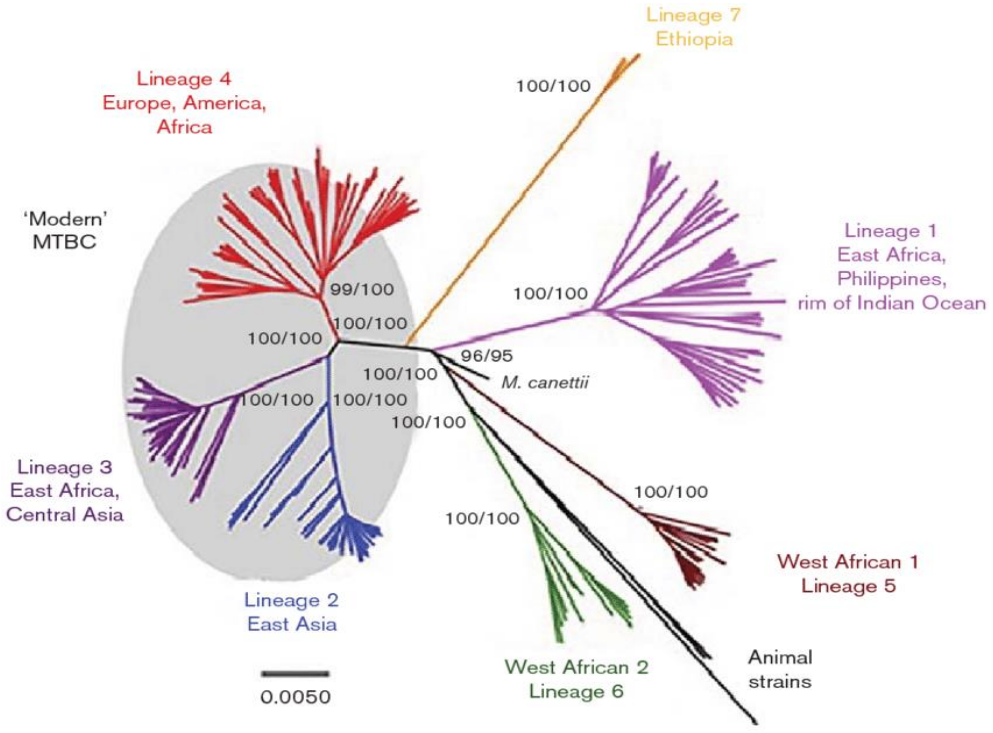


Figure 1 Phylogeny of the MTB complex based on genome-wide studies. [15]

Throughout history, tuberculosis has gone by a number of names, including phthisis pulmonalis, white plague, and consumption.[11] Hippocrates in approximately 460 B.C identified TB as a commonly lethal illness. He cautioned physicians against providing medical care to individuals with advanced-stage TB, as their inevitable demise could tarnish their professional standing.[16]

In 1679, the Dutch scientist Sylvius de la Boe made pathological and anatomical observations of tuberculosis and identified the presence of tubercles as pathological feature in TB patients. [17]The author's findings were subsequently published in Opera Medica, detailing the typical impact of the disease on the lungs, which later advanced to the formation of hollow areas and pockets of pus. He was the first to establish a connection between phthisis, a lung disease, and scrofula, affecting the lymph nodes in the neck.[18] Spinal tuberculosis was initially documented by a British surgeon named Percivall Pott in 1779.[17] TB can impact any organ, but it most commonly affects the lungs.[19]

Milestones in diagnosis and treatment of PTB

Benjamin Marten was the first person to identify tuberculosis (TB) as a communicable disease, characterizing it as an illness caused by "little living things." Furthermore, he stated that contracting the disease requires extra and intimate contact with an individual infected with TB.[16] In 1865, Jean-Antoine Villemin, a French military physician, demonstrated the transmission of tuberculosis (TB) from humans to cattle. [20]

On 24th March, 1882 Robert Koch, one of the founders of bacteriology, discovered the bacillus that causes tuberculosis and is considered as the World TB Day and he devised a staining method to detect Mycobacterium tuberculosis (MTB) and explained that the mode of transmission is through droplets during sneezing or coughing. [20]

Discovery of Tuberculosis Vaccine and Treatment

The French physicians Camille Guerin and Leon Charles Albert Calmette created the BCG vaccine in 1906 & data revealed that while this vaccine may effectively prevent severe forms of tuberculosis in children, it does not produce the same result in adults. Therefore, the main approach continues to be focused on preventing the spread of tuberculosis and administering anti-tubercular drugs to individuals with active cases. The Medical Research Council was founded in 1913 in Great Britain, primarily dedicated to addressing tuberculosis. [11]

Before the development of anti-TB drugs, people would isolate patients as a therapy method and focus on improving their nutritional health in absence of definitively treatment. Patients had limited medical choices and were primarily offered surgical interventions such as plombage and pneumonectomy. "Plombage" is a surgical technique used to treat cavitary PTB in the upper lobe of the lung and an inert substance is placed in the pleural space to compress the cavitary lesion.[21]

Advances in chemotherapy of tuberculosis[22]

1. Sulphonamides' bacteriostatic action was first observed in 1940, when animals infected with MTB were studied. It was observed that promin (glucosulfone sodium), a dapsone derivative, was a chemotherapeutic agent that could halt the progression of tuberculosis in guinea pigs that would otherwise be fatal.[22]
2. When Waksman isolated streptomycin in 1944 and it. Discovery of streptomycin by Waksman in 1944 from Streptomyces griseus had a curative effect on experimental TB in animal model.[22]

3. It was found in 1949 that when administered streptomycin and PAS were used together, it resulted in reduced development of resistance. Since then, it's established that the combination of two or more medications is necessary for the effective treatment of tuberculosis.[22]

4. **Isoniazid's** antitubercular properties were discovered in 1952. It was first made 40 years ago and still is an important part of our DS-TB regimens because of its action, affordability, and lack of major side effects. [22]

5. In 1956, shocking tests were done in **Madras** that showed ambulatory and domiciliary treatment worked very well and did not make family members more likely to get sick. These results led to a dramatic shift from conventional sanatorium care and created new opportunities for national treatment initiatives in developing nations.[22]

6. **Rifampicin** was found to be possibly the most successful treatment for tuberculosis in the late 1960s. The use of rifampicin, a broad-spectrum antibiotic primarily used to treat tuberculosis, resulted in the development of contemporary and successful short-course regimens.[22]

7. In 1964, **intermittent regimens** were found to be equally effective when compared to daily regimens, providing benefit of being easily accessible and under direct supervision.[22]

8. Short-course chemotherapy regimens were proposed by the **British Medical Research Council** after adequate research.[22]

- 6- and 8-month regimens are successful in producing a high rate and a low relapse rate.

- Even patients with smear-positive cavitary illness can benefit from short-term, effective treatment with regimens combining rifampicin.
- Pyrazinamide is only required for the first part of treatment, although rifampicin remains essential in regimens lasting six and eight months.

9. From 1995 to 2008, the **DOTS program** successfully treated over 36 million patients with tuberculosis, preventing around 6 million deaths.

The DOTS program consists of five essential components:

- Political commitment with adequate financing.
- Prompt case detection through quality tests.
- Uniform and supervised treatment with adequate patient support.
- Monitoring and evaluation of program and impact measurement.

Pulmonary tuberculosis

EPIDEMIOLOGY

In 1993, the WHO classified TB as a pandemic, impacting approximately one-third of the world-wide population and it still remains a prominent cause of worldwide mortality & morbidity among patients with medical conditions.[23]

The global mortality toll from TB was 1.3 million in 2022, with 167,000 of those fatalities among HIV positive people. Overtaking HIV and AIDS, TB remains the second leading infectious killer worldwide. TB prevention has saved 75 million lives worldwide since 2000.[1]

In 2022, WHO's report on regional TB prevalence revealed that "South-East Asian Region (46%), African Region (23%), and Western Pacific (18%) regions had the newest TB cases. The WHO attributes more than 50% of the worldwide TB burden to four countries: India contributing to 28% of cases followed by Indonesia (9.2%), China (7.4%) and the Philippines (7.0%)."[1]

BURDEN IN INDIA

Although there was a temporary decrease in TB notifications in 2020 and 2021, NTEP successfully recovered and surpassed these figures. With 24.2 lakh cases reported in 2021, India achieved a significant milestone in 2022 in its efforts to treat tuberculosis. This translates into a notification rate of approximately 172 cases per lakh individuals. 7.3 lakh private tuberculosis case notifications, the highest number to date, were also received during this time. In 2022, 63,801 cases of MDR/RR were diagnosed. Increased efforts to find unidentified TB patients through active and passive case finding, which kept the progress

going, made the aforementioned accomplishment possible. From 763 in 2021 to 1281 per lakh population in 2022, the nation's presumptive TB examination rate climbed by 68%.[24]

AETIOLOGY

M. tuberculosis is a small, aerobic, non-motile, weakly gram-positive bacillus and exhibit acid fastness. It has a complex cell wall structure which is rich in long-chain fatty acids [25] and it contains peptidoglycans and complex lipids contributing to its pathogenicity. The capsule & outer layer of the cell wall, helps the bacterium live and cause disease. [26]

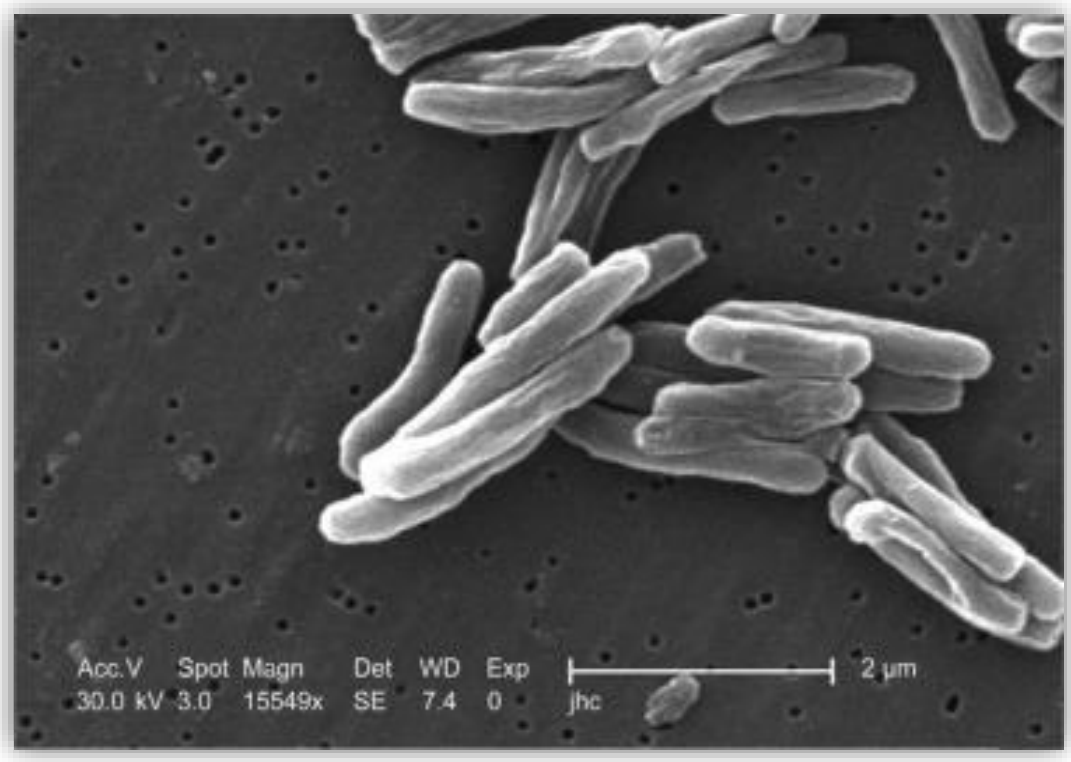


Figure 2 Electron microscopy of Mycobacterium tuberculosis

TRANSMISSION [27,28]

MTB is primarily transmitted by air droplets and the risk of MTB infection after exposure depends on three factors:

- The number of infectious droplet nuclei produced by a case.
- The volume of air in which droplets are suspended.
- The period of exposure to air containing these droplet nuclei.

There is a difference in the risk of infection between exposure outside and indoors. When indoors, especially with inadequate ventilation, infectious droplet nuclei become confined. Outside, on the other hand, they quickly spread throughout air and are quickly destroyed by sunlight.[28]

PATHOGENESIS

Tuberculosis (TB) is spread through aerosol droplets of infected person and these droplets are released into the air when individuals with active TB sneeze, cough & talk. Once host inhales the bacilli, it migrates through the respiratory tract to the lung. After this host's natural immune system becomes active to suppress the infection, and the bacilli are taken in by macrophages. When these macrophages cannot eliminate the infection, the bacilli reproduce inside intracellular environment of macrophages and then gets released. The released bacilli are eaten by other macrophages and increase in number.[29] Lymphocytes initiate a CMI response wherein immune cells arrives and aim to isolate the bacilli and restrict further proliferation.[30] The host does not develop symptomatic, and the TB bacilli may die or enter dormant state in the granuloma but if immunity is weakened, the disease promptly becomes active.[31]

Role of cytokines

In certain patients with NRAMP1 gene variants, the disease may advance without an effective immune response. **"(NRAMP1** is a transmembrane ion transport protein found in endosomes and lysosomes that contribute to the microbial killing.)" [32]

Dendritic cells and macrophages present these MTB antigens to CD4 cells in draining lymph nodes which is 3 weeks after exposure. [32] Macrophage secrete IL-12 which induces TH1 CD4+ T cells and these cells secrete IFN-γ. IFN-γ which drives macrophage activation which release mediators and also upregulate genes, which recruits monocytes, and differentiate into "epithelioid histiocytes".[32]

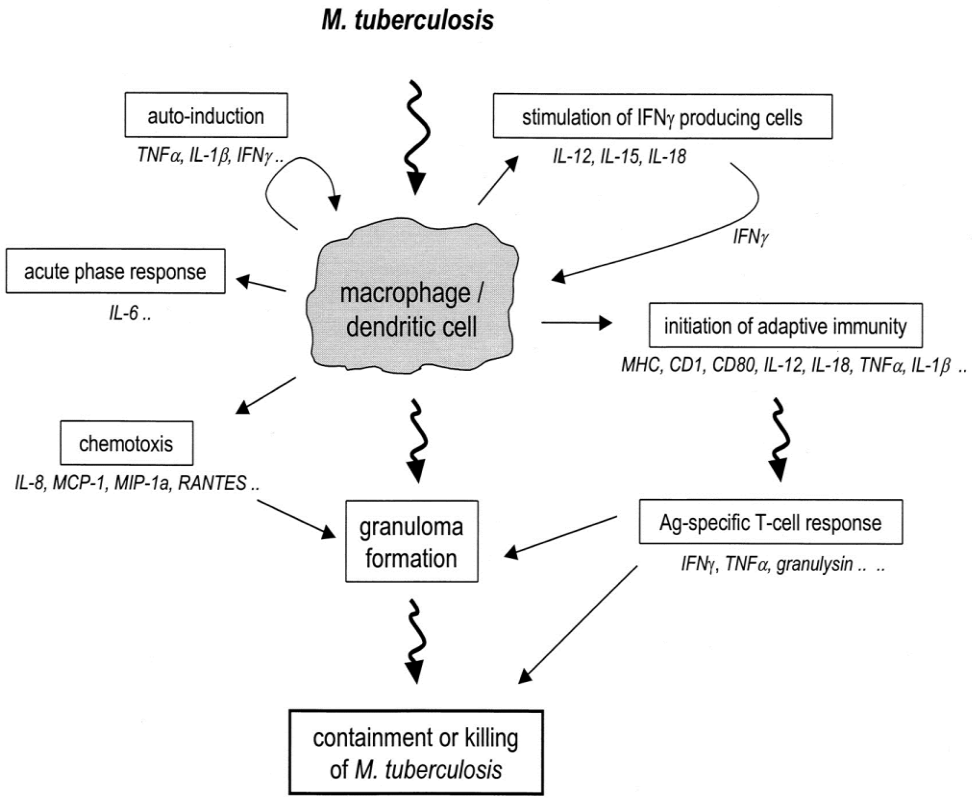


Figure 3 Pathogenesis of TB. (Adapted from Robbins basic pathology 9th ed)

The granuloma is a characteristic of PTB which consists of disorganized cluster of macrophages with purpose of limiting the dissemination of bacteria in body.[31] When the

granuloma cannot remove the pathogen, it captures the bacilli preventing illness in immunocompetent people. However, blocking phagolysosome fusion and evading the CMI allows the bacteria to survive in host. MTB can survive for decades in this friendly habitat by influencing the immune system and slowing replication.[30]

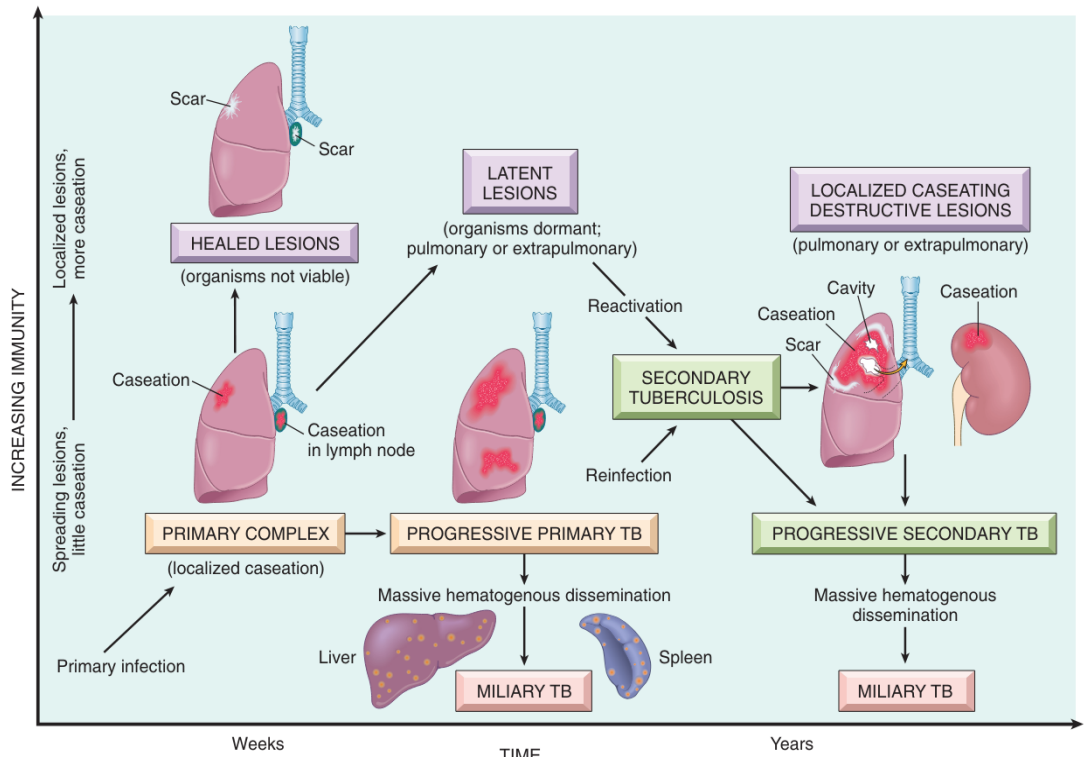


Figure 4 Natural history and spectrum of tuberculosis disease. (Robbins basic pathology 9th ed)

Macrophages differentiate into foamy macrophages as the granuloma matures. [33] A caseous granuloma, also called caseum, forms when host immune cells break down microscopically, necrotizing its core[34], which closely resembles cheese. Foamy macrophages, characterized by the accumulation of lipid droplets, are found in the vicinity of the necrotic areas of the granuloma.[35] Additionally, mycolic acids which is the main lipid elements of MTB's cell wall, helps macrophages become foam cells.[36]

These granulomas serve as reservoirs, shielding the bacilli that maintain a dormant form.[37] With progression of MTB infection, its caseous core softens and cavity develops, allowing the bacilli to reappear. As a result, the patient develops symptoms of active tuberculosis, which leads to the transmission of the bacilli to a healthy individual.[33,37] Host's immunity greatly limits bacterial replication and prevent active infection.[30] The granuloma liquefies and cavitates during compromised immune system, causing the dormant bacilli to reactivate and multiply.[35] Ultimately, bacilli disperse throughout the lung and body through blood. This stage of the disease manifests symptoms and becomes highly contagious as active TB infection. During active disease, these granulomas appear at distinct stages in lung histology, suggesting that they promote tuberculosis recurrence. [38]

Granulomas can be histologically classified into three types: solid, necrotic, and caseous. Histologically, solid granulomas are associated with both inflammation and bacillary containment because they develop early in the disease and also cause tissue damage. T lymphocytes keep the infection under control inside the structure, which frequently has a fibrotic barrier around it and no central necrotic tissue. [38,39]

Solid granulomas are more prevalent in LTBI due to reduced MTB load. The solid granuloma core necroses as the disease progresses and dormant bacteria becomes active when the necrotic core grows and melts forming a caseous granuloma. [38,39]

FATE OF TUBERCULOSIS INFECTION

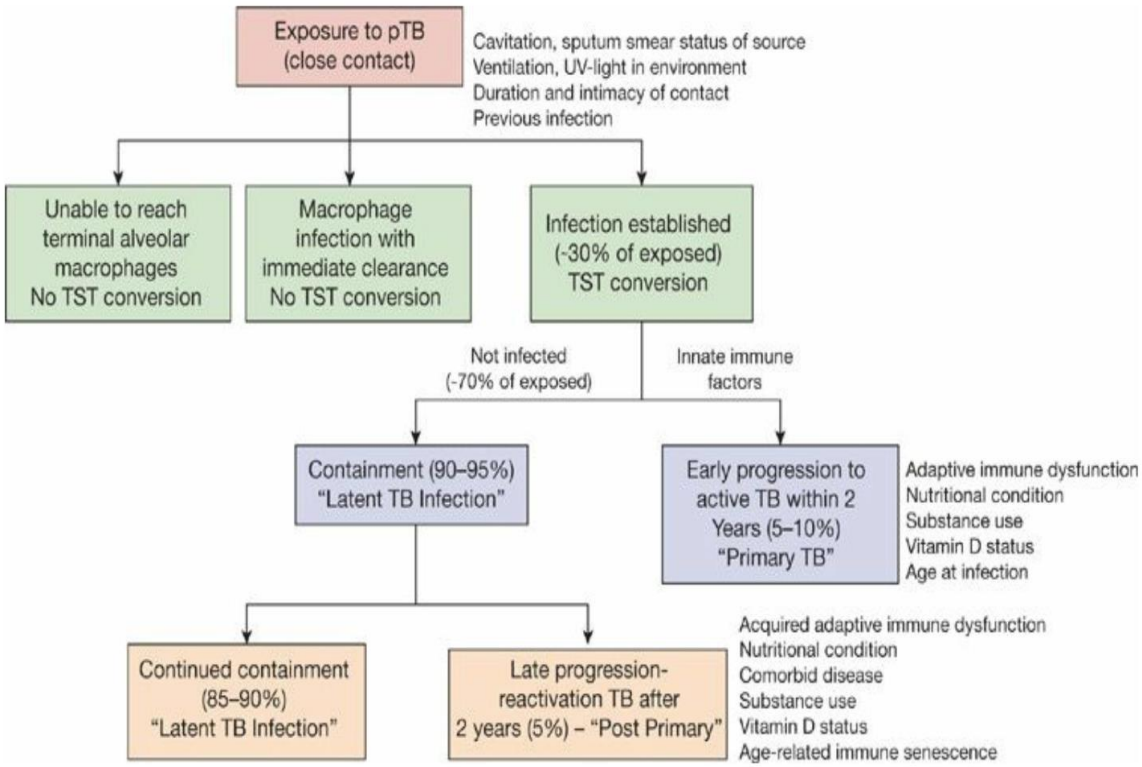


Figure 5 Natural history of TB infection.

CLINICAL FEATURES OF PULMONARY TUBERCULOSIS

Most patients remain asymptomatic after initial infection. Most of these individuals lead to complete resolution of infection. However, it can also enter a "dormant" stage with possibility of "reactivation" at a later stage. [39] Approximately 10 percent of individuals who show symptoms develop a primary infection and experience dispersion to other organs. This is particularly common in patients with compromised immune systems, e.g., HIV.[40]

"Only one-third of patients with lung involvement experience respiratory symptoms, so the most frequently reported symptom is prolonged fever. The fever usually follows a diurnal pattern; it increases as the day goes and subsides at night, sometimes associated with night sweat." [41] Respiratory symptoms are mild cough with expectoration, chest pain and

shortness of breath. However, during the advancement of the disease, it may progress to hemoptysis and extrapulmonary symptoms may manifest, including lymphadenopathy and fatigue[42], and in advanced cases, individuals may experience anorexia with a significant weight loss.[43]

TB CASE DEFINITIONS[44]

To characterize a TB case, uniform standards are required for:

- Appropriate case notification and patient registration.
- Choosing suitable treatment protocol.
- Uniform the data collection process for tuberculosis control.
- Calculate the percentage of cases based on treatment history and bacteriology.
- Analysis of treatment results using a cohort.
- Accurate trend tracking and efficacy evaluation of TB initiatives.

Case definitions [44]

"For the purpose of tuberculosis control programs, a "**case**" is therefore defined as a patient in whom tuberculosis has been confirmed bacteriologically or diagnosed by a clinician."[45]

Pulmonary cases are sputum smear-positive or negative (or unknown). Smear-negative sputum samples can be culture-positive, culture-negative, or unavailable for MTB. [45]

There are three ways to define a case of tuberculosis based on the **level of certainty of the diagnosis**.

- **Tuberculosis suspect** – "Any individual exhibiting signs or symptoms suggestive of tuberculosis (TB). The most common symptoms include a productive cough lasting longer than two weeks, which may be combined with constitutional symptoms (loss of appetite, weight loss, fever, and night sweats) or other respiratory symptoms (shortness of breath, chest pain, haemoptysis)." [46]

- **Case of tuberculosis**- "A definite case of TB (defined below) or one in which a health worker (clinician or other medical practitioner) has diagnosed TB and has decided to treat the patient with a full course of TB treatment."[46]

(Note- "Any person given treatment for TB should be recorded as a case. Incomplete "trial" TB treatment should not be given as a method for diagnosis.") [46]

- **Definite case of tuberculosis**- "A patient with MTB complex identified from a clinical specimen, either by culture or molecular line probe assay. In countries that lack the laboratory capacity to routinely identify MTB, a pulmonary case with one or more initial sputum smear examinations positive for acid-fast bacilli (AFB) is also considered to be a "definite" case, provided that there is a functional external quality assurance (EQA) system with blind rechecking."[46]

As per **global tuberculosis report 2023**[1] by WHO:

1. "People diagnosed with TB using rapid molecular tests recommended by WHO, lateral flow urine lipoarabinomannan (LF-LAM) assays, sputum smear microscopy or culture are defined as **"bacteriologically confirmed" cases** of TB."[1]
2. "The microbiological detection of TB is critical because it allows people to be correctly diagnosed and started on the most effective treatment regimen as early as

possible. People diagnosed with TB in the absence of bacteriological confirmation are classified as **"clinically diagnosed" cases** of TB."[1]

History of previous treatment[1]

Patients who have received past treatment should be evaluated with MTB culture & DST at the start of therapy. Tracking the TB pandemic necessitates distinguishing between new and treated patients and their subgroups and evaluate them for emergence of drug resistance.

"New patients are those who have not received TB treatment or have taken anti-TB medications for less than one month. Patients can have illness at any anatomical site with either positive or negative bacteriology." [44]

"Previously treated patients are those who have received 1 month or more of anti-TB medications, may have positive or negative bacteriology, and may have disease at any anatomical site." [44]

DIAGNOSIS

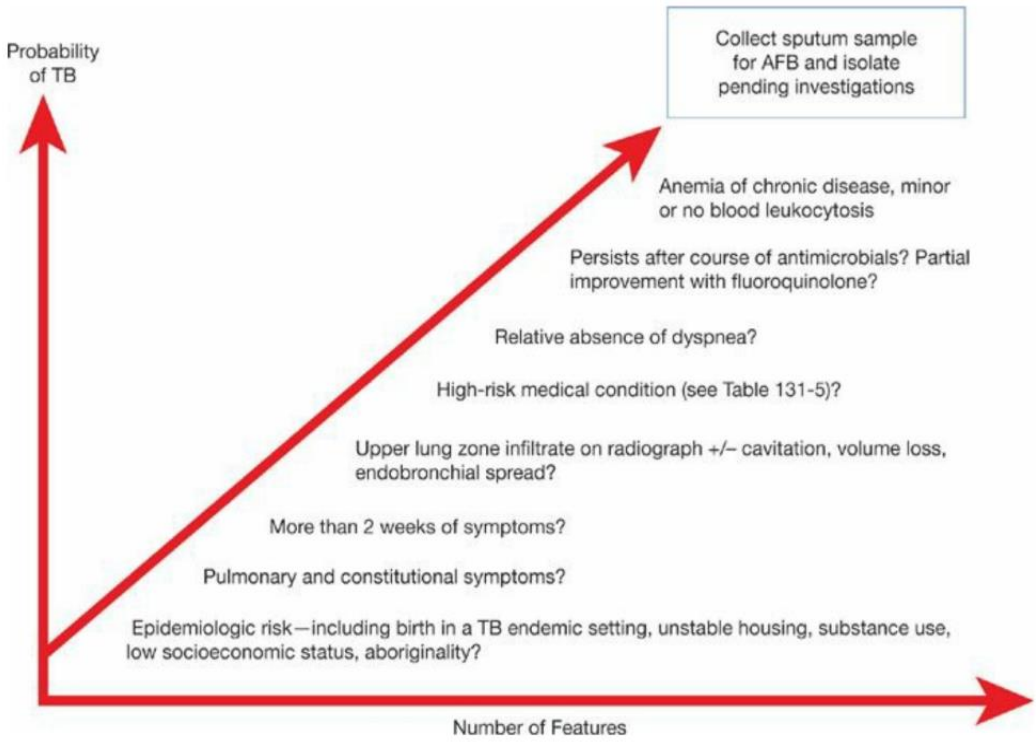


Figure 6 Clinical factors and increasing suspicion for tuberculosis. (Fishman pulmonary disease and disorders 5th ed)

AFB Smear microscopy and Culture

Sputum is the most important specimen for pulmonary tuberculosis laboratory investigation and is available in most primary health-care laboratories at the health-center level.[47] Microscopy of two consecutive early morning sputum samples has shown to diagnose 95% of smear-positive PTB patients.[48,49] The World Health Organization (WHO) revised its recommendation on case detection by microscopy as a new TB patients but this approach of diagnosing PTB is implemented where there is EQA and high-quality sputum microscopy.[49] Compared to Ziehl-Neelsen, conventional fluorescence microscopy is faster and has a higher sensitivity of 10%. However, its pricey mercury vapor light sources, upkeep, and dark chamber limits its use.[4]

LED made fluorescence microscopy cheaper and more sensitive than Ziehl-Neelsen, improving quality, operations, and finances. According to these findings, the World Health Organization (WHO) suggests replacing conventional fluorescence microscopy with LED microscopy and gradually introducing it as an alternative to conventional light microscopy.[50]

Nevertheless, sputum examination, despite its specificity, exhibits limited and fluctuating sensitivity, rendering it incapable of detecting drug-resistant strains. HCW should get MTB culture confirmation whenever possible as it confirms the diagnosis and provides DST. [47]

Culture Media are classified into solid media and liquid media. Solid media allows observation of mixed culture and contaminant colonies, while liquid media accelerates mycobacteria growth. The agar contains following malachite green, coagulated eggs, glycerol, potato starch, asparagine, and mineral salt solution, which promote the growth of bacilli.[51] MTB culture from microscopy-positive material can show growth within 2-4 weeks and 4-8 weeks in those with microscopy-negative material. [51]

Selective media for MTB culture are:

- Egg-based culture media: eg- Gruft modification of LJ media and Mycobactosel LJ
- Agar-based culture media: Selective 7H11 (Mitchison's medium), containing carbenicillin, amphotericin B, polymyxin B and trimethoprim as selective agent

Liquid culture media are:

- BACTEC MGIT 960 system
- ESP Culture System II
- MB/BacT.

Thus, liquid media is still suggested for MTB isolation because to its faster reporting time of 10 to 14 days and better isolation capabilities as compared to solid culture media which takes about 4 to 6 weeks to show growth and further delaying DST by 10 days. Case yield of liquid culture is 10% greater and its low latency and high sensitivity are some of its advantages. [19] However, liquid systems are more prone for contamination which causes 5%-10% of specimen failure. Avoiding cross-contamination, which occurs when bacteria move from positive to negative specimens, requires strict technique.[52] Solid culture & DST is simpler and less vulnerable to contamination than liquid culture media making it important to eliminate NTM contamination. Several laboratory tools can automatically identify MTB development, including the Bactec "Mycobacterial development Indicator Tube 960" ("MGIT 960; Becton-Dickinson, Sparks, MD, USA) and the MB/Bact Alert 10 3D". These automated incubators are expensive, slow, and unable to distinguish mycobacterial species or mixed or contaminated cultures.[53]

Tuberculin Skin Testing (TST)

Traditional tuberculin pure protein derivative (PPD) testing detects type IV hypersensitivity. Patients with MTB can create sensitized T cells that identify MTB antigens. MTB antigens again stimulate these cells, releasing soluble lymphokines that cause inflammation and induration. [27]

The TST results are obtained 72 h after PPD injection, and average diameter of induration is measured. A response of ≥5 mm is regarded as positive, while an average diameter of induration of less than 5 mm is taken as negative.[27]

Radiology in Pulmonary tuberculosis

Chest X-ray remains the main radiologic test for suspected or confirmed PTB. It also gives crucial patient care and follow-up information. A useful screening tool. It cannot make a definitive diagnosis without microbiological tests.

1. **Primary pulmonary tuberculosis-** In 70% of children and 90% of adults, radiographs show parenchymal infection with patchy or lobar consolidation. In primary TB, localized infection usually produces a caseating granuloma that calcifies into Ghon lesion. Cavitation is rare seen. Ipsilateral hilar and contiguous mediastinal lymphadenopathy, usually right-sided, is more prevalent in children. [54,55] 30-40% of adults have pleural effusions, while its seen only in 5-10% of children. [54]
2. **Post-primary TB-** Reactivation or secondary tuberculosis happens years later, often in the context of a weakened immune system. Most lesions are in posterior upper lobes and superior lower lobes and lymphadenopathy is uncommon. [54]Cavitation is a characteristic feature of post-primary tuberculosis and is present in approximately 50% of all patients. Frequently, there is uneven and indistinct consolidation seen.[56]

The limited value of chest X-ray lies in its ability to only confirm the stability of a lesion, without providing information on the presence of active bacilli within stable lesions.[55]

Computed tomography (CT) of chest is usually necessary to identify lesions that may not be detected on a chest X-ray. [57] and it's also useful to detect bacterial activity. Branching opacities, cavitation, and consolidation indicate active TB, but bacilli in sputum must prove diagnosis. "The "tree-in-bud" pattern on chest CT shows many branching linear

structures that suggest bronchogenic disease spread with caseating necrosis in the respiratory and terminal bronchioles".[58]

Other pneumonias can also cause tree-in-bud opacities and the suspicion of PTB should arise when detected with cavity or nodular infiltrates in the upper and/or posterior lung segments.[57] Although computed tomography (CT) of the chest can be helpful in explaining unclear results, its effects on patient management have not been demonstrated to be statistically significant. As a result, microbiological culture-based TB identification should come after this test.[54]

Molecular methods

Nucleic acid amplification testing

In sputum smear-negative patients, where clinical diagnosis is questionable and conclusive results are needed, nucleic acid amplification (NAA) assays increase diagnosis accuracy but lack the sensitivity to clearly eliminate disease possibility. [59]

Two advantages distinguish NAA testing from AFB smear microscopy. For AFB smear-positive specimens in NTM-endemic areas, it has a higher positive predictive value (>95%). Second, it detects MTB quickly in 50% to 80% of sputum smear negative, culture positive patients. For 80% of suspected patients, it can detect MTB several weeks before culture report comes and these culture tests corroborated the results.[60,61] The initial diagnosis of probable tuberculosis requires NAA testing, but routine ordering is not necessary because NAA tests have a PPV of less than 50% for such instances.[62]

CDC recommends Nucleic Acid Amplification (NAA) testing on respiratory specimens. [60] A single NAA test report should not be used to rule out PTB and HCW should interpret all laboratory findings along with patient's clinical condition and not use them to diagnose TB alone[59, 63] as these tests are not effective for monitoring treatment progress; however, they can help to differentiate between MTB and NTM.[59]

Line probe assay

"Rapid DST of isoniazid and rifampicin or rifampicin alone utilizing molecular technologies is suggested above conventional testing in sputum smear-positive or culture-proven MDR-TB patients, such as previously treated patients."[64] LPA can detect gene markers linked with rifampicin or isoniazid resistance and it's usually used for quick DST. Mutations in katG, InhA and ahp C promoter area are the main causes of MDR-TB. Approximately 96% of rif resistant MTB isolates also had a rpoB mutations. [65]

LPA involves extraction of DNA from MTB isolates or clinical specimens, amplifying the resistance-determining region of gene using PCR then hybridizing labelled PCR products with nucleotide probes immobilized on a strip, and colorimetrically locating the probes. The innovative, quick, automated, cartridge-based NAA. Systematic reviews and meta-analyses indicate that LPA (line probe assays) show sensitive and specificity of ≥97% and ≥99% respectively in detecting rif resistance in MTB isolates or clinical specimens, alone or in combination with isoniazid resistance. [63,64]

CBNAAT/ Xpert MTB/RIF (Cepheid)

The innovative, quick, automated, cartridge-based NAA, Xpert MTB/RIF assay detects TB and rif resistance and gives results within 2 hours. GeneXpert cartridges are pre-loaded for processing, DNA extraction, amplification, and detection of the rpoB B gene target. High test accuracy with low hands-on technical time is a big benefit. [66]

Same-day diagnosis and therapy are possible with its 2-hour results. This is the quickest turnaround time of any method, compared to 72 hrs. in LPA, 2.5 months in liquid culture and DST, and four months in solid culture with DST. About 5% of rifampicin-resistant organisms have mono-resistance, but a significant number (~95%) also have concurrent isoniazid resistance. Thus, rifampicin resistance can indicate multidrug-resistant tuberculosis.[67]

Samples which can be processed are sputum, aseptically collected specimens in normal saline and fine needle aspirates and specimens contaminated by commensals which are not collected aseptically like gastric lavage, bronchial washings and pus. [67]

Cartridge-based Nucleic Acid Amplification Test[68] (CBNAAT) is used to "detect MTB and rif-resistance using GeneXpert IV Dx system and the Xpert MTB/ RIF cartridge." CBNAAT has automatic sample processing followed by nucleic acid amplification and detection of MTB and rif resistance. This uses single use and disposable CBNAAT cartridges to hold PCR reagents and host the process.[68]

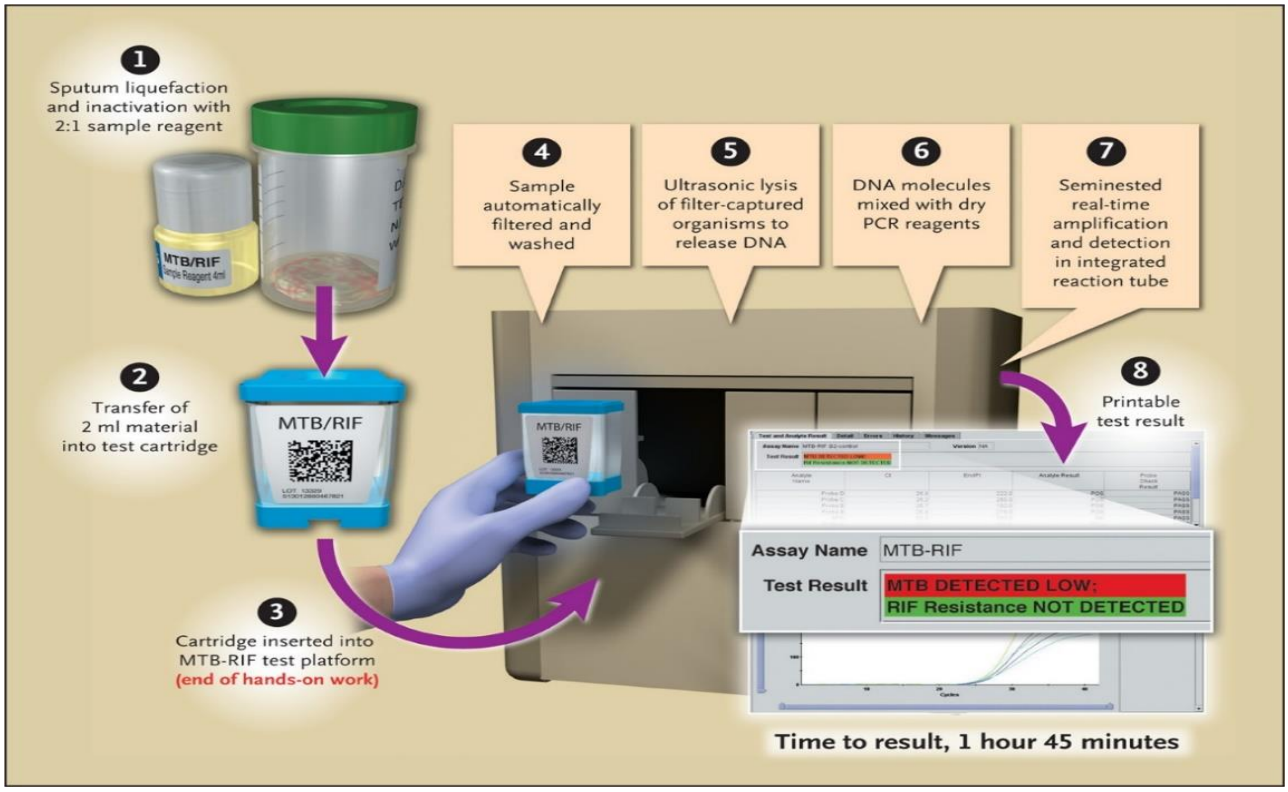


Figure 7 CBNAAT Procedure for MTB.

The process of CBNAAT involves following steps:[68]

1. **"Sample processing:** Specimens are processed by adding the sample reagent at 2:1 (v/v) ratio to the sputum sample, mixing and incubation for 15 minutes at room temperature. The sputum sample get liquified after the processing step."

2. **"Loading sample into cartridge:** Liquefied sample is added to the cartridge using a transfer pipette."

3. **"Setting up the machine to run the test:** After switching on the system, "Create Test" is clicked in the GeneXpert Dx system window, test details are added, the barcode label of the cartridge is scanned and "Start Test" is clicked to initiate testing."

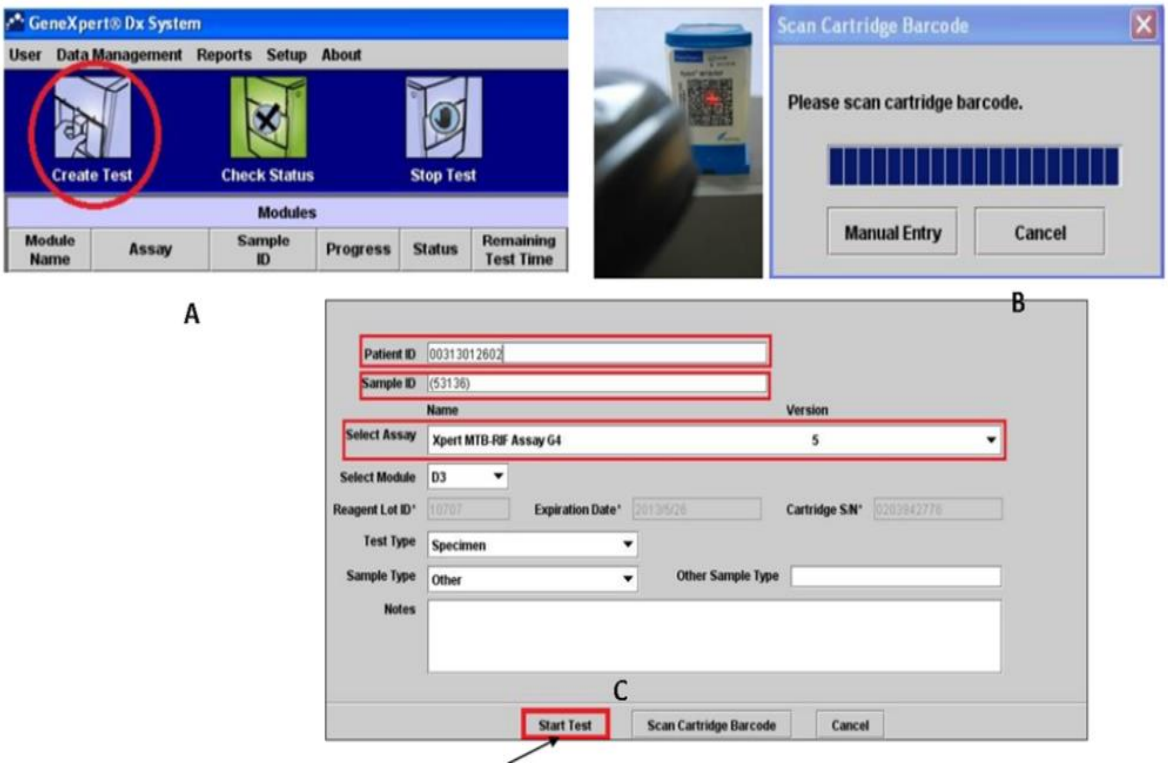


Figure 8 Setting the machine to run CBNAAT assay by creating test (A), adding test details, scanning barcode (B) to start test (C); (Source: GLI Training Package for CBNAAT).

The WHO issued preliminary guidelines particularly for individuals who are suspected of having MDR-TB by incorporating CBNAAT testing as mandatory step after smear microscopy.[67] Despite having high sensitivity for MTB in smear-positive patients, CBNAAT can be useful as an add-on test after smear microscopy in smear-negative patients. The International Standards for TB Care advocated CBNAAT and/or sputum cultures for patients suspected of PTB and negative smear microscopy. When rif resistance is rare, CBNAAT's PPV is much lower, especially below 5%.[66] In low-risk MDR-TB patients, a repeated test on a fresh material can detect rifampicin resistance, according to a recent WHO regulation.[8]

Serological Diagnosis

Antibody Detection- These tests rely on recognition of antibodies against MTB antigens by the humoral immune response and the WHO recommends against use of serologic tests for the diagnosis of PTB.

QuantiFERON-TB-Gold® In Tube (QFT-GIT) test (Cellestis, Carnegie, Australia)

QFT evaluates CMI responses to peptide antigens which mimick mycobacterial proteins, which are "ESAT-6, CFP-10, and TB7.7". These proteins are not found in BCG vaccine strains or NTM, except for Mycobacterium kansasii, Mycobacterium szulgai, & Mycobacterium marinum. [69]

Lymphocytes that are capable of identifying these antigens which are usually present in the blood of an individual infected with MTB complex organisms. IFN-γ is released and produced as an immune response, and the QFT test counts and measures IFN-γ in order to identify infection. So, in BCG vaccinated contacts of infectious cases, IGRA correlates better to MTB exposure than the TST. [69]

Antigen Detection- Clinical specimens like sputum, serum, and urine can be used to identify the presence of circulating antigens. One unique element of the MTB cell envelope is lipoarabinomannan (LAM), which may one day be used as a biomarker to diagnose tuberculosis. "A lateral flow LAM test for urine is called Fuji LAM. For TB in adults, Fuji Lam's sensitivity and specificity are 70% and 93%, respectively, whereas for TB in children, they are 51% and 87%, respectively. It functions more effectively and has a higher diagnostic sensitivity in individuals who have HIV infection or a CD4+ T cell count of less than 200 cells/μL."

New techniques [59]

- Next-Generation Sequencing (NGS)
- Mass Spectrometry
- Artificial Intelligence (AI)

DIAGNOSTIC ALGORITHMS FOR SPUTUM NEGATIVE PULMONARY TUBERCULOSIS

The diagnosis and treatment of sputum smear negative patients relies on clinical symptoms, but 20% are asymptomatic. Since a doctor is trying to establish tuberculosis, starting empirical ATT or using other methods can be difficult and time-consuming. One may over diagnose or underestimate TB based on clinical and radiological findings. Smear-negative TB cases account for 13–20% of transmissions and are 20–25% more infectious. Identifying sputum smear-negative, culture-positive tuberculosis cases is crucial, since they have a 14.1% mortality rate. Particularly for smear-negative TB, the performance of current TB diagnostics is still subpar. Since TB can manifest in a wide range of ways, the first challenge in diagnosis of smear-negative TB.

Whenever feasible, a medical history and clinical examination should be combined with radiological, microbiological and molecular tests to diagnose sputum smear negative PTB.[70] These patients should undergo MTB culture for confirmation of diagnosis and correct culture utilization can improve diagnostic sensitivity and allow early case diagnosis. The diagnostic path followed by physicians to diagnose sputum smear negative PTB often prefer different diagnostic tests like induced sputum analysis,[71] chest CT imaging,[58] bronchoscopy with BALF analysis,[72] and lung tissue biopsy [73].

Even in patients who test negative for smear-negative tuberculosis, CBNAAT testing is recommended as next step for diagnosis of PTB. With its low PPV and moderate (35–80%) sensitivity, smear microscopy is still the gold standard for diagnosis of PTB in limited resource setting. Despite being the gold standard, MTB culture typically requires technical expertise and yields results in 2–6 weeks. A chest X-ray can be helpful screening tool, but it is not a precise d for diagnosis of PTB. Furthermore, symptoms and unusual radiologic findings associated with tuberculosis may be difficult to differentiate from community-acquired pneumonia. Timely and accurate diagnosis of TB and its DST are essential for initiating treatment and managing the illness successfully. [59,62]

The rapidity and cost-effectiveness of CBNAAT make it a potentially revolutionary test for PTB diagnosis and treatment. Because it targets the rpoB gene of MTB—the crucial gene linked to rif resistance. It uses 3 distinct primers and 5 molecular probes which makes CBNAAT an extremely specific test for MTB diagnosis. The physicians must treat the patient as having TB and start anti-TB medication while awaiting the results of the culture if the CBNAAT is positive and sputum smear for AFB is negative. [59,62]

New diagnostic tests can significantly reduce turn-around time and improve accuracy, but they cannot replace MTB culture as gold standard.[59] Nevertheless, cultures may come back negative in some sputum smear negative PTB cases and additional issues may arise from sampling errors or technical difficulties. In cases of negative MTB culture, clinicians may assess response to treatment, clinical findings, or future positive culture in decision making. Thus, TB diagnosis in this population is likely to be made individually.[70]

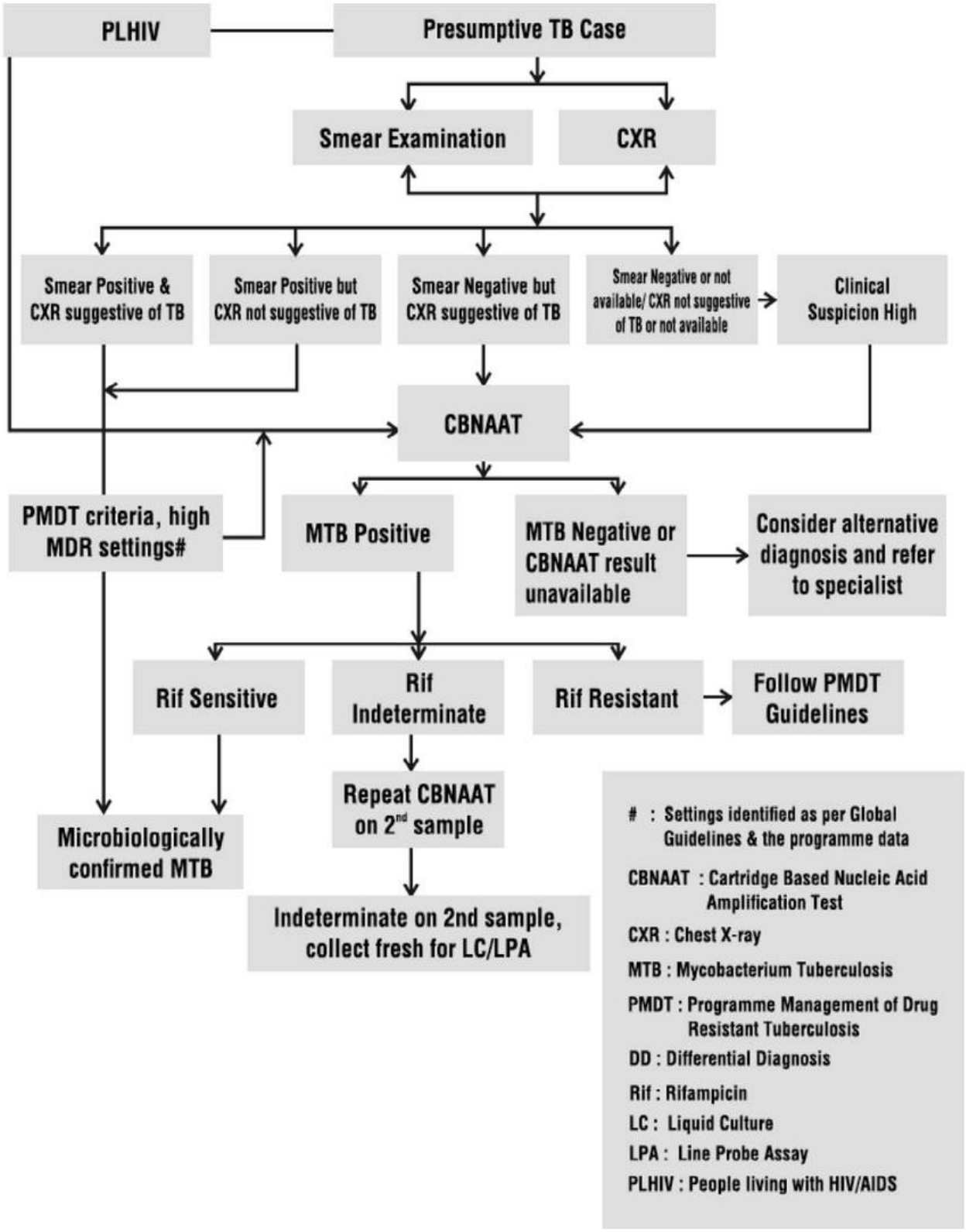
A significant drawback when the MTB load is low is that the majority of techniques used to diagnose TB still involve isolating the pathogen. Due to these factors, the diagnosis of sputum smear negative PTB is frequently made solely on the patient's response to empirical ATT rather than microbiological confirmation.[63,70] For smear negative patients, samples for MTB culture should be obtained along with active follow-up of these patients.[59]

Using antibiotic response-based diagnostic steps has several drawbacks. Fluoroquinolones and aminoglycosides should be avoided because they are active against MTB and may cause a temporary improvement in TB patients. Adherence to the algorithm's sequential steps may cause patients illness to rapidly get worse before receiving the proper care.[74]

BRONCHOALVEOLAR LAVAGE

Suboptimal management of TB results from diagnostic hurdles when a patient has high clinical & radiological suspicion of PTB but is unable to produce adequate sputum for analysis. Therefore, alternative techniques for identification of MTB in suspected PTB patients can be applied, one such method is video bronchoscopy, which can be used to collect adequate respiratory sample for analysis. [75] The three most common bronchoscopy-based techniques are (BAL, bronchial washing, and TBLB.[76] Among these bronchoscopic samples, BALF is the best diagnostic material for the diagnosis of PTB with diagnosis rate of up to 86.6% with minimal complication in trained hands.[75]

Figure 9 Diagnostic algorithm for PTB.



METHODOLOGY

Study design

A cross-sectional study

Study population

1. Patients with clinical or radiological features suggestive of pulmonary tuberculosis and sputum smear negative for acid fast bacilli.
2. Patients who visited outpatient department or admitted under department of respiratory medicine, KLES Dr. Prabhakar Kore hospital and MRC, Belagavi.

Study Time:

Research study was conducted for 12 months from October 2022 to October 2023.

Source of data:

The source for the present study is from patients, who visited outpatient department or admitted under care of department of Respiratory medicine, KLES Dr. Prabhakar Kore hospital and MRC, Belagavi with clinical or radiological features suggestive of PTB with sputum smear negative for AFB.

Inclusion criteria

- Patients more than 18 years old with symptoms suggestive of PTB like cough more than 2 weeks, night sweats, loss of weight, hemoptysis, dyspnea and chest pain.

- Patients with radiological signs suggestive of PTB like consolidation, cavity, fibrosis and infiltrates.
- Patients with 2 sputum samples smear negative (spot sample and consecutive early morning sputum sample) for AFB.

Exclusion criteria

- Diagnosed case of PTB on anti-tubercular treatment.
- Sputum smear positive for acid fast bacilli.
- Patients not fit for the bronchoscopy procedure. E.g.- patients diagnosed with unstable angina, coagulopathies, hypoxemia, etc
- Consent withdrawn.

Sample size

Sample size was calculated by using the sensitivity of BAL CBNAAT in diagnosis of Sputum Negative Pulmonary TB from the study by **Archana B et al.**[77] using the formula

$n = [Z\alpha^2 * Sn * (1 – Sn)]/ (d^2 * p)$[78]

Z = Standard normal value at 95% Confidence level

Sn = Sensitivity = 96.5%

100 – Sn = 3.5%

d = desired absolute precision = 5%

p = prevalence = 50% [From the study by **Alnour TM et al**[79]]

By considering above values

n = 104 subjects with Sputum Smear Negative Pulmonary TB were included in the study

Considering a non-response rate of 10%, 104 + 11 = 115 subjects were included in the study.

	At 5% Alpha Error
Z	1.96
Sensitivity	0.965
100 - Sensitivity	0.035
Precision (d)	0.05
Prevalence	0.5
N	**103.800**

Sampling Technique

After obtaining approval and clearance from the institutional ethics committee, the individuals fulfilling the inclusion criteria were enrolled for the study after obtaining informed consent (Annexure) and demographic data was collected by the principal researcher.

Patients fulfilling the inclusion criteria were asked to collect early morning sputum sample which was sent for CBNAAT for MTB. If the sputum CBNAAT for MTB comes negative, then the patient was subjected to bronchoscopy after obtaining a separate informed consent for the procedure.

Bronchoscopy was performed by an expert pulmonologist and it was done using OLYMPUS CX-190 ***EVIS EXERA III Therapeutic video bronchoscope*** in bronchoscopy suite under conscious sedation and broncho-alveolar washings were obtained by instilling 20 ml of 0.9% normal saline at room temperature into a segment of affected bronchus and aspirating available fluid, maximum up to 60 ml fluid was instilled. The obtained broncho-alveolar aspirate was subjected to CBNAAT for MTB and MGIT culture.

Ethical Consideration

Ethical clearance was obtained from the Ethical Committee of KLES Dr Prabhakar Kore hospital and MRC, Belagavi before conducting the study and revised clearance was obtained for change in dissertation title.

Statistical Analysis

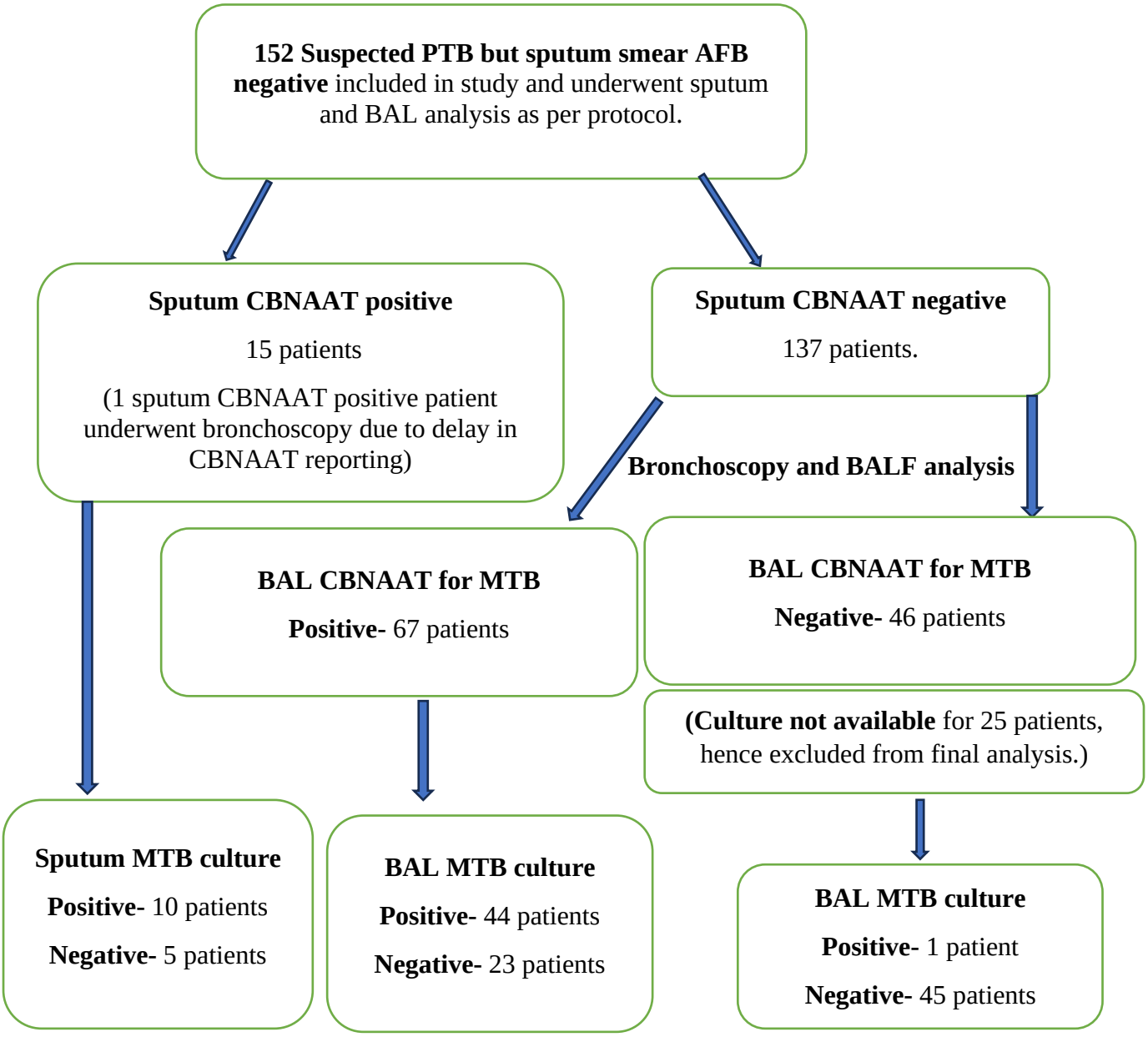
Data was entered into Microsoft excel data sheet and analyzed using SPSS 22 version software (IBM SPSS Statistics, Somers NY, USA). Categorical data was represented in the form of frequencies and proportions. Pie diagrams and Bar diagrams were used to represent data graphically.

Chi-square test or Fischer's exact test [If Chi-square test was invalid] was used as test of significance for Categorical data. Students t test was used for the test of significance for Quantitative data.

RESULTS

This present cross-sectional study was conducted in the department of respiratory medicine at KLES Dr. Prabhakar Kore Hospital and MRC, Belagavi, Karnataka for a period of one year.

This study was undertaken to study and compare sensitivity & specificity of sputum and broncho-alveolar aspirate CBNAAT for MTB in diagnosis of sputum smear negative PTB. A total of 152 sputum smear negative but suspected PTB patients were included in the present study and 54 (35.5%) patients had final diagnosis of PTB based on MTB culture.



Demographic Characteristics of patients

Table 1 Gender distribution of patients.

Gender	No. of patients	Percentage
Female	58	38.2
Male	94	61.8
Total	152	100.0

Among the 152 suspected PTB patients, there were 58 females (38.2%) and 94 (61.8%) males.

Figure 1 Gender distribution of patients.

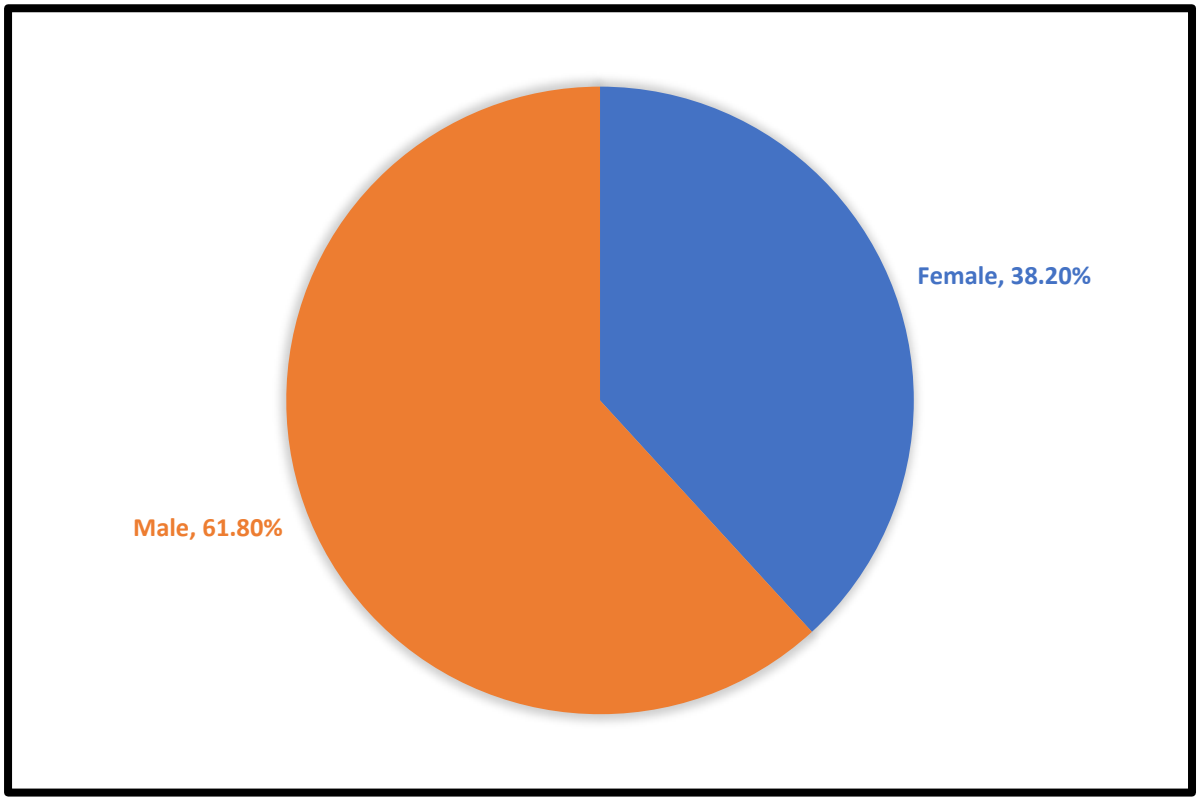


Table 2 Age distribution of patients. (age in years)

Age groups	No. of patients	% of patients
<40	61	40.1
41-60	50	32.9
>60	41	27.0
Total	152	100.0

This study included a total of 152 sputum smear negative patients and 61(40.1%) of the patients were under the age of 40 yrs. This distribution indicates a balanced age range among the study participants, with a significant representation of individuals in each age group.

Figure 2 Age distribution of patients.(age in years)

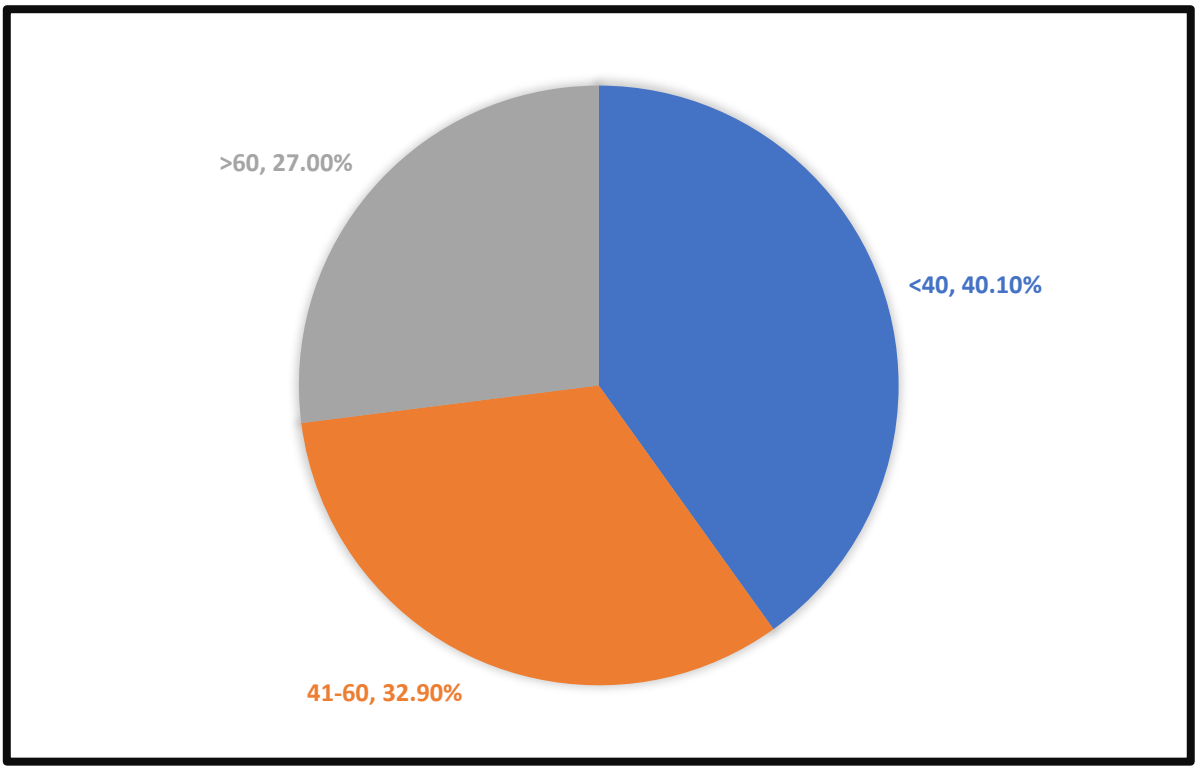


Table 3 Radiological Findings.

Radiological Findings	Present	Percentage
Cavity	24	15.8
Nodular Infiltrates	71	46.7
Fibrosis	24	15.8
Consolidation	27	17.8

The predominant radiological finding noted was nodular infiltration in 71 patients (46.8%), followed by consolidation in 27 participants (17.8%), cavity in 24 (15.8%) and fibrosis in 24 (15.8%).

Figure 3 Radiological Findings.

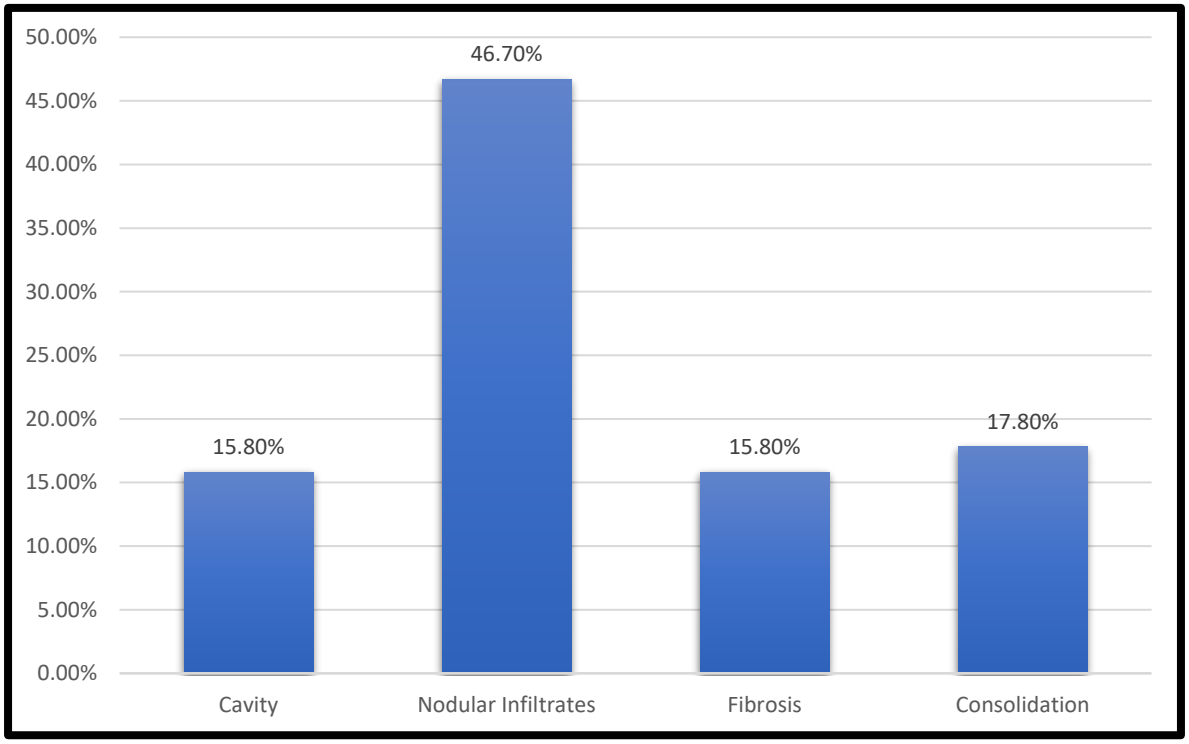


Table 4 Co-morbid conditions.

Co-morbidities	Frequency	Percent
Type 2 DM	17	11.2
Hypertension	8	5.3
IHD	4	2.6
Post TB OAD	5	3.3
Carcinoma Lung	1	.7
CKD	2	1.3
COPD	2	1.3
HBsAg	2	1.3
Malignancy	1	.7
NAFLD	2	1.3
Rheumatoid arthritis	1	.7
HIV	3	2.0

Type 2 DM was the most prevalent comorbidity in 17 (11.2%) among the, followed by hypertension in 8 (5.3%) and IHD in 4 (2.6%) patients.

Figure 4 Co-morbid conditions.

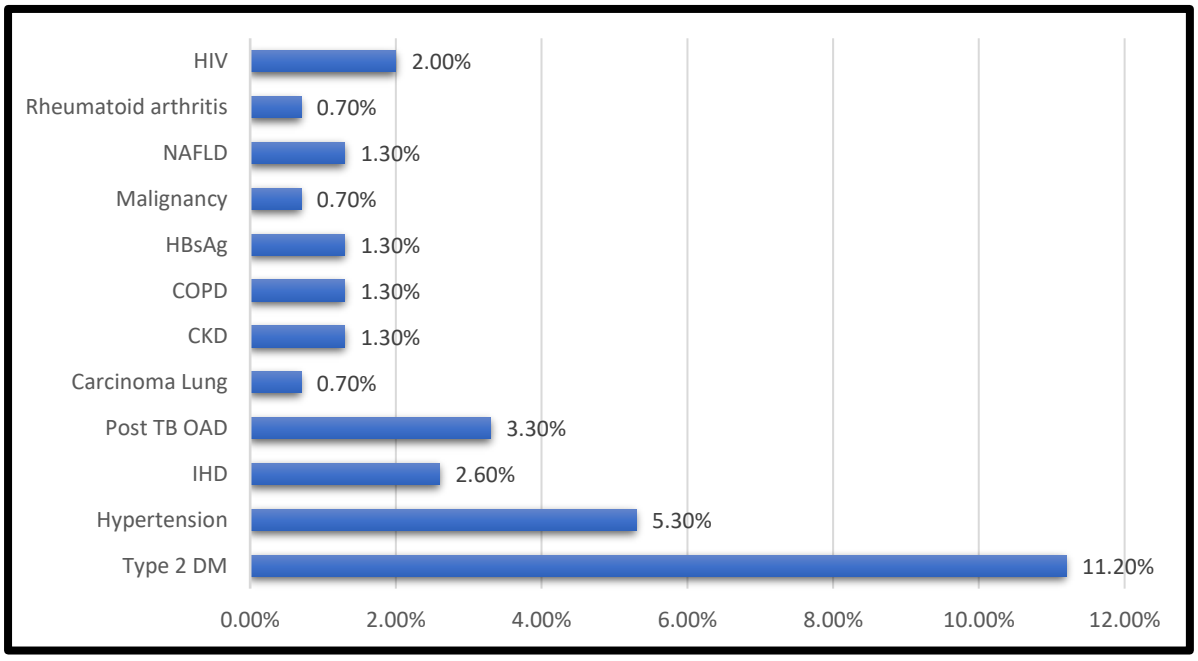


Table 5 Sputum CBNAAT for MTB.

Sputum CBNAAT for MTB	No. of patients	Percentage
Positive	15	9.9
Negative	137	90.1
Total	152	100.0

Out of 152 sputum smear AFB negative PTB, sputum CBNAAT was positive in 15 (9.9%) and 137 (90.1%) patients tested negative.

This indicates a relatively low positivity rate for MTB in sputum smear negative samples using the CBNAAT method.

Figure 5 Sputum CBNAAT for MTB

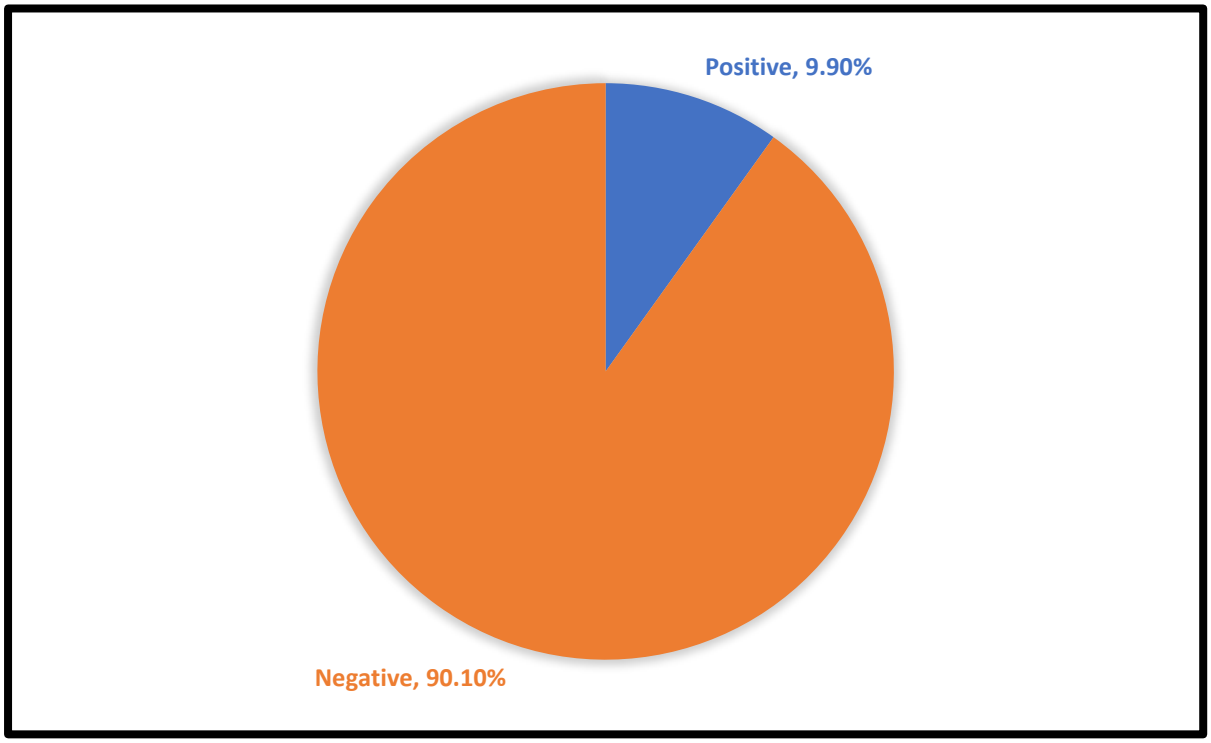


Table 6 BAL CBNAAT for MTB.

BAL CBNAAT for MTB	No. of patients	Percentage
Positive	67	44.1
Negative	71	46.7
Not performed	14	9.2
Total	152	100.0

BAL CBNAAT for MTB was positive in 67 (44.1%), negative in 71 (46.7%) and procedure wasn't performed for 14 (9.2%) patients since their sputum CBNAAT was positive. One sputum CBNAAT positive patient underwent bronchoscopy due to delay in sputum CBNAAT reporting.

The positivity rate for CBNAAT for MTB was significantly higher in BAL samples compared to sputum samples, suggesting BAL may be more effective in detecting MTB.

Figure 6 BAL CBNAAT for MTB.

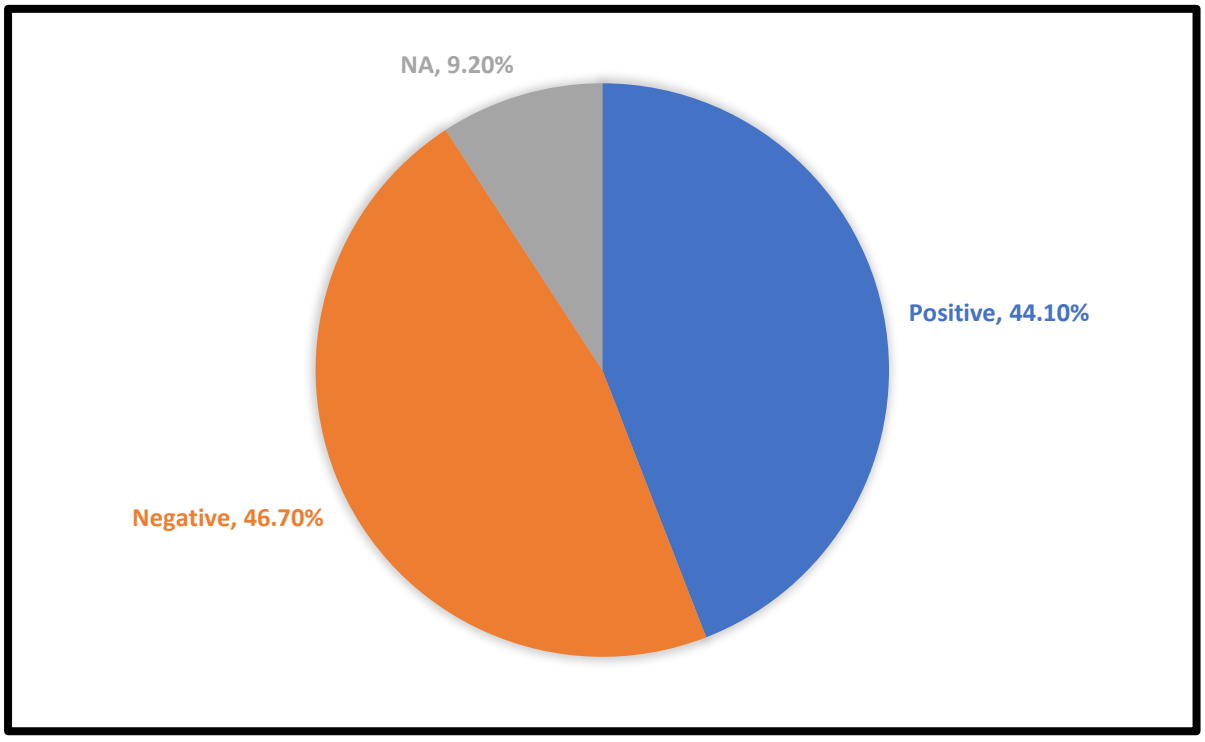


Table 7 MTB Culture in all patients. (Sputum CBNAAT and BAL CBNAAT)

MTB Culture	No. of patients	Percentage
Positive	54	35.5
Negative	73	48.0
NA	25	16.4
Total	152	100.0

152 sputum smear negative samples were analyzed and MTB culture was positive in 54 (35.5%) and negative in 73(48.0%).

25 (16.4%) patients did not have MTB culture reports available; hence they are excluded from MTB culture analysis.

Figure 7 MTB Culture in all patients. (Sputum CBNAAT and BAL CBNAAT).

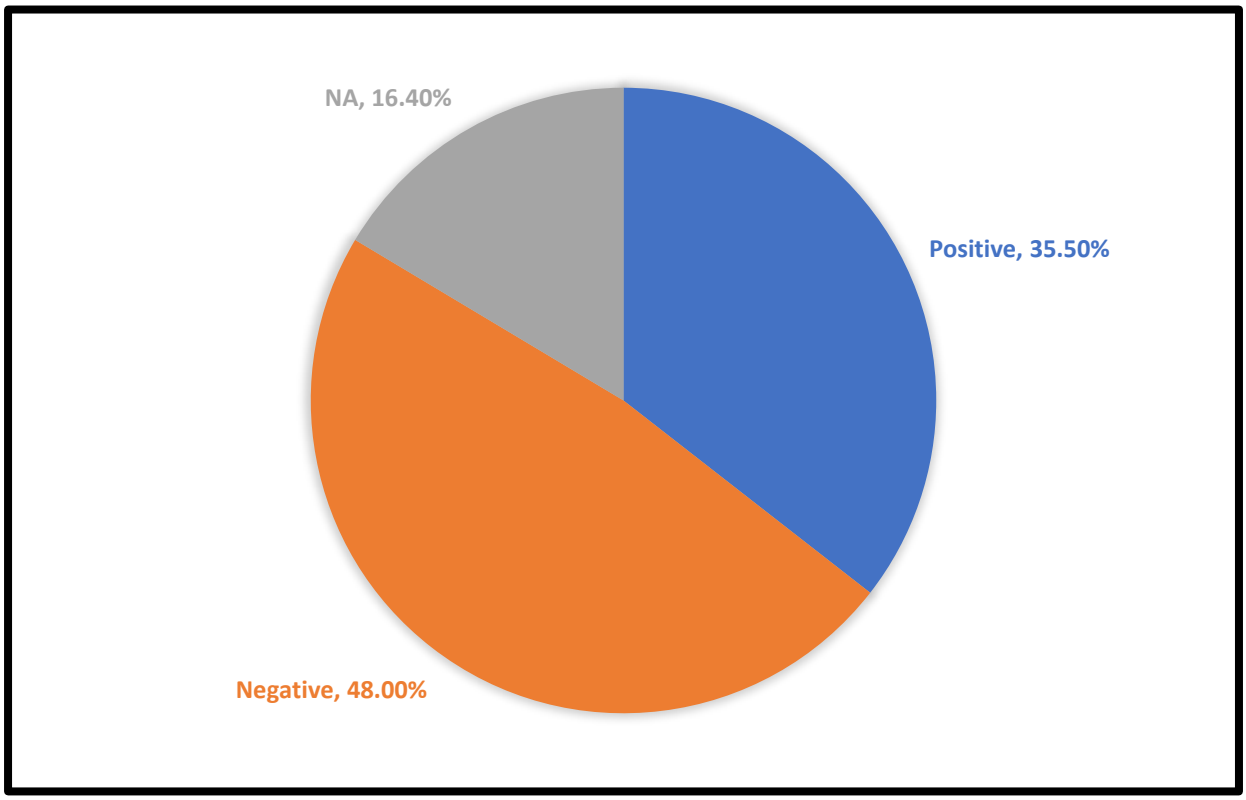


Table 8 Agreement between detection of MTB by Sputum CBNAAT and Culture method.

		MTB Culture				Total
		Positive	%	Negative	%	
Sputum CBNAAT for MTB	Positive	10	66.6	5	33.3	15
	Negative	44	39.2	68	60.7	112
Total		54	42.5	73	57.4	127

Kappa = 0.129, P value = 0.044 (Significant)

There was a statistically significant association found between sputum CBNAAT and MTB culture for diagnosis of MTB; (Kappa = 0.129, p = 0.044).

The Kappa value of 0.129 indicates only a **fair agreement** between the two tests, suggesting that there are a notable number of cases where the tests disagree.

Table 9 Sensitivity and specificity of sputum CBNAAT for MTB as compared to culture method.

	Value	95% CI
Sensitivity	18.52%	9.25% to 31.43%
Specificity	93.15%	84.74% to 97.74%
Positive Predictive Value	66.67%	42.04% to 84.65%
Negative Predictive Value	60.71%	57.29% to 64.03%
Accuracy	61.42%	52.37% to 69.92%

Sensitivity of sputum CBNAAT is 18.52% (95% CI: 9.25% to 31.43%) which means that sputum CBNAAT misses a significant proportion of TB cases, and a negative result should be interpreted with caution. It is important to follow up with additional diagnostic tests, such as MTB culture, if TB is still suspected in sputum smear negative patient.

Specificity of sputum CBNAAT was 93.15% (95% CI: 84.74% to 97.74%). This mean that the test was very good at correctly identifying people who did not have TB. A high specificity is important for ruling out TB in patients with symptoms that could be caused by TB or other diseases.

Positive predictive value (PPV) of sputum CBNAAT was 66.67% (95% CI: 42.04% to 84.65%) which suggest that a positive result from sputum CBNAAT might not always confirm the presence of TB. **Negative predictive value (NPV) of sputum CBNAAT was 60.71%** (95% CI: 57.29% to 64.03%) which means that a negative test result accurately reflects the absence of TB, but a negative result may not be definitive and further testing may be warranted if TB is still suspected.

The **accuracy of sputum CBNAAT is 61.42%** (95% CI: 52.37% to 69.92%) which measures the overall correctness of the test.

Table 10 Agreement between detection of MTB in BAL CBNAAT and Culture method.

		MTB Culture				Total
		Positive	%	Negative	%	
BAL CBNAAT for MTB	Positive	44	65.6	23	34.3	67
	Negative	1	0.02	45	97.8	46
Total		45	39.8	68	60.1	113

Kappa = 0.591, P value = 0.001 (Significant)

There was a statistically significant *agreement* between BAL CBNAAT and MTB culture results. (Kappa = 0.591, p = 0.001)

The **Kappa value of 0.591** indicates a moderate to good agreement between the two tests which suggests that the tests generally agree, but there are still some cases where they disagree.

Table 11 Sensitivity & specificity of BAL CBNAAT for MTB compared to MTB culture method.

Statistic	Value	95% CI
Sensitivity	97.78%	88.23% to 99.94%
Specificity	66.18%	53.68% to 77.21%
Positive Predictive Value	65.67%	57.77% to 72.79%
Negative Predictive Value	97.83%	86.54% to 99.68%
Accuracy	78.76%	70.07% to 85.89%

Sensitivity of BAL CBNAAT was 97.78% (95% CI: 88.23% to 99.94%) which means that BAL CBNAAT could correctly identify a very high proportion of TB cases.

Specificity of BAL CBNAAT was 66.18% (95% CI: 53.68% to 77.21%) which means that the test had a limited ability to correctly identify people who do not have TB. There is a chance that a positive test result could be a false positive.

As we can see in Table 10 that 23 (34.3%) patients of the positive BAL CBNAAT group were culture negative indicating that CBNAAT can detect the presence of dead MTB bacilli, hence MTB culture remains the gold standard method in diagnosis of PTB.

PPV of BAL CBNAAT was 65.67% (95% CI: 57.77% to 72.79%) which suggests that there was a good chance that a positive result accurately reflected the presence of MTB in sputum smear negative patients**. NPV of BAL CBNAAT was 97.83%** (95% CI: 86.54% to 99.68%) and it means that there is a very high chance that a negative test result accurately reflects the absence of TB.

Table 12 Rif Sensitivity and resistance pattern in all patients. (Sputum CBNAAT and BAL CBNAAT)

Rif Sensitivity	No. of patients	Percentage
Positive	74	48.7
Negative	72	47.3
Resistant	6	3.9
Total	152	100.0

Rifampicin Sensitivity analysis showed rif sensitivity in 74 (48.7%), rif resistance was detected in (3.9%) patients and not detected in 72 (47.8%)

Figure 8 Rif Sensitivity and resistance in all patients. (Sputum CBNAAT and BAL CBNAAT)

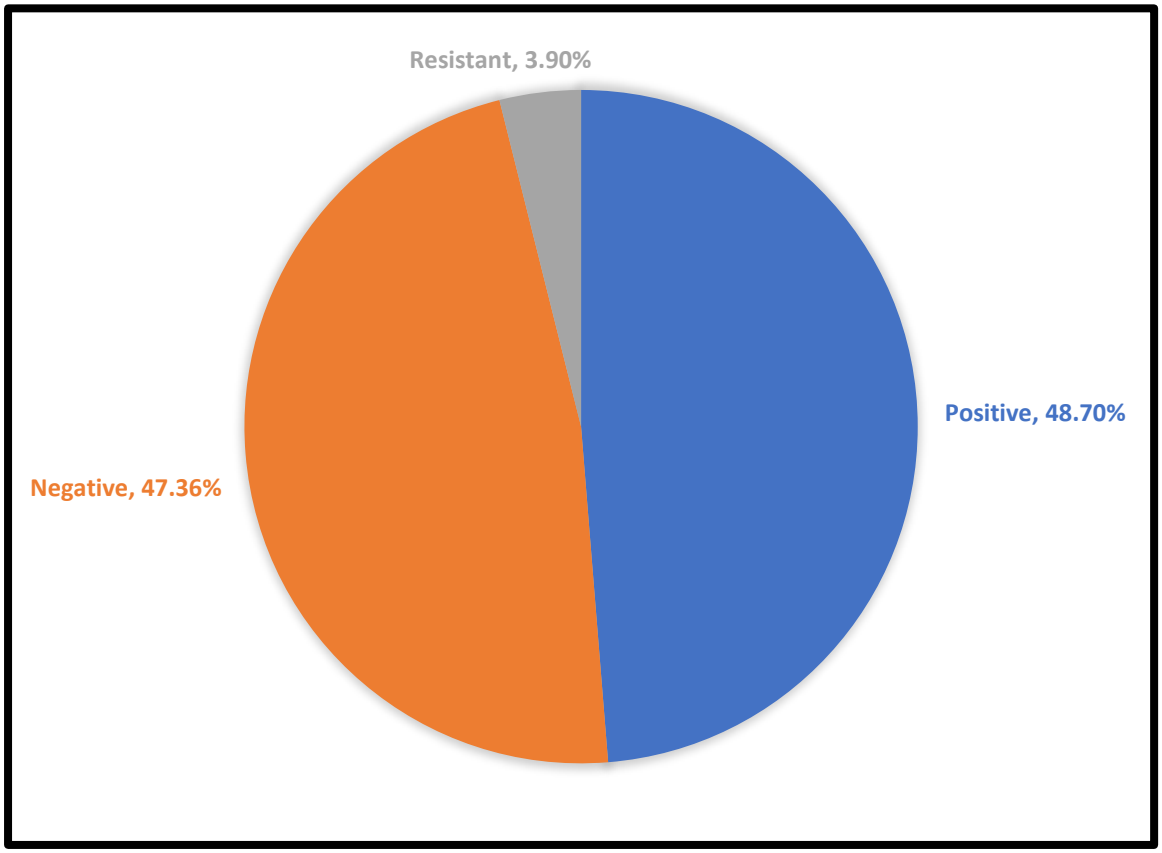


Table 13 Agreement between cavity and BAL CBNAAT for MTB

			BAL CBNAAT for MTB		Total
			Positive	Negative	
Cavity	Present	Count	16	7	23
		%	69.6%	30.4%	100.0%
	Absent	Count	51	64	115
		%	44.3%	55.7%	100.0%
Total		Count	67	71	138
		%	48.6%	51.4%	100.0%

Chi square = 4.879, P value = 0.027 (S)

BAL CBNAAT vs. Cavity: Statistically significant association (p=0.027) was observed between BAL CBNAAT results and the presence of a cavity on radiologic examination. This indicates that BAL CBNAAT might be more effective in diagnosing PTB in patients with cavitary lesions compared to those without.

Table 14 Agreement between consolidation and sputum CBNAAT

			Sputum CBNAAT for MTB		Total
			Positive	Negative	
Consolidation	Present	Count	3	24	27
		%	11.1%	88.9%	100.0%
	Absent	Count	12	113	125
		%	9.6%	90.4%	100.0%
Total		Count	15	137	152
		%	9.9%	90.1%	100.0%

Chi square = 0.057, P value = 0.811 (NS)

Table 15 Agreement between nodular infiltrations and BAL CBNAAT for MTB.

			BAL CBNAAT for MTB		Total
			Positive	Negative	
Nodular infiltrations	Present	Count	32	36	68
		%	47.1%	52.9%	100.0%
	Absent	Count	35	35	70
		%	50.0%	50.0%	100.0%
Total		Count	67	71	138
		%	48.6%	51.4%	100.0%

Chi square = 0.119, P value = 0.730 (NS)

Table 16 Agreement between fibrosis and BAL CBNAAT for MTB.

			BAL CBNAAT for MTB		Total
			Positive	Negative	
Fibrosis	Present	Count	9	11	20
		%	45.0%	55.0%	100.0%
	Absent	Count	58	60	118
		%	49.2%	50.8%	100.0%
Total		Count	67	71	138
		%	48.6%	51.4%	100.0%

Chi square = 0.118, P value = 0.731 (NS)

Table 17 Agreement between consolidation and BAL CBNAAT for MTB.

			BAL CBNAAT for MTB		Total
			Positive	Negative	
Consolidation	Present	Count	10	15	25
		%	40.0%	60.0%	100.0%
	Absent	Count	57	56	113
		%	50.4%	49.6%	100.0%
Total		Count	67	71	138
		%	48.6%	51.4%	100.0%

Chi square = 0.894, P value = 0.344 (NS)

BAL CBNAAT vs Radiological findings (except cavity)- No statistically significant association was found between Bal CBNAAT and consolidation, nodular infiltration and fibrosis.

Table 18 Agreement between cavity and Sputum CBNAAT for MTB.

			Sputum CBNAAT		Total
			Positive	Negative	
Cavity	Present	Count	1	23	24
		%	4.2%	95.8%	100.0%
	Absent	Count	14	114	128
		%	10.9%	89.1%	100.0%
Total		Count	15	137	152
		%	9.9%	90.1%	100.0%

Chi square = 0.420, P value = 0.517 (NS)

Table 19 Agreement between nodular infiltrations and sputum CBNAAT for MTB

			Sputum CBNAAT for MTB		Total
			Positive	Negative	
Infiltrations	Present	Count	3	68	71
		%	4.2%	95.8%	100.0%
	Absent	Count	12	69	81
		%	14.8%	85.2%	100.0%
Total		Count	15	137	152
		%	9.9%	90.1%	100.0%

Chi square = 3.654, P value = 0.056 (NS)

Table 20 Agreement between fibrosis and sputum CBNAAT for MTB

			Sputum CBNAAT for MTB		Total
			Positive	Negative	
Fibrosis	Present	Count	4	20	24
		%	16.7%	83.3%	100.0%
	Absent	Count	11	117	128
		%	8.6%	91.4%	100.0%
Total		Count	15	137	152
		%	9.9%	90.1%	100.0%

Chi square = 0.712, P value = 0.399 (NS)

Table 21 Agreement between consolidation and sputum CBNAAT for MTB

			Sputum CBNAAT for MTB		Total
			Positive	Negative	
Consolidation	Present	Count	3	24	27
		%	11.1%	88.9%	100.0%
	Absent	Count	12	113	125
		%	9.6%	90.4%	100.0%
Total		Count	15	137	152
		%	9.9%	90.1%	100.0%

Chi square = 0.057, P value = 0.811 (NS)

Sputum CBNAAT vs. Radiological Findings: No statistically significant association was found between sputum CBNAAT results (positive/negative) and any of the radiological findings (consolidation, cavity, infiltrations, or fibrosis) in PTB suspects. This suggests that sputum CBNAAT alone may not be sufficient for diagnosis in sputum smear-negative PTB, especially in cases with specific radiological patterns.

Table 22 Agreement between radiological finding of Consolidation and MTB Culture.

			MTB Culture		Total
			Positive	Negative	
Consolidation	Present	Count	7	15	22
		%	31.8%	68.2%	100.0%
	Absent	Count	47	58	105
		%	44.8%	55.2%	100.0%
Total		Count	54	73	127
		%	42.5%	57.5%	100.0%

Chi square = 1.247, P value = 0.264 (Not significant)

54 (42.5%) patients had consolidation and among these patients, only 7 (31.8%) had a positive MTB culture, confirming TB.

Figure 9 Radiological finding of Consolidation and MTB Culture

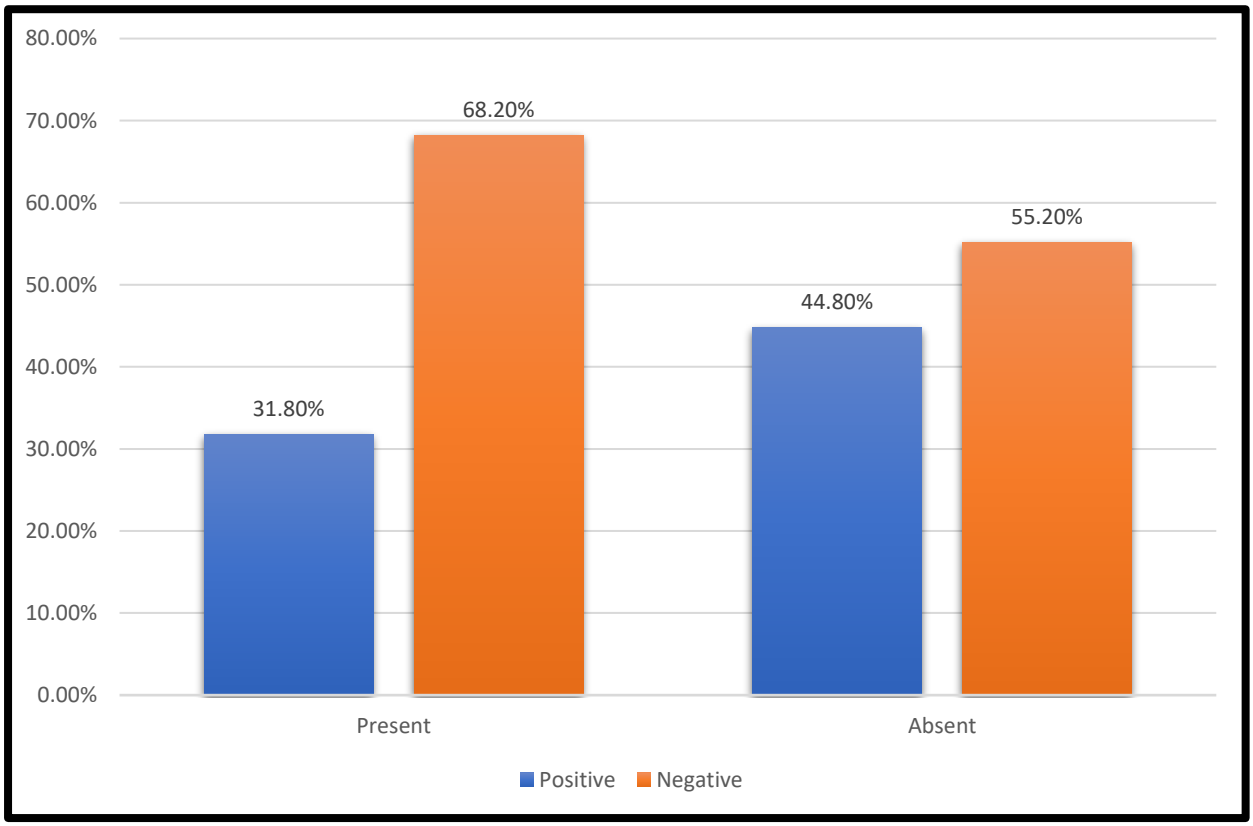


Table 23 Agreement between radiological finding of fibrosis and MTB culture.

			MTB Culture		Total
			Positive	Negative	
Fibrosis	Present	Count	11	10	21
		%	52.4%	47.6%	100.0%
	Absent	Count	43	63	106
		%	40.6%	59.4%	100.0%
Total		Count	54	73	127
		%	42.5%	57.5%	100.0%

Chi square = 1.001, P value = 0.317 (not significant)

Out of 127 patients, 54 (42.5%) had fibrosis as radiological finding and among these patients, 11 (52.4%) tested positive for TB, while 10 (47.6%) tested negative by MTB culture.

In the group of 73 patients without fibrosis, 43 (40.6%) tested positive for TB, while 63 (59.4%) tested negative.

Figure 10 Radiological finding of fibrosis and MTB culture.

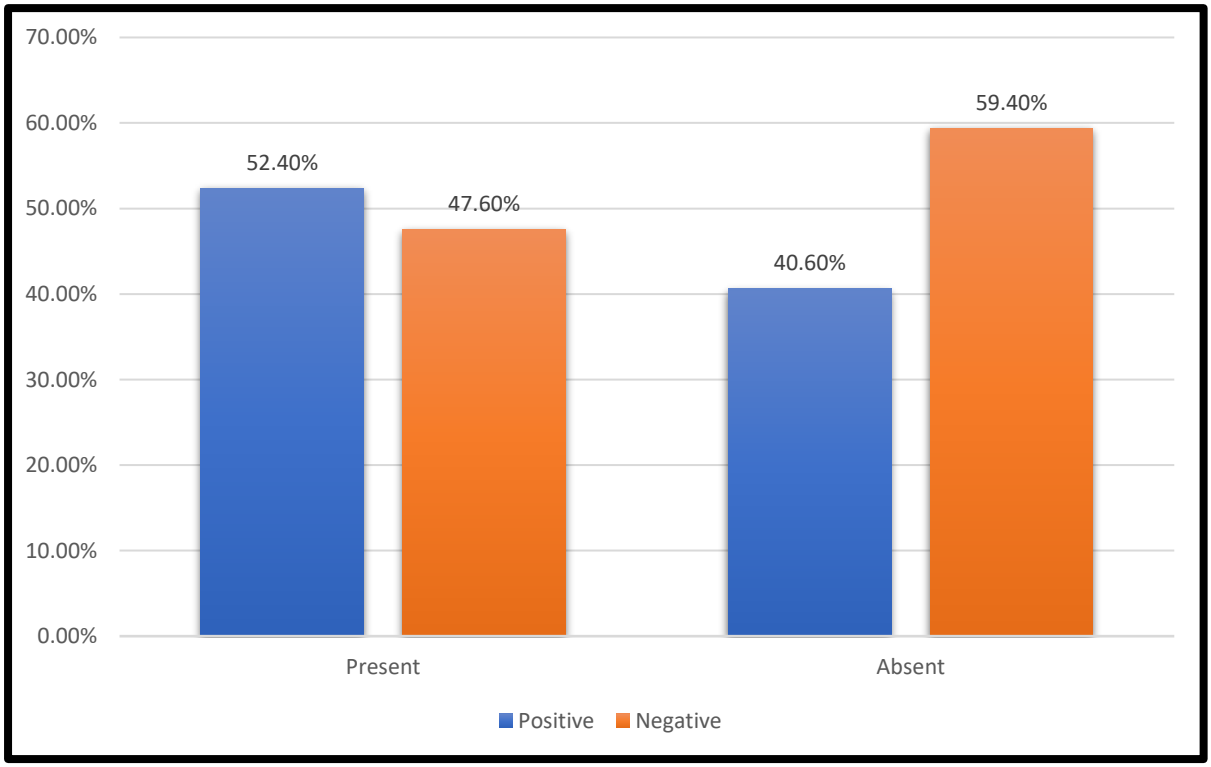


Table 24 Agreement between radiological finding of Nodular Infiltrates and MTB Culture.

			MTB Culture		Total
			Positive	Negative	
Infiltrations	Present	Count	24	35	59
		%	40.7%	59.3%	100.0%
	Absent	Count	30	38	68
		%	44.1%	55.9%	100.0%
Total		Count	54	73	127
		%	42.5%	57.5%	100.0%

Chi square = 0.153, P value = 0.696 (not significant)

54 (42.5%) patients had nodular infiltrates as radiological finding and among these patients, 24 (40.7%) tested positive for TB, while 35 (59.3%) tested negative by MTB culture.

Figure 11 Radiological finding of nodular infiltrates and MTB culture.

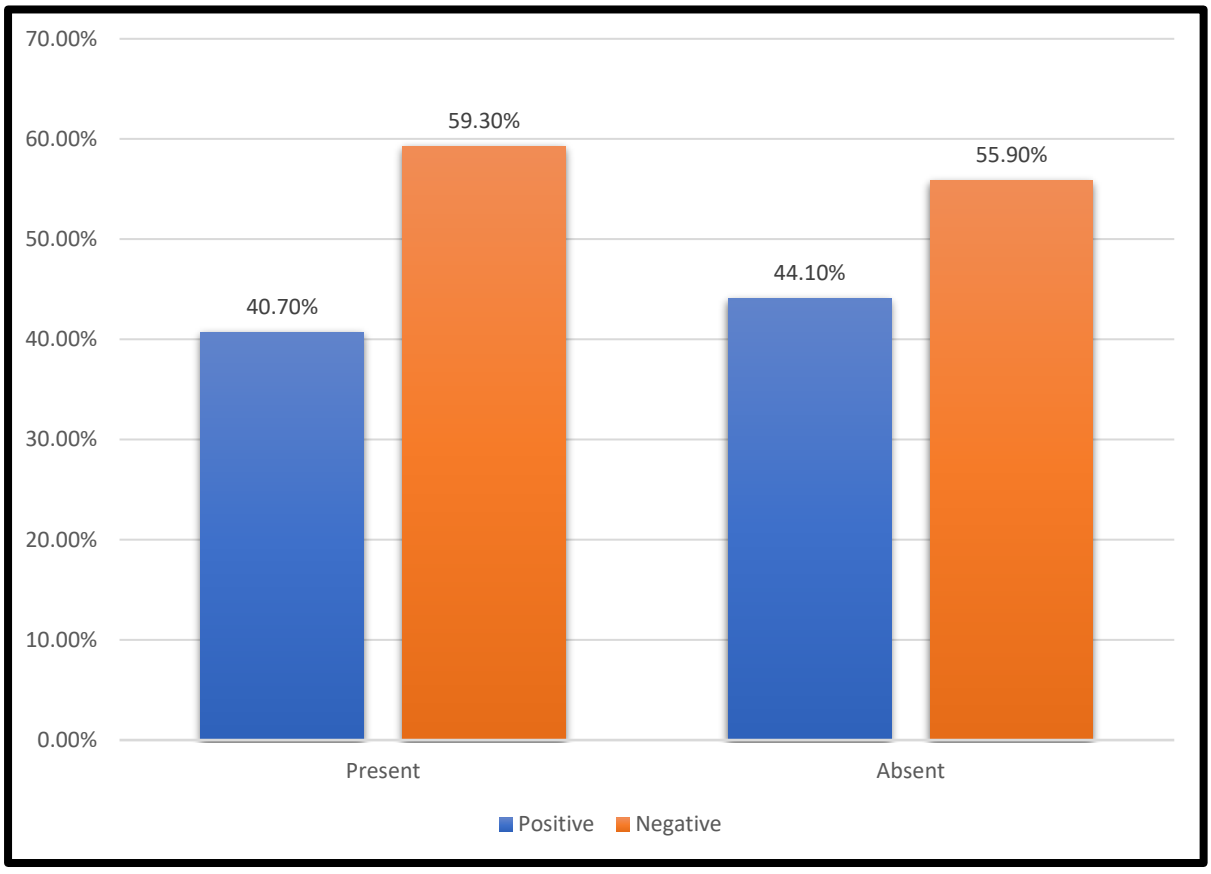


Table 25 Agreement between radiological finding of Cavity and MTB Culture.

			MTB Culture		Total
			Positive	Negative	
Cavity	Present	Count	10	11	21
		%	47.6%	52.4%	100.0%
	Absent	Count	44	62	106
		%	41.5%	58.5%	100.0%
Total		Count	54	73	127
		%	42.5%	57.5%	100.0%

Chi square = 0.268, P value = 0.605 (Not significant)

54 (42.5%) had cavity as radiological feature and among these patients, 10 (47.6%) tested positive for TB, while 11 (52.4%) tested negative by MTB culture. Among 73 patients without cavities, 44 (41.5%) tested positive for TB, while 62 (58.5%) tested negative.

Chi-square test was performed between radiological features and MTB culture results and it had not statistically significant association.

Figure 12 Radiological finding of Cavity and MTB Culture

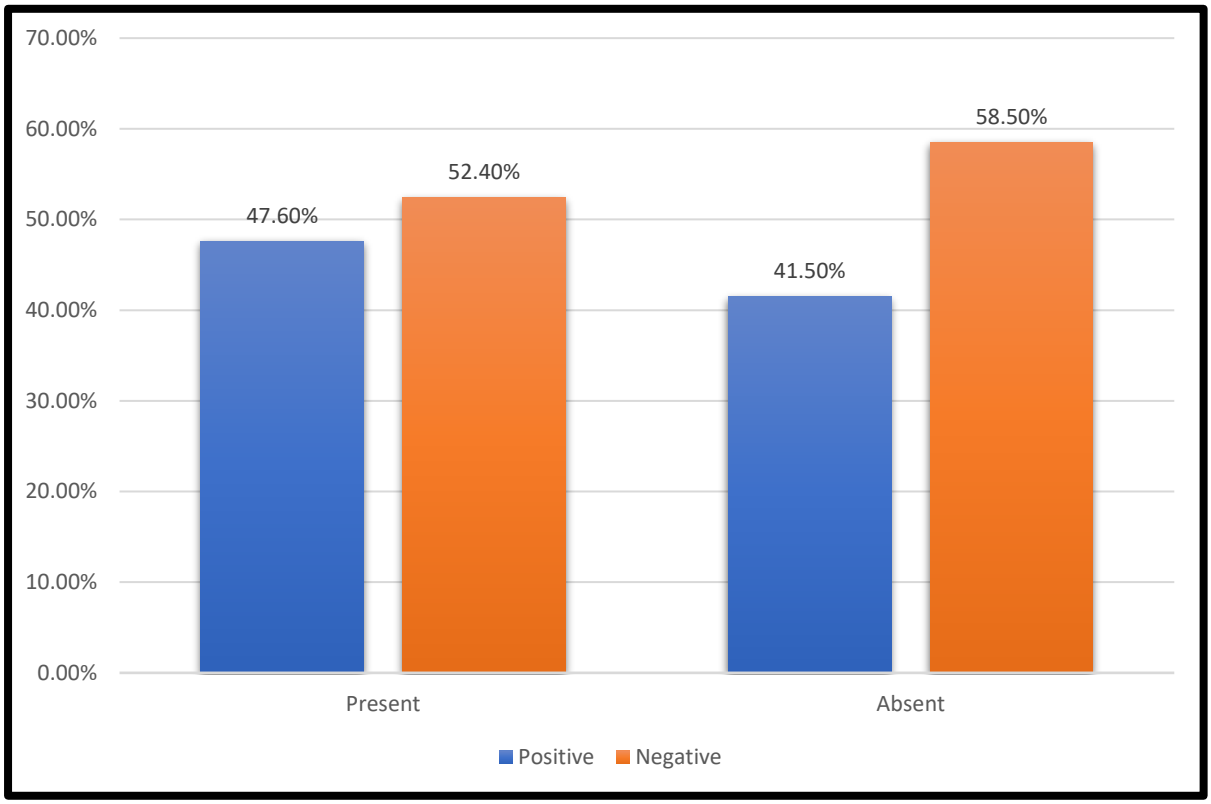


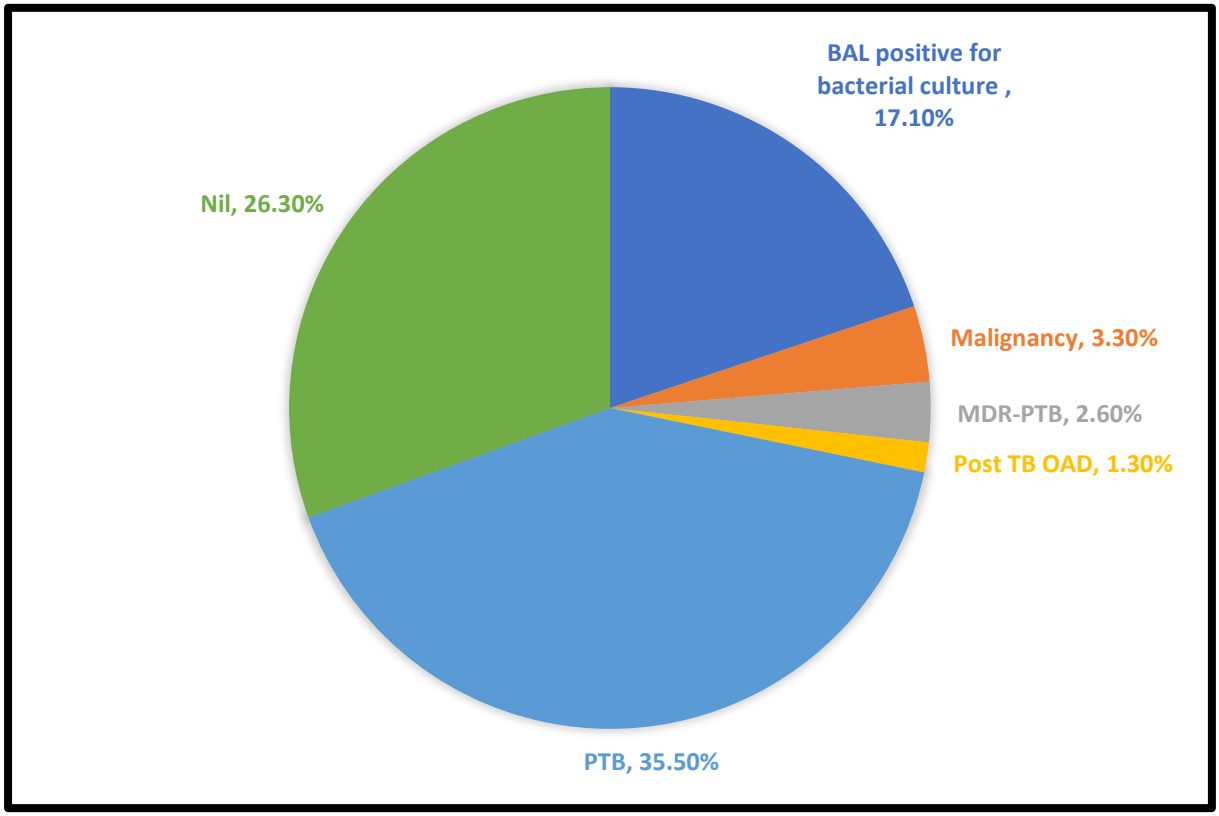
Table 26. Final Diagnosis.

Final Diagnosis	Frequency	Percentage
BAL positive for bacterial culture	26	17.1
Malignancy	5	3.3
MDR-PTB	4	2.6
Post TB OAD	2	1.3
PTB	54	35.5
Nil	40	26.3
Total	152	100.0

BAL was positive for bacterial growth in 26 (17.1%) patients, malignancy was diagnosed in 5 (3.3%) patients, and pulmonary tuberculosis (PTB) was the most common diagnosis, affecting 54 (35.5%) patients. MDR-PTB was diagnosed in 4 patients (2.6%).

Pulmonary Tuberculosis (PTB) was the most prevalent final diagnosis among the participants.

Figure 13 Final Diagnosis



DISCUSSION AND REVIEW OF RELATED ARTICLES

This present study was a cross-sectional, prospective and non-randomised study conducted to compare sensitivity and specificity of sputum and bronchoalveolar aspirate CBNAAT for MTB in diagnosis of sputum smear AFB negative patients, and also the significance of BAL CBNAAT and culture positivity.

This study included a total of 152 patients with sputum smear negative for AFB, but clinically and radiologically highly suspicious of pulmonary tuberculosis. Among these 152 patients, 15 patients were sputum CBNAAT positive and 67 patients were BAL CBNAAT positive. Out of all the positive CBNAAT samples, 54 patients were diagnosed as PTB after culture reports and 1 sputum CBNAAT negative patient came BAL MTB culture positive.

This study was comparable to a study done by Niraj Puri et al.[80] in terms of number of patients in a single centre study and the diagnostic yield of fiberoptic bronchoscopy. The diagnostic yield of present study was 45% which is significantly high compared to this study. However, the bronchial aspirate samples were subjected to ZN staining method in their study whereas CBNAAT method in present study. This substantiates the fact that yield obtained by BAL samples after bronchoscopy remains an important tool for diagnosis of PTB.

Of the 152 probable PTB patients, 38.2% were female and 61.8% male. 40.1% of patients were under 40, 32.9% were 41-60, and 27.0% were above 60. On radiographs, nodular infiltration (46.8%), consolidation (17.8%), cavity (15.8%), and fibrosis (15.8%) were most common. The most common comorbidities were type 2 diabetes mellitus (11.2%), hypertension (5.3%), and ischemic heart disease (2.6%).

Out of 152 sputum smear AFB negative but suspected PTB patients were included and CBNAAT and Culture for MTB results in present study are as follows:

1. Sputum CBNAAT for MTB: Positive in 9.9% and negative in 90.1% of patients respectively.
2. BAL CBNAAT for MTB: positive in 44.1%, negative in 46.7%, and not performed in 9.2% of patients respectively.
3. MTB Culture results were positive in 35.5% of patients and negative in 48.0%. It was negative in 23 (34.3%) MTB-positive BAL CBNAAT samples and positive in 1 (0.002%) negative sample.
4. The sensitivity of sputum CBNAAT for MTB compared to the culture method was 18.52%, and a specificity of 93.15%. The PPV was 66.67%, and NPV was 60.71%. This indicates that sputum CBNAAT missed a significant proportion of TB cases, and a negative result should be interpreted with caution.
5. The sensitivity and specificity in our study of BAL CBNAAT was 97.78% and 66.18% which means that BAL CBNAAT could correctly identify a very high proportion of TB cases, the reason for low specificity can be presence of dead bacilli in sputum sample which are detected by CBNAAT. PPV of BAL CBNAAT was 65.67% and NPV of BAL CBNAAT was 97.83%.
6. The positivity rate for CBNAAT was significantly higher in BAL samples compared to sputum samples, suggesting that BAL may be more effective in detecting MTB.

Table 1 COMPARISON OF PRESENT STUDY WITH SIMILAR STUDIES.

Study	Sample Size (n)	Diagnostic Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Nikbakhsh et al[75] (2015)	290	BAL- AFB smear with culture	60	91	89	64
Tiwari et al.[81] (2020)	76	BAL- CBNAAT with culture	92.31	66.76	79.32	87.5
Dubey et al.[82] (2021)	100	BAL- CBNAAT with culture	78.8	93.2	64.7	96.39
Badr et al. [76](2022)	330	BALF CBNAAT with culture	72.9	100	100	75.3
Uddin et al. [83](2022)	210	BAL Xpert MTB/RIF assay with culture	88.9	91.8	61.5	98.2
Sivaji et al. (2023) [84]	50	Diagnostic yield of sputum and BAL CBNAAT	Sputum CBNAAT yield- 46%	BAL CBNAAT yield- 85%	-	-
Oh et al. [85](2022)	937	Bronchoscopy and microbiological confirmation	Outcome- 49.1% positive by BAL	-	-	-
Imtiaz and Prasad et al.[86] (2022)	49	Diagnostic yield of BAL- PCR and culture	PCR positive- 23 (47%)	Culture positive- 39 (80%)	-	-
Present Study (2024)	**152**	**Sputum and BAL CBNAAT with MTB culture**	**Sputum CBNAAT- 18.15** **BAL CBNAAT- 97.78**	**Sputum CBNAAT- 93.15** **BAL CBNAAT- 66.18**	**Sputum CBNAAT- 65.6** **BAL CBNAAT- 65.6**	**Sputum CBNAAT- 60.7** **BAL CBNAAT- 97.8**

Nikbakhsh et al.[75] (2015) tested fibreoptic bronchoscopy (FOB)-guided (BAL) in 290 patients for identifying PTB in patients with negative sputum smears. The study included 290 patients with clinical and radiological signs of PTB but negative AFB sputum smears. FOB and BAL were performed on all patients, and BAL was stained and cultured. 38% of the

total patients had BAL AFB positive and had 60% sensitivity and 91% specificity for PTB diagnosis with PPV of 89% and NPV of 64% respectively. MTB was grown in 25% of sputum samples by LJ medium.

The results of this study were comparable to our study in terms of detection of MTB in BAL samples, however the culture positivity rate in our study was more (35.5%) due to use of MGIT liquid culture and an advantage of our study was agreement between MTB CBNAAT and culture method. (kappa=0.591, p value= 0.001)

A study done by **Niraj Puri et al**[80] in 2021 included 150 patients and found that the yield from the fiberoptic bronchoscope was 70.07% as compared to conventional bronchoscopy. The greatest bronchial wash yield was 22.78% for Squamous cell cancer and 21.5% for pulmonary tuberculosis. The greatest endobronchial biopsy yield was 41.21% for Squamous cell cancer and 31.42% for Adenocarcinoma.

The inference of this study is at par to our current study and concludes that bronchoscopy stays an essential diagnostic tool to obtain good quality and adequate samples for analysis which helps clinicians to arrive at a correct diagnosis and enables prompt treatment initiation.

A prospective observational study by **Tiwari et al.** [81]**(2020)** compared the sensitivity of ZN staining and CBNAAT using MTB culture as the gold standard in BALF samples in sputum smear-negative or non-sputum-producing patients with suspected PTB. The study included 76 suspected PTB patients as per clinical and radiological features. A flexible FOB was used to obtain BALF samples and sent for ZN staining, CBNAAT and MTB liquid culture. ZN staining was positive in 17.1% of BALF samples, CBNAAT for MTB in 68.72%. The ZN staining method had sensitivity of 23.08%, specificity of 89.19%, with PPV of

69.23%, NPV of 52.38%, and accuracy of 55.26% respectively. The BAL CBNAAT had a sensitivity of 92.31%, specificity of 66.76%, PPV of 79.23%, NPV of 87.50%, and accuracy of 75% respectively.

The sensitivity and specificity in our study of BAL CBNAAT was 97.78% and 66.18% respectively which means that BAL CBNAAT could correctly identify a very high proportion of TB cases, the reason for low specificity can be presence of dead bacilli in sputum sample which are detected by CBNAAT. PPV and NPV of BAL CBNAAT was 65.67% and 97.83% respectively. The above study is at par with results of this study.

Dubey et al. [82] **in 2021** conducted a prospective observational study and examined the diagnostic yield of bronchoalveolar lavage (BAL) in presumptive tuberculosis (TB) patients with negative sputum AFB and CBNAAT. They included 100 study participants with an average age 47.31 yrs and chest radiograph of these patients showed 40% alveolar opacities, 24% inhomogeneous, and 20% cavitary lesions. MTB was found in 15% of BAL CBNAAT tests and 2% were rifampicin-resistant. AFB was found in 10% of BAL samples by Ziehl-Neelsen (ZN) staining and out of total 14% were culture positive. The study concluded that ZN staining of BAL fluid was 42.86% sensitive and 95.35% specific respectively. BAL CBNAAT had 78.57% sensitivity and 93.02% specificity respectively. 10% of bronchoscopies resulted in complications and the most common were bronchospasm and hypoxia.

These results are strongly comparable to our study and additional analysis in our study is the agreement of sputum CBNAAT with MTB culture results in sputum smear AFB negative patients.

Uddin et al. (2022) [83] performed Xpert MTB/RIF assay (CBNAAT), culture, and AFB microscopy on BAL fluid from patients with probable pulmonary tuberculosis (PTB) who could not produce sputum. The study included 210 study participants. When compared to a CRS (composite reference standard), the CBNAAT for MTB assay demonstrated great sensitivity (92.9%) compared to culture (64.3%) and AFB microscopy (28.6%).

The results of this study are at par to our study results. This study emphasized the role of bronchoscopy specially in patients who cannot produce sputum spontaneously.

Sivaji et al[84] **in 2022** analysed sputum CBNAAT and BAL CBNAAT as helpful adjuncts for detecting pulmonary tuberculosis (PTB) in patients with negative sputum smear findings**.** Using sputum CBNAAT, 46% of the 50 patients tested positive for PTB, while BAL CBNAAT yielded positive results in 85% of cases. In this patient group, BAL CBNAAT had a greater diagnostic yield, nevertheless.

The sample size of this study was small but the results observed in this study are in line with present study.

A retrospective analysis by Oh et al.[85] **in 2022** evaluated the effectiveness of bronchoscopy in diagnosing microbiologically negative TB in a Korean cohort. The study involved 937 patients out of which 319 underwent bronchoscopy. The primary outcome was proportion of microbiological diagnoses made after bronchoscopy, and the study found that 157 (49.1%) of patients achieved microbiological confirmation. 105 patients had additional MTB culture-positive results while only 52 patients were confirmed only by PCR.

The study concluded that bronchoscopy is a valuable tool for diagnosing TB and detecting drug resistance. This study confirms the findings of our study and are comparable.

A large sample size of compared cohort adds weight to the hypothesis that BAL is beneficial in diagnosis of sputum smear negative PTB cases.

Badr et al.[76] **in 2022** assessed 330 patients suspected of PTB and evaluated the diagnostic yield of transbronchial lung biopsies and bronchial lavage fluid (BALF). BALF culture, CBNAAT and AFB smear showed a sensitivity of 80.7%, 72.9% and 21.1% respectively. When culture was the gold standard, BALF CBNAAT had 89.7% sensitivity and 99.5% specificity, while BALF AFB had 26% and 100%. In 132 patients with positive BALF CBNAAT for MTB, radiographic diagnoses varied: 38.6% as consolidation, 32.6% as centrilobular nodules/tree-in-bud, 18.9% as cavitary lesions, and 5.3% as numerous nodules ($p<0.001$). TB diagnosis is faster and more accurate with bronchoscopic specimens, according to studies.

Statistically significant association ($p=0.027$) was observed in our present study between BAL CBNAAT positivity and presence of cavity on radiology. This study findings are in comparison to our present study in emphasis on bronchoscopy and its utility in diagnosis of sputum smear AFB patients.

A retrospective analysis by **Imtiaz and Batubara et al.**[86] **(2022)** found that bronchoscopy and BALF analysis were an effective diagnostic tool in patients with sputum-negative PTB. BALF for MTB culture and MTB PCR were positive in 71% and 47% of patients, respectively. The results of this study are comparable to our present study.

Moreover, they also found that combined BAL MTB PCR and transbronchial lung biopsies provided a rapid diagnosis in 57% of patients, achieving an overall diagnostic yield of 90%. Upper lobe lavage and cavities on chest imaging were associated with higher diagnostic yields. In corelation with the study mentioned above by Badr[76] et al., this study too

revealed higher diagnostic yield of BALF for MTB in patients with positive findings on radiological imaging.

Prasad and Singh [87] **et al. (2019)** investigated the usefulness of bronchoscopy in the detection of TB, when sputum smear microscopy is negative. The literature search of the studies on significance of bronchoscopy had varying diagnostic yields ranging from 30% to 90%. Their meta-analysis on role of bronchoscopy in diagnosing EBTB and its use for therapeutic purposes to treat fibro-stenotic or tumorous varieties of EBTB by dilation or ablation techniques to restore airway patency. They also proved that bronchoscopic approaches like TBNA and EBUS-TBNA can be useful for collecting samples for smear microscopy and culture with sensitivity of 65.0–100.0 % and 70–80%, respectively, with a specificity of 100.0%.

Otto et al[88] compared the diagnostic yield between a regular sputum, induced sputum, and bronchoscopy in the diagnosis of TB. They observed that bronchoscopy had a significantly higher diagnostic yield when compared with multiple sputum samples after induction. This study also reported that bronchoscopy gave an additional 10% diagnostic yield with BAL culture positivity as gold standard in smear-negative pulmonary TB. These cases would have been otherwise missed by AFB smear and CBNAAT testing of sputum and induced sputum alone.

Ahmad et al. [89] **in 2019** evaluated the diagnostic value of BAL in patients with suspected PTB who had negative sputum smear and culture results unlike other studies who only evaluated smear AFB. The study included 190 patients who underwent bronchoscopy and BAL and results showed that BAL was able to detect PTB in 32.1% of patients, with mycobacterial culture and polymerase chain reaction having the highest diagnostic yield.

BAL is a useful diagnostic tool for PTB cases when sputum smear and culture are negative and this conclusion is comparable to our study highlighting the importance of BAL to obtain good quality sample for testing.

A study by **Archana B et al**. [77]**(2020)** examined the effectiveness of the BAL CBNAAT in diagnosing sputum smear-negative PTB. The study involved 112 patients with PTB but negative sputum smear results and results showed that BAL CBNAAT was positive in 75.8% of patients, while BAL culture was positive in 76.7%. The sensitivity and specificity of CBNAAT were 79.4% and 100.0%, respectively, making it a valuable tool for early PTB diagnosis.

The sensitivity and specificity of BAL CBNAAT and culture positivity is at par in the study as compared to our present study.

Bhatia et al. [90]**(2021)** conducted a prospective observational study in a tertiary care hospital in New Delhi to compare molecular and conventional diagnostic methods for detecting pulmonary tuberculosis (PTB) in BAL fluid from sputum smear-negative or scarce patients. Fibreoptic bronchoscopy and BAL collection were performed in 175 clinically suspected PTB patients and BALF was sent for ZN staining, Xpert MTB/RIF CBNAAT and MTB liquid culture. The gold standard for PTB diagnosis was MGIT culture plus a CRS. MGIT had 50.0% sensitivity using CRS. Sputum CBNAAT was more sensitive than ZN stain but had same sensitivity. BAL CBNAAT was 79.4% sensitive and 100% specific. The findings of this study are in line to our study.

Rasool et al. [91] **in 2019** found that the CBNAAT assay is a rapid and accurate tool for detecting Mycobacterium tuberculosis (MTB) in sputum smear-negative PTB suspects. The study involved 168 participants with negative sputum smear AFB. The CBNAAT detected

MTB in 28.57% of sputum smear negative cases, while MTB culture detected it in 34.52%. It also identified one case of multidrug-resistant TB.

This study did not use bronchoscopic lavage, hence the percentage of CBNAAT positivity and culture positivity among sputum negative cases is low in contrast to the present study which again justifies the bronchoscopy.

McWilliams [92]**et al. in 2002** studied 129 sputum smear AFB negative patients. 27 patients (96.3%) were identified by induced sputum testing, 14 patients (51.9%) by bronchoscopy and 1 case was diagnosed only by bronchoscopy. The authors discovered that induced sputum was more sensitive than bronchoscopy for identifying active PTB and came to the conclusion that when examining smear-negative pulmonary tuberculosis, bronchoscopy should be replaced with three induced sputum tests. **Chang et al**[93] **(2008)** studied a cohort of 219 patients and discovered that in order to diagnose PTB in patients with sputum smear AFB negative, induced sputum with hypertonic saline significantly outperformed supervised sputum collection. This raises a need for further research on induced sputum to diagnose PTB in resource liming setting. A study by **Prakash et al. (2016)** [94] conducted at a tertiary care hospital in Agra, India and examined the diagnostic efficacy of induced sputum and BAL in the diagnosis of smear-negative PTB. Induced sputum and BALF analysis were carried out in 50 suspected sputum smear negative PTB patients. Induced sputum detected PTB by culture more accurately (83.3%) than BAL (77.1%) and induced sputum had a higher sensitivity (90%) than BAL (83.3%). Induced sputum is a good substitute for smear-negative PTB and may increase diagnostic sensitivity, according to the authors' conclusion.

This warrants for potential research to evaluate the diagnostic yield of induced sputum samples, as we know PTB is more prevalent in low socio-economic status group.

LIMITATIONS

- Diagnostic efficacy of induced sputum was not analysed in this study.
- The sample size in this study was relatively small.
- There can be referral bias in the study as it was conducted at a tertiary care hospital.
- The sensitivity and specificity of AFB smear in BAL sample was not analyzed.
- Bronchoscopy procedure related complications were not evaluated in our present study.

CONCLUSION

Bronchoscopic aspirate for CBNAAT is more sensitive and specific than sputum CBNAAT for MTB in negative sputum smear AFB samples for diagnosis of PTB. With increasing incidence and prevalence of sputum smear negative patients, bronchoscopy plays a crucial role in diagnosis of patients who cannot produce sputum. The findings of our study shows that bronchoscopy is a better modality for obtaining respiratory samples in patients who cannot produce sputum. Patients with cavity as radiological finding and sputum smear AFB negative should be subjected to bronchoscopy and patients with positive sputum CBNAAT samples should be interpreted with caution as the chances of detection of dead bacilli are high (false positive patients) and culture takes time to show mycobacterial growth. On statistical analysis, BAL CBNAAT and MTB culture had a better agreement for diagnosis of sputum smear negative patients.

Two major concerns in the programmatic management of tuberculosis cases is the detection of cases which are missed by the diagnostic algorithm as it contains sputum analysis only and also establishment of cure in MDR-TB patients is based on conventional sputum culture.

All these findings support the conclusion of our study that bronchoscopy stays an important tool in diagnosis of patients who are unable to produce adequate sputum samples, especially those with positive radiological findings suspicious of PTB. However, analysis of these patients is challenging in resource-limited areas due to availability, cost, and logistics which may restrict bronchoscopy's application in TB diagnosis and future research should examine how new bronchoscopic diagnostic and therapeutic methods can improve early TB detection and appropriate treatment.

SUMMARY

- This present study was conducted at KLES Dr. Prabhakar Kore Hospital and MRC, Belagavi, Karnataka for a duration of one year.
- The aim was to compare sensitivity & specificity of sputum and broncho-alveolar aspirate CBNAAT for MTB diagnosis, and CBNAAT and MTB culture positivity.
- The study included 152 sputum smear negative but suspected PTB patients.
- 61 patients (40.1%) were under 40 years.
- Most common radiological finding was nodular infiltration in 71 patients (46.8%), followed by consolidation in 27 participants (17.8%), cavity in 24 (15.8%), and fibrosis in 24 (15.8%).
- Sputum CBNAAT positive: 15 (9.9%), 137 (90.1%) patients tested negative.
- BAL CBNAAT positive in 76 (44.1%), negative in 71 (46.7%) patients and procedure wasn't performed for 14 (9.2%) patients and 1 sputum CBNAAT positive patient underwent bronchoscopy due to delay in reporting of sputum CBNAAT.
- 152 sputum smear negative samples were analysed and MTB culture was positive in 54 (35.5%) and negative in 73 (48.0%) patients and 25 (16.4%) patients did not have MTB culture reports, hence excluded from MTB culture analysis.
- Sensitivity and specificity of sputum CBNAAT was 18.52% and 93.15% respectively.
- Sensitivity and specificity of BAL CBNAAT was 97.78% and 66.18% respectively.
- MTB culture was taken as gold standard method for diagnosis of PTB.
- There were no deaths noted during the study period.

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ANNEXURE-I

INFORMED CONSENT FORM

"Evaluating the diagnostic utility of sputum and bronchoalveolar aspirate CBNAAT For Mycobacterium tuberculosis in diagnosis of sputum negative pulmonary tuberculosis-One-year tertiary hospital based cross sectional study.**"**

Name of Student/Principal Investigator: REG NO. BR0121001

Name of Guide/Co Investigators:

Explanation of procedure: After signing the consent form, necessary personal information and detailed medical history will be taken by the Investigator. After this basic blood investigations will be sent and you will be subjected to CBNAAT of sputum sample and video Bronchoscopy to obtain samples to test for pulmonary tuberculosis. Video Bronchoscopy will be Performed by Consultant Pulmonologist and Bronchoscopy Specialist. Samples collected will be Sent for investigations to NTEP.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will/will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact: "Name of student/PI, mobile number, email ID" If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

Consent statement:

I am making a voluntary decision to participate in the study "Evaluating the diagnostic utility of sputum and bronchoalveolar aspirate CBNAAT For Mycobacterium tuberculosis in diagnosis of sputum negative pulmonary tuberculosis- One-year tertiary hospital based cross sectional study". My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant: Signature:

Name of the witness: Signature:

Name of the investigator: Signature:

Place:

Date:

Phone no.:

ANNEXURE-II

PROFORMA

Case No.	
Name	
Age	
Sex	Male Female
Height	
Weight	
Chief Complaints	
Past medical history	
Viral markers	
Radiology findings	

RESULTS

Investigation	**Sputum**	**Bronchoalveolar aspirate**
CBNAAT for MTB		
MTB Culture		

ANNEXURES III - MASTER CHART

Name	Age (in years)	Sex	Co-morbidity						Radiolgical findings				Sputum AFB	Sputum CBNAAT for MTB	BAL CBNAAT for MTB	Rif Sensitivity	MTB Culture	Final Diagnosis
			Type 2 DM	HTN	IHD	Post TB OAD	Others	HIV	cavity	infiltrations	fibrosis	consolidation						
suresh naik	39	m	a	a	a	a	a	r	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
Gopal megeri	72	m	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
Manjula mastiholimath	64	f	a	a	a	a	a	nr	a	a	a	p	n	n	n	n	n	BAL positive for bacterial culture
Subhash Jadhav	52	m	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	nil
satish	58	m	p	a	a	a	a	nr	a	a	a	p	n	n	n	n	n	BAL positive for bacterial culture
shodhan manolkar	25	m	p	a	a	a	a	nr	p	a	a	a	n	n	p	p	p	PTB
narayan shastri	38	m	a	a	a	p	a	nr	p	a	p	a	n	n	n	n	n	post tb oad
laxmi	39	f	a	a	a	a	ckd	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
sharada jadhav	46	F	a	a	a	a	Rheumatoid arthritis	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
dastagirsab	70	m	a	a	a	a	a	nr	a	a	a	p	n	n	p	p	p	PTB
Niyatha Raj	19	F	a	a	a	a	a	nr	a	a	a	p	n	n	n	n	n	BAL positive for bacterial culture
malika soudhagar	24	F	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
anad durgappa gadiwar	35	M	a	a	a	a	a	nr	p	a	a	a	n	n	p	p	n	nil
jagadish	24	M	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
savita menasappagol	21	F	a	a	a	a	a	nr	p	a	a	a	n	n	p	p	n	nil
kalakappa	38	M	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
jayalakshmi khode	40	F	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
Raghavandra kannoli	44	M	a	a	a	a	a	nr	p	a	a	a	n	n	p	p	p	PTB
siddappa ajjni	60	M	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	n	nil
abdulsab a pathan	80	M	a	a	a	a	a	nr	p	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
savitri	62	F	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
gousapak shekh	35	M	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	n	nil
nanasaheb jadhav	71	M	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	malignancy
sushma tavadare	25	F	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
krishna shetty	70	M	a	a	a	a	a	nr	a	a	a	p	n	n	n	n	n	Nil
kasturi patil	55	F	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	malignancy
afreen banu kanchagar	32	F	a	a	a	a	a	nr	p	a	a	a	n	n	p	p	p	ptb
shivanand	55	M	a	a	a	a	a	nr	a	p	p	a	n	n	p	p	p	PTB
devendra gupta	67	M	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	n	nil

32	Meera patil	57	f	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	Nil
33	nadanbi sanadi	67	F	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
34	nagaraja joshi	67	M	a	a	a	a	a	nr	a	a	a	p	n	n	n	n	n	malignancy
35	swayam chavan	19	F	a	a	a	a	a	nr	p	a	a	p	n	n	n	n	n	malignancy
36	mehamood begum	34	M	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
37	vinayak kore	34	M	a	a	a	a	a	nr	a	a	a	a	n	n	p	p	n	nil
38	ranjana shrinivas joshi	36	F	a	a	a	a	a	nr	p	a	a	a	n	n	p	resistant	p	MDR-PTB
39	yogesh rajpurohit	28	M	a	a	a	a	a	nr	a	a	a	p	n	n	p	p	n	nil
40	marayann jamdar	38	F	a	a	a	a	a	nr	a	a	a	a	n	n	n	n	n	Nil
41	priyanka	25	F	a	a	a	p	a	nr	p	a	a	p	n	n	p	resistant	n	MDR-PTB
42	shama rao kulkarni	44	F	a	a	a	a	a	nr	p	a	a	p	n	n	n	n	n	malignancy
43	prashant krishnaji satardekar	51	M	p	a	a	a	NAFLD	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
44	SAVITRI BAI	70	F	a	p	a	a	COPD	nr	a	p	a	a	n	n	p	p	n	BAL positive for bacterial culture
45	bhimappa pundalik boraganve	46	M	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	n	BAL positive for bacterial culture
46	samad bagawan	62	M	a	a	p	a	Malignancy	nr	a	a	a	p	n	n	n	n	n	BAL positive for bacterial culture
47	mahalaxmi angadi	23	F	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
48	siddhartha	23	M	a	a	a	a	a	nr	p	p	a	a	n	n	p	p	p	PTB
49	basavar halolli	36	M	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	n	BAL positive for bacterial culture
50	basavaraj mutagi	42	M	p	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
51	prahlad meti	32	M	a	a	a	a	a	nr	a	a	p	a	n	n	n	n	n	nil
52	santosh badiger	36	M	a	a	a	p	a	nr	a	a	p	a	n	n	n	n	n	post tb oad
53	jyotheppa pujer	63	M	a	a	a	p	a	nr	p	a	p	a	n	n	p	p	n	nil
54	krishna ghodake	75	F	p	p	a	a	a	nr	a	p	p	a	n	n	p	resistant	p	mdr tb
55	rahul guntakal	26	M	a	a	a	a	a	nr	a	a	a	a	n	n	n	n	n	Nil
56	athmaram	42	M	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
57	prema muttalamuri	49	F	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
58	sanjay patil	52	M	a	a	a	a	a	nr	p	a	a	a	n	n	p	p	n	nil
59	gunavati	35	F	a	a	a	p	a	nr	a	p	a	a	n	n	p	n	n	nil
60	pradeep patil	34	M	a	a	a	a	a	nr	p	a	a	a	n	n	p	p	p	PTB
61	ganaraj chougale	39	M	a	a	a	a	a	nr	a	a	p	a	n	n	n	n	n	nil
62	sangappa vannur	40	M	a	a	a	a	a	nr	a	a	a	a	n	n	p	p	n	NIL
63	shritant mudalagi	56	M	a	a	a	a	hbsag	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture

64	kavita patil	31	F	a	a	a	a	hbsag	nr	a	p	a	a	n	n	p	p	p	PTB
65	durgappa gadiwaddar	28	M	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
66	mehaboob jamakhandi	51	M	a	a	a	a	a	nr	a	a	p	a	n	n	n	n	n	BAL positive for bacterial culture
67	nagavva pujer	50	F	a	a	a	a	a	nr	a	a	a	p	n	n	p	resistant	p	PTB
68	ramachandra bhajantri	76	M	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
69	balappa gadagalli	37	M	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
70	basavaraj shiramagond	59	M	p	p	a	a	nafld	nr	a	a	a	p	n	n	n	n	n	BAL positive for bacterial culture
71	parwati waghamode	70	F	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	nil
72	parvatevva hiremath	67	F	p	p	a	a	a	nr	a	p	a	a	n	n	n	n	n	BAL positive for bacterial culture
73	laxman bhajantri	50	M	a	a	a	a	a	nr	a	a	a	p	n	n	p	p	n	NIL
74	shivaleela v k	35	F	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
75	manohar	55	M	p	a	a	a	a	nr	p	a	a	a	n	n	p	p	p	PTB
76	mandira patil	37	F	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	n	nil
77	bharamappa kankanwadi	75	M	a	a	a	a	a	nr	a	a	a	p	n	n	p	p	p	PTB
78	nagendra khajjidoni	58	M	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
79	sundravva soundatti	63	F	a	a	a	a	a	nr	p	a	a	a	n	n	p	p	p	PTB
80	isabel fernandes	50	F	a	a	a	a	ca lung	nr	a	p	a	a	n	n	p	p	p	PTB
81	kalappa badiger	52	M	a	a	a	a	copd	nr	a	a	a	p	n	n	n	n	n	nil
82	hanamantappa rathod	35	M	a	a	a	a	a	nr	a	a	a	p	n	p	p	p	p	PTB
83	tanuja kolkar	49	F	a	a	a	a	ckd	nr	a	a	p	a	n	n	n	n	n	nil
84	arjun nesarikar	71	M	a	a	a	a	a	nr	a	a	a	a	n	n	n	n	n	nil
85	jyoti rathod	25	F	p	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
86	madiwalappa nandgad	48	M	p	a	a	a	a	nr	a	a	a	a	n	n	p	p	p	PTB
87	Ramappa desai	53	m	p	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
88	Ashok Naik	72	m	a	p	a	a	a	nr	a	a	a	a	n	n	p	p	p	PTB
89	madhukar kamkar	29	m	a	a	a	a	a	nr	a	a	p	a	n	n	p	p	p	PTB
90	Devendra Gupta	69	m	p	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
91	Shrimanthai kamble	66	f	p	p	p	a	a	nr	a	a	p	a	n	n	n	n	n	nil
92	Basappa naikar	52	m	a	a	a	a	a	nr	a	a	a	a	n	n	n	n	n	nil
93	Ranjana Joshi	45	f	a	a	a	a	a	nr	a	a	a	a	n	n	p	resistant	p	MDR- TB
94	Shewanta sasane	62	f	p	a	a	a	a	nr	p	a	a	a	n	n	p	p	p	PTB
95	Vinubhai parmar	55	m	p	p	p	a	a	nr	p	a	a	a	n	n	p	p	p	PTB

96	Mohan Madar	45	m	p	a	a	a	a	nr	a	a	a	a	n	p	na	p	p	PTB
97	Vishalaxi Gadagin	32	M	a	a	a	a	a	nr	a	a	a	a	n	p	na	p	p	PTB
98	Ningouda Patil	40	p	a	p	a	a	a	nr	a	a	a	p	n	p	na	p	n	NIL
99	Lingayya Basayya	65	M	p	a	p	a	a	nr	a	p	a	a	n	p	na	p	p	PTB
100	Rudresh Madar	21	M	a	a	a	a	a	nr	a	a	p	a	n	p	na	p	p	PTB
101	Shantawwa Naik	55	F	a	a	a	a	a	nr	a	a	a	a	n	p	na	p	n	NIL
102	Reshma Kaki	35	F	a	a	a	a	a	nr	a	p	a	a	n	p	na	p	p	PTB
103	Akkawwa Naik	48	F	a	a	a	a	a	nr	a	a	p	a	n	p	na	p	p	PTB
104	Raju Naik	40	M	a	a	a	a	a	nr	a	p	a	a	n	p	na	p	n	NIL
105	Mohamad umar Domani	31	M	a	a	a	a	a	nr	p	a	a	a	n	p	na	p	n	NIL
106	Mallikarjun chidanand	29	M	a	a	a	a	a	nr	a	a	p	a	n	p	na	p	n	NIL
107	Areefa Riyaz Adimani	25	F	a	a	a	a	a	nr	a	a	p	a	n	p	na	p	p	PTB
108	Ninganagouda Patil	41	M	a	a	a	a	a	nr	a	a	a	a	n	p	na	p	p	PTB
109	Kalpana Sambargi	19	F	a	a	a	a	a	nr	a	a	a	p	n	p	na	p	p	PTB
110	Madina bepari	28	m	a	a	a	a	a	nr	a	a	p	a	n	n	n	n	p	PTB
111	Vasima Killedar	37	m	a	a	a	a	a	nr	a	a	p	p	n	n	p	p	p	PTB
112	Yamanappa gadivaddar	40	m	a	a	a	a	a	nr	a	a	p	a	n	n	p	p	p	PTB
113	Chaitra patil	22	f	a	a	a	a	a	nr	a	a	p	a	n	n	p	p	p	PTB
114	kalakappa	51	m	a	a	a	a	a	nr	a	a	a	p	n	n	p	p	p	PTB
115	Abubakar shaikh	21	m	a	a	a	a	a	nr	a	a	p	a	n	n	p	p	P	PTB
116	imamsab musamiya	67	m	a	a	a	a	a	nr	a	a	p	a	n	n	p	p	N	NIL
117	balu bhogan	62	m	a	a	a	a	a	nr	a	a	a	p	n	n	p	p	N	NIL
118	sangappa badavadagi	44	m	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	N	NIL
119	maruti lavage	77	m	a	a	a	a	a	nr	a	a	a	a	n	n	p	p	N	NIL
120	raju jyadi	40	m	a	a	a	a	a	r	a	p	a	a	n	n	p	p	N	NIL
121	Vishwanath hebbali	44	m	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
122	Gulappa walikar	52	m	a	a	a	a	a	nr	a	p	a	a	n	n	p	resistant	p	PTB

123	Santosh tarki	36	m	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
124	Shabbir mulla	35	m	a	a	a	a	a	r	a	p	a	a	n	n	p	p	p	PTB
125	Rakshita rudrappure	23	f	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	p	PTB
126	kallappa hanamasagar	55	m	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	N	NIL
127	amarappa kurbar	68	m	a	a	a	a	a	nr	p	a	a	a	n	n	p	p	N	NIL
128	sakashi bafna	24	f	a	a	a	a	a	nr	a	p	a	a	n	n	n	n	N	nil
129	Mairun thased	30	f	a	a	a	a	a	nr	a	p	a	a	n	n	p	p	P	PTB
130	basappa irappa	63	m	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
131	Lakshmanrao vasamsetti	80	m	a	a	a	a	a	nr	p	a	a	a	n	n	n	n/a	n/a	nil
132	Mallari Jadhav	55	m	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
133	Abdukrazak	69	m	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
134	ningappa naganur	55	m	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
135	Sumati arage	40	m	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
136	Bhimara bangari	56	m	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
137	Balawwa	62	f	a	a	a	a	a	nr	p	a	a	a	n	n	n	n/a	n/a	nil
138	Satish gundhar	60	m	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
139	Mahadev Patil	63	m	a	a	a	a	a	nr	a	a	p	a	n	n	n	n/a	n/a	nil
140	Saraswati	67	f	a	a	a	a	a	nr	a	a	a	p	n	n	n	n/a	n/a	nil
141	savitri kalakeru	64	f	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
142	Parawwatti patil	70	f	a	a	a	a	a	nr	p	a	a	a	n	n	n	n/a	n/a	nil
143	khairunbi	75	f	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
144	Basavaneppa kadathal	80	m	a	a	a	a	a	nr	a	a	p	a	n	n	n	n/a	n/a	nil
145	Godavva patil	67	f	a	a	a	a	a	nr	a	a	a	a	n	n	n	n/a	n/a	nil
146	Sudha honagekar	49	f	a	a	a	a	a	nr	a	a	a	p	n	n	n	n/a	n/a	nil
147	Veerabhadrappa	65	f	a	a	a	a	a	nr	a	a	a	p	n	n	n	n/a	n/a	nil
148	Jangalima	48	f	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
149	Ranjana Yadav	48	f	a	a	a	a	a	nr	a	a	a	a	n	n	n	n/a	n/a	nil
150	sarita patil	50	f	a	a	a	a	a	nr	a	a	p	a	n	n	n	n/a	n/a	nil
151	Girija more	48	f	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
152	Shabana Patel	47	f	a	a	a	a	a	nr	a	a	a	p	n	n	n	n/a	n/a	nil
153	afraid Bikaji	19	M	a	a	a	a	a	nr	a	p	a	a	n	n	n	n/a	n/a	nil
154	Chandrayya Hiremath	57	M	a	a	a	a	a	nr	a	a	a	p	n	n	n	n/a	n/a	nil

ANNEXURE-IV

KEY TO MASTER CHART

A	-Absent
P	- Present
R	- Reactive
NR	- Non reactive
M	- Male
F	-Female
HIV	-Human immunodeficiency Virus
HTN	-Hypertension
Type 2 DM	-Type 2 diabetes mellitus