Monaldi Archives for Chest Disease

eISSN 2532-5264 https://www.monaldi-archives.org/

Monaldi Arch Chest Dis 2025 [Online ahead of print]

To cite this Article:
Sontakke A, Bundale R, Kolte S, et al. **Unveiling the role of cartridge-based nucleic acid amplification test in extrapulmonary tuberculosis diagnosis: insights from a cross-sectional study.** *Monaldi Arch Chest Dis* doi: 10.4081/monaldi.2025.3238

Unveiling the role of cartridge-based nucleic acid amplification test in extrapulmonary tuberculosis diagnosis: insights from a cross-sectional study

Anil Sontakke, Rajasi Bundale, Sagar Kolte, Nidhi Girdhar,
Hrishikesh Gaikwad, Harshal Ital

Department of Respiratory Medicine, NKP Salve Institute of Medical Sciences and Lata Mangeshkar Hospital, Nagpur, Maharashtra, India

Correspondence: Rajasi Bundale, Department of Respiratory Medicine, NKP Salve Institute of Medical Sciences and Lata Mangeshkar Hospital, Nagpur, Maharashtra, India.
Tel.: +917769806660. E-mail: rajasi1803@gmail.com

Contributions: AS, takes responsibility for the content of the manuscript, including the data and analysis, and the integrity of the data and the accuracy of the data analysis; RB, SK, NG, contributed substantially to the study design, data analysis and interpretation; RB, SK, NG, HG, HI, contributed significantly to the writing of the manuscript; AS, has contributed to the drafting of the article and revising it critically for important intellectual content. All the authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors have no conflict of interest to declare.

Ethics approval and consent to participate: ethical approval (office letter no. NKPSIMS & RC and LMH/IEC/1/2024) has been obtained from the Institutional Ethics Committee.

Informed consent: written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Patient consent for publication: obtained.

Availability of data and materials: all data generated or analysed during this study are included in this published article.

Funding: none.

Abstract

Extrapulmonary tuberculosis (EPTB) is a multifaceted disease that could potentially impact nearly all organs. Current global data indicate a significant variation in the proportion of EPTB among all tuberculosis cases, ranging from 15% to 53%. Clinicians in India express concerns about the efficacy of the cartridge-based nucleic acid amplification test (CBNAAT) in EPTB, as its yield frequently does not align with the findings of the World Health Organization meta-analysis. Hence, the present study was conducted to evaluate the diagnostic yield of CBNAAT in EPTB at a tertiary care hospital.

Specifically, the present hospital-based observational cross-sectional study was conducted at the Department of Respiratory Medicine at a tertiary care hospital from February 2024 to April 2024. A total of 52 patients with presumptive EPTB were enrolled. Demographic information, clinical history, and clinical examination findings were recorded with the help of a standard, semi-structured, pre-validated case record proforma. A composite reference standard (CRS), which was defined by clinical, radiological, laboratory, and histopathological findings and treatment response to antitubercular therapy along the course, was considered. The statistical software, namely SPSS 22.0, was used for the analysis of the data.

Among a total of 52 patients, the mean age of patients was 37.42±16.18 years, with the proportion of males being 59.62%. The majority of patients had tuberculosis pleural effusion (63.46%). The pooled diagnostic yield of CBNAAT showed sensitivity and specificity of CBNAAT compared to CRS of 22.22% and 100%, respectively.

Culture had the highest sensitivity in diagnosing EPTB in the study population, as compared to CBNAAT, thereby emphasizing the importance of diagnosis by culture method.

Key words: composite reference standard, Xpert MTB/RIF, lymph node TB, TB pleural effusion, abdominal TB, bone and joint TB.

Introduction

Tuberculosis (TB) is a communicable disease primarily affecting the lungs, caused by the bacterium Mycobacterium tuberculosis (MTB) [1]. Historically, it was posited that pulmonary tuberculosis accounts for approximately 85% of total tuberculosis cases, while the remaining 15% are attributed to extrapulmonary tuberculosis (EPTB) cases [2].

Extrapulmonary tuberculosis is a multifaceted disease that could potentially impact nearly all organs. It is prevalent among the lower socioeconomic demographic [3]. The impact of tuberculosis is more pronounced with increased involvement of extrapulmonary organs in this at-risk population [4,5]. Current global data indicate a significant variation in the proportion of EPTB among all tuberculosis cases, ranging from 15% to 53% [6,7].

In April 2017, Indian guidelines for EPTB were established as evidence-based practices for the suspicion, diagnosis, and management of EPTB within medical care services. CBNAAT (Xpert MTB/RIF) is a commercially accessible assay for M. tuberculosis complex (MTB) that employs polymerase chain reaction (PCR) to analyse specimens for genetic material specific to MTB while concurrently identifying a gene (rpoB) associated with rifampicin resistance [8,9].

In recent years, CBNAAT has become a significant diagnostic instrument due to its superior sensitivity and diagnostic yield. EPTB continues to present a considerable challenge in diagnosis owing to its varied clinical manifestations and the constraints of traditional diagnostic techniques. While microbiological confirmation is essential for TB diagnosis, obtaining a specimen for EPTB is often challenging. As a result, neither CBNAAT nor culture can always serve as the definitive gold standard for diagnosing EPTB. Therefore, the Composite Reference Standard (CRS), which encompasses clinical, radiological, laboratory, and histopathological findings is utilised for the initiation of Antitubercular Therapy (ATT). CRS also helps in monitoring response to ATT.

Clinicians in India express concerns about the efficacy of CBNAAT in EPTB, as its yield frequently does not align with the findings of the WHO meta-analysis. Hence, the present study was conducted to evaluate the diagnostic yield of CBNAAT in EPTB at tertiary care hospital.

Materials and Methods

This investigation was designed as a cross-sectional study at a tertiary care hospital's Department of Respiratory Medicine and conducted over three months, from February to April 2024. The study aimed to assess the diagnostic accuracy of the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in patients suspected of having EPTB.

Study participants

Patients presenting with clinical symptoms indicative of EPTB were included after obtaining informed consent. Participants were selected based on inclusion criteria, such as symptoms suggestive of EPTB, and were excluded if they were already receiving anti-tubercular therapy or declined participation. Ultimately, 52 eligible patients were enrolled.

Data collection and processing

Demographic details, clinical history, and physical examination findings were systematically recorded using a validated proforma. Biological specimens, such as pleural fluid, lymph node aspirates, and bone biopsy tissues, were collected based on the suspected site of disease. These samples underwent CBNAAT testing alongside AFB culture and other diagnostic procedures like histology.

Diagnostic framework

The diagnostic performance of CBNAAT was evaluated against a Composite Reference Standard (CRS). A CRS typically integrates several diagnostic components, including:

- Clinical Evaluation: Comprehensive assessment of patient symptoms and medical history like cough, evening rise of temperature, night sweats, weight loss.
- Radiological Imaging: Use of imaging techniques like x-rays, ultrasonography to detect signs indicative of EPTB.
- Laboratory Investigations: Analysis of biological samples for markers associated with tuberculosis infection like Adenosine Deaminase (ADA) levels of pleural fluid.
- Histopathological Examination: Microscopic evaluation of tissue samples to identify pathological changes consistent with tuberculosis like necrotizing granulomas, caseous necrosis, giant cells, etc.
- Microbiological Tests: Procedures such as culture and staining to detect the presence of Mycobacterium tuberculosis like Ziehl-Neelson staining, Solid media or liquid culture methods.
- Therapeutic Response: Monitoring patient response to anti-tubercular therapy in terms of symptomatic or radiological improvement, which can support the diagnosis when other evidence is inconclusive.

A CRS serves as a surrogate benchmark in the absence of a definitive 'gold standard' test, combining multiple diagnostic methods to establish a more accurate reference.

Laboratory analysis

All diagnostic tests were conducted in National Accreditation Board for Testing and Calibration Laboratories (NABL)-accredited facilities. The CBNAAT analyses were performed using the GeneXpert MTB/RIF platform, ensuring strict adherence to quality control protocols. AFB culture was done by either BACTEC mycobacterial growth indicator tube (MGIT) or Solid media (Lowenstein-Jensen) according to resource availability. In the case of pleural TB, our methodology was limited by the inability to perform parietal pleura biopsy due to resource constraints at our center. Consequently, we were unable to perform histologic assessments or rapid molecular tests recommended by WHO.

Ethical approval and statistical analysis

The study protocol received approval from the Institutional Ethics Committee (approval number: NKPSIMS & RC and LMH/IEC/1/2024). Participant confidentiality was maintained throughout, with data anonymized before analysis. Statistical evaluations were conducted using SPSS version 22.0. Descriptive statistics summarized the data, while sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to compare CBNAAT with CRS. Associations were tested using chi-squared analysis, with significance set at $p < 0.05$.

Results

Demographic characteristics

This research included 52 individuals who were suspected to have EPTB. The participants had an average age of 37.42 years (± 16.18), with the largest proportion (48.08%) being between 31 and 50 years of age. Age-wise, 15.38% were in the 18-30 years category, 30.77% were between 51-70 years, and 5.77% were older than 70 years. Males represented 59.62% (n=31) of the study group, while females accounted for 40.38% (n=21) (Table 1).

Comorbidities and medical history

Diabetes mellitus emerged as the most prevalent comorbidity, affecting 17.31% of the participants, followed by hypertension at 11.54%. A history of tuberculosis was reported by 7.69% of the individuals, and one participant (1.92%) tested positive for HIV. The vast majority (98.07%) tested negative for HIV infection (Table 1).

Distribution of EPTB cases

Pleural effusion was the most frequently diagnosed type of EPTB, affecting 63.46% (n=33) of the patients. Other types included bone and joint TB (15.38%, n=8), abdominal TB (13.46%, n=7), lymph node TB (5.77%, n=3), and endometrial TB (1.92%, n=1) (Table 2).

Diagnostic performance and yield

The diagnostic capabilities of CBNAAT and culture were assessed in comparison to the Composite Reference Standard (CRS). CBNAAT exhibited a sensitivity of 22.22% and a specificity of 100%, indicating its ability to correctly identify all positive cases without any false positives. The positive predictive value (PPV) for CBNAAT was 100%, though its negative predictive value (NPV) was lower at 16.67%. Culture showed a sensitivity of 33.33% and a specificity of 85.71%, with a PPV of 93.75% and an NPV of 16.67%. While culture identified a greater number of true positives than CBNAAT, it had a slightly higher incidence of false positives (Table 3).

Diagnostic yields by EPTB type

The effectiveness of CBNAAT and culture varied significantly among different forms of EPTB. For lymph node TB, both tests yielded a diagnostic success rate of 66.67%. For bone and joint TB, CBNAAT identified 25% of cases, whereas culture was more effective, detecting 75%. In cases of pleural effusion, CBNAAT diagnosed 15.15% while culture detected 9.09%. Similarly, abdominal TB showed higher diagnostic success with culture at 57.14% compared to CBNAAT's 14.29% (Table 4). Neither CBNAAT nor culture was able to detect endometrial TB. These variations underline the site-specific differences in test performance and the necessity of combining diagnostic methods for improved accuracy.

Comparative diagnostic yields

Lymph node TB had the highest diagnostic yield using CBNAAT (66.67%), followed by bone and joint TB (25%). Pleural effusion and abdominal TB demonstrated lower diagnostic yields with CBNAAT. Culture, on the other hand, showed superior performance for bone and joint TB (75%) and abdominal TB (57.14%), underscoring its importance in diagnosing these specific forms of EPTB (Table 4).

Discussion

The current observational cross-sectional study aimed to assess the diagnostic yield of CBNAAT in EPTB at a tertiary care hospital. The study included 52 patients with suspected EPTB.

Among the 52 participants, the majority (48.08%) were between 31 and 50 years of age, with an average age of 37.42 ±16.18 years. Male patients constituted 59.62% of the study population. Studies often indicate that EPTB is more common in females, especially among younger women. Lymph node TB is commonly reported in females whereas pleural TB has higher prevalence in males. Diabetes mellitus emerged as the most common comorbidity, present in 17.31% of cases. Studies indicate that in settings with lower HIV prevalence, diabetes mellitus often emerges as the leading comorbidity due to its global prevalence and its impact on immune function. Similar demographic patterns were observed in studies by Nishal N et al. [10] and C. Mohan Rao et al. [11].

In this study, the most prevalent form of EPTB was tuberculous pleural effusion (63.46%), followed by bone and joint TB (15.38%). This finding contrasts with the studies conducted by Nishal N et al. [10] and C. Mohan Rao et al. [11], where lymph node TB was the predominant form of EPTB. This disparity may be attributed to certain limitations in the current study, such as its setting at a tertiary care center. Patients presenting with cervical swelling often consult the ENT outpatient department initially and are subsequently referred to the surgery department for excision procedures, likely resulting in a smaller sample size for lymph node TB.

The diagnostic performance of CBNAAT in this study revealed a sensitivity of 22.22% and a specificity of 100% compared to the composite reference standard (CRS). The highest diagnostic yield was observed for lymph node TB (66.67%), followed by bone and joint TB (25%).

A study by Jose et al. [12] in North Kerala (n=1145) reported a CBNAAT sensitivity of 12.6%. In contrast, Vadwai et al. [13] (n=283) reported a sensitivity of 81% against CRS, while S. Suzana et al. [14] (n=494) found a sensitivity of 62%. Krishna V et al. [15] observed a CBNAAT sensitivity of 68.5% in their study. A meta-analysis conducted by the WHO on the use of Xpert MTB/RIF for lymph node aspirates in diagnosing EPTB reported pooled sensitivity and specificity values of 83.7% and 99.2%, respectively. The variability in CBNAAT sensitivity for EPTB stems from multiple factors. These include differences in the types of samples collected, as pleural fluid and cerebrospinal fluid often have lower bacterial loads compared to lymph node aspirates, impacting detection rates. Additionally, studies have shown significant heterogeneity in sample processing techniques, such as whether concentration steps are employed. Variations in reference standards, with some using composite reference standards and others relying solely on culture, further contribute to this inconsistency. The heterogeneity in study designs, including differences in smear positivity, sample freshness, and the proportion of HIV-positive patients, also influences sensitivity outcomes [16].

In the present study, CBNAAT demonstrated higher diagnostic efficacy for lymph node aspirates and pus, whereas it failed to detect EPTB in pleural fluid, cerebrospinal fluid, and peritoneal fluids. This phenomenon could be explained by the limited bacterial load in pleural spaces, reflecting a hypersensitivity reaction mediated by cell-mediated immunity. Previous studies have shown that pleural fluid cultures yield positive results in only 20-40% of patients with confirmed tuberculous pleuritis [17,18]. Since our study relied solely on pleural fluid for diagnosing suspected TB pleural effusion, the sensitivity of molecular and microbiologic methods may have been lower than if biopsy specimens had been included, potentially impacting diagnostic accuracy.

The World Health Organization (WHO) recommends the use of nucleic acid amplification tests (NAATs), such as Xpert MTB/RIF and Xpert Ultra, as initial diagnostic tools for tuberculosis (TB). These tests are strongly recommended for both pulmonary and extrapulmonary TB due to their high specificity and ability to simultaneously detect rifampicin resistance. They are particularly beneficial in resource-limited settings and offer faster results compared to traditional culture-based methods. These recommendations extend to patients of all ages and include individuals with HIV [19].

One of the major limitations of this study was the absence of a definitive gold standard for EPTB diagnosis, which could have led to false positive or false negative results within the study cohort. This inherent challenge affects not only this study but also any research focused on EPTB diagnosis. Additionally, the small sample size restricts the generalizability of the findings to the larger population. Also some forms of EPTB were not included in the analysis, further limiting the scope of the study.

Conclusions

This study underscores the critical role of CBNAAT in diagnosing EPTB, particularly due to its high specificity and utility in resource-limited settings. Although CBNAAT's sensitivity is modest, its integration with complementary methods like culture and histopathology significantly enhances diagnostic accuracy.

A notable strength of this research is its evaluation of CBNAAT across various EPTB forms, offering insights into its site-specific efficacy. Despite a limited sample size, the findings contribute valuable data supporting a multimodal diagnostic approach. Future studies on larger populations are recommended to validate these results and improve diagnostic sensitivity. CBNAAT remains a vital component in the framework for effective EPTB diagnosis.

References

1. Doucette K, Cooper R. Tuberculosis. In: Grippi M, Elias J, Fishman J, Kotloff R, Pack A, Senior R, eds. Fishman's pulmonary diseases and disorders. 5th ed. New York, NY, USA: McGraw Hill; 2015. pp. 2012-31.
2. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res 2004;120:316-53.
3. Olson NA, Davidow AL, Winston CA, et al. A national study of socioeconomic status and tuberculosis rates by country of birth, United States, 1996-2005. BMC Public Health 2012;12:365.
4. Golden MP, Vikram HR. Extrapulmonary tuberculosis: an overview. Am Fam Physician 2005;72:1761-8.
5. Central TB Division. TB India 2017: RNTCP status report. New Delhi: Ministry of Health and Family Welfare; 2017.
6. Kruijshaar ME, Abubakar I. Increase in extrapulmonary tuberculosis in England and Wales 1999-2006. Thorax 2009;64:1090-5.
7. Ministry of Health, Family Welfare-Government of India. RNTCP annual status report 2023. New Delhi, India: Ministry of Health and Family Welfare; 2024.
8. Lawn SD, Mwaba P, Bates M, et al. Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis 2013;13:349-61.
9. Sharma SK, Ryan H, Khaparde S, et al. Index-TB guidelines: guidelines on extrapulmonary tuberculosis for India. Indian J Med Res 2017;145:448-63.
10. Nishal N, Arjun P, Arjun R, et al. Diagnostic yield of CBNAAT in the diagnosis of extrapulmonary tuberculosis: a prospective observational study. Lung India 2022;39:443-8.
11. Rao CM, Thakur B, Thakur S, et al. Pattern of extrapulmonary tuberculosis among urban population with special reference to CBNAAT as a diagnostic tool: a retrospective study at a tertiary care hospital of Odisha, India. Int J Res Med Sci 2018;6:3375-80.
12. Jose BP, Kumar AP, Mohan A. Evaluation of extrapulmonary tuberculosis in a tertiary care centre in Kerala, South India, utilising GeneXpert. Int J Res Rev 2020;7:551-6.
13. Vadwai V, Boehme C, Nabeta P, et al. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis?. J Clin Microbiol 2011;49:2540-5.
14. Suzana S, Ninan MM, Gowri M, et al. Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis—an experience from a tertiary care centre in South India. Trop Med Int Health 2016;21:385-92.
15. Krishna V, Gopalakrishnan R, Tarigopula A, et al. Utility of Xpert MTB/RIF in the diagnosis of extrapulmonary tuberculosis. Indian J Med Microbiol 2019;37:448-9.

16. World Health Organization. Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Available from: https://iris.who.int/bitstream/handle/10665/112472/9789241506335_eng.pdf?sequence=1.
17. Berger HW, Mejia E. Tuberculosis pleurisy. Chest 1973;63:88-92.
18. Scharer L, McClement JH. Isolation of tubercle bacilli from needle biopsy specimens of parietal pleura. Am Rev Respir Dis 1968;97:466-8.
19. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, 3rd edition. Available from: https://www.who.int/publications/i/item/9789240089488.

Table 1. Demographic distribution among patients.

Demographic characteristics		Frequency (n=52)	Percentage
Age (years)	18-30	08	15.38
	31-50	22	48.08
	51-70	14	30.77
	>70	03	5.77
Gender	Male	31	59.62
	Female	21	40.38
Co-morbidity	Diabetes Mellitus	09	17.31
	Hypertension	06	11.54
	Past H/O TB	04	7.69
HIV status	Positive	01	1.92
	Negative	51	98.07

H/O TB, history of tuberculosis; HIV, human immunodeficiency virus.

Table 2. Type of EPTB distribution among patients:

Type of EPTB	Frequency (n=52)	Percentage
TB pleural effusion	33	63.46
Bone & Joint	08	15.38
Abdominal TB	07	13.46
Lymph Node	03	5.77
Endometrial TB	01	1.92
Total	52	100

EPTB, extrapulmonary tuberculosis; TB, tuberculosis,

Table 3. Diagnostic yield of cartridge-based nucleic acid amplification test and culture in extrapulmonary tuberculosis among study population.

Diagnostic test		CRS- EPTB		Total
		Positive (n=45)	Negative (n=07)	
CBNAAT	**Positive**	10 (22.22%)	00 (00%)	10 (19.23%)
	Negative	35 (77.78%)	07 (100%)	42 (80.77%)
Culture	**Positive**	15 (33.33%)	01 (14.29%)	16 (30.77%)
	Negative	30 (66.67%)	06 (85.71%)	36 (69.23%)

CBNAAT, cartridge-based nucleic acid amplification test; EPTB, extrapulmonary tuberculosis; CRS, composite reference standard.

Table 4. Type of extrapulmonary tuberculosis and yield by various modalities among patients.

Type of EPTB	CBNAAT (%)	AFB culture (%)
TB pleural effusion (n=33)	5 (15.15)	3 (9.09%)
Bone & Joint (n=8)	2 (25.00)	6 (75.00)
Abdominal TB (n=7)	1 (14.29)	4 (57.14)
Lymph Node (n=3)	2 (66.67)	2 (66.67)
Endometrial TB (01)	00 (00)	00 (00)
Total (n=52)	10 (22.22)	15 (33.33)

EPTB:, extrapulmonary tuberculosis; CBNAAT, cartridge-based nucleic acid amplification test; AFB culture, acid fast bacilli culture; TB, tuberculosis.