Section: Pathology

Review Article

MOLECULAR AND CYTOLOGICAL APPROACHES IN THE DIAGNOSIS OF TUBERCULAR LYMPHADENITIS: A NARRATIVE REVIEW (2000–2023)

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ABSTRACT

Background: Tubercular lymphadenitis (TBLN) is the most common form of extrapulmonary tuberculosis, yet its diagnosis remains challenging due to nonspecific clinical and cytological presentations. Conventional tools such as fine-needle aspiration cytology (FNAC), Ziehl–Neelsen (ZN) staining, and mycobacterial culture are widely used but often lack sensitivity in paucibacillary cases. **Aim:** This narrative review summarizes and critically analyses studies published between 2000 and 2023 that compared conventional cytological and culture-based methods with molecular techniques (Polymerase Chain Reaction (PCR), GeneXpert) in diagnosing TBLN.

Materials and Methods: Literature was retrieved from PubMed, Scopus, and Google Scholar using the keywords "tubercular lymphadenitis," "FNAC," "PCR," "GeneXpert," and "molecular diagnosis." Studies in English from 2000 to 2023 that compared at least one molecular diagnostic modality with conventional techniques were included.

Results: FNAC continues to be a sensitive, rapid, and cost-effective frontline tool, but its specificity is limited. PCR consistently demonstrated higher diagnostic yield than smear microscopy and culture, particularly in smear-negative and paediatric cases. GeneXpert MTB/RIF further improved sensitivity while enabling rifampicin resistance detection. Integrated diagnostic strategies combining FNAC with PCR/GeneXpert yielded the best outcomes across multiple studies.

Conclusion: Evidence over the last two decades highlights the complementary value of cytological and molecular approaches. FNAC should remain the initial screening tool, but molecular assays are indispensable for confirmation, especially in challenging or atypical presentations. A combined algorithm offers the highest diagnostic accuracy, supporting timely treatment initiation and improved patient outcomes.

Keywords: FNAC, GeneXpert, molecular diagnosis**,** PCR.

INTRODUCTION

Tubercular lymphadenitis (TBLN) is the most common form of extrapulmonary tuberculosis, particularly affecting cervical lymph nodes, and remains major diagnostic challenge.[1] Conventional tests such as fine-needle aspiration cytology (FNAC), Ziehl–Neelsen staining, and culture are widely used,

but their sensitivity is often poor in paucibacillary disease.[2] Molecular techniques have improved diagnostic accuracy. Early studies demonstrated that polymerase chain reaction (PCR) could detect Mycobacterium tuberculosis DNA in aspirates missed by cytology or culture.[3,4] In-house assays targeting IS6110 or hupB genes further increased sensitivity over conventional methods.[5,6] More recently, cartridge-based nucleic acid amplification tests such as Xpert MTB/RIF have shown higher yield than smear microscopy and good specificity, even in HIV-positive and paediatric populations.[7,8,9] Despite these advances, no single test is definitive. An integrated diagnostic approach combining cytology, culture, and molecular assays provides the best balance of sensitivity, specificity, and timeliness in diagnosing TBLN.

MATERIALS AND METHODS

Methods of Review

An electronic search of PubMed, Scopus, and Google Scholar was conducted to identify studies between 2000 and 2023 assessing diagnostic modalities for TBLN using keywords tubercular lymphadenitis, FNAC, PCR, GeneXpert, molecular diagnosis and extrapulmonary TB. Only studies that directly compared at least one molecular modality with conventional methods (FNAC, ZN stain, culture) in suspected TBLN cases were included.

Research Question

What is the comparative diagnostic accuracy of molecular methods (PCR, real-time PCR, and GeneXpert MTB/RIF) versus conventional cytological and microbiological techniques (FNAC, Ziehl–Neelsen staining, and culture) in the detection of tubercular lymphadenitis?

Inclusion Criteria

Studies included in this narrative review were published between 2000 and 2023 and focused on patients with clinically suspected tubercular lymphadenitis (TBLN). Only studies that compared at least one molecular diagnostic method, such as PCR, real-time PCR, or GeneXpert MTB/RIF, with conventional diagnostic methods including fine-needle aspiration cytology (FNAC), Ziehl–Neelsen staining, or mycobacterial culture were considered. The review included peer-reviewed original research articles, prospective or retrospective observational studies, cross-sectional studies, and clinical trials. Furthermore, studies were required to report adequate details on sample size, methodology, and diagnostic outcomes, including sensitivity, specificity, or concordance with culture.

Exclusion Criteria

Studies were excluded if they were case reports, editorials, commentaries, letters to the editor, or conference abstracts without complete data. Research focusing solely on pulmonary tuberculosis without lymph node involvement, animal or in-vitro studies, or studies that did not provide a comparative assessment between molecular and conventional diagnostic methods was also excluded.

REVIEW OF LITERATURE (2000–2023)

Narrative Review

The diagnostic approach to tubercular lymphadenitis (TBLN) has advanced steadily over the last two decades, from early demonstrations of PCR's feasibility on lymph node material to field-friendly cartridge and isothermal nucleic acid amplification technology (NAATs). The body of evidence supports the routine use of molecular assays as adjuncts to cytology and culture, particularly in paucibacillary or smear-negative cases.

Singh K et al. provided one of the earliest comparisons of in-house PCR against conventional techniques, showing that PCR could detect Mycobacterium tuberculosis DNA in several cases missed by cytology and culture and thereby establishing the potential role of molecular tests in granulomatous lymphadenopathy.[1] Baek and colleagues and other contemporaneous groups supported this pattern, demonstrating that PCR applied to the residual aspirate after FNAC increases diagnostic yield.[2]

Sen MK et al. confirmed that PCR applied to lymph-node aspirates can identify M. tuberculosis where FNAC and culture are inconclusive.[3] Mohapatra PR et al. reviewed the clinical approach to tuberculous lymphadenitis and emphasized limitations of conventional tests while endorsing molecular adjuncts for improved diagnostic certainty.[4]

Two studies clarified both the possibilities and limits of molecular diagnostics in lymph node material. Chantranuwat C et al. demonstrated that IS6110 PCR can be successful even from archival Papanicolaou-stained FNA smears a useful approach when fresh specimens are unavailable.[5] Osores F et al. evaluated a commercial 16S rRNA (Amplicor) PCR test on aspirates and biopsies and reported moderate sensitivity but high specificity, with higher sensitivity in AFB-positive or high-bacillary samples.[6]

Subsequent studies refined gene targets and validated in-house assays. Verma P et al,[7] assessed hupB as a target and reported improved sensitivity on FNA material, while Sharma M et al,[8] validated an in-house IS6110 assay with higher yield than smear and culture. Patwardhan SA et al. compared cytology, culture, and PCR and found PCR substantially increased diagnostic yield when used adjunctively.[9] Derese Y et al. in Ethiopia confirmed higher sensitivity of PCR vs culture in FNA samples, noting that specimen handling and inhibitors may affect results.[10] Mehta PK et al. synthesized evidence across extrapulmonary sites and highlighted that gene target, assay format, and pre-analytic processing determines PCR reliability.[11]

Later, evaluations of cartridge-based NAATs became prominent. Dhasmana DJ et al. evaluated Xpert on EBUS-TBNA mediastinal samples and reported good sensitivity and excellent specificity, particularly when combined with cytology.[12] Ghariani A et al. evaluated GeneXpert on a large series of lymph node

specimens and reported high detection rates, with Xpert outperforming smear microscopy and confirming its value in rapid diagnosis.[13] Several field studies in Africa and Asia around this time reached similar conclusions: cartridge NAATs improved speed and diagnostic yield compared with conventional tests, although sensitivity remained variable depending on sample type and bacillary load.[14,15,16]

Alternative amplification strategies were also explored. Kawano S et al. demonstrated the feasibility of loop-mediated isothermal amplification (LAMP) for diagnosis in ulcerated lymphadenitis lesions, indicating isothermal amplification as a practical, resource-friendly alternative in some settings.[17] Head-to-head comparisons across the decade consistently showed that NAATs shortened time-to-diagnosis and detected cases missed by microscopy or culture, though sensitivity was imperfect in low-bacillary aspirates.

More recently, a systematic review and meta-analysis focused on Xpert in paediatric lymph-node TB and reported moderate sensitivity but high specificity, supporting its role as a rapid rule-in test while highlighting its limitations in culture-negative disease.[18] Jha H et al. reported a large Indian cohort where PCR showed high sensitivity and specificity and identified additional smear-negative cases, reaffirming the value of molecular assays as routine adjuncts to FNAC and culture in contemporary practice.[19]

Taken together, these studies demonstrate that molecular assays whether conventional PCR, real-time PCR, cartridge NAATs such as Xpert, or isothermal methods like LAMP consistently increase diagnostic yield when used alongside FNAC and culture. Important insights include the impact of gene target and assay format on performance, the need for careful pre-analytic handling, and the practical advantages of cartridge-based systems for rapid, specific results. In resource-limited settings, validated in-house PCR and LAMP can serve as alternatives if standardized appropriately. An evidence-based diagnostic pathway combining FNAC for rapid triage, microscopy and culture for confirmation and molecular NAATs for rapid detection and smear-negative cases offers the best balance of accuracy, timeliness, and feasibility. **[Table-1]**

RESULTS

Across the 15+ included studies, FNAC consistently demonstrated high sensitivity and utility as an initial diagnostic tool but lacked specificity, particularly in differentiating TBLN from other granulomatous diseases. PCR including conventional and real-time formats, repeatedly outperformed ZN staining and culture, especially in smear-negative and paediatric cohorts. GeneXpert not only improved detection but also allowed rapid rifampicin resistance identification, an advantage absent in older methods. However, a few studies (e.g., Mustafa et al. 2022) reported higher yield with FNAC than PCR, reflecting variability due to technical expertise and bacillary load.

FUTURE RESEARCH PRIORITIES

Despite significant advances in molecular diagnostics for tubercular lymphadenitis, several areas require further investigation to optimize patient care. Future research should focus on large, multicentric prospective studies to validate the diagnostic accuracy of emerging molecular assays across diverse populations, including paediatric and immunocompromised patients. There is a need to assess cost-effectiveness, turnaround time, and accessibility of PCR and GeneXpert in resource-limited settings, where conventional FNAC remains the primary diagnostic tool. Additionally, studies should explore the integration of rapid molecular diagnostics with point-of-care platforms and the development of standardized protocols for sample collection, processing, and interpretation. Comparative research evaluating novel biomarkers, next-generation sequencing, and multiplex PCR approaches may further enhance sensitivity and specificity, especially in paucibacillary and smear-negative cases. Overall, these efforts will help refine diagnostic algorithms, improve early case detection, and guide timely therapeutic interventions, ultimately contributing to reduced tuberculosis burden globally.

Table 1: Structured Summary of Verified Studies (2000–2023)

Author (Year)	Sample Size	Diagnostic Modalities Compared	Key Findings
Singh K et al. (2000),[1]	55	FNAC, smear, culture, in-house PCR	PCR detected M. tuberculosis DNA in cases negative by conventional methods; improved sensitivity.
Baek CH et al. (2000),[2]	41	FNAC aspirates, PCR	PCR on FNA material improved detection compared with cytology; supported feasibility.
Sen MK et al. (2005),[3]	Case series	FNAC, culture, PCR	PCR identified M. tuberculosis in aspirates negative by other methods.
Chantranuwat C et al. (2006),[5]	Archival smears	IS6110 PCR on Pap-stained FNA smears	Demonstrated feasibility of PCR on archival cytology slides.
Osores F et al. (2006),[6]	112	Amplicor 16S rRNA PCR, culture, histology	PCR showed moderate sensitivity but high specificity; better in AFB-positive samples.

Mohapatra PR et al. (2009),[4]	–	FNAC, smear, culture, PCR (review)	Highlighted limitations of conventional methods; recommended PCR as adjunct.
Verma P et al. (2010),[7]	60	FNAC, smear, culture, hupB-PCR	hupB PCR more sensitive than conventional tests, detecting additional cases.
Sharma M et al. (2010),[8]	50	FNAC, smear, culture, IS6110 PCR	In-house IS6110 PCR had higher diagnostic yield than smear and culture.
Patwardhan SA et al. (2011),[9]	82	FNAC, culture, PCR	PCR increased diagnostic yield when combined with cytology and culture.
Linasmita P et al. (2012),[16]	91	Real-time PCR (16S rRNA), FNAC, culture	Real-time PCR improved detection of cervical TBLN in high-burden setting.
Derese Y et al. (2012),[10]	132	FNAC, culture, PCR	PCR more sensitive than culture; specificity high but affected by inhibitors.
Mehta PK et al. (2012),[11]	–	Review of PCR targets and extrapulmonary TB	Emphasized role of gene target selection and assay standardization.
Biadglegne F et al. (2014),[15]	96	FNAC, smear, culture, Xpert MTB/RIF	Xpert had higher sensitivity than smear, especially in HIV-positive patients.
Dhasmana DJ et al. (2014),[12]	150	Cytology, culture, Xpert MTB/RIF	Xpert improved diagnosis of mediastinal lymphadenitis; high specificity.
Kawano S et al. (2014),[17]	Case report	LAMP vs conventional	Successful diagnosis with LAMP from ulcerated lesion aspirates.
Tadesse M et al. (2015)[14]	143	FNAC (concentrated), Xpert MTB/RIF	Xpert improved yield vs smear; useful in resource-limited settings.
Ghariani A et al. (2015),[13]	152	FNAC, culture, smear, Xpert MTB/RIF	Xpert detected more positives than microscopy; recommended for rapid LNTB diagnosis.
Chen HK et al.(2022),[18]	12 studies	Xpert MTB/RIF vs culture	Meta-analysis: moderate sensitivity, high specificity in paediatric lymph-node TB.
Jha H et al.(2023),[19]	100	FNAC, smear, culture, PCR	PCR had highest sensitivity and specificity; detected additional smear-negative cases.

DISCUSSION

This two-decade review underscores the complementary nature of cytological and molecular approaches in diagnosing TBLN. While FNAC remains indispensable due to its rapid turnaround and cost-effectiveness, molecular assays significantly enhance diagnostic accuracy. In contrast to FNAC's limitations in specificity, PCR and GeneXpert offer confirmatory power and can detect cases missed by smear or culture. Similarly, GeneXpert ability to identify drug resistance extends its clinical value.

In paediatric populations and smear-negative cases, molecular methods consistently outperformed cytology, highlighting their critical role in these challenging groups. Conversely, in resource-limited settings, FNAC continues to serve as the backbone of diagnosis, with molecular assays best reserved for problem-solving or confirmation.

The synthesis of evidence from 2000 to 2023 thus supports an integrated diagnostic pathway: FNAC as the frontline tool, molecular methods for confirmation, and culture/histopathology for gold-standard validation where feasible. Such an approach balances sensitivity, specificity, turnaround time, and cost, ultimately leading to earlier treatment initiation and reduced morbidity.

CONCLUSION

From 2000 to 2023, multiple studies have consistently demonstrated the limitations of conventional diagnostic modalities in TBLN, particularly in smear-negative disease. FNAC, while highly useful as a frontline investigation, requires molecular confirmation in challenging cases. PCR and GeneXpert significantly enhance diagnostic yield, improve early detection, and in the case of GeneXpert, provide resistance data essential for therapy. The weight of evidence strongly supports an integrated diagnostic algorithm combining FNAC, PCR/GeneXpert, and culture/histopathology to achieve timely, accurate, and comprehensive diagnosis of tubercular lymphadenitis.

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