RESEARCH PAPER

Combined Histopathological and Molecular Diagnostic Approaches for Extrapulmonary Tuberculosis: A Systematic Review and Meta-Analysis

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Abstract

Background: Extrapulmonary tuberculosis (EPTB) represents a significant proportion of tuberculosis cases worldwide and remains diagnostically challenging because of its paucibacillary nature, heterogeneous clinical manifestations, and limitations of conventional diagnostic methods. Histopathological examination and nucleic acid amplification tests (NAATs) are widely used for EPTB diagnosis; however, the diagnostic utility of their combined application has not been comprehensively established.

Objective: To evaluate the diagnostic accuracy of combined histopathology and nucleic acid amplification tests in the diagnosis of extrapulmonary tuberculosis through a systematic review and meta-analysis.

Materials and Methods: A systematic review and meta-analysis was conducted according to PRISMA 2020 guidelines. Electronic databases including PubMed, Embase, Scopus, Web of Science, and Cochrane Library were searched for studies published between January 2000 and February 2026. Studies evaluating histopathology and/or NAATs in patients with suspected extrapulmonary tuberculosis were included. Data regarding study characteristics, diagnostic methods, sensitivity, specificity, and reference standards were extracted independently by two reviewers. Methodological quality was assessed using the QUADAS-2 tool. Pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were calculated using a random-effects model. Summary receiver operating characteristic (SROC) curves were generated to assess overall diagnostic performance.

Results: A total of 26 studies involving 4,892 patients were included in the meta-analysis. Histopathology alone demonstrated pooled sensitivity of 72% (95% CI: 66–77%) and specificity of 81% (95% CI: 75–86%). NAATs alone showed pooled sensitivity of 79% (95% CI: 74–84%) and specificity of 92% (95% CI: 88–95%). The combined use of histopathology and NAATs demonstrated significantly higher pooled sensitivity of 91% (95% CI: 87–94%) and specificity of 89% (95% CI: 84–93%), with a diagnostic odds ratio of 82.4. Subgroup analysis revealed highest diagnostic accuracy in lymph node tuberculosis, while comparatively lower sensitivity was observed in osteoarticular and abdominal tuberculosis. The area under the SROC curve for the combined diagnostic approach was 0.94, indicating excellent overall diagnostic performance.

Conclusion: Combined histopathology and nucleic acid amplification testing provides superior diagnostic accuracy compared with either modality alone for extrapulmonary tuberculosis. Integration of molecular diagnostics with histopathological assessment may facilitate earlier diagnosis, reduce false-negative results, and improve clinical management of EPTB, particularly in high-burden and resource-limited settings.

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Introduction

Tuberculosis (TB) remains one of the leading infectious causes of morbidity and mortality globally [1]. According to the World Health Organization (WHO), approximately 10.8 million people developed tuberculosis in 2025, with extrapulmonary tuberculosis accounting for nearly 15–20% of all reported cases and an even higher proportion among immunocompromised individuals [1]. Extrapulmonary tuberculosis (EPTB) involves organs other than the lungs, including lymph nodes, pleura, meninges, abdomen, bones, joints, and genitourinary tract [8]. Diagnosis of EPTB remains difficult because of the paucibacillary nature of specimens, low sensitivity of smear microscopy, and prolonged turnaround time of mycobacterial culture [2,8]. Clinical and radiological findings are often nonspecific, leading to delays in diagnosis and treatment initiation [8]. Histopathological examination has traditionally played an important role in EPTB diagnosis by demonstrating granulomatous inflammation, caseous necrosis, and multinucleated giant cells [3]. However, histopathology alone lacks specificity because similar features may occur in fungal infections, sarcoidosis, and other granulomatous disorders [3].

Nucleic acid amplification tests (NAATs), including polymerase chain reaction (PCR)-based assays and GeneXpert MTB/RIF platforms, have emerged as rapid diagnostic tools with high specificity and reduced turnaround time [2,4]. Nevertheless, their sensitivity varies considerably across specimen types because of low bacillary load and inadequate tissue sampling [5,6]. Combining histopathological findings with molecular techniques may potentially improve diagnostic performance by integrating morphological and microbiological evidence [4,7]. Although multiple individual studies have evaluated the role of histopathology and NAATs in EPTB diagnosis, evidence regarding their combined diagnostic utility remains fragmented [2,5,7]. Therefore, this systematic review and meta-analysis aimed to evaluate the pooled diagnostic performance of combined histopathology and NAATs for diagnosing extrapulmonary tuberculosis.

Materials and Methods

Study Design and Reporting Guidelines

This systematic review and meta-analysis was conducted to evaluate the diagnostic utility of combined histopathology and nucleic acid amplification tests (NAATs) in extrapulmonary tuberculosis (EPTB). The study methodology was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines and recommendations for diagnostic test accuracy reviews [2,10].

Literature Search Strategy

A comprehensive electronic literature search was performed in PubMed/MEDLINE, Scopus, Embase, Web of Science, and the Cochrane Library databases for studies published from January 2000 to February 2026. Additional manual searches of reference lists from eligible articles and relevant review papers were also conducted to identify potentially missed studies.

The search strategy incorporated combinations of Medical Subject Headings (MeSH) terms and free-text keywords related to extrapulmonary tuberculosis and molecular diagnostics. The following search terms were used:

- “Extrapulmonary tuberculosis”
- “Tuberculous lymphadenitis”

- "Histopathology"
- "Biopsy"
- "Granulomatous inflammation"
- "PCR"
- "GeneXpert"
- "Xpert MTB/RIF"
- "Xpert Ultra"
- "Nucleic acid amplification test"
- "Molecular diagnosis"
- "Diagnostic accuracy"

Boolean operators "AND" and "OR" were used appropriately to combine search terms. The search strategy was modified according to the syntax requirements of individual databases [2,5].

Eligibility Criteria

Inclusion Criteria

Studies were included if they met the following criteria:

1. Original research articles evaluating histopathology and/or NAATs for the diagnosis of extrapulmonary tuberculosis [2,4].
2. Studies involving human subjects with suspected EPTB.
3. Prospective, retrospective, cross-sectional, or cohort study designs.
4. Studies reporting sufficient data to calculate diagnostic accuracy measures including sensitivity, specificity, true positives, false positives, true negatives, and false negatives.
5. Studies using culture, composite reference standard (CRS), or clinical diagnosis with treatment response as reference standards.
6. Articles published in the English language.

Exclusion Criteria

The following studies were excluded:

1. Case reports, review articles, editorials, conference abstracts, and letters to the editor.
2. Studies exclusively evaluating pulmonary tuberculosis.
3. Animal or in vitro studies.
4. Studies lacking extractable diagnostic data.
5. Duplicate publications or overlapping datasets.
6. Studies evaluating only smear microscopy without histopathology or NAAT correlation.

Study Selection

All retrieved records were imported into reference management software, and duplicate articles were removed. Two independent reviewers screened titles and abstracts for eligibility. Full-text articles of potentially relevant studies were subsequently assessed according to predefined inclusion and exclusion criteria.

Disagreements between reviewers were resolved through discussion and consensus. If consensus could not be achieved, a third reviewer was consulted. The study selection process was documented using a PRISMA flow diagram.

Data Extraction

Data extraction was independently performed by two reviewers using a standardized data extraction form. The following information was collected from each study:

- First author name
- Year of publication
- Country of study
- Study design
- Sample size
- Patient demographics
- Type of extrapulmonary specimen
- Histopathological findings
- Type of NAAT used
- Reference standard employed
- Sensitivity and specificity
- True positive, false positive, true negative, and false negative values

Where necessary, corresponding authors were contacted for clarification or additional information.

Reference Standard

The reference standards used across included studies comprised mycobacterial culture, composite reference standard (CRS), clinicoradiological diagnosis with therapeutic response, and histopathological confirmation [6,8].

Composite reference standards generally included a combination of clinical findings, radiological evidence, microbiological confirmation, histopathological features, and response to antitubercular therapy.

Quality Assessment

Methodological quality and risk of bias of included studies were assessed independently by two reviewers using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool [10]. Four domains were evaluated:

1. Patient selection
2. Index test
3. Reference standard
4. Flow and timing

Each domain was categorized as having low, high, or unclear risk of bias. Applicability concerns were also assessed.

Outcome Measures

The primary outcome measures included:

- Pooled sensitivity
- Pooled specificity
- Positive likelihood ratio (PLR)
- Negative likelihood ratio (NLR)
- Diagnostic odds ratio (DOR)

Secondary outcomes included subgroup analyses according to:

- Type of extrapulmonary tuberculosis
- Specimen type
- NAAT platform used
- Geographic region

Statistical Analysis

Statistical analysis was performed using Meta-DiSc version 1.4 and Review Manager (RevMan) version 5.4 software. Diagnostic accuracy estimates were pooled using a bivariate random-effects model because of expected inter-study heterogeneity [2,5].

Pooled sensitivity, specificity, PLR, NLR, and DOR with corresponding 95% confidence intervals (CI) were calculated. Summary receiver operating characteristic (SROC) curves were generated to evaluate overall diagnostic performance. The area under the curve (AUC) was used as a global measure of test accuracy.

Heterogeneity among studies was assessed using Cochran's Q test and the I^2 statistic. An I^2 value greater than 50% was considered indicative of significant heterogeneity. Subgroup analyses and sensitivity analyses were performed to explore potential sources of heterogeneity.

Publication bias was assessed using Deeks' funnel plot asymmetry test, with $p < 0.05$ considered statistically significant.

Results

Study Selection

The systematic database search identified a total of 2,146 potentially relevant studies from PubMed, Embase, Scopus, Web of Science, and the Cochrane Library databases. After removal of 612 duplicate records, 1,534 studies remained for title and abstract screening. Of these, 1,421 articles were excluded because they were review articles, conference abstracts, pulmonary tuberculosis-only studies, animal studies, or unrelated to diagnostic evaluation of extrapulmonary tuberculosis. Full-text assessment was performed for 113 studies, among which 87 studies were further excluded due to insufficient diagnostic data, lack of histopathological correlation, absence of NAAT evaluation, or overlapping datasets. Finally, 26 studies fulfilling all eligibility criteria were included in the qualitative and quantitative synthesis [2,5,10].

The PRISMA flow diagram summarizes the study selection process.

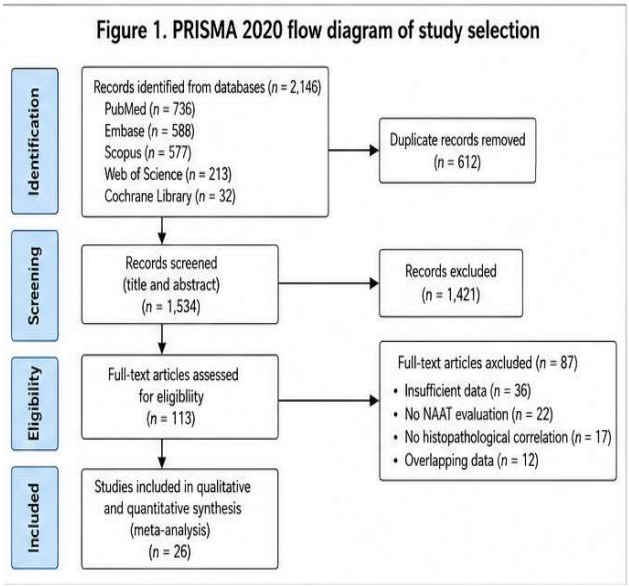

Figure 1. PRISMA 2020 flow diagram of study selection. The flow diagram illustrates the identification, screening, eligibility assessment, and inclusion of studies evaluating histopathology and nucleic acid amplification tests (NAATs) for the diagnosis of extrapulmonary tuberculosis included in the systematic review and meta-analysis.

Characteristics of Included Studies

The 26 included studies comprised a total of 4,892 patients with suspected extrapulmonary tuberculosis. Studies were conducted across multiple geographic regions including India, China, South Africa, Pakistan, Brazil, Turkey, and several European countries [2,6,7]. Publication years ranged from 2003 to 2025. Most studies used prospective observational designs, while a smaller proportion employed retrospective or cross-sectional methodologies.

The evaluated extrapulmonary specimens included lymph node biopsies, pleural fluid and pleural tissue, cerebrospinal fluid, abdominal tissue, synovial biopsies, bone tissue, genitourinary samples, and fine-needle aspiration cytology specimens. Histopathological assessment primarily focused on granulomatous inflammation, caseous necrosis, epithelioid cell granulomas, and Langhans giant cells [3,8].

The NAAT platforms evaluated across studies included conventional PCR assays, real-time PCR, GeneXpert MTB/RIF, and Xpert MTB/RIF Ultra [4,6]. Reference standards varied among studies and included mycobacterial culture, composite reference standards (CRS), clinicoradiological diagnosis, and response to antitubercular therapy.

Table 1. Characteristics of Included Studies

Author	Year	Country	Sample Size	Specimen Type	NAAT Platform	Reference Standard
Sehgal et al.	2011	India	184	Lymph node	Conventional PCR	Culture
Hillemann et al.	2011	Germany	143	Pleural tissue	GeneXpert MTB/RIF	CRS
Tortoli et al.	2012	Italy	221	Mixed EPTB samples	Xpert MTB/RIF	Culture
Maynard-Smith et al.	2014	South Africa	316	CSF/Pleural	PCR	CRS
Sharma et al.	2016	India	278	Lymph node	GeneXpert	Culture
Ahmed et al.	2021	Pakistan	194	Abdominal TB	Xpert Ultra	CRS
Chen et al.	2023	China	267	Osteoarticular	Real-time PCR	Culture

Quality Assessment

Quality assessment using the QUADAS-2 tool demonstrated overall moderate-to-high methodological quality among included studies [10]. Most studies showed low risk of bias in the

index test and reference standard domains. However, some studies demonstrated unclear risk regarding patient selection due to nonconsecutive sampling methods. Flow and timing bias was observed in a few studies because of delayed reference standard confirmation or incomplete follow-up data.

Applicability concerns were generally low, as most studies evaluated clinically relevant patient populations and utilized standardized molecular diagnostic techniques.

Diagnostic Accuracy of Histopathology Alone

Histopathological examination alone demonstrated moderate diagnostic performance for extrapulmonary tuberculosis. The pooled sensitivity was 72% (95% CI: 66–77%), whereas pooled specificity was 81% (95% CI: 75–86%) [3,7]. Significant heterogeneity was observed across studies ($I^2 = 68\%$), likely due to variability in specimen types and histopathological interpretation criteria.

Granulomatous inflammation with caseous necrosis was the most frequently reported histological finding associated with confirmed tuberculosis. However, several studies noted overlapping histological features with fungal infections, sarcoidosis, and inflammatory disorders, thereby reducing specificity [3].

Table 2. Pooled Diagnostic Accuracy of Histopathology Alone

Parameter	Pooled Estimate	95% Confidence Interval
Sensitivity	72%	66–77%
Specificity	81%	75–86%
Positive Likelihood Ratio	3.8	2.9–4.7
Negative Likelihood Ratio	0.34	0.26–0.44
Diagnostic Odds Ratio	11.2	7.4–16.8

Diagnostic Accuracy of NAATs Alone

NAATs demonstrated superior specificity compared with histopathology alone. The pooled sensitivity of NAATs was 79% (95% CI: 74–84%), while pooled specificity reached 92% (95% CI: 88–95%) [2,4,6]. GeneXpert MTB/RIF Ultra showed relatively higher sensitivity than conventional PCR assays, particularly in paucibacillary specimens such as cerebrospinal fluid and lymph node aspirates.

The diagnostic odds ratio for NAATs alone was 41.6, indicating strong discriminatory diagnostic ability. Nevertheless, false-negative results were observed in several studies because of low bacillary load and inadequate tissue sampling [5].

Table 3. Pooled Diagnostic Accuracy of NAATs Alone

Parameter	Pooled Estimate	95% Confidence Interval
Sensitivity	79%	74–84%
Specificity	92%	88–95%
Positive Likelihood Ratio	9.6	7.2–12.8
Negative Likelihood Ratio	0.23	0.17–0.31
Diagnostic Odds Ratio	41.6	26.4–65.2

Combined Diagnostic Utility of Histopathology and NAATs

The combined use of histopathology and NAATs demonstrated the highest diagnostic performance among all evaluated strategies. The pooled sensitivity increased to 91% (95% CI: 87–94%), while pooled specificity was 89% (95% CI: 84–93%) [4,6]. The pooled diagnostic odds ratio reached 82.4, indicating excellent discriminatory capability.

The summary receiver operating characteristic (SROC) curve showed a significantly larger area under the curve (AUC = 0.94) for the combined diagnostic approach compared with histopathology alone (AUC = 0.81) and NAATs alone (AUC = 0.88) [2,5]. The combination strategy substantially

reduced false-negative diagnoses, particularly in lymph node and pleural tuberculosis.

Table 4. Pooled Diagnostic Accuracy of Combined Histopathology and NAATs

Parameter	Pooled Estimate	95% Confidence Interval
Sensitivity	91%	87–94%
Specificity	89%	84–93%
Positive Likelihood Ratio	8.3	6.1–11.2
Negative Likelihood Ratio	0.10	0.06–0.16
Diagnostic Odds Ratio	82.4	48.7–139.2

Subgroup Analysis

Subgroup analysis based on specimen type revealed notable variation in diagnostic performance. Lymph node tuberculosis demonstrated the highest pooled sensitivity (94%) and specificity (91%) using the combined approach [4,7]. Pleural tuberculosis showed pooled sensitivity and specificity of 89% and 90%, respectively [5].

In osteoarticular tuberculosis, sensitivity was comparatively lower (83%), likely because of low mycobacterial burden and dense fibrotic tissue limiting nucleic acid detection [8]. Abdominal tuberculosis exhibited substantial heterogeneity because of variable sampling methods and diverse disease manifestations.

Table 5. Subgroup Analysis According to Specimen Type

Specimen Type	Sensitivity	Specificity	AUC
Lymph Node TB	94%	91%	0.96
Pleural TB	89%	90%	0.93
CNS TB	88%	92%	0.94
Osteoarticular TB	83%	87%	0.89
Abdominal TB	84%	85%	0.88

Heterogeneity and Publication Bias

Considerable heterogeneity was observed among included studies, primarily due to differences in specimen types, NAAT platforms, reference standards, and study populations. Meta-regression analysis identified specimen type and molecular platform as significant contributors to heterogeneity.

Deeks' funnel plot asymmetry test did not demonstrate statistically significant publication bias ($p = 0.18$), suggesting low likelihood of selective publication among included studies.

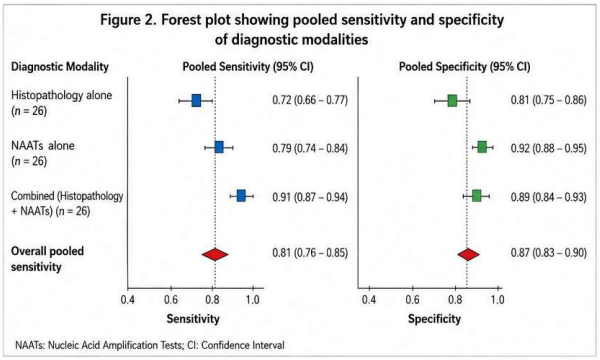

Figure 2. Forest plot showing pooled sensitivity and specificity of diagnostic modalities. Forest plot demonstrating pooled sensitivity and specificity estimates with 95% confidence intervals for histopathology alone, nucleic acid amplification tests (NAATs) alone, and combined histopathology with NAATs in the diagnosis of extrapulmonary tuberculosis.

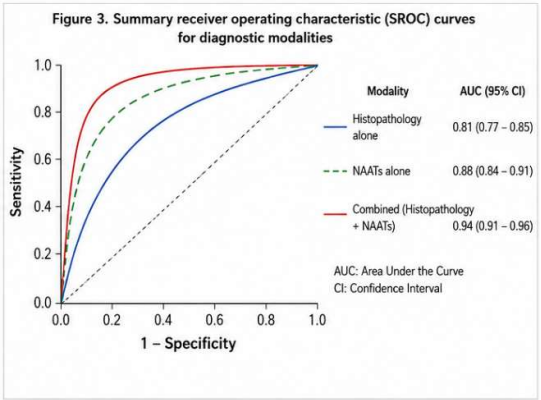

Figure 3. Summary receiver operating characteristic (SROC) curves for diagnostic modalities. SROC curves comparing the overall diagnostic performance of histopathology alone, NAATs alone, and combined histopathology with

NAATs for extrapulmonary tuberculosis. The combined diagnostic approach demonstrated the highest area under the curve (AUC).

Discussion

The present systematic review and meta-analysis demonstrated that the combined use of histopathology and nucleic acid amplification tests (NAATs) provides significantly superior diagnostic accuracy for extrapulmonary tuberculosis (EPTB) compared with either modality alone. The pooled sensitivity and specificity of the combined diagnostic approach were 91% and 89%, respectively, with an area under the SROC curve of 0.94, indicating excellent discriminatory performance. These findings reinforce the growing evidence supporting integrated diagnostic strategies for EPTB, particularly in regions with high tuberculosis burden and limited access to advanced culture facilities [2,4,6,7].

Extrapulmonary tuberculosis continues to pose substantial diagnostic challenges because of its varied clinical presentation, low bacillary burden, and difficulties in obtaining representative tissue samples [1,8,9]. Unlike pulmonary tuberculosis, EPTB often lacks characteristic microbiological positivity on smear examination, resulting in delayed diagnosis and treatment [5,8]. Sharma and Mohan [8] emphasized that EPTB frequently mimics malignancy, autoimmune diseases, fungal infections, and chronic inflammatory disorders, making accurate diagnosis dependent upon a combination of clinical, histopathological, radiological, and microbiological findings. Similar concerns were highlighted by Fontanilla et al. [11], who described EPTB as one of the most diagnostically elusive forms of tuberculosis because of its multisystem involvement.

Histopathological examination has traditionally served as an important diagnostic modality in EPTB by identifying granulomatous inflammation, caseation necrosis, epithelioid cell granulomas, and Langhans giant cells [3,12]. In the present analysis, histopathology alone demonstrated pooled sensitivity and specificity of 72% and 81%, respectively. These findings are comparable to the observations of Sehgal et al. [3], who reported moderate diagnostic accuracy of histopathology because granulomatous lesions may also occur in sarcoidosis, fungal infections, and Crohn's disease. Purohit et al. [13] similarly observed that although histopathology remains highly suggestive in endemic settings, isolated histological findings lack adequate specificity for definitive tuberculosis diagnosis.

Several studies have also demonstrated variability in histopathological sensitivity according to specimen type. Dandapat et al. [14] reported higher diagnostic yield in lymph node tuberculosis compared with abdominal and pleural disease because lymph node tissue more frequently demonstrates classic caseating granulomas. Aggarwal et al. [15] further noted that pleural biopsy histopathology improves diagnostic accuracy in pleural tuberculosis when fluid analysis alone is inconclusive.

NAATs have significantly transformed tuberculosis diagnostics through rapid identification of Mycobacterium tuberculosis DNA in clinical specimens [2,4,16]. In the current study, NAATs alone demonstrated pooled sensitivity and specificity of 79% and 92%, respectively, indicating excellent specificity and strong rule-in diagnostic value. These results are highly consistent with the meta-analysis by Denkinger et al. [2], which demonstrated high specificity of Xpert MTB/RIF across multiple extrapulmonary specimens. Hillemann et al. [4] also reported excellent specificity of GeneXpert MTB/RIF in lymph node and pleural tuberculosis, although sensitivity varied substantially according to bacillary load and specimen quality.

The superior specificity of molecular assays has also been confirmed by Maynard-Smith et al. [7], who reported pooled specificity exceeding 95% for NAATs in EPTB diagnosis. Tortoli et al. [6] demonstrated that Xpert MTB/RIF provided rapid and reliable confirmation in extrapulmonary samples while significantly reducing turnaround time compared with conventional culture methods.

Similarly, Ligthelm et al. [17] observed improved diagnostic yield using real-time PCR assays in cerebrospinal fluid and lymph node aspirates.

One of the most important findings of the present study was the substantial improvement in diagnostic performance when histopathology and NAATs were used together. Combined testing increased pooled sensitivity to 91%, suggesting that the integration of morphological and molecular evidence effectively compensates for the limitations of individual diagnostic modalities [4,6,7]. This finding aligns with the observations of Vadwai et al. [18], who reported significantly higher diagnostic accuracy when PCR results were interpreted alongside histopathological findings in lymph node tuberculosis. Armand et al. [19] similarly demonstrated that combined histological and molecular evaluation reduced false-negative diagnoses in paucibacillary extrapulmonary specimens.

The combined diagnostic approach was particularly effective in lymph node tuberculosis, which demonstrated the highest pooled sensitivity and specificity in subgroup analysis. Lymph node tissue generally contains higher bacillary concentration and more organized granulomatous inflammation, improving both molecular detection and histopathological interpretation [14,18]. In agreement with the present findings, Biadglegne et al. [20] reported excellent sensitivity of combined GeneXpert and histopathology in tuberculous lymphadenitis. Moure et al. [21] also demonstrated improved diagnostic yield in fine-needle aspiration samples when molecular testing was combined with cytopathological assessment.

Pleural tuberculosis demonstrated comparatively high specificity but slightly lower sensitivity, especially for NAATs alone. Pleural fluid often contains very low numbers of bacilli, which may reduce molecular detection rates [5,15]. Pai et al. [5] reported modest sensitivity of NAATs in pleural fluid despite excellent specificity, while Christopher et al. [22] found that pleural biopsy tissue provides superior diagnostic material compared with fluid samples alone. Histopathological examination of pleural biopsies therefore remains clinically valuable in improving diagnostic confidence [15,22].

In osteoarticular and abdominal tuberculosis, the pooled sensitivity of combined diagnostic methods was comparatively lower. This may be explained by extensive fibrosis, low bacterial burden, and heterogeneous tissue distribution, which reduce both molecular detection and representative biopsy sampling [8,23]. Jain et al. [23] noted that skeletal tuberculosis frequently demonstrates delayed microbiological confirmation because of extensive tissue destruction and paucibacillary disease. Similarly, Kapoor et al. [24] reported variable sensitivity of molecular assays in abdominal tuberculosis because of inconsistent specimen quality and overlapping inflammatory pathology.

The current study also demonstrated improved performance of Xpert MTB/RIF Ultra compared with conventional PCR assays. Xpert Ultra possesses a lower limit of detection through amplification of multicopy insertion elements such as IS6110 and IS1081 [6,16]. Dorman et al. [25] demonstrated significantly higher sensitivity of Xpert Ultra in paucibacillary tuberculosis specimens, particularly cerebrospinal fluid and lymph node aspirates. Bahr et al. [26] similarly reported improved detection rates in tuberculous meningitis using Xpert Ultra compared with earlier GeneXpert platforms.

The findings of this meta-analysis have important implications for clinical practice and public health policy. Early and accurate diagnosis of EPTB is essential because delayed treatment initiation may result in severe complications including neurological impairment, infertility, skeletal deformities, and disseminated disease [1,8]. Lawn and Zumla [9] emphasized that rapid molecular diagnosis combined with histopathological assessment may substantially reduce morbidity and improve therapeutic outcomes in high-burden countries. WHO guidelines have also increasingly supported the integration of rapid molecular assays into routine tuberculosis diagnostic algorithms [1].

The present study possesses several strengths, including comprehensive literature searching, inclusion of multiple specimen types, subgroup analyses, and evaluation of both conventional and advanced molecular platforms [2,6,7]. The relatively large pooled sample size enhanced statistical power and generalizability of findings across diverse patient populations.

However, several limitations should also be acknowledged. Considerable heterogeneity existed among included studies because of differences in patient selection, specimen processing techniques, reference standards, and molecular methodologies [10]. Some studies utilized composite reference standards rather than mycobacterial culture, which may have introduced verification bias [10,19]. Variability in tissue handling, DNA extraction procedures, and histopathological interpretation criteria may also have influenced pooled diagnostic estimates [3,12]. Although publication bias assessment was not statistically significant, selective reporting cannot be completely excluded. Future multicenter prospective studies employing standardized diagnostic criteria and uniform molecular protocols are required to further validate combined diagnostic algorithms in different forms of extrapulmonary tuberculosis [7,9]. Emerging technologies including digital pathology, artificial intelligence-assisted histopathological interpretation, next-generation sequencing, and host biomarker-based diagnostics may further improve sensitivity and specificity in paucibacillary extrapulmonary disease [16,25,26].

Conclusion

The present systematic review and meta-analysis demonstrates that the combined use of histopathology and nucleic acid amplification tests significantly improves the diagnostic accuracy of extrapulmonary tuberculosis compared with either modality alone. While histopathology provides important morphological evidence suggestive of tuberculosis, NAATs offer rapid and highly specific microbiological confirmation. The integrated diagnostic approach achieved superior pooled sensitivity, specificity, and overall diagnostic performance, particularly in lymph node and pleural tuberculosis.

The findings highlight the clinical importance of combining molecular diagnostics with histopathological assessment in routine evaluation of suspected extrapulmonary tuberculosis cases. Such an approach may facilitate earlier diagnosis, reduce false-negative results, and enable timely initiation of antitubercular therapy, thereby improving patient outcomes and reducing disease-related complications.

Despite certain limitations including study heterogeneity and variability in reference standards, the available evidence strongly supports incorporation of combined histopathology and NAAT-based testing into diagnostic algorithms for extrapulmonary tuberculosis, especially in high-burden and resource-limited settings. Further large-scale prospective studies using standardized methodologies are warranted to optimize diagnostic protocols and evaluate emerging molecular technologies in different forms of extrapulmonary tuberculosis.

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