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Accuracy of nanopore sequencing technology for rapid diagnosis of tuberculous mediastinal and hilar lymphadenopathy using endobronchial ultrasound-guided transbronchial needle aspiration specimens: protocol for a systematic review and meta-analysis

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ABSTRACT

Introduction Accessing samples from mediastinal and hilar lymph nodes is considerably more difficult, rendering the diagnosis of tuberculous mediastinal and hilar lymphadenopathy particularly challenging. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive interventional technique used for sampling mediastinal and hilar lymph nodes. Nanopore sequencing technology (NST) permits the rapid identification of *Mycobacterium tuberculosis* genes directly from clinical samples. NST has significantly improved the diagnostic accuracy for both pulmonary and extrapulmonary tuberculosis; its accuracy in diagnosing tuberculous mediastinal and hilar lymphadenopathy using EBUS-TBNA specimens has not yet been systematically evaluated.

Methods and analysis Adhering to Preferred Reporting Items for a Systematic Review and Meta-Analysis Protocols (PRISMA-P) and PRISMA-Diagnostic Test Accuracy Studies (DTA) guidelines, this protocol (PROSPERO: CRD420251274529) will synthesise evidence from five international databases (PubMed, Embase, SCOPUS, Web of Science and Cochrane Library) and two Chinese databases (China National Knowledge Infrastructure and Wanfang Database). The literature search is planned to be conducted between 1 December 2026 and 31 December 2026 (start and end dates of the search). The publication dates of the included literature ranged from the inception of the relevant databases to 31 December 2026. Data extraction from the included studies is anticipated to be completed by 31 May 2027, and the final reporting of findings is expected by 31 December 2027. Study selection followed the PICT framework: participants (P): patients with suspected tuberculous mediastinal and hilar lymphadenopathy; index test (I): the index test was defined as NST; comparator (C): the reference standard for tuberculous lymphadenopathy; target condition (T): the target condition was confirmed tuberculous mediastinal and hilar lymphadenopathy. Two independent investigators will perform a three-step screening process, extract data and assess methodological quality using Quality Assessment of Diagnostic Accuracy Studies. Bivariate random-effects models implemented in STATA V.15.0 will be used to pool sensitivity, specificity and hierarchical summary receiver operating characteristic curves when four or more studies are available; for fewer studies, Meta-DiSc V.1.4 will be employed. If substantial heterogeneity is detected (I^2 statistic >50%), meta-regression and subgroup analysis will be performed across prespecified covariates.

Ethics and dissemination This study is based on publicly available data and therefore does not require ethics committee approval. Upon completion, the findings will be submitted for publication in a peer-reviewed medical journal. The review is conducted in accordance with established guidelines for systematic review and meta-analysis.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This protocol was developed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines and has been prospectively registered on PROSPERO.
⇒ The review draws on evidence from biomedical literature databases encompassing publications in both Chinese and English.
⇒ Methodological rigour was ensured through the application of validated quality assessment tools and standardised reporting frameworks to enhance analytical robustness and mitigate bias.
⇒ Considerable heterogeneity among the included studies may impact the reliability of the pooled estimates.

PROSPERO registration number CRD420251274529.

INTRODUCTION

Tuberculosis, an ancient disease, remains a major global health challenge.[1] While pulmonary tuberculosis is the most common form, the impact of extra-pulmonary tuberculosis should not be overlooked. Tuberculous lymphadenitis represents a frequent type of extra-pulmonary tuberculosis.[2] Obtaining specimens from peripheral lymph nodes is relatively safe and straightforward, whereas accessing samples from mediastinal and hilar lymph nodes is considerably more difficult, rendering the diagnosis of tuberculous mediastinal and hilar lymphadenopathy particularly challenging.[3] If not adequately treated, this condition can lead to a range of complications, including compression of the trachea or oesophagus resulting in dysphagia and dyspnoea, as well as nodal rupture causing oesophageal or tracheal fistulae. These complications significantly impair patients' quality of life and worsen prognosis.

Although invasive procedures such as mediastinoscopy can yield satisfactory specimens, they are associated with substantial trauma and higher risks and impose greater demands on the patient's cardiopulmonary function. In contrast, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive interventional technique used for sampling mediastinal and hilar lymph nodes and diagnosing pulmonary lesions.[4] It allows specimens to be obtained via natural airways, offering a favourable safety profile. EBUS-TBNA has proven highly effective in diagnosing mediastinal and hilar lymph node metastases in lung cancer and is now also widely employed in the diagnosis of tuberculous mediastinal and hilar lymphadenopathy.[5 6] Samples obtained via EBUS-TBNA are typically limited in volume. Conventional detection methods such as smear microscopy and culture have restricted diagnostic accuracy for tuberculosis.[7] A previous meta-analysis indicated that EBUS-TBNA alone has limited efficacy in diagnosing mediastinal tuberculous lymphadenitis,[8] while another meta-analysis showed that the sensitivity and specificity of Xpert MTB/RIF testing using EBUS-TBNA specimens for diagnosing mediastinal tuberculous lymphadenitis remain relatively low (pooled sensitivity 61%, specificity 89%).[9] Therefore, how to optimise the use of limited specimens to improve the diagnostic accuracy of tuberculous mediastinal and hilar lymphadenopathy warrants careful consideration.

Nanopore sequencing technology (NST) permits the rapid identification of *Mycobacterium tuberculosis* and its antibiotic resistance genes directly from clinical samples, leveraging its long-read technology and capacity for real-time analysis.[10] While NST has significantly improved the diagnostic accuracy for both pulmonary and extrapulmonary tuberculosis,[11] its accuracy in diagnosing tuberculous mediastinal and hilar lymphadenopathy using EBUS-TBNA specimens has not yet been systematically evaluated. To address this gap, we plan to conduct a systematic review and meta-analysis to comprehensively assess the diagnostic performance of NST for this condition based on EBUS-TBNA samples. Prior to conducting the meta-analysis, we have designed this study protocol to ensure the rigorous and standardised execution of the review. To date, no systematic review or meta-analysis has evaluated the diagnostic accuracy of NST for tuberculous mediastinal and hilar lymphadenopathy using EBUS-TBNA specimens. This protocol provides a pre-specified, transparent methodology to synthesise the available evidence and to address anticipated sources of heterogeneity that are particularly relevant to this emerging technology.

METHODS

Protocol development and registration

To ensure methodological rigour, a study protocol was formulated and prospectively registered on PROSPERO (CRD420251274529). The protocol was authored in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines.[12] Upon completion of the meta-analysis, findings will be reported following the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement for diagnostic test accuracy studies.[13] As the protocol relied exclusively on publicly available data, ethical approval was not required.

Eligibility criteria

Study selection followed the PICT framework.[14]

Participants (P): patients with suspected tuberculous mediastinal and hilar lymphadenopathy were included, regardless of sex, age or ethnic background.

Index test (I): the index test was defined as NST. A positive index test result is defined a priori as the detection of *M. tuberculosis complex* (MTBC) DNA in an EBUS-TBNA specimen using NST, according to the following minimum criteria: at least one unique read mapping to MTBC with a read length ≥500 base pairs and a mapping quality score ≥30, after removal of host sequences and quality filtering (eg, Q-score ≥7).[15] For targeted sequencing approaches (eg, amplicon-based or capture-based panels), a positive result is defined as ≥10 mapped reads specific to MTBC, or the threshold reported by the study authors if higher.[16] Studies that do not report sufficient detail on their positivity criteria will be included, but the lack of clarity will be recorded as an 'unclear' risk of bias in the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) index test domain. We will not apply a single universal threshold across studies; rather, we will use each study's reported positivity criterion as the basis for constructing its 2×2 table.

Comparator (C): the reference standard for tuberculous mediastinal and hilar lymphadenopathy is defined as a composite of microbiological confirmation (positive

smear, culture or molecular detection of *M. tuberculosis* (with the specific assay type recorded, eg, Xpert MTB/RIF, Xpert Ultra, other commercial PCR, in-house PCR, line probe assay)), histopathological evidence (caseating granulomas), immunological evidence (positive PPD and/or interferon-gamma release assay), compatible imaging findings and clinical response to anti-tuberculosis therapy. We acknowledge that variation in the components of this composite reference standard across studies may introduce heterogeneity and potential bias.

To address this, we will categorise the reference standard used in each included study into two types:

Microbiological reference standard: studies that require microbiological confirmation (culture, smear or molecular test) as part of the reference standard for at least a subset of patients.

Composite clinical reference standard: studies that rely on a combination of histopathological, immunological, imaging and/or clinical criteria without microbiological confirmation.

All decisions regarding reference standard categorisation will be made by two independent reviewers, with disagreements resolved by discussion or third-party arbitration.

Target condition (T): the target condition was confirmed tuberculous mediastinal and hilar lymphadenopathy.

Study types: this review will include diagnostic accuracy studies (prospective or retrospective cross-sectional or cohort designs) that evaluate NST against a composite reference standard for tuberculous mediastinal and hilar lymphadenopathy using EBUS-TBNA specimens. Case-control studies, case series, case reports, narrative reviews, systematic reviews, meta-analysis, conference abstracts, editorials, letters, and animal or in vitro studies will be excluded.

Eligible studies were required to report, or allow derivation of, sensitivity and specificity of NST against the reference standard. Inclusion also required that data could be extracted or calculated to populate a 2×2 diagnostic contingency table (true-positive, false-positive, false-negative, true-negative). Studies with potentially retrievable missing data through author correspondence were also considered.

Exclusion criteria: studies meeting any of the following criteria will be excluded: inability to extract or obtain via author correspondence the necessary data (true-positive, false-positive, false-negative, true-negative) for constructing a 2×2 diagnostic table; publication types such as conference abstracts, case reports, reviews or meta-analysis; unavailable full texts.

Information sources

Eligible studies will be identified through a systematic search of five international databases (PubMed, Embase, SCOPUS, Web of Science and Cochrane Library) and two Chinese databases (China National Knowledge Infrastructure (CNKI) and Wanfang Database). The literature search is planned to be conducted between 1 December 2026 and 31 December 2026 (start and end dates of the search). The publication dates of the included literature ranged from the inception of the relevant databases to 31 December 2026. Data extraction from the included studies is anticipated to be completed by 31 May 2027, and the final reporting of findings is expected by 31 December 2027. Furthermore, the reference lists of pertinent systematic review and meta-analysis will be manually examined to identify any potentially eligible publications not captured by the electronic search.

Search strategy

Database-specific search strategies will be developed collaboratively by GY and K D (online supplemental file 1). The search will be limited to records in English or Chinese. For PubMed, Embase and other international databases, language filters (English or Chinese) will be applied. For Chinese databases (CNKI and Wanfang Database), no additional language filter is needed as the content is primarily in Chinese. This decision is based on the linguistic proficiency of the review team (native Chinese speakers with fluent English reading and writing skills) and the feasibility of accurate data extraction. Restricting to English and Chinese is unlikely to introduce substantial bias for this research question, because the majority of original studies on nanopore sequencing for tuberculosis diagnosis are published in these two languages. Any potential impact of this language restriction will be discussed as a limitation in the final review. A draft search strategy for PubMed is presented below:

#1 “Tuberculous mediastinal lymphadenopathy” OR “Tuberculous mediastinal lymphadenitis” OR “Tuberculosis of the mediastinal lymph nodes” OR “Mediastinal tuberculous lymphadenitis” OR “Mediastinal tuberculous lymphadenopathy” OR “Mediastinal lymph nodes tuberculosis” OR “Tuberculous mediastinal and hilar lymphadenopathy” OR “Tuberculous hilar lymphadenopathy” OR “Tuberculous hilar lymphadenitis” OR “Tuberculosis of the hilar lymph nodes” OR “Hilar tuberculous lymphadenitis” OR “Hilar tuberculous lymphadenopathy” OR “Hilar lymph nodes tuberculosis”

#2 “endobronchial ultrasound-guided transbronchial needle aspiration” OR EBUS-TBNA OR “Endoscopic Ultrasound-Guided Needle Aspiration”

#3 #1 AND #2

#4 “Third-Generation Sequencing” OR “TGS” OR “Single-Molecule Real-Time Sequencing” OR “SMRT sequencing” OR “Oxford Nanopore Sequencing” OR “Nanopore sequencing” OR “Long-read sequencing” OR “PacBio sequencing” OR “Complete genome sequencing”

#5 “High-Throughput Nucleotide Sequencing”[Mesh:-NoExp] OR “Sequencing, Third-Generation” OR “Third generation sequencing”

#6 #4 OR #5

#7 #3 AND #6

#8 English[Language] OR Chinese[Language]

#9 #7 AND #8

Literature screening and selection

In accordance with PRISMA guidelines, the screening process will proceed as follows. Records will be de-duplicated automatically in EndNote V.9.2 (Clarivate Analytics). Two independent investigators (GY and KD) will perform a three-step screening: (1) initial title-based exclusion of clearly irrelevant studies; (2) abstract review for protocol relevance and (3) full-text review against the predefined eligibility criteria. Disagreements will be resolved by discussion and, if necessary, by arbitration from a senior investigator (FZ). Reasons for full-text exclusion will be documented systematically.

Data extraction

After final study selection, two investigators (GY and KD) will independently extract data using a standardised form. The following variables will be collected:

Study characteristics: first author name, publication year, country, study design, enrolment method (consecutive/non-consecutive).

Patient profile: sample size, age group (child/adult), HIV status.

Diagnostic parameters: reference standard used (based on diagnostic criteria for tuberculous mediastinal and hilar lymphadenopathy), type of molecular test (eg, Xpert MTB/RIF vs other PCR), specimen type (eg, aspirate), pre-analytical processing (decontamination, homogenisation), specimen state (fresh/frozen), anatomical locations (lymph node stations) accessible by/for EBUS-TBNA (mediastinal or hilar).

NST details: sequencing platform (eg, Oxford Nanopore MinION, GridION, PromethION; other long-read platforms if applicable). Sequencing approach (metagenomic vs targeted). Read depth (median or mean reads per sample, or total reads). Positivity threshold used (eg, minimum read count, coverage depth or presence/absence). Quality filtering parameters (eg, minimum read length, Q-score, mapping quality). Contamination control measures (eg, negative controls, no-template controls, extraction blanks, threshold for background noise). Laboratory accreditation.

Performance metrics: true-positive, false-positive, false-negative, true-negative values stratified by the reference standard.

Disagreements will be resolved by re-examination and, if needed, arbitration by a third investigator (FZ). To ensure accuracy, a random 20% of the extracted data will be re-extracted independently, with an acceptable error threshold of ≤5%.

Quality evaluation

The methodological quality of included studies will be appraised using the QUADAS-2 tool.[17] This instrument evaluates risk of bias across four domains: patient selection, index test, reference standard and flow/timing. Two independent reviewers will rate each domain as 'low', 'high', or 'unclear' risk, providing study-based justification for each judgement. Disagreements in ratings will be resolved through discussion and, if necessary, by third-party arbitration (FZ).

Publication bias

In accordance with PRISMA-DTA recommendations, formal assessment of publication bias will not be performed. Currently available methods for detecting such bias in diagnostic accuracy meta-analyses—such as funnel plot asymmetry—are of limited validity in this context, largely due to heterogeneous threshold effects and variations in disease spectrum across the included studies.

Evidence evaluation

The certainty of evidence for critical outcomes will be evaluated using the Grading of Recommendations Assessment, Development and Evaluation framework.[18] The evidence for each outcome will be graded as high, moderate, low or very low, based on consideration of five domains: risk of bias, inconsistency, indirectness, imprecision and publication bias.

Data synthesis and statistical analysis

Diagnostic accuracy measures—including pooled sensitivity, specificity and the area under the hierarchical summary receiver operating characteristic (HSROC) curve—will be derived from 2×2 contingency data using bivariate random-effects models. Results will be presented as point estimates with 95% CIs and displayed using forest plots and HSROC curves.

In diagnostic test accuracy meta-analysis, heterogeneity may arise from several sources, including differences in study populations, reference standards and particularly threshold effects (variation in the cut-off or positivity criterion used to define a positive nanopore sequencing result). The conventional I^2 statistic will be calculated to quantify overall heterogeneity in sensitivity and specificity separately. However, I^2 alone is insufficient for detecting threshold effects. Therefore, we will specifically evaluate threshold effects using three complementary methods:

Spearman correlation—the correlation between the logit of sensitivity and the logit of (1-specificity) will be calculated across studies. A strong positive correlation (Spearman's $\rho>0.6$) suggests the presence of a threshold effect.

Receiver operating characteristic (ROC) space visualisation—study-specific pairs of sensitivity and 1-specificity will be plotted in ROC space. A curvilinear (shoulder-shaped) pattern indicates threshold effects.

HSROC model parameters—the HSROC model estimates a shape parameter (beta). A beta significantly different from zero ($p<0.05$) provides statistical evidence of asymmetry due to threshold effects.

If a strong threshold effect is identified (eg, Spearman $\rho>0.6$ and significant beta from HSROC), we will present the HSROC curve as the primary graphical summary rather than a single summary point, avoid reporting a

pooled sensitivity and specificity without clearly displaying the accompanying HSROC curve and consider subgroup analysis based on clinically meaningful threshold categories (eg, different read count cut-offs for NST positivity), provided at least four studies are available per subgroup. If the number of included studies is insufficient to reliably fit bivariate or HSROC models (<4 studies), we will use fixed-effect exact binomial methods in Meta-DiSc V.1.4 and will clearly state that threshold effects could not be formally assessed.

Non-threshold heterogeneity (eg, differences in patient characteristics, reference standard or study design) will be quantified with the inconsistency index (I^2), interpreted as follows: 0–50% representing negligible heterogeneity and >50% representing substantial heterogeneity. If substantial heterogeneity (I^2 >50%) is present, exploratory subgroup analysis and meta-regression will be performed. The following covariates will be examined: reference standard type (microbiological vs composite clinical reference standard); type of molecular test (eg, Xpert MTB/RIF vs other PCR); study design (prospective/retrospective); enrolment method (consecutive/convenience sampling); host factors (HIV status, child/adult); anatomical locations (mediastinal or hilar); specimen characteristics (fresh/frozen, homogenisation protocol); NST factors (platform (such as Oxford Nanopore), sequencing depth (<50× vs ≥50×), targeted vs metagenomic approach). A leave-one-out sensitivity analysis will be conducted to identify studies disproportionately influencing heterogeneity.

For analyses involving four or more studies, bivariate modelling will be performed in STATA V.15.0 (StataCorp LP) using the MIDAS and METANDI packages, supplemented by HSROC framework extensions to account for threshold variability. For fewer than four studies, fixed-effect estimates with exact binomial CIs will be calculated using Meta-DiSc V.1.4.

DISCUSSION

This systematic review and meta-analysis will evaluate the diagnostic accuracy of NST for tuberculous mediastinal and hilar lymphadenopathy using EBUS-TBNA specimens. The findings may provide valuable evidence to guide clinical decisions, particularly in settings where rapid and accurate diagnosis of extrapulmonary tuberculosis is essential. The results of this review may inform future research directions. If NST demonstrates high accuracy, prospective studies validating its cost-effectiveness and turnaround time in routine clinical workflows would be warranted. Additionally, head-to-head comparisons between NST and other molecular methods (eg, Xpert MTB/RIF, line probe assays) using EBUS-TBNA samples would help clarify the optimal diagnostic strategy. If heterogeneity proves substantial, standardised protocols for specimen collection, processing and NST application should be developed to improve comparability across studies. In summary, this systematic review will synthesise currently available evidence on the accuracy of NST for diagnosing tuberculous mediastinal and hilar lymphadenopathy from EBUS-TBNA specimens. The findings will help clinicians and policymakers understand the potential role of this rapid molecular technology in a challenging diagnostic scenario, while also highlighting evidence gaps that require further investigation.

Limitations

This systematic review and meta-analysis will have several methodological limitations that should be considered when interpreting the findings. First, the reference standard for tuberculous mediastinal and hilar lymphadenopathy is not a single definitive test but a composite of microbiological, histopathological, imaging, immunological and clinical response criteria. This composite reference may introduce verification bias because the components are not uniformly applied across studies. Moreover, the lack of a universally accepted gold standard could lead to misclassification of the target condition. Second, substantial clinical and methodological heterogeneity is anticipated across the included studies. Differences in study design (prospective vs retrospective), patient populations (HIV status, age groups, prevalence of tuberculosis), specimen handling (fresh or frozen, with or without homogenisation) and NST protocols (targeted vs metagenomic sequencing, sequencing depth, platform type) may influence diagnostic accuracy estimates. While we plan to explore heterogeneity through subgroup analyses and meta-regression, these exploratory analyses are limited by the number of available studies and may not fully explain observed variability. Third, the number of eligible studies is expected to be small because EBUS-TBNA is a relatively specialised procedure and NST is an emerging technology for tuberculosis diagnosis. A limited number of studies may reduce the precision of pooled estimates and preclude reliable subgroup analyses. If fewer than four studies are identified, we will use simpler fixed-effect models, which assume homogeneity and may produce overly narrow CIs. Fifth, the review will include only studies published in English or Chinese. This language restriction may introduce language bias and could exclude relevant studies published in other languages, particularly from high tuberculosis burden countries. Sixth, EBUS-TBNA specimens typically yield small volumes of aspirate, which may limit the sensitivity of NST. Studies that do not report details on sample adequacy or DNA extraction methods may have undetected performance bias. Our ability to assess this limitation depends on the completeness of reporting in the original studies. Finally, our review only assesses the accuracy of nanopore sequencing for diagnosing tuberculous mediastinal and hilar lymphadenopathy (detection of *M. tuberculosis* DNA). We will not analyse its ability to detect antibiotic resistance. Consequently, this review cannot comment on the value of nanopore sequencing for resistance testing, which is a separate research question that requires further investigation.

ETHICS AND DISSEMINATION

This study is based on publicly available data and therefore does not require ethics committee approval. This study is in accordance with the Declaration of Helsinki. Upon completion, the findings will be submitted for publication in a peer-reviewed medical journal. The review is conducted in accordance with established guidelines for systematic review and meta-analysis.

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Contributors GY: the author will design search strategies, search databases, select literatures, manage data, assess quality, feedback, and draft and revise the manuscript. KD: the author will design search strategies, search databases, select literatures, manage data and evaluate quality. YS: the author will provide statistical expertise and draft and revise the manuscript. YL: the author will supervise and revise the manuscript. FZ: the author will provide method guidance and statistical expertise, read, draft and revise the manuscript, and approve the final manuscript. FZ is the guarantor of the review. GY and KD contributed equally to this work as co-first authors.

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