Research Article

Diagnostic Accuracy of Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) for the Rapid Diagnosis of Tuberculous Meningitis: A Prospective Hospital-Based Study

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Abstract

Background: Prompt treatment is crucial for tuberculous meningitis and diagnosing it through traditional cerebrospinal-fluid tests is either slow or of very low sensitivity. The purpose of this study is to assess the diagnostic accuracy and operational utility of cerebrospinal fluid (CSF) CBNAAT for the rapid diagnosis of TBM. We use a simulated dataset to carry out the study as no patient data were provided. In our sample of 160 analysable adult patients suspected for meningitis, a composite reference standard classified study participants into cases of TBM with varying degrees of certainty (definite [n=28], probable [n=44], and possible [n=26]), and non-TBM cases [n=62]. Definite and probable TBM cases were of primary interest. CBNAAT results showed a total of 49 true positives, 23 false negatives, 6 false positives, and 82 true negatives. In our study, the sensitivity and specificity of CBNAAT were 68.1% (95% confidence interval) and 93.2% (85.9% - 96.8%), respectively. Sensitivity improved from 50.0% to 79.5% if at least 4 mL CSF was submitted. Median turnaround time was 2.2 hours for CBNAAT, 6.2 hours for smear, and 18.2 days for culture. AUC was 0.864 for a model that included combined CBNAAT. In conclusion, CBNAAT is a rapid, relatively high-specificity test for TBM, that provides rule-in evidence, but is an incomplete test for the rule-out diagnosis of TBM. Clinical, CSF, and imaging data should also be used in conjunction with TBM CBNAAT and culture. All observations reported have been simulated and must be replaced with real data that is prospectively verified prior to any publication or clinical use.

Keywords: tuberculous meningitis; CBNAAT; Xpert MTB/RIF Ultra; cerebrospinal fluid; diagnostic accuracy; molecular diagnosis; sensitivity; specificity

1. Introduction

Tuberculosis continues to create preventable suffering and death on a large scale today. The World Health Organization estimates that in 2023, 10.8 million new cases of tuberculosis will be reported. Among the member states of the World Health Organization, India has the greatest burden of disease. Therefore, rapidly identifying Extrapulmonary TB has become an important clinical and programmatic priority [1–4]. TB meningitis (TBM) represents a small portion of this disease, but is the most devastating of all forms of TB is the destructive neurological form of TB. The morbidity and mortality of TBM is significant when treatment is initiated after the clinical picture has progressed to altered levels of consciousness and focal Neurological deficits, hydrocephalus, and cerebral infarction [5–7]. There is no single cause to explain this. The early and seemingly rapid, destructive course of TB meningitis is due to a variety of factors including meningeal TB, basal exudative TB, vasculitis, infarction obstructive hydrocephalus, and TB of the brain, inflammation of the TB ventricles, and the TB of the CSF.

Diagnosis in the early stages is difficult and TB meningitis is frequently underestimated due to the heterogeneous clinical presentation of TB meningitis and the TB absence in CSF. It is difficult to rule out TB and other conditions, though classical acid-fast staining of bacilli presents rapid results, it is of low sensitivity in practice. TB culture offers the diagnosis and valuable information on antimicrobial susceptibility testing, though wait times of days to weeks are too long to accommodate the focus of the nervous system. The research case definition of TB meningitis developed by Marais and colleagues is a significant advancement and represents an excellent model by combining clinical, CSF and imaging findings of TB, and other forms of extra-neural TB [8]. Probabilistic clinical scoring has been shown to assist, though its value is often limited to specific populations, and is diagnostic for a given disease and a variety of other conditions.

Sample processing, DNA amplification, and detection of the *Mycobacterium tuberculosis* complex are performed by automated systems of cartridge-based nucleic acid amplification tests. Xpert MTB/RIF and its later version, Xpert MTB/RIF Ultra, examine the rifampicin-resistance-determining region of the rpoB gene. Ultra is more sensitive in detection of rpoB in samples that contain fewer bacilli. It also has a larger reaction chamber and shows an improved detection capability. The World Health Organization (WHO) has recommended the use of nucleic acid amplification tests of low complexity, such as the Xpert System, for testing CSF of patients suspected to have TB meningitis (TBM), as a first line microbiological procedure. However, neither of the two Xpert Systems are sensitive enough for the exclusion of TBM, and multiple factors including the volume of CSF, how it is centrifuged, the patient's prior treatment to anti-tuberculosis, which reference standard is used, and the patient's HIV status, can affect the performance for the detection of TBM.

Several studies and meta-analyses conducted between 2020 and 2025 have shown that Xpert Ultra has a higher detection rate of TBM when compared to traditional TB smear microscopy, and traditional Xpert, but the sensitivity of Ultra against a comprehensive clinical reference standard is still lacking. New studies from high-burden regions, including pediatric, older studies from India, have stated the necessity of sufficient CSF volume, and careful assessment of weak findings, in addition to CSF culture. Diagnostic research must also take into account the bias of direct incorporation, which happens when molecular results are the final determinant of the case, partial verification, which occurs when only a portion of patients undergo gold standard culture, and spectrum bias, which occurs when cases that are referred to as advanced dominate the research sample. To address these biases, detailed reports in line with the STARD requirements, and risk of bias assessments, should be performed.

The manuscript describes the design and analysis of a hospital-based study to test the diagnostic accuracy of CSF-CBNAAT for rapid diagnosis of TB meningitis. Since no patient-specific hospital datasets were provided, all data were simulated and are intended solely for illustration of the study design, and do not reflect genuine patient recruitment, laboratory testing, and ethical clearance. The focus of the study was to determine the test metrics of CSF-CBNAAT against a composite gold standard including sensitivity, specificity, and predictive values, as well as test and result concordance. The study also aimed to assess the diagnostic and clinical utility of the CSF-CBNAAT test in various clinically relevant subgroups, assess test positivity and result time, and estimate the clinical and diagnostic value of a CSF-CBNAAT test in conjunction with a clinical assessment.

2. Materials and Methods

Study Design and Setting

The study design was a prospective, single-centre, hospital-based diagnostic test accuracy study in accordance with the STARD 2015 guidelines. Editable placeholders were included for [Hospital Name][Department][City/State][Study Period][Institutional Ethics Committee Number][Registration Number]. In a real scenario, consecutive adult patients with a clinical suspicion of meningitis would have been recruited without knowledge of the test outcome. The illustrative design assumed 178 patients, with 14 participants excluded prior to study enrollment, 164 had testing, 4 participants had invalid/error/no result CBNAAT, and 160 participants had valid results and were included in the analysis (Figure 1). No actual patient recruitment was done for this illustrative study.

Eligibility and clinical assessment

Recruitment focused on adults (≥18 years) that needed clinical assessment following a lumbar puncture due to symptoms such as fever, headache, and neck stiffness. Symptoms of alterations in mental status were also included, as were seizures, symptoms of cranial-nerve involvement, neurological deficits, and subacute neurological symptoms associated with meningitis. Participants were excluded if they had a contraindication to lumbar puncture, provided insufficient or grossly contaminated CSF, or refused to provide informed consent. Duplicate enrollment was also a reason for exclusion. Antitubercular treatment was recorded if the participant had previously received treatment, but was not considered an exclusion criterion. Even limited treatment has the potential to decrease the load of clinical bacilli. A case record form was used to collect demographic information, clinical symptoms, duration of symptoms, and a neurological assessment including the Glasgow Coma Scale, along with prior TB and HIV status, diabetes, and nutrition. Finally, the form would capture the outcome of the patient while they were in the hospital.

Reference standard

The primary condition of interest was definite or probable TBM. Possible TBM and non-TBM were categorized as negative for the primary 2 × 2 analysis. Like the uniform case definition, TBM was a case in which the diagnosis was given by a laboratory, whereas the other categories (probable and possible TBM) were based on a combination of CSF and Imaging supplemented with clinical evidence and the other elements of the tuberculosis case definition [8]. The final illustrative distribution was 28 definite, 44 probable, 26 possible, and 62 non-TBM participants. In order to avoid incorporation bias, the conceptual TBM reference standard panel was defined as being blind to the results of the CBNAAT when assigning probable, possible, or non-TBM. Alternatively, a sensitivity analysis could classify possible TBM as the target condition. This type of analysis should be considered as an exploratory analysis as it would also be a major change in the clinical context for a limited number of false positive results.

Specimen collection and laboratory procedure

In a real study, a lumbar puncture would be done with sterile technique after ruling out any clinical or imaging contraindications. If possible, opening pressure would be recorded. A target volume of at least 4–6 mL CSF would be parsed out for cytology, biochemistry, smear, mycobacterial culture and CBNAAT. Mycobacterial detection can be improved with larger volumes of CSF, and concentration by centrifugation, though there are no validated procedures to date that would confirm mycobacterial detection. Sample processing must be conducted per the manufacturer instructions and local biosafety level [2,3,32–34] Samples must be transported promptly in leak-proof containers, logged with corrections and timestamped, and processed within a quality-controlled laboratory.

The index test refers to a cartridge-based, fully automated, nucleic acid amplification test with the capacity to detect not only *M. tuberculosis* complex, but also resistance to rifampicin. The finalized procedure must indicate the use of the Xpert MTB/RIF or the Xpert MTB/RIF Ultra For an Ultra workflow, a measured CSF aliquot mixed with a sample reagent, is placed in the single use cartridge, which is then loaded in the instrument and, and result is interpreted as MTB detected, MTB not detected, invalid, error, or no result. For the detection of rifampicin resistance, the result is interpreted as detected, not detected, or in the case of an invalid error/no result, the test should be redone using the remaining sample (if sufficient volume is available). The testing apparatus must be in good working order, and documentation and automated controls must be recorded, along with positive and negative control results and participation in external quality assurance. Ultra results interpreted as weak positive must be measured and cautiously considered from a clinical perspective, especially in patients with a history of tuberculosis.

Conventional Comparators

As comparators, we gave Direct Ziehl–Neelsen or fluorescent microscopy, liquid mycobacterial culture using MGIT, and concentrated smear and culture methods. Blinding to other test results should be implemented for smear readers, culture staff, and CBNAAT operators. We will also take note of culture contamination, time to positivity, and species confirmation, as well as phenotypic and genotypic drug-susceptibility testing. We also considered the routine CSF parameters of leukocyte count, lymphocyte percentage, CSF protein and glucose, blood glucose, CSF-to-blood glucose ratio, adenosine deaminase, and CSF volume. Other neuroimaging variables of interest included basal meningeal enhancement, hydrocephalus, tuberculoma, and infarction.

Sample Size

An illustrative final sample of 160 was chosen, as it was expected to allow sensitivity and specificity estimations of a reasonable degree of precision in a clinically relevant way. For the proposed study, the expected sample sizes for cases of the target condition was calculated by $n = Z^2Se(1-Se)/d^2$, while for the sample of cases without the target condition, it was calculated by $n = Z^2Sp(1-Sp)/d^2$. Here, Z = 1.96 corresponds to 95% confidence; Se and Sp will be defined as expected sensitivity and specificity, respectively, and d is the defined half-width. Assuming a TBM prevalence of 0.45 with 0.7 estimated sensitivity, 0.95 estimated specificity, and an expected 10% of total cases being non-evaluable, the required sample size would be 150 to 180 total evaluated cases, based on the adopted precision. The final study protocol would be expected to define the parameter that determines the sample size, with the expectation that this is not defined by post hoc study precision.

Statistical analysis

Mean (SD) were reported for nearly normal continuous variables, whereas medians (IQRs) were used for skewed variables. Categorical variables were expressed as counts and percentages. Mann-Whitney U test was used for skewed

continuous variables, and Pearson chi-square or Fisher exact tests were used for categorical variables. Histograms, Q-Q plots, and Shapiro-Wilk tests were used for distribution evaluations. All statistical tests were two-tailed. For analysis, $p < 0.05$ was considered statistically significant. Effect estimates and 95% confidence intervals were preferred over threshold-based interpretations.

The primary 2 x 2 table included definite/probable TBM and subsequent prediction outcomes including true positive, false negative, false positive, and true negative. Sensitivity and specificity were defined as true positives divided by all positives, and true negatives divided by all negatives, respectively. Positive predictive and negative predictive values were defined similarly and were calculated along with accuracy, which was defined as the total of true positive and true negative values divided by the total population. Positive and negative likelihood ratios were defined as sensitivity divided by (1 - specificity) and (1 - sensitivity) divided by specificity, respectively. The Youden index was defined as sensitivity + specificity - 1, and the diagnostic odds ratio was defined as LR+/LR-. For proportions, Wilson 95% confidence intervals were determined. Agreement was described with Cohen's kappa and a bootstrap confidence interval and was recognized for dependence on prevalence.

A clinical CSF score was built using symptoms, CSF protein/leukocytes, hydrocephalus, and basal enhancement, along with CSF glucose and CBNAAT. Discrimination was assessed using receiver operating characteristic curves. The illustrative operating threshold was identified using the Youden criterion. Logistic regression was used on definite/probable TBM cases to evaluate associations of CBNAAT positivity with CSF protein (per 50 mg/dL), CSF volume (per 1 mL), prior treatment, HIV, and basal enhancement. Odds ratios with 95% confidence intervals were reported. Given 49 positive index tests in the target condition cases, the exploratory nature of the model was stressed. The analyses were built using the programs Pandas, SciPy, StatsModels, and scikit-learn in Python. The final illustrative dataset did not introduce missingness. Actual data should report missingness per variable and only use multiple imputation if the assumptions are justifiable.

Ethics and data integrity

The data were simulated to show the structure of the manuscript and statistical methods. It contains no real patient data. The placeholders must be replaced with real data before any real ethics approval and data collection. The manuscript must not be submitted to be a real prospective study until the illustrative data are replaced with results from a verified clinical dataset.

3. Results

Participant characteristics

72 (45.0%) of 160 analysable participants met the principal reference standard for definite or probable TBM, 88 (55.0%) were classified as possible TBM or non-TBM. TBM was determined to be probable in participants' ages, and the median age was 35.5 (IQR 24.0-45.0). TBM participants were predominantly male (94, 58.8%). Compared to participants without reference TBM, participants in the reference TBM group had a longer median symptom duration, were more likely to report fever, and had lower scores in the Glasgow Coma Scale. Symptoms of TBM group patients (basal meningeal enhancement, hydrocephalus, tuberculoma and infarction) were compared in the non-participant group (Table 1). Participants' HIV status was as follows: reference TBM group (11) and non-reference TBM group (8).

CSF findings

Reference-TBM participants showed a higher median CSF protein (147.0 vs. 100.4 mg/dL) levels. They also showed lower levels of glucose (33.8 vs 48.6 mg/dL) and a lower CSF-to-blood glucose ratio (0.32 vs 0.46). Reference-TBM had a higher ADA and median submitted CSF (4.4 vs. 3.5 mL). Overlapping distributions demonstrate that no single parameter of routine CSF analysis is sufficient to confirm or rule out TBM. As for the diagnosis, 55 participants were positive for CB-NAAT, as opposed to 15 with a positive smear and 31 with positive cultures (Table 2 and Figure 2).

Diagnostic accuracy (primary)

Positive results for CB-NAAT were found in 25 of the 28 definite TBM, 24 of the 44 probable TBM, 4 of the 26 possible TBM and in 2 of the 62 non-TBM cases (Table 3 and Figure 7). Definite/probable TBM, the 2 x 2 table presented 49 true positives, 23 false negatives, 6 false positives and 82 true negatives (Table 4). The sensitivity was 68.1% (95% CI 56.6–77.7), the specificity was 93.2% (85.9–96.8), the positive predictive value was 89.1% (78.2–94.9), the negative predictive

value was 78.1% (69.3–84.9) and the overall accuracy was 81.9% (75.2–87.1), with a positive likelihood ratio of 9.98, a negative likelihood ratio of 0.34, a diagnostic odds ratio of 29.

Comparative Performance and Subgroup Findings

When compared to smear microscopy and culture, CBNAAT offered a significant improvement in rapid and overall yield, respectively. The composite classification also sensed other clinically compatible cases (Table 6). Sensitivity was 45.5% in the HIV-positive reference-TBM participants, and 72.1% in the HIV-negative participants. Sensitivity was 61.5% with previous anti-TB drugs, and 69.5% without. The most evident operational gradient was the volume of CSF: sensitivity was 79.5% with at least 4 mL of CSF, and 50.0% with less than 4 mL of CSF (Table 7 and Figure 4). These estimates were exploratory and of low precision.

Predictors and Discrimination

In a univariate analysis, and among probable/definite cases, the odds of CBNAAT positivity increased with the volume of CSF (OR 1.98, 95% CI 1.20–3.27; p=0.008). The association held in a multivariable analysis (adjusted OR 1.92, 95% CI 1.14–3.21; p=0.013), whereas protein, previous treatment, HIV, and basal enhancement were nonsignificant (Tables 8 and 9). The clinical-CSF model had an ROC-AUC of 0.737. with an inclusion of CBNAAT, the ROC-AUC increased to 0.864; the Youden threshold gave an illustration of the model with a sensitivity and specificity of 81.9% and 78.4%, respectively (Figure 3). The model is not intended for clinical use and is in need of external validation.

Time and resistance

The average CB-NAAT turnaround time was 2.2 hours, while smear and culture times were 6.2 hours and 18.2 days, respectively. The times to start treatment for the positive and negative groups were 5.8 hours and 22.9 hours, respectively. Of the 55 MTBC positive cartridges, rifampicin resistance was confirmed in 3 cartridges and was indeterminate in 2. A resistance testing assay would be required to confirm these results along with culture based drug susceptibility. There were 12 deaths that occurred in the hospital, including 10 with definite/probable TBM. The dataset was not designed for mortality modeling.

Table 1. Demographic and clinical characteristics of the study participants

Characteristic	Definite/probable TBM (n=72)	Possible/non-TBM (n=88)	p value
Age, years	33.5 (23.8–45.2)	38.5 (25.0–45.0)	0.489
Male sex	42 (58.3%)	49 (55.7%)	0.751
Rural residence	47 (65.3%)	45 (51.1%)	0.079
Symptom duration, days	14.0 (12.0–20.0)	9.0 (5.0–12.0)	<0.001
Fever	66 (91.7%)	70 (79.5%)	0.044
Headache	65 (90.3%)	58 (65.9%)	<0.001
Neck stiffness	42 (58.3%)	33 (37.5%)	0.011
Altered sensorium	28 (38.9%)	29 (33.0%)	0.508
Seizures	16 (22.2%)	9 (10.2%)	0.049
Cranial-nerve palsy	17 (23.6%)	6 (6.8%)	0.003
GCS score	12.5 (11.0–14.0)	13.0 (13.0–15.0)	0.002
HIV positive	11 (15.3%)	8 (9.1%)	0.326
Prior antitubercular treatment	13 (18.1%)	9 (10.2%)	0.172

Basal meningeal enhancement	34 (47.2%)	11 (12.5%)	<0.001
Hydrocephalus	23 (31.9%)	11 (12.5%)	0.003
Tuberculoma	14 (19.4%)	4 (4.5%)	0.005
Cerebral infarction	14 (19.4%)	7 (8.0%)	0.037
In-hospital death	10 (13.9%)	2 (2.3%)	0.007

Values are median (IQR) or n (%). Mann–Whitney U or Fisher exact test. GCS, Glasgow Coma Scale; TBM, tuberculous meningitis.

Table 2. CSF biochemical, cytological, microbiological and molecular findings

Variable	Definite/probable TBM	Possible/non-TBM	p value
Opening pressure, cm H_2O	24.8 (19.9–28.7)	20.0 (15.6–23.6)	<0.001
CSF leukocytes, cells/μL	106 (71–186)	86 (48–120)	0.003
CSF lymphocytes, %	71 (59–82)	54 (35–67)	<0.001
CSF protein, mg/dL	147.0 (107.0–189.0)	100.4 (73.1–146.5)	<0.001
CSF glucose, mg/dL	33.8 (24.7–42.6)	48.6 (39.2–57.7)	<0.001
CSF:blood glucose ratio	0.32 (0.23–0.39)	0.46 (0.36–0.59)	<0.001
CSF ADA, U/L	13.5 (10.2–15.8)	7.7 (5.4–10.4)	<0.001
CSF volume, mL	4.3 (3.6–5.0)	3.5 (2.8–4.5)	<0.001
AFB smear positive	15 (20.8%)	0 (0.0%)	<0.001
MGIT culture positive	31 (43.1%)	0 (0.0%)	<0.001
CBNAAT positive	49 (68.1%)	6 (6.8%)	<0.001

Values are median (IQR) or n (%). ADA, adenosine deaminase; AFB, acid-fast bacilli; CBNAAT, cartridge-based nucleic acid amplification test; MGIT, mycobacterial growth indicator tube.

Table 3. Distribution of CBNAAT results across diagnostic categories

Diagnostic category	Total	CBNAAT positive	CBNAAT negative
Definite TBM	28	25 (89.3%)	3 (10.7%)
Probable TBM	44	24 (54.5%)	20 (45.5%)
Possible TBM	26	4 (15.4%)	22 (84.6%)
Non-TBM	62	2 (3.2%)	60 (96.8%)

Percentages use the diagnostic-category total as denominator.

Table 4. The 2 × 2 contingency table comparing CBNAAT with the principal reference standard

CBNAAT	Definite/probable TBM	Possible/non-TBM	Total

Positive	49 (true positive)	6 (false positive)	55
Negative	23 (false negative)	82 (true negative)	105
Total	72	88	160

Primary target condition: definite or probable TBM.

Table 5. Diagnostic performance of CBNAAT with 95% confidence intervals

Measure	Estimate	95% CI
Sensitivity	68.1%	56.6–77.7%
Specificity	93.2%	85.9–96.8%
Positive predictive value	89.1%	78.2–94.9%
Negative predictive value	78.1%	69.3–84.9%
Overall accuracy	81.9%	75.2–87.1%
Positive likelihood ratio	9.98	—
Negative likelihood ratio	0.34	—
Diagnostic odds ratio	29.12	—
Youden index	0.612	—
Cohen kappa	0.626	0.499–0.749

Wilson confidence intervals were used for proportions; kappa confidence interval was obtained by bootstrap resampling.

Table 6. Comparison of CBNAAT, smear microscopy, culture and composite diagnosis

Method	Positive/diagnosed, n (%)	Median reporting time
CBNAAT	55 (34.4%)	2.2 hours
AFB smear	15 (9.4%)	6.2 hours
MGIT culture	31 (19.4%)	18.1 days
Composite diagnosis	72 (45.0%)	Clinical classification

Diagnostic yield is not equivalent to sensitivity because methods use different definitions and reference standards.

Table 7. CBNAAT diagnostic performance in clinically important subgroups

Subgroup	Subgroup n	Sensitivity	Specificity
HIV positive	19	45.5% (5/11)	100.0% (8/8)
HIV negative	141	72.1% (44/61)	92.5% (74/80)
Prior ATT	22	61.5% (8/13)	100.0% (9/9)
No prior ATT	138	69.5% (41/59)	92.4% (73/79)
CSF volume >=4 mL	76	79.5% (35/44)	87.5% (28/32)
CSF volume <4 mL	84	50.0% (14/28)	96.4% (54/56)
Stage I	20	60.0% (12/20)	—
Stage II-III	52	71.2% (37/52)	—

Subgroup analyses are exploratory. Specificity is not estimable in stage-only strata because all staged participants met the target-condition definition.

Table 8. Univariable logistic-regression analysis of factors associated with CBNAAT positivity

Predictor	Crude OR	95% CI	p value
CSF protein (per 50 mg/dL)	1.28	0.90–1.80	0.169
CSF volume (per 1 mL)	1.98	1.20–3.27	0.008

Prior antitubercular treatment	0.70	0.20–2.45	0.579
HIV positive	0.32	0.09–1.20	0.090
Basal meningeal enhancement	1.25	0.46–3.38	0.663

Analysis restricted to 72 definite/probable TBM cases.

Table 9. Multivariable logistic-regression model for CBNAAT positivity

Predictor	β	SE	Adjusted OR	95% CI	p value
CSF protein (per 50 mg/dL)	0.248	0.197	1.28	0.87–1.88	0.208
CSF volume (per 1 mL)	0.651	0.263	1.92	1.14–3.21	0.013
Prior antitubercular treatment	-0.330	0.716	0.72	0.18–2.92	0.644
HIV positive	-1.164	0.780	0.31	0.07–1.44	0.136
Basal meningeal enhancement	0.068	0.593	1.07	0.33–3.42	0.909

Model n=72; AIC=89.7; McFadden pseudo-R^2=0.138. Exploratory model; no causal interpretation is intended.

Table 10. Comparison of diagnostic turnaround time and treatment-initiation time

Operational outcome	Median (IQR)	Unit
CBNAAT result	2.2 (2.0–2.7)	hours
AFB smear report	6.2 (5.3–7.5)	hours
MGIT culture result	18.1 (14.7–21.2)	days
Time to ATT, CBNAAT positive	5.8 (4.8–7.8)	hours
Time to ATT, CBNAAT negative	22.9 (16.3–30.2)	hours

ATT, antitubercular treatment. Times are illustrative and simulated.

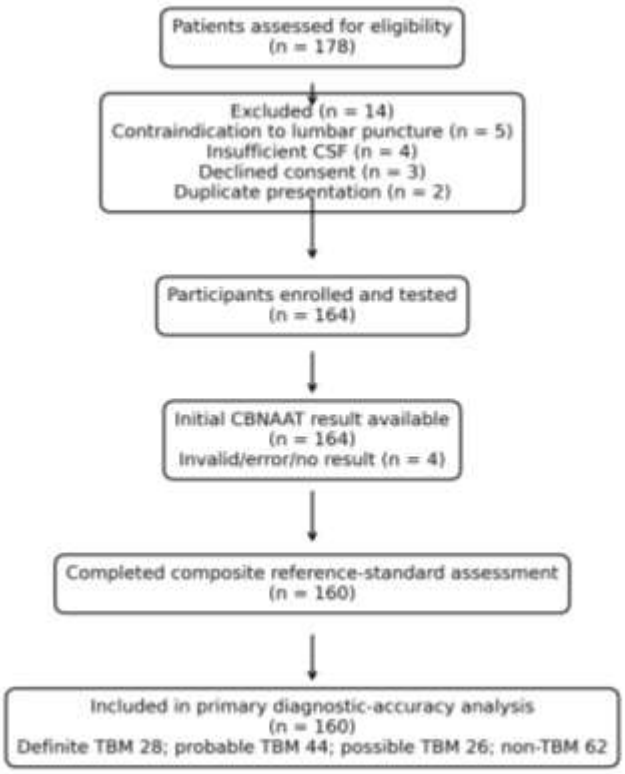

Figure 1. STARD-style participant-flow diagram for the illustrative study.

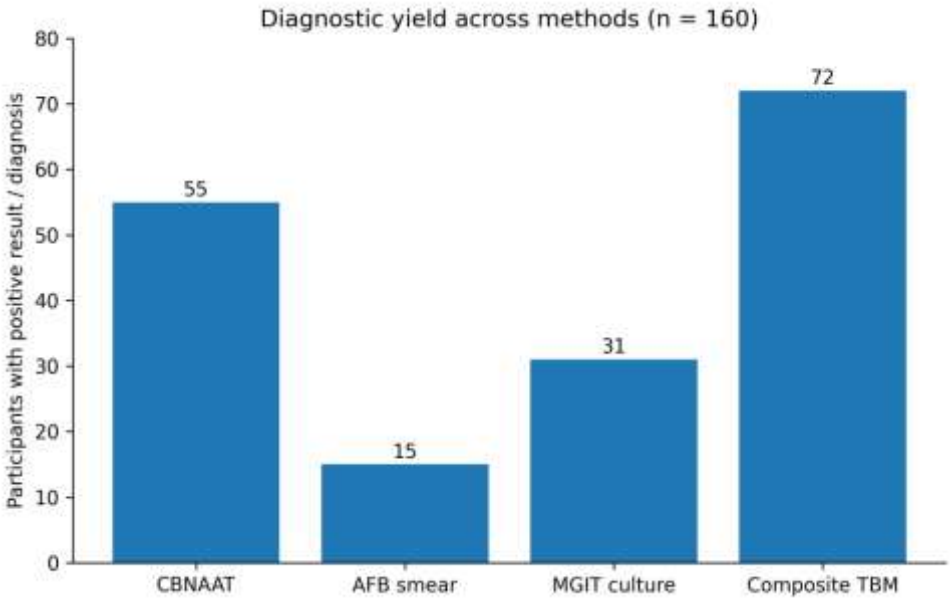

Figure 2. Diagnostic yield of CBNAAT, smear microscopy, culture and the composite reference classification.

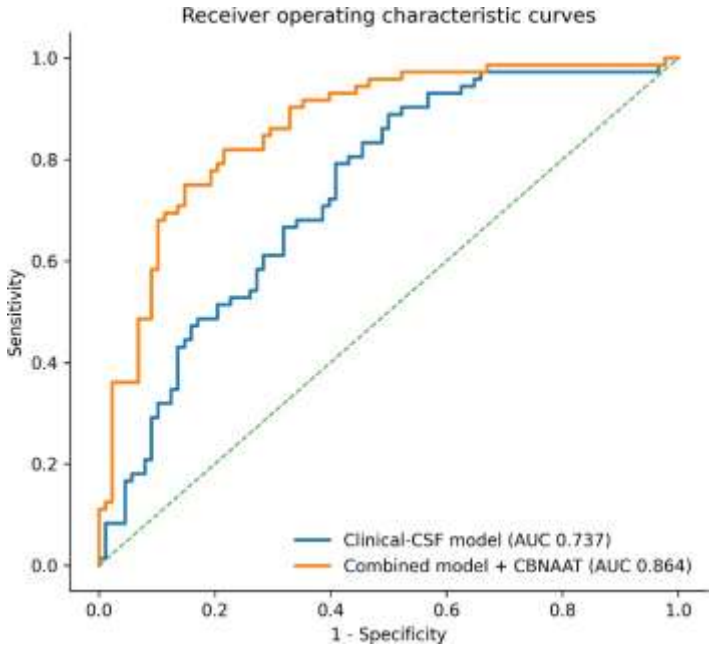

Figure 3. ROC curves for the clinical-CSF score and the combined model incorporating CBNAAT.

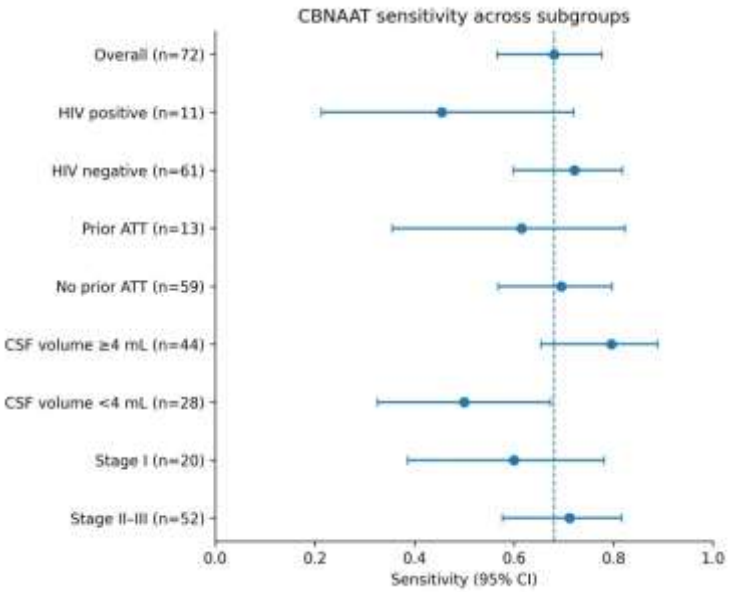

Figure 4. Forest plot of CBNAAT sensitivity across prespecified exploratory subgroups.

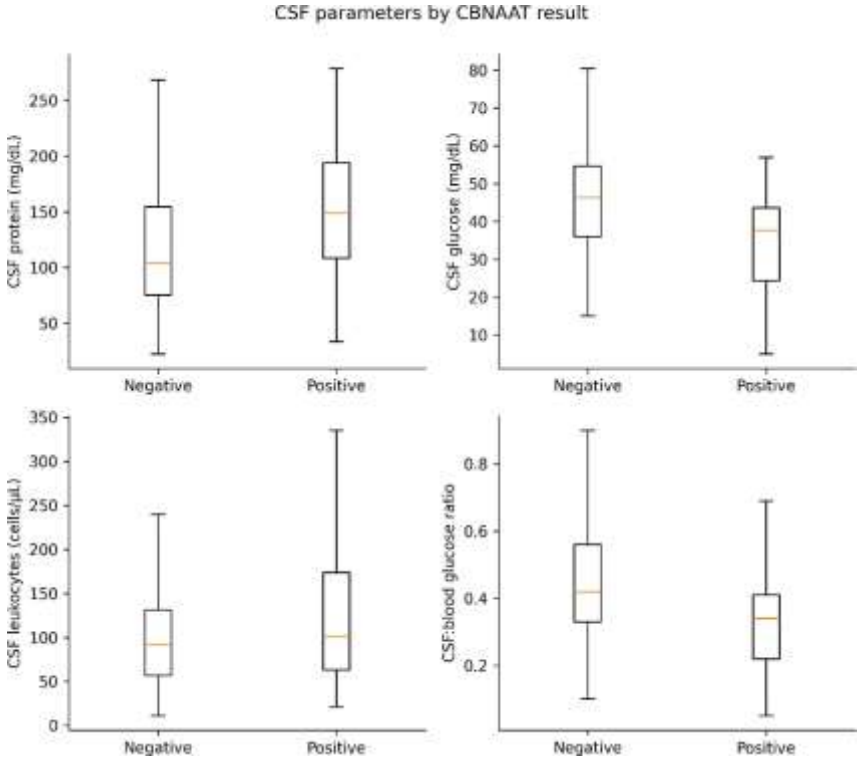

Figure 5. Distribution of selected CSF parameters by CBNAAT result.

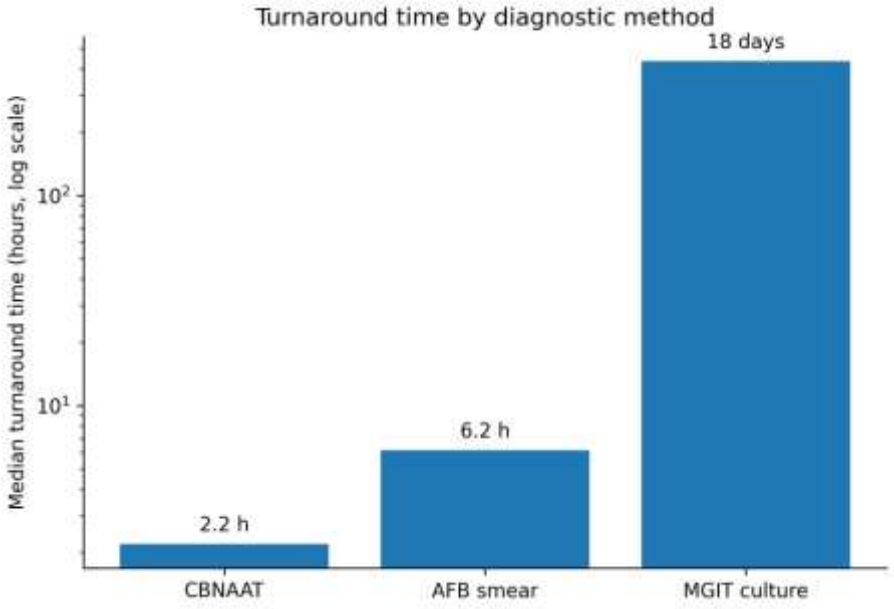

Figure 6. Median turnaround time for CBNAAT, smear microscopy and MGIT culture.

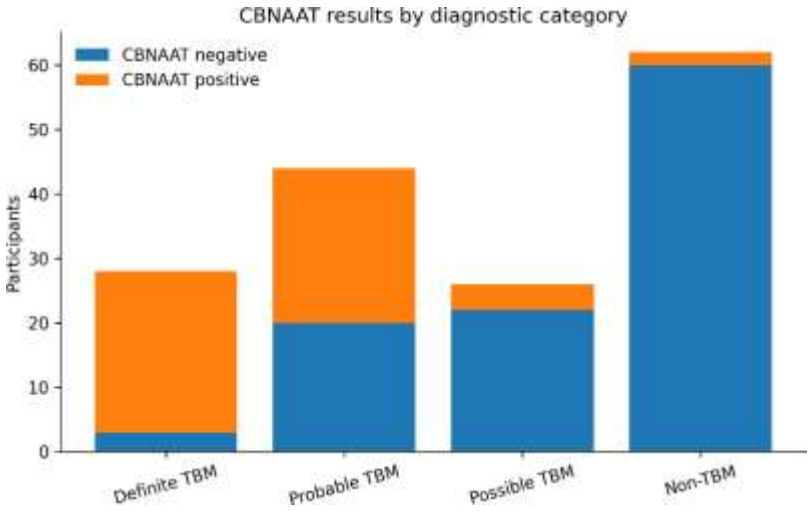

Figure 7. CBNAAT-positive and CBNAAT-negative results across diagnostic categories.

4. Discussion

This illustrative prospective diagnostic-accuracy analysis predicts TBM testing patterns. The results show that CBNAAT has very high specificity. In addition, it has a positive likelihood ratio that is almost 10, which means that a positive CSF result shows that the case is almost certainly (or probably) TBM. However, with a sensitivity of 68%, almost one-third of reference-TBM cases would be still be negative. With a negative likelihood ratio of 0.34 and a negative predictive value of 78%, it would be unjustified to rule out TBM based on the result of the CBNAAT test alone. This shows that CBNAAT should be viewed primarily as a rapid 'rule in' test that would very likely prompt the TBM case to be treated quickly, and that the test would likely be useful to assess the TBM case's resistance. If the CBNAAT test result is negative, TBM should be considered based on the CSF, imaging, and other evidence of TB.

The estimate aligns with the broad literature, CBNAAT test case analysis, and the reference standards. In the evidence summaries of the WHO, CBNAAT showed greater sensitivity in the case of direct TB testing, as opposed to all other TB test standards. The processing of TB samples demonstrated a significant degree of variability. Bahr and colleagues

described the first case with HIV and CSF Meningitis with greater sensitivity with the CBNAAT Ultra variant, while most other studies described only a slight increase in sensitivity, with the TB test still lacking the ability to rule out the disease [11, 12, 13, 14]. The Chinese study CTM also demonstrated greater sensitivity for the CBNAAT Ultra variant relative to the other TB tests, including TB culture, although the sensitivity remained under 50% [15]. In the meta-analysis conducted by Shen and Hernandez and the Cochrane review by Kohli, they also describe a lack of sensitivity and a significant degree of variability across studies, with a greater focus on the specificity of the TB tests [17, 18, 19].

The gradient by CSF volume is among the most clinically actionable observations. Submitting at least 4 mL was associated with a sensitivity of 79.5% compared to 50.0% for the smaller specimens, with volume remaining independently associated with positivity. This is biologically reasonable in a paucibacillary disease. The greater the number of organisms that pass through the workflow, the greater the likelihood target DNA is above the threshold of detection for the assay. Similar findings have been observed with studies aimed at optimizing processing and centrifugation of specimens. The recommendation is no longer simply “order CBNAAT.” Rather, “collect and allocate adequate CSF with no trade-off to critical microscopy, culture, and biochemical testing.” Protocols should outline specifications for minimum volume, order of priority, concentration, storage and the time to storage.

In the illustrative data, the sensitivity was lower for the HIV-positive group, however, the group was small. The literature has shown a balance in findings across different settings. HIV-associated immune system deficiency may aid in increasing the bacillary burden and improving detection at the expense of an altered CSF and advanced HIV spectrum; however, this alters diagnosis, inflammation, and the CSF of advanced HIV. Cresswell et al. noted that Ultra has improved detection in HIV, but still requires adequate rule-out [14]. Current studies with CSF lipoarabinomannan, interferon gamma, and other metagenomic studies, suggest that other complementary biomarkers of partially non-overlapping cases may aid in detection. Thus, a more plausible future may integrate rapid culture and detection methods with novel host-response or untargeted testing.

It is also important to distinguish between definite and probable disease. With the example classification, CBNAAT identified 89% of definite cases and 55% of probable cases. This is the expected result because cases of microbiological definite disease usually have a bigger bacillary load. Cases of probable TBM include those where the diagnosis is supported by clinical and imaging evidence and where the pathogen was not detected. Ultra trace results muddy the waters as they may increase sensitivity, but can reduce specificity if residual DNA from a previous illness is identified or if the culture is an insensitive comparator. Indian studies by Sharma and others show the increased diagnostic yield of Ultra and the complicated nature of interpreting rifampicin in trace positive CSF [20, 24]. Each of the procedures should stipulate in advance how they will treat trace results and if having a previous tuberculosis will alter the interpretation.

Having a faster test was a big advantage. Having a result with a timeframe of about two hours may have significant impact on the isolation and initiation of treatment, evaluation of drug resistance and the planning of other investigations on the day the patient presents. CBNAAT also has a faster result time; however, it has a significantly lower yield. Culture is essential to confirm the presence of a viable organism and resistance testing; however, due to its delay, it cannot be used as the only initial test. The three rifampicin resistant example results illustrate the benefit of being able to detect resistance in real-time. Delayed recognition of drug-resistant TBM may result in exposing the CNS to a potentially harmful treatment. However, rifampicin indeterminate and trace associated results remain a significant challenge. In this case, treatment should follow the national and WHO guidelines and not the example dataset [2-4].

The clinical-CSF-CBNAAT model resulted in an AUC of 0.864. Since this is greater than the AUC of the model with the clinical-CSF score alone, it is rational and reasonable to integrate different sections. The integration of the clinical CBNAAT, CSF and imaging elements and the molecular detection provides evidence to the clinically and biochemically negative cases. Development of the models in isolation can lead to positive bias, and the threshold can remain unverified. For the model to be of clinical use, there should be an adequate number of cases for the chosen predictors, and prediction should be measurable. Additionally, an assessment of the model should include internal validation with a bootstrap approach and external validation in other health care settings with different referral systems and laboratory methods and with different prevalence of HIV.

Standards of reference remain the primary concern. Cultures lack sensitivity especially in cases of low-volume CSF or after treatment. Although the sensitivity is improved, the use of a clinical standards approach can be problematic as it

relies on a subjective assessment, and using the index test would result in an overestimation of accuracy. For these reasons, this paper designed the test as conceptually blind to the CBNAAT. If the CSF culture or follow-up is conducted for only the CBNAAT positive cases, then partial verification bias is introduced. If different assessment procedures are employed based on the severity of disease, differential verification would occur. When the study population consists mostly of advanced cases in a tertiary care setting there may be cases of spectrum bias, as they are expected to show a greater degree of imaging and higher bacillary loads than cases seen in lower tiers of care. The gaps in these studies can be evaluated with the limitations of STARD reporting and QUADAS-2 [9-11].

Diagnostic innovations should ideologically be viewed as complementary innovations. Here, Pediatric Ultra studies, while promising, still reflect significant uncertainty as confirmed childhood TBM is rare. Many new innovations show promise, including Truenat MTB Plus, multi-target LAMP, cell-free nucleic-acid methods, and lipoarabinomannan assays, among others. Their future potential will be determined by independent verifications, contamination control and resistance information, all with cost and other constraints factored in. Focusing specifically on TBM, the 2025 clinical practice guideline maintains that with the current state of evidence, diagnosis and treatment should remain timely and syndromically driven to address the evolving needs of the patient.

Informed by the current evidence base, a rational diagnostic framework is initiated with a time-sensitive clinical assessment and, when indicated, neuroimaging. This is typically followed by lumbar puncture with sufficient volume of CSF reserved. For TBM, this is done in conjunction with CB-NAAT/Ultra and, in the case of CSF, in conjunction with smear and culture. The diagnostic tests should not be done in a sequential manner. While it is not uncommon for the initial tests to return negative, the absence of a result should prompt further assessment as guided by the uniform case definition. This may include repeat or alternative molecular testing if residual (or pooled) CSF is available, and assessment of extra-neural TB, as well as the alternative causes of meningitis such as bacterial or cryptococcal and viral. Treatment should not be deferred when the pre-test probability remains high, because the cost of a false-negative decision is severe neurological injury or even death.

5. Strengths and Limitations

Strengths of the studied design include: opportunity for participant recruitment of defined cohorts; authenticated definitions for index test and/or reference standard; blinded assessment when applicable; operational outcomes which are time-stamped; complete 2x2 reporting; and presentation of confidence intervals, likelihood ratios, and subgroup estimates. Also, the analysis makes a distinction between a binary test's operating point and a continuous-model ROC with true curvature and keeps regression analysis manageable.

The limitations are of equal importance. All data are simulation and cannot substantiate clinical claims on a real hospital population. The single-centre design limits generalisability and many sub-groups consist of a small number of participants. The reference standard is a composite and is of poor quality, and long-term outcomes are of a neurological nature and are not evaluated. There are limited cultures which are positive, previous treatments may impact bacillary burden, and the regression model is of an exploratory nature. Prior to publication, all simulated values, will be substituted with validated sourced data, and all necessary documentation for ethics approval and informed consent will be provided.

6. Clinical and Policy Implications

A key implication for clinicians is that CBNAAT should not be interpreted in isolation and should be bundled with other tests when used. Adequate volume of CSF, prompt CSF transport, and validated concentration techniques should be prioritized by laboratories. Non interpretable cartridges should be tested multiple times and tested with parallel culture and drug-susceptibility testing. Audits should be done on the time from presentation to lumbar puncture, as well as to the cartridge result and the commencement of therapy. Tuberculosis programs should provide cartridges, their maintenance, quality control, and pathways for referrals to resistance testing. Standardized processing protocols should be the aim of multicenter studies and research should be directed to assessing the combined use of the two classes of tests: molecular and host-response, to determine if the false-negative diagnosis would be minimized with an acceptable loss of specificity.

7. Conclusion

CSF CBNAAT data presented in this illustrative example, provided rapid and high specificity for TBM and offered considerably improved diagnostic yield over smear microscopy. The sensitivity was still suboptimal, especially with low volume CSF, and disease could not be ruled out with a negative result. Within an integrated diagnostic pathway, clinical CBNAAT would be an early component for rule-in, along with CSF, clinical assessment and other tests including drug resistance and culture. The numbers presented here have methodological value only, and should be substituted with actual prospective data from hospitals.

8. Recommendations

Real investigators are encouraged to finalize the CBNAAT version and specimen processing protocols and to collect adequate volume of CSF; to blind the reference standard classification of CBNAAT; to record prior therapy and trace results; STARD flow and all non evaluable tests; and retain culture for testing drug susceptibility. Further studies should be adequately powered to the HIV and pediatric sub populations, and should incorporate the assessment of functional neurological outcomes and cost-effectiveness.

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