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Using cerebrospinal fluid nanopore sequencing assay to diagnose tuberculous meningitis: a retrospective cohort study in China

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ABSTRACT

Objective This study aimed to evaluate the efficiency of nanopore sequencing for the early diagnosis of tuberculous meningitis (TBM) using cerebrospinal fluid and compared it with acid-fast bacilli (AFB) smear, mycobacterial growth indicator tube culture and Xpert *Mycobacterium tuberculosis* (MTB)/rifampicin (RIF).

Design Single-centre retrospective study.

Setting The Tuberculosis Diagnosis and Treatment Center of Zhejiang Chinese and Western Medicine Integrated Hospital.

Participants We enrolled 64 adult patients with presumptive TBM admitted to our hospital from August 2021 to August 2023.

Methods We calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of AFB smear, culture, Xpert MTB/RIF and nanopore sequencing to evaluate their diagnostic efficacy compared with a composite reference standard for TBM.

Results Among these 64 patients, all tested negative for TBM by AFB smear. The sensitivity, specificity, PPV and NPV were 11.11%, 100%, 100% and 32.2% for culture, 13.33%, 100%, 100% and 2.76% for Xpert MTB/RIF, and 77.78%, 100%, 100% and 65.52% for nanopore sequencing, respectively.

Conclusion The diagnostic accuracy of the nanopore sequencing test was significantly higher than that of conventional testing methods used to detect TBM.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ In our study, we evaluated the diagnostic efficacy of four assays for tuberculous meningitis (TBM) patients and compared with each other.
⇒ Our study showed that the nanopore sequencing technology had the best diagnostic efficacy and could identify other bacteria in the cerebrospinal fluid (CSF) sample.
⇒ The results of the study confirmed that the nanopore sequencing technology to detect *Mycobacterium tuberculosis* in CSF sample can provide more targeted suggestions for the management of TBM patients.
⇒ Main disadvantage of this study is that the study is not a randomised controlled clinical trials and is only the single-centre retrospective cohort.

INTRODUCTION

Tuberculous meningitis (TBM) is a severe infection of the central nervous system (CNS) caused by *Mycobacterium tuberculosis* (MTB) that crosses the blood–brain barrier to invade the skull. TBM can cause severe disability and mortality in approximately 50% of all affected patients.[1] According to a previous study, early definitive diagnosis and timely antituberculosis treatment (ATT) are crucial factors in the prognosis of TBM.[2]

Nevertheless, early identification of TBM is difficult because of its diverse and nonspecific clinical presentation and the limited availability of laboratory tests.[3 4] Currently, the diagnosis of TBM relies on clinical symptom detection, laboratory assay results and radiological examinations, and it is challenging to achieve a definitive pathogenic diagnosis.

The earliest and most commonly used method for the diagnosis of tuberculosis is Ziehl-Neelsen (ZN) staining, which directly detects acid-fast bacilli (AFB) in the sample. Although the AFB smear is a convenient, rapid and cheap approach, it has a relatively low positive rate of detection. The modified ZN staining method offers improved detection rates of AFB in respiratory samples; however, its sensitivity remains low (8%) in body fluid specimens, which hinders the early detection of TBM.[5 6] Positive MTB culture in cerebrospinal fluid (CSF) is definite evidence for verifying the diagnosis of TBM; however, MTB culture is associated with a long lead time of approximately 2–4 weeks, which is not conducive to early diagnosis. Numerous studies have reported that the positivity rate ranges from 36% to 81.8% based on the quality and timing of the CSF sample obtained.[7–9] Therefore, the low sensitivity and time-consuming nature of the classical microbiology screening

process do not meet clinical expectations, and faster molecular and microbiological diagnostic techniques are warranted.

Xpert MTB/rifampicin (RIF) (Cepheid, Sunnyvale, California, USA) is a rapid, fully automated, encapsulated nucleic acid amplification PCR assay system that provides results in 2 hours. In 2010, the WHO recommended the use of this method for detecting tuberculosis in regions with high MTB burden. In 2018, this test was used to diagnose TBM in adults infected with HIV. Its sensitivity is approximately 43% and rapid report of bacterial susceptibility to RIF,[10] which is vastly superior to that of traditional AFB smear and culture testing methods. Scientists from various countries have reported sensitivities of 45%–86% for the estimation of TBM in HIV-negative patients. The diagnostic accuracy of this test may vary with specimen volume, and volume of ≥6 mL CSF is associated with frequent detection of MTB.[11] Conversely, the aforementioned reports have suggested that negative results obtained using Xpert MTB/RIF do not sufficiently confirm the absence of TBM.

Recently, next-generation sequencing (NGS) has partially replaced numerous traditional biochemical or molecular detection technologies because it can provide comprehensive data with unmatched accuracy.[12] It is an emerging method for the identification of pathogenic micro-organisms based on nucleic acid detection sequences.[13 14] It is imperative to exclude environmental micro-organisms and human commensal bacteria while interpreting results.[15] Non-respiratory samples, such as CSF, have fewer bacteria and may, therefore, result in less contamination during the test. Therefore, it is valuable for the diagnosis of CNS infections.[16] Notably, the whole genome provided by the Oxford Nanopore MinION platform has developed a third-generation portable nanopore sequencing device. This device can generate long sequencing reads with an average yield of 10–30 Gb per 48 hours of runtime, which is comparable to that of the Illumina MiSeq platform. Moreover, this device can clarify complex microbial genome structures after analysing the data from multiple perspectives; therefore, it can be advantageous to obtain a comprehensive collection of sequence information and increase pathogen classification accuracy. Furthermore, long reads and the availability of analysis software have renewed the interest in its clinical application. Fast and accurate test results are particularly suitable for detecting infectious diseases such as severe respiratory infections, bloodstream infections and unexplained fever.[17–19] However, the high diagnostic efficacy of this emerging technology for suspected TBM is unclear. Therefore, we conducted a retrospective study to evaluate the accuracy of nanopore sequencing technology in the early diagnosis of presumptive TBM patients and compare its results with those of AFB smear, culture and Xpert MTB/RIF assay.

MATERIALS AND METHODS

Study design

We retrospectively screened patients with clinically presumptive TBM admitted to the Tuberculosis Diagnosis and Treatment Center of Zhejiang Chinese and Western Medicine Integrated Hospital from August 2021 to August 2023. The inclusion criteria were as follows: (1) patients aged 18–80 years; (2) those presenting with the symptoms of presumptive TBM (such as headache, high fever, nausea and vomiting or altered consciousness) or signs of neurological disease; (3) those who completed lumbar puncture and (4) those whose CSF samples were used for all four tests (AFB smear, culture, Xpert MTB/RIF and nanopore sequencing). Patients whose CSF samples were not obtained for relevant testing and those lost to follow-up were excluded.

Our final clinical diagnosis of TBM was based on the patient's clinical symptoms and neurological signs, lumbar puncture findings, cranial MRI and the presence of MTB infection elsewhere.[10 20] In accordance with the TBM international standard,[21] TBM cases in our study were classified into four groups: definite, probable, possible and non-TBM. Definite TBM with positive CSF pathogenicity is defined by a positive result obtained using CSF AFB smear, culture or one or more molecular diagnostic methods such as Xpert MTB/RIF. Patients with probable TBM develop symptoms and signs of one or more types of meningitis, have abnormal lumbar puncture and cerebral imaging results and have confirmed tuberculosis infection at other sites. Possible TBM manifests symptoms and signs of meningitis, but lumbar puncture or cerebral imaging results remain normal. Non-TBM cases are identified using an alternative diagnosis method or their response to therapy, in which their disease status improves after anti-infective treatment but not after ATT.

Patient and public involvement

This study was patient-centred and the research questions and outcome measures took into account the patients' priorities, experiences and preferences. The steps were as follows: patients were informed by their doctors about the treatment programmes, precautions, and possible benefits and disadvantages of the different screening methods and treatment options, and they voluntarily chose the screening methods and options.

Patients will be informed face to face by their doctor during stay in the hospital about how they will be recruited for this study and it is up to the patient to decide. The doctor informed the patient in detail about the four tests and the final clinical diagnostic criteria and explained the results to the patients. The patients were also informed of the additional cost of choosing the nanopore sequencing technique.

Clinical sample collection and handling

In this study, all patients underwent a lumbar puncture performed by the supervising physician before ATT. The asepsis principle was followed during the lumbar

puncture procedure. Fresh clinical CSF samples were collected from the patients, which were directly dropped into sterile test tubes and then sealed. Next, fresh samples were equally distributed for AFB smear, culture, Xpert MTB/RIF and nanopore sequencing tests.

AFB smear

Overall, 1 mL CSF was centrifuged, and the precipitate was used for acid-fast staining. After performing fluorescent staining, the samples were observed under a microscope at 400×magnification; the results were clarified by performing ZN staining.

Culture

BACTEC mycobacterial growth indicator tube (MGIT) 960 culture test was performed following the manufacturer's (Becton Dickinson Life Sciences, USA) instructions. Briefly, 0.5 mL of the treated sample and 0.8 mL of MGIT additive were simultaneously inoculated into the MGIT culture tube. Subsequently, the culture tube was placed into the fully automated Bactec 960 system instrument, which automatically and continuously monitors the changes in fluorescence intensity displayed in the growth indicator tube medium at regular intervals to detect the growth of MTB.

Xpert MTB/RIF

The CSF sample (1 mL) and sample processing solution (2 mL) were mixed, incubated for 15 min at 16–30 centigrade and vortexed for 8 min. Then, a 2 mL of liquid was transferred to a plastic kit and placed into the GeneXpert full containment test apparatus. After DNA release, it was mixed with PCR reaction reagents and subjected to seminested real-time amplification and fluorescence signal detection. The results were obtained automatically after 2 hours.

Nanopore sequencing

CSF sample processing

First, sterile CSF samples were centrifuged at 12 000 rpm. Subsequently, protease, lysozyme and zirconium oxide were added to the precipitate to obtain a ground homogenate. An equal amount of anhydrous ethanol solution was added, 400 µL of each CSF sample was liquefied, centrifuged and the supernatant was discarded. Subsequently, a 20 µL of magnetic beads was added, which were left undisturbed and then centrifuged; following this, the supernatant was discarded. Further, 600 µL of 1×WB solution and 800 mL of 80% ethanol were successively added, mixed and centrifuged. The liquid precipitated at the bottom was completely aspirated using a pipette. Finally, 80 mL of eluent was added, thoroughly mixed and cultured at room temperature for 5 min. It was then transferred to a new sterile test tube for nucleic acid extraction.[22]

DNA extraction and PCR

We extracted MTB genomic DNA (gDNA) directly from CSF samples using a previously reported method.[23] A Qubit V.4.0 fluorometer (Life Technologies, USA) was used for quality control and a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) was used to assess the purity of nucleic acid. Based on the instructions provided by ShengTing Bioinformatics Institute, we prepared PCR mixtures comprising 20 ng of gDNA extract, 5 µL forward and 10 µL reverse primers and 15 µL LongAmp Taq 2×Master mix. The mixture was added to the PCR instrument for cycling, and the temperature and time were maintained according to the PCR operation procedure. After finishing the purification and quality control, 100 ng of the final prepared library was used for sequencing.

Nanopore sequencing and data analysis

The prepared DNA library was placed into an R V.9.4 flow cell (ONT) instrument. After the completion of sequencing, we used MinKnow V.3.6.5 software for data analysis. All raw data were quality filtered using an in-house programme to remove sequenced fragments <200 bp and host DNA, and the remaining reads were compared with reference values obtained from the National Center for Biotechnology Information (NCBI) database (ftp://ftp.ncbi.nlm.nih.gov/genomes/). The NCBI reference sequence number of MTB is NC_00962.3. Owing to the intracellular nature of MTB and low likelihood of CSF contamination, a positive result is considered when at least one read is detected for either species or genus, confirming the diagnosis of TBM.

Statistical analysis

The Statistical Package for the Social Sciences V.25.0 software (IBM) was used for statistical analysis. To assess diagnostic accuracy, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different methods were calculated using MedCalc Statistical V.15.2.2 software (MedCalc Software Bvba, Ostend, Belgium; http://www.medcalc.org). The final clinical diagnosis was considered as the reference diagnostic criteria. McNemar and χ^2 tests were used to evaluate and compare the diagnostic performance of nanopore sequencing for TBM with that of culture and MTB/RIF Xpert assay. Venn diagrams were drawn to show the internal connection between the three methods (http://www.xiantaozi.com/literatures). P values of <0.05 were considered statistically significant.

RESULTS

Clinical characteristics of the participants

After screening 73 patients, 9 with incomplete data were excluded and 64 patients with complete clinical data were enrolled. The average age of the participants was 47±19 years, and 39 (60.94%) were male. All patients tested negative for blood HIV antibody tests, whereas 53 (82.81%) patients tested positive for T-SPOT.TB assay. Based on the international standards,[21] 45 patients were diagnosed with TBM, including 18 definite and 27 probable cases. The remaining 19 patients were not diagnosed

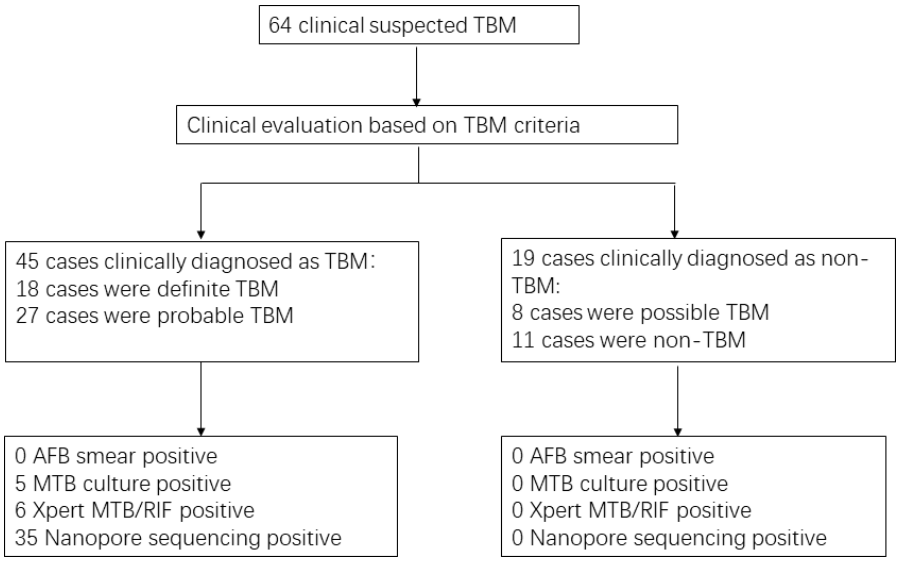

Figure 1 Flow diagram presenting the classification of patients in the present study. AFB, acid-fast bacillus; MTB, *Mycobacterium tuberculosis;* RIF, rifampicin; TBM, tuberculous meningitis.

with TBM, including 8 possible and 11 non-TBM cases (figure 1). The reads of nanopore sequencing samples in patients with and without TBM diagnosis were 0–24509 (mean, 1594) and 0, respectively.

Diagnostic accuracy of nanopore sequencing, AFB smear, culture and Xpert MTB/RIF assays for the detection of TBM

The CSF AFB smear test was negative for all patients. Using the clinical diagnosis as the reference standard, the sensitivity, specificity, PPV and NPV for nanopore sequencing were 77.78% (95% CI 62.91% to 88.80%), 100% (95% CI 82.35% to 100.0%), 100% (95% CI 90.00% to 100.0%) and 65.52% (95% CI 45.67% to 82.06%), respectively. The corresponding values for culture were 11.11% (95% CI 3.71% to 24.05%), 100% (95% CI 82.35%% to 100.0%), 100% (95% CI 47.82% to 100.0%) and 32.20% (95% CI 20.62% to 45.64%), respectively. Further, corresponding values for Xpert MTB/RIF were 13.33% (95% CI 5.05% to 26.79%), 100% (95% CI 82.35%% to 100.0%), 100% (95% CI 54.07% to 100%) and 32.76% (95% CI 21.01% to 46.34%), respectively. Table 1 summarises the diagnostic accuracy of the three tests. Figure 2 shows the Venn diagram of positive tests compared with the composite reference standard.

Our data suggested that the nanopore sequencing method exhibited the highest sensitivity and NPV for presumptive TBM cases among the tested approaches ($p<0.05$). Among these tests, the accuracy of culture was similar to that of Xpert MTB/RIF and the AFB smear was the least sensitive approach for the detection of presumptive TBM ($p>0.05$).

DISCUSSION

TBM is caused by the invasion of the CNS by MTB. TBM is recognised as the most devastating form of extrapulmonary TB, accounting for deaths and disabilities in approximately half of the TBM cases owing to the difficulties in its early diagnosis.[24 25] As CSF is a sterile body fluid, conventional testing methods yield a relatively low detection rate for pathogenic micro-organisms.[26 27] In this study, no positive result was observed in the CSF AFB smear, and the sensitivity of culture was only 11.11%. A previous study reported that AFB smear and culture have approximately 10% positivity rate in patients with TBM, which is in accordance with our findings.[28] The low positive test result rate may be attributed to the low absolute MTB numbers in CSF, limited volume of samples for testing, and influence of sample preparation and interpretation methods on smear test results. This considerably impacts the diagnosis of patients' infection and delays the provision of early and effective ATT. AFB smear, the most widely used clinical test, is ineffective in diagnosing patients with presumptive TBM. The major disadvantage of culture is its low bacterial detection rate

Table 1 Accuracy of MTB culture, Xpert MTB/RIF and nanopore sequencing for TBM diagnosis

Test	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)
MTB culture	11.11 (3.71 to 24.05)	100 (82.35 to 100.00)	100 (47.82 to 100.00)	32.20 (20.62 to 45.64)
Xpert MTB/RIF	13.33 (5.05 to 26.79)	100 (82.35 to 100.00)	100 (54.07 to 100.00)	32.76 (21.01 to 46.34)
Nanopore sequencing	77.78 (62.91 to 88.80)	100 (82.35 to 100.00)	100 (90.00 to 100.00)	65.52 (45.67 to 82.06)

AFB, acid-fast bacillus; MTB, *Mycobacterium tuberculosis*; NPV, negative predictive value; PPV, positive predictive value; RIF, rifampicin.

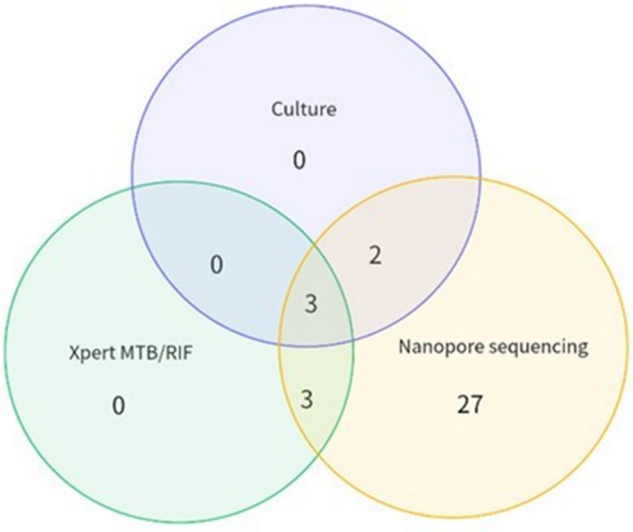

Figure 2 Venn diagram of positive tests compared with the composite reference standard. MTB, *Mycobacterium tuberculosis;* RIF, rifampicin.

and approximately 2-week waiting period before results are obtained. This delay in acquiring results can be fatal for patients with this dangerous and serious disease, especially in cases of the CNS MTB infection. Owing to these issues, both AFB smear and culture testing are not conducive to the early diagnosis and treatment of patients with TBM.

With the introduction of Xpert MTB/RIF in 2010, WHO approved its use for the clinical detection of TBM in countries where TB is endemic. This test is a fully automated assay and can provide results within 2 hours, improving the time-consuming nature of culture, is the fastest detection method. In this study, the sensitivity and NPV of Xpert MTB/RIF assay were 13.33% and 32.76%, respectively. These values are much higher than those of the AFB smear and culture test. Previous studies[29 30] have reported that the sensitivity of this assay for sputum samples is approximately 60%, whereas for CSF samples, positivity rates vary from 14.2% to 78.6%, and this value is influenced by CSF volume, CSF glucose level and decision to perform centrifugation.[31–33] The detection values reported in this study are lower than those reported in other studies. This may be attributed to the fact that after CSF samples were simultaneously sent for several other tests, only 1–2 mL sample remained for performing Xpert MTB/RIF assay; therefore, an additional centrifugation step was not performed. We found that the diagnostic efficiency of both Xpert MTB/RIF and culture was low, and they did not significantly predict MTB infection (0.5<AUC<0.7). Therefore, the use of a new detection method is warranted.

Over the last decade, metagenomic NGS (mNGS) technology has been introduced to detect pathogens with complete DNA content as a highly sensitive technology using various types of specimens such as blood, urine, CSF and sputum.[16 34 35] This technique is not dependent on clinical culture strains and can provide results within 1 day after sending the specimen, although the detection time is longer than Xpert assay, but it can detect all pathogenic micro-organisms contained in the sample, which is of better diagnostic value for patient with mixed intracranial infection. The third-generation genetic testing technology (nanopore sequencing) has been widely used in clinical practice, owing to its advantage of real-time analysis alongside long sequencing reads, low error rates, high detection rates, leading to fast and effective diagnosis and provision of timely targeted treatment.[36] The study by Yu *et al* evaluated the diagnostic accuracy of nanopore sequencing for pulmonary tuberculosis (PTB) using respiratory specimens, the sensitivity and specificity were 94.8% and 97.9%, respectively, which proved that this assay was useful for early diagnosis of PTB.[23] When the nanopore sequencing was applied to CSF samples for the diagnosis of CNS infections, 76% were positive and the sensitivity was obviously higher than that of microbial culture.[37] Our study using fresh CSF samples showed that the sensitivity and NPV values were 77.78% and 65.52%, respectively, which were higher than those of mNGS technology for presumptive TBM.[38 39] Meanwhile, nanopore sequencing had better diagnostic performance than other traditional test methods (p<0.001), indicating its potential for use as an effective diagnostic technique for the early diagnosis of TBM. The specificity and PPV of nanopore sequencing were 100.0%; therefore, it can help prevent the improper use of TB drugs. Nanopore sequencing technology yielded MTB-positive results for 29 CSF samples, whereas other tests produced negative results for all samples. It is pertinent to note that all patients enrolled in the study were eventually diagnosed with TBM. Therefore, nanopore technology can greatly increase the positive rate, improve early diagnosis, and aid in providing timely and effective treatment for TBM.

We observed that the nanopore sequencing method can effectively identify infectious species in samples obtained from patients with CNS infectious diseases, especially when other tests fail to detect the causative bacteria.[40] For rare or new pathogenic bacteria, this technology can help in providing anti-infection therapy.[41] We found that two patients enrolled in our study were infected with Enterobacteriaceae and two with Cryptococcus in the non-TBM group. After the administration of anti-infective treatment, these four patients did not have any sequelae.

CONCLUSION

The nanopore sequencing test had the highest diagnostic efficacy for the diagnosis of early-stage TBM among AFB smear, culture and Xpert MTB/RIF. We speculate that CSF nanopore sequencing should be recommended in clinical practice as a highly effective diagnostic approach for differential diagnosis of encephalitis and/or meningitis.

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Contributors LZ is the guarantor and primary contributor to the content within this manuscript. LZ and QH designed the study. LZ, XZ, YY and QH collected the data. XZ and YY were involved in data cleaning, mortality follow-up and verification. XZ and QH analysed the data. LZ drafted the manuscript. LZ and QH contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript. LZ and QH obtained funding.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Zhejiang Chinese and Western Medicine Integrated Hospital (2023-YS-031). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi:10.5061/dryad.k98sf7mfq.

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REFERENCES

1 Méchaï F, Bouchaud O. Tuberculous meningitis: challenges in diagnosis and management. *Rev Neurol (Paris)* 2019;175:451–7.
2 Ahlawat S, Chaudhary R, Dangi M, *et al*. Advances in tuberculous meningitis diagnosis. *Expert Rev Mol Diagn* 2020;20:1229–41.
3 Wang Y-Y, Xie B. Progress on diagnosis of tuberculous meningitis. *Methods Mol Biol* 2018;1754:375–86.
4 Thwaites GE, Tran TH. Tuberculous meningitis: many questions, too few answers. *Lancet Neurol* 2005;4:160–70.
5 Stadelman AM, Ssebambulidde K, Buller A, *et al*. Cerebrospinal fluid AFB smear in adults with tuberculous meningitis: a systematic review and diagnostic test accuracy meta-analysis. *Tuberculosis (Edinb)* 2022;135:102230.
6 Balabanova Y, Drobniewski F, Nikolayevskyy V, *et al*. An integrated approach to rapid diagnosis of tuberculosis and multidrug resistance using liquid culture and molecular methods in Russia. *PLOS ONE* 2009;4.
7 Thwaites GE, Chau TTH, Farrar JJ. Improving the Bacteriological diagnosis of tuberculous meningitis. *J Clin Microbiol* 2004;42:378–9.
8 Marx GE, Chan ED. Tuberculous meningitis: diagnosis and treatment overview. *Tuberc Res Treat* 2011;2011.
9 Erdem H, Ozturk-Engin D, Elaldi N, *et al*. The Microbiological diagnosis of tuberculous meningitis: results of Haydarpasa-1 study. *Clin Microbiol Infect* 2014;20:600–8.
10 Bahr NC, Nuwagira E, Evans EE, *et al*. Diagnostic accuracy of Xpert MTB/RIF ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. *Lancet Infect Dis* 2018;18:68–75.
11 Kohli M, Schiller I, Dendukuri N, *et al*. Xpert(®) MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance. *Cochrane Database Syst Rev* 2018;8.
12 Huynh J, Donovan J, Phu NH, *et al*. Tuberculous meningitis: progress and remaining questions. *Lancet Neurol* 2022;21:450–64.
13 Seth-Smith HMB, Biggel M, Roloff T, *et al*. Transition from PCR-Ribotyping to whole genome sequencing based typing of clostridioides difficile. *Front Cell Infect Microbiol* 2021;11.
14 Sun W, Lu Z, Yan L. Clinical efficacy of metagenomic next-generation sequencing for rapid detection of mycobacterium tuberculosis in smear-negative Extrapulmonary specimens in a high tuberculosis burden area. *International Journal of Infectious Diseases* 2021;103:91–6.
15 Goldberg B, Sichtig H, Geyer C, *et al*. Making the leap from research laboratory to clinic: challenges and opportunities for next-generation sequencing in infectious disease diagnostics. *mBio* 2015;6.
16 Salzberg SL, Breitwieser FP, Kumar A, *et al*. Next-generation sequencing in Neuropathologic diagnosis of infections of the nervous system. *Neurol Neuroimmunol Neuroinflamm* 2016;3.
17 Smith C, Halse TA, Shea J, *et al*. Assessing nanopore sequencing for clinical diagnostics: a comparison of next-generation sequencing (NGS) methods for Mycobacterium tuberculosis. *J Clin Microbiol* 2020;59:e00583-20.
18 Sanderson ND, Street TL, Foster D, *et al*. Real-time analysis of nanopore-based metagenomic sequencing from infected orthopaedic devices. *BMC Genomics* 2018;19.
19 Hyeon J-Y, Li S, Mann DA, *et al*. Quasimetagenomics-based and real-time-sequencing-aided detection and subtyping of salmonella enterica from food samples. *Appl Environ Microbiol* 2018;84:e02340-17.
20 Gliddon HD, Frampton D, Munsamy V, *et al*. A rapid drug resistance genotyping Workflow for Mycobacterium tuberculosis, using targeted isothermal amplification and nanopore sequencing. *Microbiol Spectr* 2021;9:e00610-21.
21 Marais S, Thwaites G, Schoeman JF, *et al*. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis* 2010;10:803–12.
22 Miller S, Naccache SN, Samayoa E, *et al*. Laboratory validation of a clinical Metagenomic sequencing assay for pathogen Detectionin cerebrospinal fluid. *Genome Res* 2019;29:831–42.
23 Yu G, Shen Y, Zhong F, *et al*. Diagnostic accuracy of nanopore sequencing using respiratory specimens in the diagnosis of pulmonary tuberculosis. *Int J Infect Dis* 2022;122:237–43.
24 Thuong NTT, Heemskerk D, Tram TTB, *et al*. Leukotriene A4 hydrolase genotype and HIV infection influence intracerebral inflammation and survival from tuberculous meningitis. *J Infect Dis* 2017;215:1020–8.
25 Thwaites GE, van Toorn R, Schoeman J. Tuberculous meningitis: more questions, still too few answers. *Lancet Neurol* 2013;12:999–1010.
26 Mai NTH, Thwaites GE. Recent advances in the diagnosis and management of tuberculous meningitis. *Curr Opin Infect Dis* 2017;30:123–8.
27 Schaller MA, Wicke F, Foerch C, *et al*. Central nervous system tuberculosis: etiology, clinical manifestations and neuroradiological features. *Clin Neuroradiol* 2019;29:3–18.
28 Chen Y, Wang Y, Liu X, *et al*. Comparative diagnostic utility of metagenomic next-generation sequencing, genexpert, modified ziehl-neelsen staining, and culture using cerebrospinal fluid for tuberculous meningitis: a multi-center, retrospective study in China. *J Clin Lab Anal* 2022;36.
29 Nhu NTQ, Heemskerk D, Thu DDA, *et al*. Evaluation of genexpert MTB/RIF for diagnosis of tuberculous meningitis. *J Clin Microbiol* 2014;52:226–33.
30 Allahyartorkaman M, Mirsaeidi M, Hamzehloo G, *et al*. Low diagnostic accuracy of Xpert MTB/RIF assay for extrapulmonary tuberculosis: a multicenter surveillance. *Sci Rep* 2019;9.
31 Rufai SB, Singh A, Singh J, *et al*. Diagnostic usefulness of Xpert MTB/RIF assay for detection of tuberculous meningitis using cerebrospinal fluid. *Journal of Infection* 2017;75:125–31.
32 Patel VB, Theron G, Lenders L, *et al*. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous meningitis in a high burden setting: a prospective study. *PLOS Med* 2013;10.
33 Sun L, Yao L, Fu G, *et al*. A comparison of the accuracy of the Capitalbio Mycobacterium real-time polymerase chain reaction and the Xpert MTB/RIF assay for the diagnosis of tuberculous meningitis. *Int J Infect Dis* 2021;104:92–6.
34 Miao Q, Ma Y, Wang Q, *et al*. Microbiological diagnostic performance of Metagenomic next-generation sequencing when applied to clinical practice. *Clin Infect Dis* 2018;67:S231–40.
35 Liu Q, Liu X, Hu B, *et al*. n.d. Diagnostic performance and clinical impact of blood metagenomic next-generation sequencing in ICU patients suspected Monomicrobial and Polymicrobial bloodstream infections. *Front Cell Infect Microbiol*13:1192931.
36 Dippenaar A, Goossens SN, Grobbelaar M, *et al*. Nanopore sequencing for mycobacterium tuberculosis: a critical review of the literature, new developments, and future opportunities. *J Clin Microbiol* 2022;60:e00646-21.

37 Fu Y, Gu J, Chen L-J, *et al*. A prospective study of nanopore-targeted sequencing in the diagnosis of central nervous system infections. *Microbiol Spectr* 2024;12:e03317-23.
38 Yu G, Wang X, Zhu P, *et al*. Comparison of the efficacy of metagenomic next-generation sequencing and Xpert MTB/RIF in the diagnosis of tuberculous meningitis. *J Microbiol Methods* 2021;180:106124.
39 Lin A, Cheng B, Han X, *et al*. Value of next-generation sequencing in early diagnosis of patients with tuberculous meningitis. *J Neurol Sci* 2021;422:117310.
40 Horiba K, Torii Y, Aizawa Y, *et al*. Performance of nanopore and Illumina Metagenomic sequencing for pathogen detection and transcriptome analysis in infantile central nervous system infections. *Open Forum Infect Dis* 2022;9:ofac504.
41 Chen SC-A, Korman TM, Slavin MA, *et al*. Antifungal therapy and management of complications of cryptococcosis due to cryptococcus Gattii. *Clin Infect Dis* 2013;57:543–51.