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ORIGINAL RESEARCH

Clinical Value of Metagenomic Next-Generation Sequencing Using Spinal Tissue in the Rapid Diagnosis of Spinal Tuberculosis

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Purpose: To evaluate the accuracy of metagenomic next-generation sequencing (mNGS) for rapid diagnosis of spinal tuberculosis using spinal tissue specimens.

Methods: Medical data regarding suspected spinal tuberculosis were retrospectively analyzed. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) of mNGS were calculated to determine its diagnostic efficacy, and these values were compared with culture and composite reference standard (CRS).

Results: Two hundred and three cases of spinal tuberculosis were included for analysis. The sensitivity, specificity, PPV, NPV, and AUC of mNGS compared with culture were all very good. When CRS was used for the comparison, the sensitivity of mNGS and culture were 71.2% and 73.0%; the specificity and PPV were 100% in all cases; the NPV were 74.2% and 75.4%; the AUCs were all 0.86. The sensitivity and NPV of culture were slightly higher than that of mNGS; however, the diagnostic efficacy of mNGS and culture was consistent ($P > 0.05$).

Conclusion: Spinal tissue specimens for mNGS testing had very good accuracy for diagnosing spinal tuberculosis.

Keywords: spinal tuberculosis, culture, mNGS, molecular test, diagnostic accuracy

Introduction

Mycobacterium tuberculosis (MTB) infection in humans leading to tuberculosis (TB), which is an ancient infectious disease that threatens public health.[1] TB can be categorized as extrapulmonary TB (EPTB) or pulmonary TB according to the status of MTB infection in the lungs.[2] Notably, EPTB is the collective term for all types of organ TB other than PTB, and TB of the osteoarticular system is a common occurrence in EPTB.[3] Approximately 50% of osteoarticular TB manifests as spinal TB, which is the most common form of osteoarticular TB in clinical practice.[4] Notably, the spine is an important part of the skeletal system, and spinal lesions can lead to the instability of the skeletal system, resulting in deformities.[5] Moreover, invasion or compression of the spinal cord can lead to paralysis.[6] Furthermore, spinal lesions generally require bed rest, severely affect the sufferers' life quality, and can lead to various other adverse consequences, especially in older adults.[7] In particular, spinal TB is easily misdiagnosed because of the deeper location of the lesions and the nonspecific nature of the symptoms.[8] Further, treatment delays lead to a higher incidence of adverse events. Therefore, there is an urgent clinical need to improve the early diagnosis of spinal TB; however, modalities for effective rapid diagnosis remain underdeveloped.

Despite the ease of use of the acid-fast bacilli (AFB) smear—the traditional approach for rapidly diagnosing TB—it has limited sensitivity and is ineffective in rapidly diagnosing spinal TB, particularly in the absence of an experienced microscopist.[9,10] In addition, MTB culture is the pathogenic microbiological reference for the diagnosis of TB, but the result usually takes several weeks to obtain; therefore, MTB culture is not suitable for the rapid diagnostic needs of TB.[11]

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Molecular biology diagnostic techniques are increasingly playing a role in the field of rapidly diagnosing TB, greatly improving the ability to rapidly diagnose TB cases.[12,13] Metagenomic next-generation sequencing (mNGS) is a molecular diagnostic technology that can simultaneously detect the DNA of thousands of pathogenic microorganisms.[14] In addition, mNGS can detect the genomic information on pathogenic microorganisms in various clinical specimens,[14,15] providing a strong basis for etiological diagnosis. With its ability to comprehensively analyze all genetic material present in clinical samples, mNGS has been shown to accurately diagnose TB in a variety of contexts, including EPTB. Its widespread use in TB research highlights the importance of this innovative technology in improving the accuracy and efficiency of TB diagnosis.[16,17] The clinical use of mNGS in the diagnosis of osteoarticular TB is limited.[18] Previous studies suggested that mNGS has good diagnostic efficacy in bone and joint TB.[18,19] However, the number of relevant studies determining its diagnostic efficacy in spinal TB is few,[19] and no research available at this time has examined the diagnosis of spinal TB using spinal tissue specimens for mNGS detection. Therefore, the aim of this study was to evaluate the diagnostic accuracy of rapid diagnosis of spinal TB when using spinal tissue specimens for mNGS and to provide more efficient information for patients and clinics.

Materials and Methods

Participants

Patients with suspected spinal TB who were hospitalized at our center between February 2019 and January 2022 were screened for inclusion in this study. Patients presenting with symptoms of TB-related toxicity, those with visible spinal lesions on imaging, those with positive TB-related immunological tests, and those with concomitant pulmonary TB or other EPTB were suspected of having spinal TB. Patient-related data and information were extracted from an electronic medical record system.

Index Test

The index test was mNGS.

Comparator Test

MTB culture was used as the comparator test.

Outcomes

The study outcomes were the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) of mNGS.

Study Design

A retrospective diagnostic accuracy test was conducted at Zhejiang TB Diagnostic and Treatment Centre—a regional TB diagnosis and treatment center—to assess the diagnostic validity of rapid diagnosis of spinal TB when using spinal tissue for mNGS.

Target Conditions

The study included patients in whom mNGS and culture were performed using their spinal tissue specimens. Patients without relevant test results or lacking relevant clinical information were excluded from the study. Spinal tissue specimens were collected by vertebral puncture or surgery. Fresh specimens obtained were used for MTB culture and mNGS.

Patients or their families signed the informed consent form. This study complies with the Declaration of Helsinki. The study protocol was approved by the Human Research Ethics Committee of the Affiliated Hangzhou Chest Hospital, Zhejiang University School of Medicine (2023-YANSHEN-050).

Reference Standards

Both MTB culture and composite reference standard (CRS) were used as diagnostic reference standards. CRS includes several indicators for the diagnosis of spinal TB. These indicators are related to symptoms associated with TB, changes

observed in imaging studies pertaining to spinal TB, immunological markers associated with TB, AFB smear, cultures, MTB molecular test results, and the effectiveness of anti-TB medications. Based on the comparison with CRS, the patients were classified into two groups. Patients with typical symptoms of TB poisoning, those with spine computed tomography and/or magnetic resonance imaging scans suggestive of TB, those with positive TB immunoassay results, those with positive AFB smear or culture in spine specimens, those with positive results for other TB molecular tests (such as geneXpert MTB/RIF), those with combined pulmonary TB or other EPTB, and those who received effective anti-TB treatment were diagnosed with spinal TB. Patients with negative AFB smear and culture, those with no evidence of TB infection, those without other causes of spinal lesions, and those who received ineffective anti-TB treatment were diagnosed with nonspinal TB.

MTB Culture

Fresh spinal tissue specimens were obtained for the liquid culture. Further, the spinal tissue specimens were processed for use by first slicing the tissue specimens and then grinding them and adding N-acetyl-L-cysteine–NaOH. Took 0.1 mL of the processed specimen and inoculated it aseptically into liquid medium. The inoculated medium was incubated at 37°C in a constant temperature cabinet and was considered positive and negative based on whether MTB growth was present or absent, respectively, after >6 weeks. The procedure for the use of the media was based on the relevant product instructions.

mNGS

Spinal tissue specimens were processed for use by first slicing the tissue specimens and then grinding them and adding N-acetyl-L-cysteine–NaOH. Subsequently, added 1 mL of the processed solution to an Eppendorf tube of 1.5 mL capacity, vigorous vortexed for 20 minutes, centrifuged at 8000 rpm for 1 minute (15). Further, 0.3 mL of the supernatant was removed for extraction of DNA with a TIANamp Micro DNA Kit (TIANGEN Biotech, Beijing, China) according to the product's instruction manual. Subsequently, terminal repair ligation, DNA fragmentation, and PCR amplification were used to construct DNA libraries. An Agilent 2100 Bioanalyzer system (Agilent Technologies, Santa Clara, CA, USA) was used for the DNA libraries quality control. Further, the qualified libraries after quality control were sequenced on a BGISEQ 50 platform (BGI-Tianjin, China). After removal of low quality sequences, short sequences and human host sequences (hg19), high quality sequences were obtained with the Burrows–Wheeler Alignment tool for computational subtraction. The matched data were further classified by aligning 4 microbial genetic databases (bacteria, viruses, parasites, and fungi genetic data; ftp://ftp.ncbi.nIm.nih.gov/genomes/). The reference sequences included the full genome sequences of 3446 bacterial genomes, 4152 viral taxa, 140 parasites and 206 fungi associated with human diseases. After the alignment, the list of suspected pathogenic microorganisms was reported through a comprehensive assessment of the number of strictly mapped reads and coverage rate. Notably, the turnaround time was approximately 3 days.[20]

Statistical Analysis

Microsoft Excel 2019 for storing the relevant patient information. True positive (TP), false positive (FP), false negative, and true negative were obtained by performing calculations in SPSS 24.0 (IBM Corp., Armonk, NY, USA). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) with 95% confidence interval (CI) of the relevant test to be evaluated in this study were obtained using the MedCalc statistical software v15.2.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org). Moreover, the receiver operating characteristic curves (ROCs) for the relevant tests were plotted using the MedCalc statistical software. Comparison of the differences between the two sets of paired data and proportions were done by McNemar's-test and the chi-square or Fisher's exact, respectively. Comparison of the differences between the two sets of AUCs was done by Z-test. The Venn diagram was drawn via an online tool (http://www.bioinformatics.com.cn/static/others/jvenn/example.html). $P < 0.05$ was considered a statistically significant difference between the two sets.

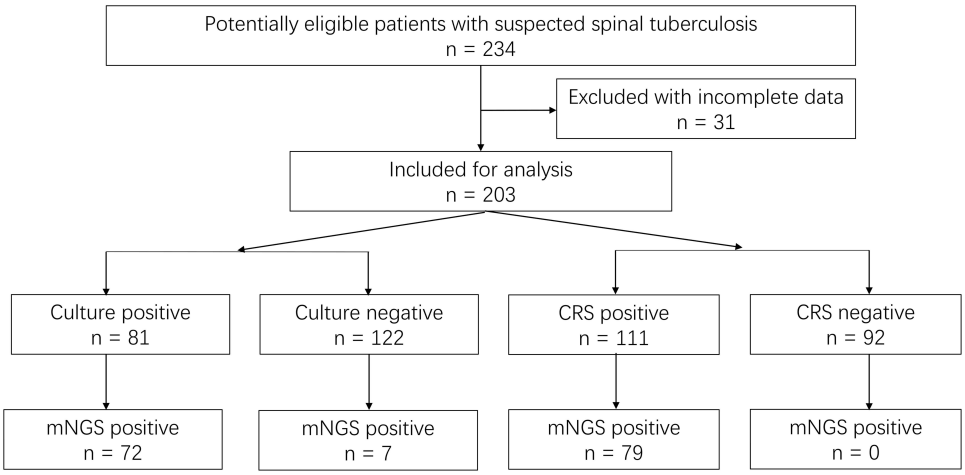

Figure 1 Diagnostic classification of the patients included in this study.
Abbreviations: CRS, composite reference standard; mNGS, metagenomic next generation sequencing.

Results

234 suspected spinal TB patients were included for initial screening, and 31 patients with missing relevant information (without the results of the relevant tests) were excluded; finally, a total of 203 patients were enrolled in this study (Figure 1). Of all patients, 111 were male and 92 were female. One tissue specimen was obtained from each patient; accordingly, 203 specimens were included. The mean age of all patients was 61.8 ± 14.8 (18–89) years. Moreover, 44 patients had concurrent pulmonary TB. The demographic and clinical characteristics of these included patients were shown in Table 1. Notably, only one patient had a positive AFB smear in the tissue specimen, and the remaining patients had a negative AFB smear. Additionally, 81 specimens with positive MTB culture results, and 79 specimens with positive mNGS results. Among the mNGS-positive specimens, the reads ranged from 1 to 4254, with a mean read of 177.4. Based on the CRS results, 111 included patients were considered to have spinal TB, and the remaining 92 were

Table 1 The Demographic and Clinical Characteristics of the Included Patients

Characteristics	All (203)	Spinal TB (111)	Non-Spinal TB (92)
Age year (Mean ± SD)	61.8 ± 14.8	59.4 ± 16.8	64.6 ± 11.4
Male (n, %)	111 (54.7)	60 (54.1)	51 (55.4)
Lesion site (n, %)			
Cervical spine	4 (1.9)	2 (1.8)	2 (2.2)
Thoracic spine	99 (48.8)	41 (36.9)	58 (63.0)
Lumbar spine	96 (47.3)	66 (59.5)	30 (32.6)
Sacral spine	4 (1.9)	2 (1.8)	2 (2.2)
Laboratory examinations			
WBC (*10^9/L, mean ± SD)	5.6 ± 1.9	5.4 ± 1.9	5.9 ± 2.1
RBC (*10^{12}/L, mean ± SD)	3.9 ± 0.6	4.1 ± 0.6	3.7 ± 0.6
Platelets (*10^{12}/L, mean ± SD)	241.7 ± 97.2	235.5 ± 101.6	254.2 ± 93.1
ESR (mm/h, mean ± SD)	58.5 ± 29.1	55.9 ± 29.4	61.7 ± 29.2

Abbreviations: TB, tuberculosis; SD, standard deviation; WBC, White blood cells; RBC, Red blood cells; ESR, erythrocyte sedimentation rate.

Table 2 Accuracy of Metagenomic Next Generation Sequencing for Spinal Tuberculosis Compared with Culture

Test		Culture		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
		Positive	Negative					
mNGS	Positive	72	7	88.9 (80.0–94.8)	94.3 (88.5–97.7)	91.1 (82.6–96.4)	92.7 (86.7–96.6)	0.92 (0.87–0.95)
	Negative	9	115					

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; mNGS, metagenomic next generation sequencing.

diagnosed with nonspinal TB. Eight patients with nonspinal TB were eventually diagnosed with nontuberculous mycobacterial (NTM) infections.

Diagnostic Accuracy of mNGS

When MTB culture was used as the diagnostic reference standard, the sensitivity, specificity, PPV, NPV, and AUC of tissue mNGS for spinal TB were shown in Table 2. The result shows that the tissue mNGS is very effective in diagnosing spinal TB. Figure 2 shows the ROC of mNGS for spinal TB using culture as the gold standard.

When CRS was as the diagnostic reference standard, the sensitivity, specificity, PPV, NPV, and AUC of culture and mNGS for spinal TB were presented in Table 3. The diagnostic accuracy of either culture or mNGS is satisfactory for spinal TB. Figure 3 shows the ROCs of these results. The sensitivity and NPV of culture were slightly higher than that of mNGS; however, the differences did not reach statistical significance ($P > 0.05$; Table 3). The overall diagnostic validity of the two tests was consistent, and there was the absence of statistical differences ($P > 0.05$; Table 3).

Discussion

Spinal TB can adversely affect spinal stability and cause serious consequences. Early and rapid diagnosis of such cases can guide precise treatment and thus lead to a better prognosis for patients. Rapid diagnosis of spinal TB is important but remains difficult to date.[21] The AFB smear has a weak ability to rapidly diagnose TB, including spinal TB.[22] In the present study, we identified only one specimen with a positive AFB smear, indicating that the AFB smear was almost

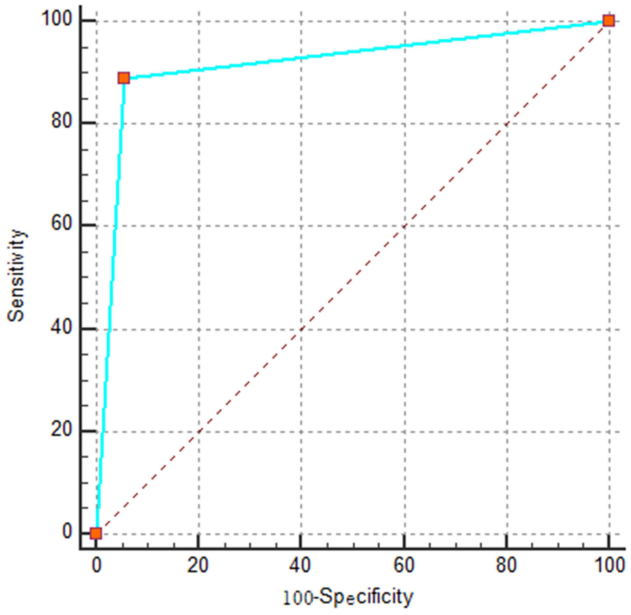

Figure 2 The receiver operating characteristic curve of metagenomic next generation sequencing for the diagnosis of spinal tuberculosis using culture as the reference standard.

Table 3 Accuracy of Metagenomic Next Generation Sequencing and Culture for Spinal Tuberculosis Compared with Composite Reference Standard

Test		CRS		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
		Positive	Negative					
mNGS	Positive	79	0	71.2 (61.8–79.4)	100.0 (96.1–100.0)	100.0 (95.4–100.0)	74.2 (65.5–81.6)	0.86 (0.80–0.90)
	Negative	32	92					
Culture	Positive	81	0	73.0 (63.7–81.0)[a]	100.0 (96.1–100.0)	100.0 (95.6–100.0)	75.4 (66.8–82.8)[a]	0.86 (0.81–0.91)[a]
	Negative	30	92					

Notes: Compared mNGS and culture, [a]P>0.05.
Abbreviations: CRS, composite reference standard; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; mNGS, metagenomic next generation sequencing.

ineffective for diagnosing spinal TB. Further, the results of the present and previous studies suggested that AFB smear was less effective for spinal TB;[23] however, the rate of positive AFB smear results in our study was lower than that in previous studies.[24] This might be attributed to the fact that this research included spinal tissue specimens and the MTB load in bony tissue specimens may have been lower. In contrast, bony tissues are more difficult to handle than soft tissues, and inadequate handling of such tissues might have affected the performance of the AFB smear. These results suggest that AFB smear should not be the method of choice for limited specimens.

MTB culture is the most classic diagnostic reference for TB; however, patients with TB may have negative culture, especially in cases of EPTB. Thus, CRS, which involves multiple evaluation indicators, is often used in TB diagnosis. In spinal TB, tissue specimen culture may be an inefficient gold standard and might lead to misclassification of the diagnosis.[25] With the use of culture as the diagnostic gold standard, the sensitivity may be overestimated, whereas the specificity may be underestimated. In contrast, CRS is a composite criterion that incorporates multiple factors associated with TB, including culture, and it helps determine the final diagnosis through a comprehensive assessment. Further, the CRS criterion helps redistribute FP and TP for the evaluation test, resulting in a decrease in sensitivity and an increase in specificity. Studies based on two reference standards may provide a more credible interval for the clinical sensitivity and specificity of the test.[26] Therefore, two reference standards were

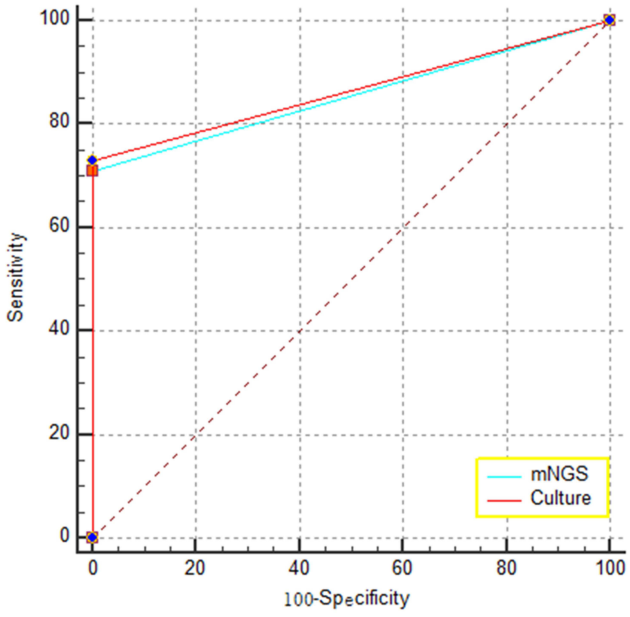

Figure 3 The receiver operating characteristic curves of metagenomic next generation sequencing and culture for the diagnosis of spinal tuberculosis compared with composite reference standard.
Abbreviation: mNGS, metagenomic next generation sequencing.

used in the present study, which are commonly used to diagnose EPTB and have been used in previous studies. In the present study, 81 specimens were MTB culture positive. With the use of CRS as the gold standard, the sensitivity, specificity, PPV, NPV, and AUC of culture for spinal TB were satisfactory. These results indicated that the effectiveness of culture for spinal TB remains very good and is essential. However, culture is not quickly available and usually takes several weeks—a time that is crucial and cannot to be ignored in patients with spinal TB, especially in those with a critical illness. Thus, while waiting for culture results, it is important to perform other rapid and effective diagnostic tests. Relying solely on culture results to diagnose TB may lead to underdiagnosis and misdiagnosis. Compared with AFB smear, culture has the advantage of differentiating between TB and NTM infections, although it is time consuming. In the present study, eight patients were eventually diagnosed with NTM infection by culture, suggesting that there is no substitute for culture for diagnosing *Mycobacterium* infections at present.

mNGS provides a new pathway for diagnosing spinal TB rapidly.[18] The findings of this study indicated that regardless of the gold standard used for comparison, mNGS demonstrated excellent diagnostic accuracy, suggesting that the role of mNGS for spinal TB is significant and not affected by the reference standard. Moreover, the diagnostic efficacy of mNGS and culture was consistent when CRS was the reference standard, suggesting that mNGS was valid for the diagnosis of spinal TB. In this study, out of the thirty-two patients who tested positive for CRS and negative for mNGS, nine of them were identified as culture-positive but still negative for mNGS. It is possible that this could be due to the fact that clinical specimens were used instead of strains, leading to a higher prevalence of false negatives. Additionally, even in culture-positive clinical specimens, there may be some negative ones due to the uneven distribution of MTB in the specimens, which could impact the sensitivity of any molecular test. Therefore, it is important to consider these limitations when interpreting the results of molecular tests in clinical specimens. mNGS is used more frequently in the diagnosis of PTB,[27] relatively less frequently in osteoarticular TB,[18] and even more rarely in spinal TB.[19] Notably, the accuracy of mNGS for PTB diagnosis was high.[28] Similar to PTB, previous studies had suggested that mNGS had good diagnostic performance in spinal TB.[19] These results were comparable to the findings of this study. Studies using spinal tissue alone for mNGS testing to diagnose spinal TB were unprecedented. Nonetheless, the results of this study may provide valid clinical implications. mNGS for mycobacterial infections can distinguish between TB and NTM infections in a way that some tests used to detect TB alone,[29] such as Xpert MTB/RIF, cannot. In the present study, eight patients were eventually diagnosed with NTM infection by culture. Moreover, mNGS detected NTM in five tissue specimens, suggesting that it might have good diagnostic accuracy for NTM infection; however, the limited number of specimens may make the results unreliable, and further confirmation is needed in studies with large specimens.

Inevitably, there were a number of shortcomings in this study. First, this study was retrospectively conducted at a regional TB diagnosis and treatment center in a TB endemic area, which might have resulted in biased patient screening, thereby rendering the study results of limited relevance to other areas with low TB burden. Second, when CRS was the diagnostic reference standard, the diagnostic factors could have been different for each patient, which may have additionally introduced bias.

Conclusion

The use of tissue specimens for mNGS testing has very good diagnostic accuracy in spinal TB. If conditions permit, mNGS might be the priority method for rapid diagnosis of spinal TB.

Data Sharing Statement

Relevant data can be requested from the corresponding author if there is a reasonable need.

Ethics Approval and Consent to Participate

Patients or their families signed the informed consent form. This study complies with the Declaration of Helsinki. The study protocol was approved by the Human Research Ethics Committee of the Affiliated Hangzhou Chest Hospital, Zhejiang University School of Medicine.

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Disclosure

The authors declare that there is no conflict of interest.

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