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Enhancing the Accuracy of Peripheral Pulmonary Tuberculosis Diagnosis: A Comparative Evaluation of CapitalBio™ *Mycobacterium* Nucleic Acid Detection Test and *Mycobacterium tuberculosis* Isothermal RNA Amplification Assay Using Endobronchial Ultrasonography with Guide Sheath

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Purpose: The diagnosis of tuberculosis located in the peripheral zone remains challenging and requires ultrasound bronchoscopy-guided maneuvers. We assessed the precision of CapitalBio™ *Mycobacterium* nucleic acid detection test (CapitalBio MTB test) and *Mycobacterium tuberculosis* isothermal RNA amplification test (MTB-RNA) using endobronchial ultrasonography with a guide sheath (EBUS-GS) for peripheral pulmonary tuberculosis (PTB) and compared it with those of acid-fast bacilli (AFB) smear and MTB culture tests.

Patients and Methods: This retrospective analysis included 287 patients suspected of peripheral pulmonary tuberculosis who underwent EBUS-GS examinations, medical examination results of AFB smears, MTB culture, CapitalBio MTB test, and MTB-RNA were analyzed. We evaluated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the receiver operating characteristic curve (AUC), and its diagnostic accuracy for peripheral PTB was evaluated in comparison with the final clinical diagnosis.

Results: The overall sensitivity, specificity, PPV, NPV, and AUC of CapitalBio MTB test were 44.83%, 100.00%, 100.00%, 54.07%, and 0.72, respectively; those of MTB-RNA were 22.99%, 100.00%, 100.00%, 45.75%, and 0.61, respectively, and those for parallel test (CapitalBio MTB test or MTB-RNA) were 46.55%, 100.00%, 100.00%, 54.85%, and 0.73, respectively. These values for AFB smear were 9.2%, 97.35%, 84.21%, 41.04%, and 0.53, respectively, and those of MTB culture were 31.03%, 100.00%, 100.00%, 48.50%, and 0.69, respectively.

Conclusion: The CapitalBio MTB test showed the best diagnostic performance compared with AFB smear, MTB culture, and MTB-RNA assays and was similar to the parallel test (CapitalBio MTB test or MTB-RNA). The CapitalBio MTB test combined with EBUS-GS had satisfactory diagnostic accuracy for diagnosing peripheral PTB.

Keywords: sensitivity, specificity, receiver operating characteristic curve, endobronchial ultrasonography, polymerase chain reaction, nucleic acid molecular testing

Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) that continues to pose a significant risk to human health.[1] TB is classified into two types: pulmonary TB (PTB) and extrapulmonary TB (EPTB), depending on the

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part of the body affected by the MTB infection.[2] PTB is responsible for almost 80% of all TB cases, and patients with PTB are the main source of TB transmission, thus, it is essential to diagnose and treat patients as early as possible.

However, in the very early stages of tuberculosis bacterial infection, if the infection is located in the peripheral zones of the lungs, which we call peripheral pulmonary tuberculosis (PTB), diagnosis is often difficult. Diagnosing peripheral PTB is particularly challenging compared with central lesions.[3]

Generally, test samples can be acquired through bronchoscopy or lung puncture biopsy. Bronchoscopy is the least invasive of the two methods and has a lower incidence of adverse reactions. However, lesions located in the periphery of the lungs have a significantly lower positive diagnostic rate because of the subpleural bronchioles' slenderness and the difficulty of bronchial penetration.[4] The use of radial endobronchial ultrasound has substantially improved the precision of bronchoscopy for peripheral pulmonary lesions, resulting in an overall diagnostic yield of 70.6%.[5–7] Endobronchial ultrasonography with guide sheath (EBUS-GS) is a tool that consists of a small circular ultrasound probe placed inside a guiding sheath. Medical professionals use it to reach Grade 8–9 bronchi. Once reached, the GS can establish pathways for ultrasound-guided biopsies, allowing direct access to lavage and biopsy samples.[8]

Advancements in nucleic acid testing have significantly improved timely and swift detection of TB, overcoming limitations of traditional diagnostic methods[9] that could impede the diagnostic process and impact the overall prognosis.[10,11] Several molecular detection methods are currently being studied for diagnosing TB, including the CapitalBio™ *Mycobacterium* nucleic acid detection test (CapitalBio MTB test) and the *Mycobacterium tuberculosis* isothermal RNA amplification test (MTB-RNA), are widely used in TB hospitals in China to diagnose TB rapidly.[12,13] The CapitalBio MTB test is a commercial test that uses real-time fluorescent polymerase chain reaction (PCR) and TaqMan probe technology. In contrast, MTB-RNA thermostatic amplification real-time detection technology uses thermostatic amplification of 16S rRNA as a target to generate a fluorescent signal to detect the presence of viable bacteria. Up to now, there are limited reports on certain methods for diagnosing peripheral PTB, particularly in obtaining bronchoalveolar lavage fluid (BALF) samples. It is unclear whether combining EBUS-GS with these methods is effective for evaluating peripheral PTB. Therefore, this study aims to determine the accuracy of these two methods in detecting nucleic acids in EBUS-GS BALF samples, providing healthcare professionals with further insights into the diagnosis of peripheral PTB.

Materials and Methods

Study Participants and Design

A retrospective analysis was conducted at the TB Diagnosis and Treatment Center at Zhejiang Chinese and Western Medicine Integrated Hospital. The clinical data of patients who underwent EBUS-GS operations and bronchial lavages between June 1, 2020 and November 30, 2023 were reviewed. All patients demonstrated a solitary nodular lesion measuring <3 cm in diameter, located in the outer region of the lungs. Because of its location, traditional bronchoscopy could not access the distant bronchial areas, making it necessary to use EBUS-GS to determine whether the patient had PTB. The doctor was fully informed the patients about the procedure and possible risks and signed written consent form about the EBUS-GS and bronchial lavage. This was a retrospective study, the study did not involve patient privacy or harm to patients, informed consent from participants was not required, and the Ethics Committee of Zhejiang Chinese and Western Medicine Integrated Hospital agreed to waive informed consent for patients (2024-YS-067).

We based our diagnosis on the People's Republic of China Health Industry Standard for PTB Diagnosis (WS 288–2017) (National Health Commission of the People's Republic of China. 2018). We used the composite reference standard (CRS) as the final diagnosis. PTB was considered confirmed when there was a positive result of MTB culture; or molecular biology test (such as CapitalBio MTB, MTB-RNA, Xpert MTB/RIF) positive and imaging results are consistent with PTB; or confirmation of pathological tissue changes consistent with TB. Probable PTB is indicated by a positive blood immunological test, characteristic PTB changes on chest CT images, and a positive response to anti-tuberculosis treatment. Non-PTB was diagnosed when there was no evidence of MTB infection and lung lesions resolved without anti-tuberculosis treatment. We considered both confirmed and probable PTB as peripheral PTB in this study.

Specimen Collection and Handling

BALF was collected from the peripheral lesion site in the lung using the EBUS-GS procedure. Fresh BALF samples were evenly distributed for AFB smear, MTB culture, CapitalBio MTB test, and MTB-RNA detection.

AFB Smear

A 1-mL BALF sample was dropped on a dry and clean glass slide. It was allowed to dry naturally and stained and rinsed with successive drops of carbolic acid and methylene blue. Finally, 300 different fields on the slide were observed using a microscope at 100× magnification. If more than one antacid bacillus was found, the result was reported as positive.

MTB Culture

We added 5 mL NaOH solution to an equal volume of BALF, mixed it thoroughly, and then centrifuged. The supernatant was discarded, and phosphate-buffered saline solution was added to the sediment. Subsequently, 0.5 mL of the mixture was transferred to a 960 liquid culture tube and incubated. The BACTEC MGIT 960 Mycobacteria Culture System automatically reports positive results based on the fluorescence signal.

CapitalBio^{TM} Mycobacterium Nucleic Acid Detection Test (CapitalBio MTB Test)

An equal amount of NaOH solution was added to 1 mL of BALF sample, centrifuged, and the supernatant was discarded. The nucleic acid extract was added to the precipitate, placed in a water bath at 95°C and incubated for 10 min, centrifuged again, and the supernatant was discarded. Nucleic acid DNA within the precipitate was extracted according to the manufacturer's instructions. RT-PCR was performed using a fluorescence quantification RT-PCR instrument (SLAN-96S Real-Time PCR System ZEESAN Xiamen CN) to amplify and detect IS6110 elements for MTB. A sample was considered positive when the amplification produced an S-curve and the Ct value was <40.

Mycobacterium Tuberculosis Isothermal RNA Amplification Assay (MTB-RNA)

One milliliter of the lavage specimen was added to an equal amount of 4% NaOH solution in a sterile tube and allowed to stand for 20 min before centrifugation. The supernatant was removed, and 1 mL of saline was added to the precipitate for washing, followed by another centrifugation. Subsequently, 50 μL of dilution solution was added to complete the sample preparation. The test samples and positive and negative control solutions were subjected to ultrasonication at 300W for 15 min and then added to the amplification detection solution. The kit was then placed in a fluorescence detection instrument with a constant temperature, where PCR amplification detection was performed using FAM as the fluorescein channel (RenDu Biotechnology Company, Shanghai, China). A positive result was indicated when the dt value was ≤35.

Statistical Analysis

Data were analyzed using SPSS, version 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables that followed a normal distribution were presented as means with standard deviations, whereas categorical data were analyzed using either the chi-squared test or Fisher's exact test. MedCalc Statistical (v15.2.2, MedCalc Software bvba, Ostend, Belgium) was used to determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC), with a 95% confidence interval for the tests being evaluated. The results were compared with that of the final clinical diagnosis. McNemar's test compared variances in paired data, and the Z-test compared different AUCs. The receiver operating characteristic (ROC) curve was drawn for each test, and a Venn diagram was created using an interactive tool (http://www.bioinformatics.com.cn/static/others/jvenn/example.html). A p-value <0.05 was considered statistically significant between the two groups.

Results

Participants' Clinical Characteristics

A retrospective study was conducted on 315 patients suspected of peripheral PTB. However, 28 patients without complete data were excluded, and finally, 287 patients and their clinical information and examination results were analyzed from June 1, 2020, to November 30, 2023. Each patient provided a sample of EBUS-GS operated BALF, and

AFB smear, culture, CapitalBio MTB test, and MTB-RNA test were completed. All patients were HIV-negative, the mean patient age was 51.14 ± 16.89 years, and 65.16% were male.

Based on the CRS, 70 specimens were confirmed PTB, 54 of which were CapitalBio MTB test positive and 40 were MTB-RNA positive. Probable PTB was identified in 104 specimens. Although 25 were CapitalBio MTB test positive, none were MTB-RNA positive. Further, 113 specimens were considered confirmed non-PTB, and no one was positive for the CapitalBio MTB test or MTB-RNA (Figure 1).

Among the 113 patients with non-PTB, 62 were finally diagnosed with bacterial pneumonia, 9 with fungal pneumonia, 1 with mycoplasma pneumonia, 9 with nontuberculous mycobacteria pulmonary disease, 17 with lung cancer, and 15 with other noninfectious lung lesions.

Diagnostic Accuracy of the AFB Smear, MTB Culture, CapitalBio MTB Test, MTB-RNA, and Parallel Tests

When the CRS was used as the reference standard, the sensitivity, specificity, PPV, NPV, and AUC of AFB smear for peripheral PTB were 9.20% (5.35–14.50%), 97.35% (92.44–99.45%), 84.21% (60.42–96.62%), 41.04% (35.10–47.19%), and 0.53 (0.47–0.59). Those of MTB culture were 31.03% (24.25–38.48%), 100.00% (96.79–100.00%), 100.00% (93.40–100.00%), 48.50% (41.92–55.11%), and 0.69 (0.62–0.76). Those of the CapitalBio MTB test were 44.83% (37.30–52.54%), 100.00% (96.79–100.00%), 100.00% (95.38–100.00%), 54.07% (47.06–60.96%), 0.72 (0.67–0.78). Those of the MTB-RNA test were 22.99% (16.96–29.96%), 100.00% (96.79–100.00%), 100.00% (91.91–100.00%), 45.75% (39.42–52.18%), 0.61 (0.56–0.67). Lastly, those of the parallel test (CapitalBio MTB test or MTB-RNA) were 46.55% (38.97–54.96%), 100.00% (96.79–100.00%), 100.00% (95.55–100.00%), 54.85% (47.79–61.78%), 0.73 (0.68–0.78). The detailed results are presented in Table 1.

The CapitalBio MTB test and MTB-RNA demonstrated higher precision than the AFB smear and MTB culture detection assays, with statistical significance ($P < 0.001$). Table 2 outlines the differences in diagnostic precision among the AFB smear, MTB culture, CapitalBio MTB test, and MTB-RNA. Figure 2 shows the ROC curves for the four tests, whereas Figure 3 presents a Venn diagram depicting the positive results from each test.

Evaluation of the Precision of the CapitalBio MTB Test, MTB-RNA, and Parallel Test (CapitalBio MTB test or MTB-RNA)

The results showed that the parallel test was significantly better than the MTB-RNA detection assay ($P < 0.001$, as shown in Table 2). Using the parallel and individual tests can improve the accuracy of diagnosis and significantly increase the diagnostic efficiency compared to the MTB-RNA test alone ($P < 0.05$). However, the difference between the CapitalBio

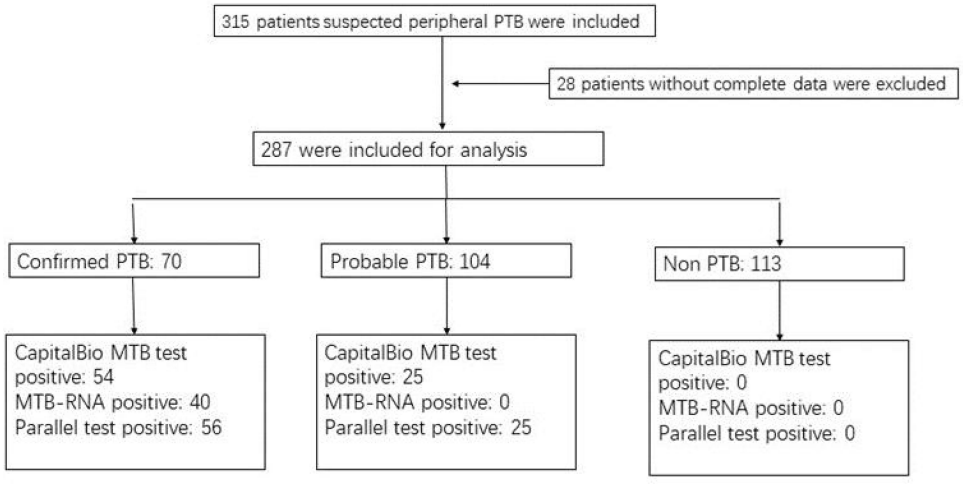

Figure 1 Diagnostic categorization of the participants in the research.

Table 1 Diagnostic Accuracy of AFB Smear, MTB Culture, CapitalBio MTB Test, MTB RNA and Parallel Test (CapitalBio MTB Test or MTB-RNA) for Rapid Diagnose Peripheral PTB Compared with a Composite Reference Standard

Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	AUC (95% CI)
AFB smear	9.20 (5.35–14.50)	97.35 (92.44–99.45)	84.21 (60.42–96.62)	41.04 (35.10–47.19)	0.53 (0.47–0.59)
MTB Culture	31.03 (24.25–38.48)	100.00 (96.79–100.00)	100.00 (93.40–100.00)	48.50 (41.92–55.11)	0.69 (0.62–0.76)
CapitalBio MTB test	44.83 (37.30–52.54)	100.00 (96.79–100.00)	100.00 (95.38–100.00)	54.07 (47.06–60.96)	0.72 (0.67–0.78)
MTB-RNA	22.99 (16.96–29.96)	100.00 (96.79–100.00)	100.00 (91.91–100.00)	45.75 (39.42–52.18)	0.61 (0.56–0.67)
Parallel test	46.55 (38.97–54.25)	100.00 (96.79–100.00)	100.00 (95.55–100.00)	54.85 (47.79–61.78)	0.73 (0.68–0.78)

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; AFB, acid-fast bacilli; MTB, *Mycobacterium tuberculosis*; CapitalBio MTB test, CapitalBio™ *Mycobacterium* nucleic acid detection test; MTB-RNA, Mycobacterium tuberculosis RNA.

Table 2 Compare the AFB Smear, MTB Culture, CapitalBio MTB Test, MTB-RNA and Parallel Test (CapitalBio MTB Test or MTB-RNA) for Rapid Diagnose Peripheral PTB

Test	Sensitivity (*P*-value)	Specificity (*P*-value)	PPV (*P*-value)	NPV (*P*-value)	AUC (*P*-value)
AFB smear vs MTB culture	<0.001	0.083	0.546	0.212	<0.001
CapitalBio MTB test vs AFB smear	<0.001	0.083	0.531	0.039	<0.001
CapitalBio MTB test vs MTB culture	<0.001	1.000	1.000	0.414	<0.001
MTB-RNA vs AFB smear	<0.001	0.083	0.561	0.418	<0.001
MTB-RNA vs MTB culture	0.0390	1.000	1.000	0.661	0.037
CapitalBio MTB test vs MTB-RNA	<0.001	1.000	1.000	0.2087	<0.001
MTB-RNA vs Parallel test	<0.001	1.000	1.000	0.1719	<0.001
CapitalBio MTB test vs Parallel test	0.0833	1.000	1.000	0.9135	0.0815

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; AFB, acid-fast bacilli; MTB, *Mycobacterium tuberculosis*; CapitalBio MTB test, CapitalBio™ *Mycobacterium* nucleic acid detection test; MTB-RNA, Mycobacterium tuberculosis RNA.

MTB and other tests was insignificant (P > 0.05; Table 2). Figure 4 shows the ROC curves for the CapitalBio MTB test, MTB-RNA and parallel test.

Discussion

Recently, the diagnosis and treatment of PTB have improved tremendously, and many patients are now able to actively seek medical treatment in the early stages of the disease, get a clear diagnosis of the disease as soon as possible, and initiate antituberculosis treatment (ATT), which is socially important for our country to actively and effectively control the spread of tuberculosis in the population. However, in patients with lesions present only in the peripheral lung bands or subpleura, it is difficult for conventional bronchoscopy to deeply penetrate into the distal bronchioles, leading to a significant decrease in the diagnostic rate. Therefore, we used EBUS-GS as an alternative approach to reach the distal end of the tracheal bundle to more efficiently diagnose peripheral PTB. This enables us to perform the lavage and biopsy with greater precision, resulting in optimal diagnostic performance with minimal patient trauma.[8]

Clinicians frequently use EBUS-GS because of its safety, effectiveness, and low bleeding risk, especially in the early diagnosis of lung cancer.[14,15] Yet, the effectiveness of EBUS-GS in evaluating solitary nodules resulting from MTB has not been thoroughly assessed. Sun et al discovered that the percentage of AFB smear positivity in BALF was just 35.2%, while the positivity rates for BALF culture and Xpert were 84.9% and 87.2%, respectively.[16] In our study, the sensitivity

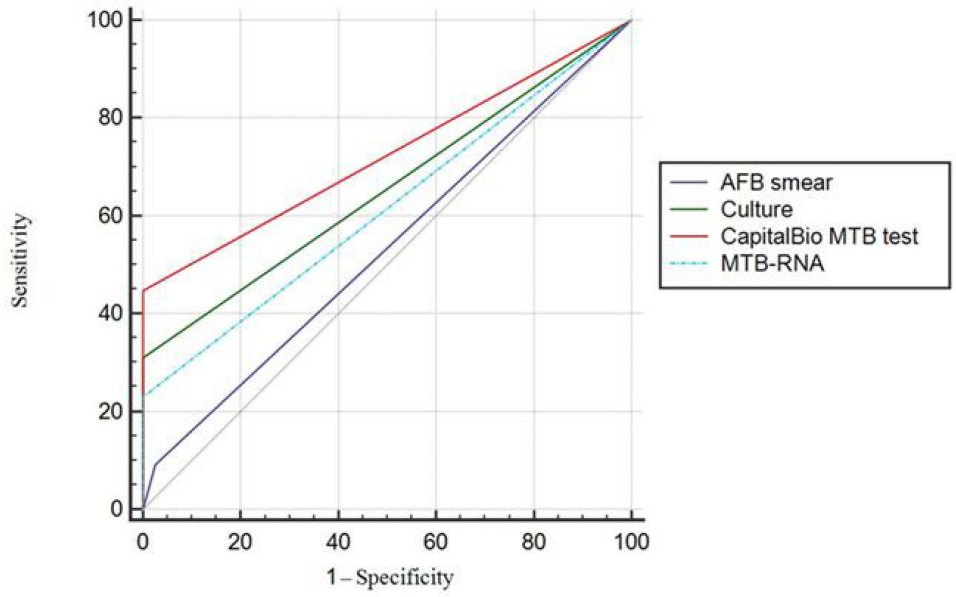

Figure 2 ROC curves for AFB smear, MTB culture, CapitalBio MTB test, and MTB-RNA tests.

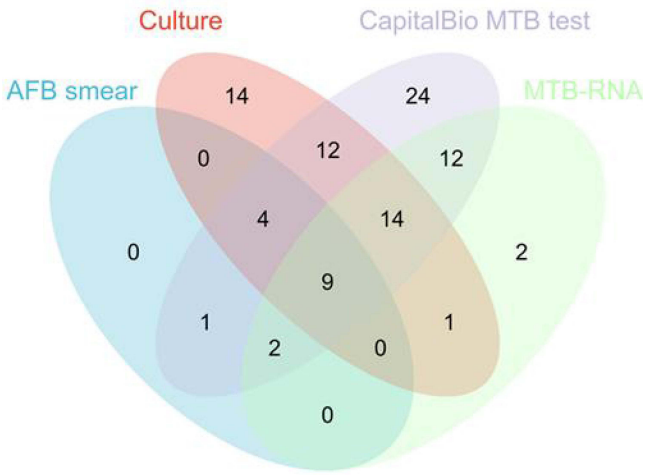

Figure 3 Venn diagram of the positive results from the four tests.

of AFB smear was only 9.2%, while that of culture was 31.03%. These lower values could be due to the small number of lung lesions in our patient sample, with many cases detected through physical examination screening in the early stages of the disease, whereas other studies were conducted in the middle or late stages. Additionally, after a few weeks, three false-positive samples of AFB smear were later confirmed to be nontuberculous mycobacteria by culture. Thus, conventional techniques for TB diagnosis, such as AFB smears and MTB culture are inadequate to meet clinical diagnostic needs.

Nucleic acid molecular testing offers a cost-effective, rapid, and accurate diagnostic method for identifying MTB. Cao et al reviewed past data and found that the accuracy of EBUS-GS combined with Xpert was notably better than BALF combined with Xpert in detecting isolated lung nodules.[5] A previous study showed that the CapitalBio MTB test had an 83.0% sensitivity for detecting TB, similar to the diagnostic precision of Xpert MTB/RIF (84.4%), costing only 1/9th as much as Xpert.[17] Bai et al found that the sensitivity and specificity of the tuberculosis bacillus DNA for BALF

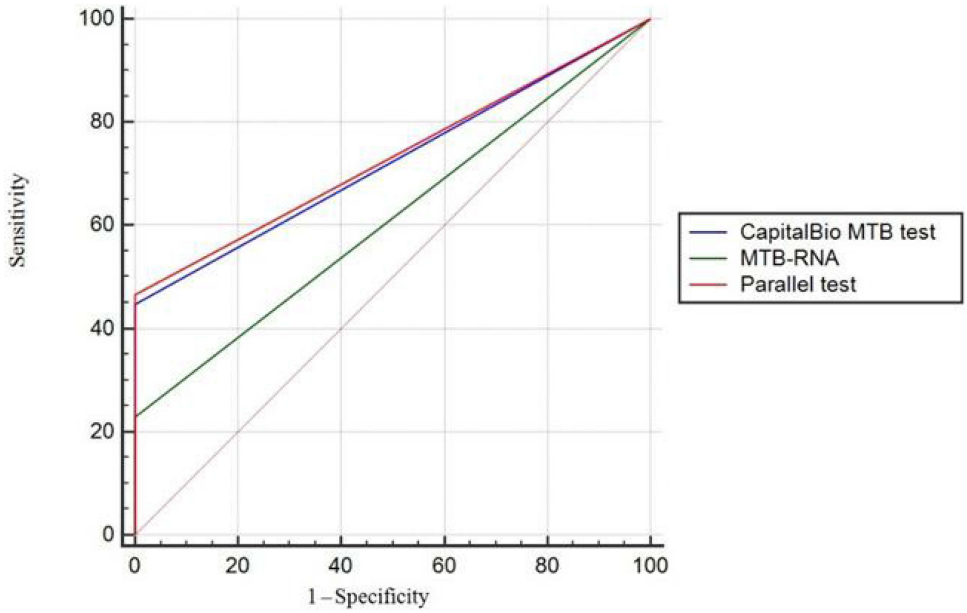

Figure 4 ROC curves of the CapitalBio MTB test, MTB-RNA tests, and parallel test.

were 67.7% and 98.9%, respectively, higher than our study findings of 44.73% sensitivity and 100.00% specificity.[18] However, Bai's research did not include imaging data, whereas our study focused on lesions in the peripheral lung bands, which are challenging to access with routine bronchoscopy.

The CapitalBio MTB test uses DNA nucleic acid testing techniques that allow the DNA nucleic acid to remain intact even after the bacteria have died and can even survive for several years. Thus, a positive test result does not necessarily indicate active PTB. In contrast, RNA is less stable than DNA, degrading within approximately one week, and exists only in living bacteria. Previous studies found that the MTB-RNA test had a sensitivity of 49.6% and 63.9% for sputum and BALF samples, respectively, and greatly increased positive rates of molecular diagnosis of PTB.[19] In this study, the MTB-RNA assay demonstrated a sensitivity of 22.99%, a specificity of 100.00%, and an AUC value of 0.61. The reason for this analysis is that the lung lesions in our study population were mild; therefore, it was not easy to obtain positive results. MTB-RNA has half the sensitivity of the CapitalBio MTB test, indicating a significant difference in diagnostic efficacy ($P < 0.001$). In our study, the diagnostic efficacy of the CapitalBio MTB test was not significantly different from the parallel test ($P > 0.05$). In clinical practice, the CapitalBio MTB test is preferred for the diagnosis of PTB, but in patients who have had PTB within the past 2–3 years and have been treated regularly, a combination of MTB-RNA test is required to determine the presence of viable MTB in order to decide whether or not to return to ATT.

This study has some limitations. First, patient selection may have been biased due to the constraints of a single retrospective study design. Second, the study was conducted in an area with a high prevalence of TB, with our department serving as a TB diagnostic facility in Zhejiang Province, where TB cases are concentrated. This may limit the generalizability of the results. Third, EBUS-GS requires a high level of technical skill, and lesions close to the subpleura may not be accessible or could result in puncture failure.

Conclusion

EBUS-GS is a method known for its precise localization and low complication rate. It is beneficial for diagnosing single nodular lesions in the outer bands of the lungs. When PTB is suspected in peripheral nodular lesions in the outer bands of the lungs, the CapitalBio MTB test is recommended in conjunction with this technique for the early diagnosis of PTB.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

Before the ultrasound bronchoscopy, the physician is fully informed about the necessity of the operating, the procedure, possible risk situations, and the patients and their family members give their informed consent and sign the consent form in writing. This is a retrospective study, patient information is confidential, and informed consent from participants is waived, these were approved by the ethical committee of the Zhejiang Chinese and Western Medicine Integrated Hospital (2024-YS-067). This study was conducted in accordance with the Declaration of Helsinki regarding ethical principles for research involving human samples.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare no conflicts of interest in this work.

References

1. Louie JK, Keh CE. Latent tuberculosis infection treatment: still a long road ahead. *Clin Infect Dis*. 2023;77(2):295–297. doi:10.1093/cid/ciad251
2. Goud KI, Kavitha M, Mahalakshmi A, et al. Molecular detection of Mycobacterium tuberculosis in pulmonary and extrapulmonary samples in a hospital-based study. *Afr Health Sci*. 2020;20(4):1617–1623. doi:10.4314/ahs.v20i4.14
3. Kim J, Lee IJ, Kim JH. CT findings of pulmonary tuberculosis and tuberculous pleurisy in diabetes mellitus patients. *Diagn Interv Radiol*. 2017;23(2):112–117. doi:10.5152/dir.2016.16157
4. Chavez C, Sasada S, Izumo T, et al. Endobronchial ultrasound with a guide sheath for small malignant pulmonary nodules: a retrospective comparison between central and peripheral locations. *J Thorac Dis*. 2015;7(4):596–602. doi:10.3978/j.issn.2072-1439.2015.03.04
5. Cao J, Gu Y, Wu XC, et al. EBUS-GS with the GeneXpert MTB/RIF assay for diagnosis of Mycobacterium tuberculosis infection of isolated pulmonary nodules. *Eur J Med Res*. 2023;28(1):370. doi:10.1186/s40001-023-01331-z
6. Zhang QC, Xuan WX, Li HL, et al. Diagnostic value of LungPoint navigation combined with EBUS-GS & ROSE in peripheral pulmonary nodules. *Indian J Med Res*. 2022;156(3):529–534. doi:10.4103/ijmr.IJMR_2114_19
7. Hong KS, Ahn H, Lee KH, et al. Radial probe endobronchial ultrasound using guide sheath-guided transbronchial lung biopsy in peripheral pulmonary lesions without fluoroscopy. *Tuberc Respir Dis*. 2021;84(4):282–290. doi:10.4046/trd.2021.0002
8. Bo L, Li C, Pan L, et al. Diagnosing a solitary pulmonary nodule using multiple bronchoscopic guided technologies: a prospective randomized study. *Lung Cancer*. 2019;129:48–54. doi:10.1016/j.lungcan.2019.01.006
9. 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. doi:10.1016/j.tube.2022.102230
10. Deng Z, Chapagain P, Leng F. Macromolecular crowding potently stimulates DNA supercoiling activity of Mycobacterium tuberculosis DNA gyrase. *J Biol Chem*. 2023;299(12):105439. doi:10.1016/j.jbc.2023.105439
11. Stephanie F, Tambunan USF, Siahaan TJM. tuberculosis transcription machinery: a review on the mycobacterial RNA polymerase and drug discovery efforts. *Life (Basel)*. 2022;12(11):1.
12. Zhang J, Bu C, Yao L, Xu K. Clinical application of the mycobacterium tuberculosis-RNA assay of pericardial tissue specimens in the diagnosis of tuberculous pericarditis. *Infect Drug Resist*. 2023;16:1875–1883. doi:10.2147/IDR.S405868

13. Yao L, Xu X, Chen G, Shen Y, Jiang W. Comparison of the accuracy of two different molecular tests for the diagnosis of tuberculous lymphadenitis using core needle biopsy specimens: a diagnostic accuracy study. *Int J Gen Med*. 2022;15:5237–5246. doi:10.2147/IJGM.S367127
14. Kajikawa S, Imai N, Okachi S, et al. Diagnostic contribution of cytological examination to endobronchial ultrasound-guided transbronchial biopsy for lung malignancies. *Nagoya J Med Sci*. 2019;81(4):613–620. doi:10.18999/nagjms.81.4.613
15. Souma T, Minezawa T, Yatsuya H, et al. Risk factors of infectious complications after endobronchial ultrasound-guided transbronchial biopsy. *Chest*. 2020;158(2):797–807. doi:10.1016/j.chest.2020.02.025
16. Sun Y, Zhang Q, Zhang Q, et al. Diagnostic Efficacy of Xpert MTB/RIF assay in bronchoalveolar lavage fluid for tracheobronchial tuberculosis: a retrospective analysis. *Front Med*. 2021;8:682107. doi:10.3389/fmed.2021.682107
17. Shen Y, Fang L, Xu X, Ye B, Yu G. CapitalBio Mycobacterium real-time polymerase chain reaction detection test: rapid diagnosis of Mycobacterium tuberculosis and nontuberculous mycobacterial infection. *Int J Infect Dis*. 2020;98:1–5. doi:10.1016/j.ijid.2020.06.042
18. Bai W, Liu L, Wu L, et al. Assessing the utility of the Xpert Mycobacterium tuberculosis/rifampin assay for analysis of bronchoalveolar lavage fluid in patients with suspected pulmonary tuberculosis. *J Clin Lab Anal*. 2022;36(1):e24154. doi:10.1002/jcla.24154
19. Qiu X, Zheng S, Yang J, Yu G, Ye Y. Comparing mycobacterium tuberculosis RNA accuracy in various respiratory specimens for the rapid diagnosis of pulmonary tuberculosis. *Infect Drug Resist*. 2022;15:4195–4202. doi:10.2147/IDR.S374826