Infection and Drug Resistance

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ORIGINAL RESEARCH

Superiority of TB-PCR Over Conventional and Immunologic Tests for Diagnosing Tuberculosis in Small Bronchoscopic Non-Malignant Specimens

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Background: In the small non-malignant specimens acquired through respiratory endoscopy, the conventional pathological examination approaches such as acid-fast staining have certain restrictions in the sensitivity of tuberculosis diagnosis.

Objective: To investigate the sensitizing effect and clinical value of polymerase chain reaction for Mycobacterium tuberculosis (TB-PCR) based on fluorescent probe nucleic acid detection technology in improving the diagnostic positive rate of non-malignant small specimens obtained by respiratory endoscopy.

Methods: A retrospective analysis was conducted on 729 patients with suspected TB who underwent respiratory endoscopy. All patients provided small non-malignant specimens for TB-PCR, acid-fast staining, and mycobacterial culture. A clinical composite diagnosis served as the gold standard. Diagnostic performance was assessed by accuracy, sensitivity, specificity, and area under the ROC curve (AUC). A subgroup of 113 patients underwent additional testing (T-SPOT. TB, TB-Ab, BALF-G-Xpert, BALF-TB) for extended comparison.

Results: The AUC, accuracy, sensitivity, specificity, PPV and NPV of TB-PCR in the diagnosis of TB were 0.88 (95% CI: 0.86–0.90), 0.88 (95% CI: 0.85–0.90), 0.99 (95% CI: 0.98–1.00), 0.78 (0.74–0.82), 0.79 (95% CI: 0.75–0.83), 0.99 (95% CI: 0.97–1.00), respectively. Among 729 patients (391 TB+, 338 TB-), TB-PCR showed significantly higher overall diagnostic efficacy (AUC: 0.88) than acid-fast staining (AUC: 0.77, $P<0.05$) and was comparable to culture (AUC: 0.87). TB-PCR also demonstrated superior accuracy (0.89 vs 0.61–0.85, $P<0.05$) compared to immunologic and BALF-based tests in the subgroup analysis, achieving nearly perfect sensitivity (0.99–1.00) and high NPV (0.99–1.00).

Conclusion: The application of TB-PCR for the detection of lung samples obtained through respiratory endoscopy holds significant clinical application value in the diagnosis of TB. Clinicians should fully recognize the merits and potential of TB-PCR technology, proactively apply it in clinical practice, and choose appropriate detection methods based on the specific conditions of patients.

Keywords: TB-PCR, tuberculosis, respiratory endoscopy, diagnostic value

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis (MTB) and has long been a major challenge to global public health.[1] According to statistics, the global prevalence of tuberculosis has remained high and shows an increasing tendency, particularly drug-resistant tuberculosis.[2] Tuberculosis can give rise to respiratory symptoms such as sputum and hemoptysis.[2] Severe cases can result in complications like massive hemoptysis, spontaneous pneumothorax, and others.[3] In addition, tuberculosis is somewhat contagious and can spread among people, causing damage to public safety.[4] Tuberculosis is the primary form of infectious tuberculosis. Its early diagnosis and treatment are of great significance in curbing the spread of the disease and improving the prognosis of patients. Acid-fast staining is a conventional microscopy approach in the diagnosis of tuberculosis.[5] It is predicated on the lipid content

Received: 5 August 2025
Accepted: 16 November 2025
Published: 10 December 2025

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within the cell wall of Mycobacterium tuberculosis, particularly mycobacterial acid, which renders it resistant to decolorization by acid dyes. Although this method has played an important role in the early diagnosis of TB in the past, it still has many diagnostic limitations such as low sensitivity and specificity, long time consuming, and limited detection range.[6] Therefore, it is of great significance to explore early, accurate, convenient and efficient new diagnostic means for TB transmission control and patient treatment.

The TB Polymerase Chain Reaction (TB-PCR) assay founded on fluorescent probe technology is a highly sensitive and highly specific molecular biological diagnostic approach that integrates the efficient amplification capacity of PCR with the real-time monitoring superiority of fluorescent probe technology and can be employed to detect MTB infection rapidly and precisely.[7] The TB-PCR assay not only can offer accurate detection outcomes within a few hours but also is capable of detecting extremely low concentrations of Mycobacterium tuberculosis DNA, demonstrating significant potential in the diagnosis of tuberculosis.[8] In addition, respiratory endoscopy techniques, such as bronchoscopic transbronchial lung biopsy (TBLB), ultrasonic bronchoscopy and thoracoscopy, have become important tools for the diagnosis of respiratory diseases.[9] These techniques can directly obtain diseased tissue or fluid samples in the respiratory tract, providing valuable materials for the etiological diagnosis of tuberculosis. Although the WHO has endorsed several diagnostic tests for tuberculosis, their comparative performance specifically in small, non-malignant biopsy specimens remains inadequately characterized.[10] Such specimens often yield low bacterial loads, posing a significant challenge for conventional tests like acid-fast staining and culture. Furthermore, while studies have evaluated these tests in sputum or bronchoalveolar lavage fluid, there is a paucity of head-to-head comparisons that include molecular tests like TB-PCR across different types of small tissue samples within the same patient cohort under a unified diagnostic standard.

Against this background, the purpose of this study was to investigate the clinical value of TB-PCR in the early diagnosis of TB, especially its sensitizing effect in the diagnosis of non-malignant small specimens based on respiratory endoscopy. To this end, a prospective database was constructed to collect clinical data from 729 patients with suspected TB who were admitted to Anhui Chest Hospital from March 2021 to March 2024. By comparing the diagnostic efficiency of TB-PCR, acid-fast staining and strain identification, this study is expected to clarify the advantages of TB-PCR in improving diagnostic sensitivity, specificity and predictive value, and provide a more reliable basis for the early diagnosis of tuberculosis. The results of this study are expected to provide clinicians with more accurate diagnostic tools to improve the early diagnosis and treatment of tuberculosis patients. In addition, the results of this study will also provide scientific basis for the optimization and improvement of tuberculosis diagnosis technology in the future.

Materials and Methods

Patient Population

This study conducted a retrospective analysis of 729 patients who came to Anhui Provincial Chest Hospital for examination and treatment with suspected tuberculosis symptoms between March 2021 and March 2024. We accessed, screened and statistically analysed the data from these patients in April 2024. All patients' test samples were obtained from small lung samples captured through respiratory endoscopy. After pathological observation of non-malignant results (including granuloma, necrosis, granulation tissue, multinucleated giant cells, epithelioid cells, etc), all patients were tested by TB-PCR, acid-fast staining, and strain identification. The study was approved by the Ethics Committee of Anhui Chest Hospital and was conducted in accordance with the ethical standards of the Declaration of Helsinki. Since the study was retrospective, the informed consent process was waived by the ethics committee. The authors did not have access to personally identifiable information of the participants during or after data collection.

Inclusion and Exclusion Criteria

All patients underwent rigorous screening. Their inclusion criteria are as follows: (a) the clinical manifestations and imaging findings were suspected of pulmonary tuberculosis; (b) did not receive anti-TB therapy prior to inclusion in the study; (c) based on the small sample of respiratory endoscopy, TB-PCR, acid-fast staining and strain identification were performed; (d) there are clear clinical comprehensive diagnosis results as the gold standard. The exclusion criteria are as follows: (a) patients with a history of tuberculosis that has been cured; (b) pathological examination indicated malignant

results; (c) patients with other lung diseases, such as pneumonia and lung cancer; (d) pregnant or nursing patients; (e) the comprehensive clinical diagnosis is unclear or absent.

Data Collection

The patient's diagnostic records were retrospectively retrieved from the hospital's electronic medical records system. All patients in this study underwent TB-PCR assay, acid-fast staining test and strain identification test. Firstly, the differences in TB diagnostic performance of these three detection methods were compared. Subsequently, to enable a broader comparison of diagnostic methods, we identified a subgroup of patients from the primary cohort who had undergone a more extensive panel of tests. Specifically, we retrospectively screened the medical record system to include patients who, in addition to the three core tests (TB-PCR, acid-fast staining, and strain identification), had also undergone both immunological tests (T-SPOT and TB-Ab) and BALF-based tests (BALF-G-Xpert and BALF-TB). The minimum criterion for inclusion in this sensitivity analysis was the availability of complete results from all seven aforementioned tests. This screening process identified 113 patients who met this criterion and constituted the subgroup for extended analysis.

Respiratory Endoscopic Sampling

In this study, all test samples were obtained based on respiratory endoscopy, including bronchoscopic biopsy, transbronchial lung biopsy (TBLB), endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and medical thoracoscopy (MT). Bronchoscopic Biopsy refers to the direct observation of the lesion by bronchoscopy and the use of biopsy forceps to pick up the lesion tissue for pathological examination. TBLB refers to the technique of inserting biopsy forceps into the lung parenchyma through bronchoscope under the guidance of X-ray fluoroscopy or CT to extract lung tissue for pathological examination. The EBUS-TBNA technology uses an ultrasound probe mounted at the front of the bronchoscope to observe ultrasound images of the trachea, bronchial wall and surrounding structures in real time, and perform accurate needle aspiration biopsy. The MT technique enables direct observation of the lesions on the pleura, lung surface, and mediastinum by inserting a thoracoscope into the chest through a small incision on the chest wall. Additionally, it allows for the performance of biopsy, lavage, and other operations. These four techniques are all important means commonly used in the detection of lung diseases, and each has its own unique advantages and scope of application.

TB-PCR Detection

Pathological tissue samples collected by respiratory endoscope were detected by TB-PCR. By specifically amplifying DNA fragments of Mycobacterium tuberculosis, TB-PCR technology combined with fluorescence detection technology can realize automatic and high-sensitivity detection of TB nucleic acid. The diathesis samples were ground using sterile instruments to expose the cell surface, and appropriate protease k was added to promote cell wall rupture and DNA release. The treated tissue samples were combined with the extraction solution, thoroughly decomposed and digested, followed by centrifugation and washing. Subsequently, the extracted DNA solution was collected into a clean centrifuge tube for quantitative and quality assessment. The PCR reaction solution was constituted with Taq enzyme, dNTPs, buffer, etc. Then, the PCR reaction solution, primer, probe, and DNA sample were mixed for amplification. The denaturation temperature was set at 95°C, the extension temperature was set at 72°C, and the number of cycles was 35. Subsequently, a fluorescent PCR detector was employed to detect the amplified products in real time. The test results were comprehensively evaluated by two clinical doctors in combination with the clinical information of the patients.

Diagnostic Criteria for TB

The definitive diagnosis for tuberculosis in this study was established using a comprehensive clinical reference standard, guided by the WS288-2017 Tuberculosis Diagnosis guideline. A positive TB diagnosis was mandatorily based on etiological and/or pathological confirmation. Etiological confirmation included a positive result from acid-fast staining, culture, or a molecular test (excluding the index TB-PCR test under evaluation). Pathological confirmation required histopathological evidence of caseating granulomas or acid-fast bacilli in biopsied tissue. Patients diagnosed solely on

clinical and radiological grounds without such confirmatory evidence were excluded to ensure the robustness of the gold standard. All diagnoses were independently verified by two clinicians, with disagreements resolved by a third.

Statistical Analysis

The variables analyzed in this study are summarized as follows. The primary quantitative variable was patient age. The primary qualitative variables included patient sex, final diagnosis (TB vs non-TB), and the binary outcomes (positive/negative) of all diagnostic tests (eg, TB-PCR, acid-fast staining). Quantitative variables were described by mean ± standard deviation, and differences were analyzed by independent sample *t*-test. Qualitative variables were described by frequency and percentage, and x^2 test was used for difference analysis. Accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were used to evaluate the diagnostic effect of different detection methods, and x^2 test was used to compare the effect of TB-PCR with other detection methods. In addition, series and parallel analysis methods were used to comprehensively evaluate the combined assay efficacy of TB-PCR, acid-fast staining and strain identification. The area under the curve (AUC) of receiver operating characteristic (ROC) was used to evaluate the diagnostic efficiency. All statistical analyses were performed using SPSS23.0. P<0.05 was considered statistically significant.

Results

General Characteristics of Patients

A total of 729 patients with suspected TB were included in this study, and their mean age was 53.54±17.22 years (Table 1). After comprehensive clinical diagnosis, there were 391 (53.64%) cases of TB patients and 338 (46.36%) cases of non-TB patients (Table 1). The mean age of TB patients was 50.01±19.32 years old, and that of non-TB patients was 57.62±13.32 years old, and the difference was statistically significant (t=6.25, P<0.001) (Table 1). There were 182 (46.55) females and 209 (53.45%) males with TB, while 158 (46.75%) females and 180 (53.25) males without TB (Table 1). There was no significant difference in the gender composition between the two groups (x^2=0.00, P=0.957) (Table 1).

Comparison of Diagnostic Results of Three Detection Methods

Among the 391 confirmed TB patients, 304, 212 and 291 cases were detected positive by TB-PCR, acid-fast staining and strain identification, respectively (Table 2). The number of TB patients with negative test results was 87, 179 and 100, respectively (Table 2). Among the 338 patients who were actually non-TB patients, 334 patients were negative by TB-PCR, acid-fast staining and strain identification, and 4 patients were positive (Table 2).

Evaluation of Diagnostic Effectiveness of Three Detection Methods

Taking the best Yoden index as the cut-off value, the accuracy, sensitivity, specificity, PPV and NPV of TB-PCR diagnostic technique in the diagnosis of TB were 0.88 (95% CI: 0.85–0.90), 0.99 (95% CI: 0.98–1.00) and 0.78 (95% CI: 0.74–0.82), 0.79 (0.75–0.83), 0.99 (95% CI: 0.97–1.00), respectively (Table 3). The accuracy, sensitivity, specificity, PPV and NPV of acid-fast staining in the diagnosis of TB were 0.75 (95% CI: 0.72–0.78), 0.99 (95% CI: 0.98–1.00), 0.54 (95% CI: 0.49–0.59), 0.65 (0.61–0.69), 0.98 (95% CI: 0.96–1.00), respectively (Table 3). In the strain identification test, the accuracy, sensitivity, specificity,

Table 1 Basic Characteristics of All Patients

Variables	Total (n = 729)	Non TB Patients (n = 338)	TB Patients (n = 391)	Statistic	*P*
Age, Mean ± SD	53.54 ± 17.22	57.62 ± 13.32	50.01 ± 19.32	t=6.25	<0.001
Sex, n(%)				χ^2=0.00	0.957
Female	340 (46.64)	158 (46.75)	182 (46.55)		
Male	389 (53.36)	180 (53.25)	209 (53.45)		

Table 2 Detection Results of TB-PCR, Acid-Fast Staining and Strain Identification

Detection Method	Diagnostic Result	Number	Clinical Comprehensive Diagnosis Results	
			TB Patients	Non TB Patients
TB-PCR	Positive	308	304 (98.70)	4 (1.30)
	Negative	421	87 (20.67)	334 (79.33)
	Total	729	391	338
Acid-fast staining	Positive	216	212 (98.15)	4 (1.85)
	Negative	513	179 (34.89)	334 (65.11)
	Total	729	391	338
Strain identification	Positive	295	291 (98.64)	4 (1.36)
	Negative	434	100 (23.04)	334 (76.96)
	Total	729	391	338

Table 3 Confusion Matrix Analysis of TB-PCR, Acid-Fast Staining and Culture

Detection Method	AUC (95% CI)	Accuracy (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cut Off
TB-PCR	0.88 (0.86–0.90)	0.88 (0.85–0.90)	0.99 (0.98–1.00)	0.78 (0.74–0.82)	0.79 (0.75–0.83)	0.99 (0.97–1.00)	0.597
Acid-fast staining	0.77 (0.74–0.79)*	0.75 (0.72–0.78)*	0.99 (0.98–1.00)	0.54 (0.49–0.59)*	0.65 (0.61–0.69)*	0.98 (0.96–1.00)	0.665
Strain identification	0.87 (0.84–0.89)	0.86 (0.83–0.88)	0.99 (0.98–1.00)	0.74 (0.70–0.79)	0.77 (0.73–0.81)	0.99 (0.97–1.00)	0.608

Note: *Compared with TB-PCR, P<0.05.

PPV and NPV were 0.86 (95% CI: 0.83–0.88), 0.99 (95% CI: 0.98–1.00), 0.74 (95% CI: 0.70–0.79), 0.77 (95% CI: 0.73–0.81), 0.99 (95% CI: 0.97–1.00), respectively (Table 3). In ROC curve analysis, AUC of TB-PCR, acid-fast staining and strain identification were 0.88 (95% CI: 0.86–0.90), 0.77 (95% CI: 0.74–0.79) and 0.87 (95% CI: 0.84–0.89), respectively (Table 3 and Figure 1A). The AUC, accuracy, specificity and PPV of TB-PCR test were significantly higher than those of acid-fast staining test (P<0.05) (Table 3). The diagnostic effect of TB-PCR was slightly better than that of strain identification, but the difference was not statistically significant (Table 3).

Combined Test of the Three Diagnostic Methods

In this study, three detection methods were used to diagnose TB according to the scheme of parallel test and series test. In parallel test, accuracy, sensitivity, specificity, PPV and NPV were 0.89 (95% CI: 0.87–0.92), 0.98 (95% CI: 0.97–1.00),

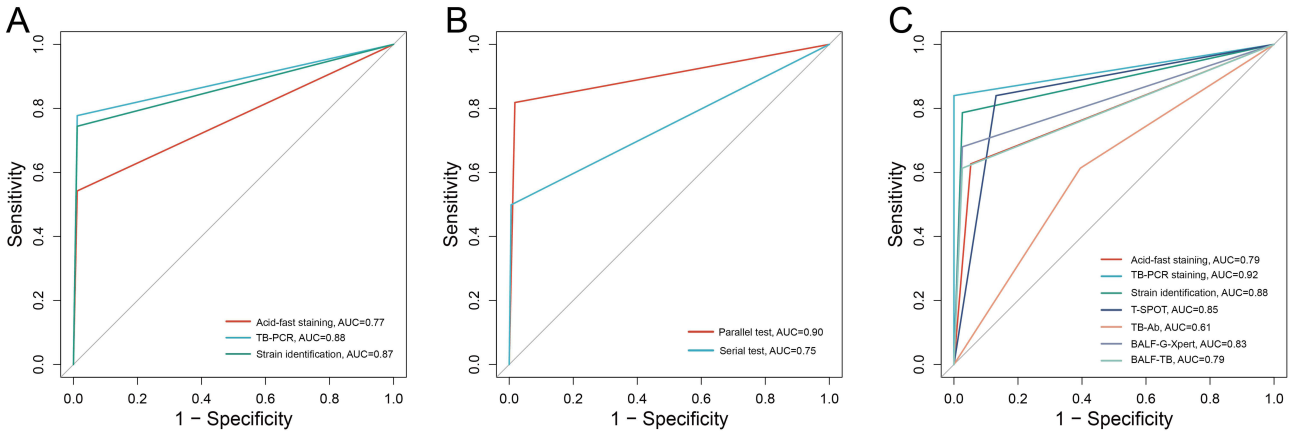

Figure 1 ROC curve analysis under different diagnostic methods. (**A**) Individual diagnostic efficacy of TB-PCR, acid-fast staining and strain identification; (**B**) combined diagnostic efficacy of TB-PCR, acid-fast staining and strain identification; (**C**) individual diagnostic efficacy of TB-PCR, acid-fast staining, strain identification, T-SPOT, TB-Ab, BALF-G-Xpert and BALF-TB.

https://doi.org/10.2147/IDR.S558492

Table 4 Combined Test Results of TB-PCR, Acid-Fast Staining and Culture

Type	AUC (95% CI)	Accuracy (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cut Off
Parallel	0.90 (0.88–0.92)	0.89 (0.87–0.92)	0.98 (0.97–1.00)	0.82 (0.78–0.86)	0.82 (0.79–0.86)	0.98 (0.97–1.00)	0.378
Series	0.75 (0.72–0.77)	0.73 (0.69–0.76)	0.99 (0.99–1.00)	0.50 (0.45–0.55)	0.63 (0.59–0.67)	0.99 (0.98–1.00)	0.679

0.82 (95% CI: 0.78–0.86), 0.82 (95% CI: 0.79–0.86), 0.98 (95% CI: 0.97–1.00), respectively (Table 4). In the string test, the accuracy, sensitivity, specificity, PPV and NPV of the combined detection scheme were 0.73 (95% CI: 0.69–0.76), 0.99 (95% CI: 0.99–1.00), 0.50 (95% CI: 0.45–0.55), 0.63 (95% CI: 0.59–0.67), 0.99 (95% CI: 0.98–1.00), respectively (Table 4). AUC for parallel and series tests were 0.90 (95% CI: 0.88–0.92) and 0.75 (95% CI: 0.72–0.77), respectively (Table 4 and Figure 1B).

Immunological Diagnosis and BALF-Based Diagnosis

Of the 729 patients, 113 were tested for TB-PCR, acid-fast staining, strain identification, T-SPOT, TB-Ab, BALF-G-Xpert, and BALF-TB. Of the 113 patients, 75 were diagnosed with TB and 38 were non-TB (Table 5). Among the 75 TB patients, 63 cases, 47 cases, 59 cases, 63 cases, 46 cases, 51 cases and 46 cases were detected positive by TB-PCR, acid-fast staining, strain identification, T-SPOT, TB-AB, BALF-G-Xpert and BALF-TB, respectively (Table 5). The accuracy, sensitivity, specificity, PPV, and NPV of the TB-PCR test were 0.89 (95% CI: 0.82–0.94), 1.00 (95% CI: 1.00–1.00), 0.84 (95% CI: 0.76–0.92), 0.76 (95% CI: 0.64–0.88), and 1.00 (95% CI: 1.00–1.00), respectively (Table 6).

Table 5 TB-PCR, Acid-Fast Staining, Strain Identification, T-SPOT, TB-Ab, BALF-G-Xpert, and BALF-TB Diagnostic Results

Detection Method	Diagnostic Result	Number	Clinical Comprehensive Diagnosis Results	
			TB Patients	Non TB Patients
TB-PCR	Positive	63	63 (100.00)	0 (0.00)
	Negative	50	12 (24.00)	38 (76.00)
	Total	113	75	38
Acid-fast staining	Positive	49	47 (95.92)	2 (4.08)
	Negative	64	28 (43.75)	36 (56.25)
	Total	113	75	38
Strain identification	Positive	60	59 (98.33)	1 (1.67)
	Negative	53	16 (30.19)	37 (69.81)
	Total	113	75	38
T-SPOT	Positive	68	63 (92.65)	5 (7.35)
	Negative	45	12 (26.67)	33 (73.33)
	Total	113	75	38
TB-Ab	Positive	61	46 (75.41)	15 (24.59)
	Negative	53	29 (54.72)	23 (43.40)
	Total	113	75	38
BALF-G-Xpert	Positive	52	51 (98.08)	1 (1.92)
	Negative	61	24 (39.34)	37 (60.66)
	Total	113	75	38
BALF-TB	Positive	47	46 (97.87)	1 (2.13)
	Negative	66	29 (43.94)	37 (56.06)
	Total	113	75	38

Table 6 Diagnostic Efficacy Analysis of TB-PCR, Acid-Fast Staining, Strain Identification, T-SPOT, TB-Ab, BALF-G-Xpert and BALF-TB (N=73)

Detection Method	AUC (95% CI)	Accuracy (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cut Off
TB-PCR	0.92 (0.88–0.96)	0.89 (0.82–0.94)	1.00 (1.00–1.00)	0.84 (0.76–0.92)	0.76 (0.64–0.88)	1.00 (1.00–1.00)	0.62
Acid-fast staining	0.79 (0.72–0.85)*	0.73 (0.64–0.81)*	0.95 (0.88–1.00)	0.63 (0.52–0.74)*	0.56 (0.44–0.68)*	0.96 (0.90–1.00)	0.698
Strain identification	0.88 (0.83–0.93)	0.85 (0.77–0.91)	0.97 (0.92–1.00)	0.79 (0.69–0.88)	0.70 (0.57–0.82)	0.98 (0.95–1.00)	0.643
T-SPOT	0.85 (0.79–0.92)	0.85 (0.77–0.91)	0.87 (0.76–0.98)*	0.84 (0.76–0.92)	0.73 (0.60–0.86)	0.93 (0.86–0.99)*	0.597
TB-Ab	0.61 (0.51–0.71)*	0.61 (0.51–0.70)*	0.61 (0.45–0.76)*	0.61 (0.50–0.72)*	0.44 (0.31–0.58)*	0.75 (0.65–0.86)*	0.656
BALF-G-Xpert	0.83 (0.77–0.89)	0.78 (0.69–0.85)*	0.97 (0.92–1.00)	0.68 (0.57–0.79)*	0.61 (0.48–0.73)*	0.98 (0.94–1.00)	0.687
BALF-TB	0.79 (0.73–0.85)*	0.73 (0.64–0.81)*	0.97 (0.92–1.00)	0.61 (0.50–0.72)*	0.56 (0.44–0.68)*	0.98 (0.94–1.00)	0.709

Note: *Compared with TB-PCR, P<0.05.

The acid-fast staining results were 0.73 (95% CI: 0.64–0.81), 0.95 (95% CI: 0.88–1.00), 0.63 (95% CI: 0.52–0.74), 0.56 (95% CI: 0.44–0.68), 0.96 (95% CI: 0.90–1.00), respectively (Table 6). The strain identification tests were 0.85 (95% CI: 0.77–0.91), 0.97 (95% CI: 0.92–1.00), 0.79 (95% CI: 0.69–0.88), 0.70 (95% CI: 0.57–0.82), and 0.98 (95% CI: 0.95–1.00), respectively (Table 6). The accuracy of T-SPOT, TB Ab, BALF-G-Xpert, and BALF-TB tests were 0.85 (95% CI: 0.77–0.91), 0.61 (95% CI: 0.51–0.70), 0.78 (95% CI: 0.69–0.85), and 0.73 (95% CI: 0.64–0.81), respectively (Table 6). The AUC of TB-PCR, acid-fast staining, strain identification, T-SPOT, TB-Ab, BALF-G-Xpert, and BALF-TB were 0.92 (95% CI). 0.88–0.96), 0.79 (95% CI: 0.72–0.85), 0.88 (95% CI: 0.83–0.93), 0.85 (95% CI: 0.79–0.92), 0.61 (95% CI: 0.71–0.71), 0.83 (95% CI: 0.71–0.71) 0.77–0.89), 0.79 (95% CI: 0.73–0.85) (Table 6 and Figure 1C). The diagnostic efficacy of TB-PCR was significantly better than that of acid-fast staining, T-SPOT, TB-Ab, BALF-G-Xpert, and BALF-TB (P<0.05), but there was no significant difference from the results of strain identification (Table 6).

Discussion

As a chronic infectious disease mainly invading lung tissue, TB poses a serious threat to individual health and social public health.[11] If TB is not treated promptly, it can result in the deterioration of lung lesions, the formation of cavities and fibrosis, severe damage to lung tissue and lung function, giving rise to symptoms such as shortness of breath, fatigue, and even death.[12] Early diagnosis of TB can not only help patients to receive standard treatment in time, improve the cure rate and prognostic effect, but also reduce the spread of mycobacterium tuberculosis effectively.[13] In addition, standardized early treatment can help reduce the occurrence of drug-resistant tuberculosis and avoid the increase in treatment difficulty and cost. Respiratory endoscopy, as a minimally invasive detection method with high safety, has been widely used in the detection of lung abnormalities. The purpose of this study was to explore the advantages of TB-PCR in the diagnosis of TB based on pulmonary samples taken by respiratory endoscopy. In the overall sample, the results showed that the AUC, accuracy, sensitivity, specificity, PPV and NPV of TB-PCR detection were 0.88, 0.88, 0.99, 0.78, 0.79 and 0.99, respectively. The efficacy of TB-PCR in the diagnosis of TB was significantly better than that of acid-fast staining test and strain identification test. In addition, in some patients who received more TB tests, TB-PCR continued to perform better than other tests (acid-fast staining, strain identification, T-SPOT, TB-AB, BALF-G-Xpert, BALF-TB). The clinical value of TB-PCR detection technology in the diagnosis of TB will be analyzed and discussed in detail below.

In the detection of pulmonary abnormalities, respiratory endoscopy can be directly inserted into the patient's airway and reach deep into the lung. Through the camera and light source on the lens, the conditions of the lung can be clearly observed, including the location, shape and extent of the lesion.[14] During the observation process, the doctor is capable of accurately collecting samples from the diseased area through the respiratory endoscopy by employing brush, clamp or puncture approaches as necessary. This sampling method is not only precise but also can prevent damage to the surrounding normal tissues.[15] Moreover, respiratory endoscopy is a minimally invasive examination method, and patients usually only need to undergo local anesthesia to tolerate the entire examination process.[16] At present, respiratory endoscopy is not only suitable for the diagnosis of pulmonary tuberculosis, but also widely used in the diagnosis of lung cancer, pulmonary nodules, pulmonary infection and other pulmonary diseases.[17]

In this study, TB-PCR technology demonstrated superior accuracy, sensitivity, specificity, PPV, NPV and AUC in the detection of lung samples taken by respiratory endoscopy than traditional acid-fast staining and strain identification. TB-PCR technology can detect extremely small amounts of Mycobacterium tuberculosis by specifically amplifying DNA fragments, making it effective in diagnosing early infections or low bacterial counts.[18,19] This advantage is particularly critical for small non-malignant respiratory endoscopy specimens, where the bacterial load is often insufficient to be detected by conventional methods—consistent with recent studies showing that even WHO-endorsed molecular assays like Xpert MTB/RIF may have reduced sensitivity in such paucibacillary samples due to dilution effects or limited target DNA.[20] This makes its sensitivity significantly higher than acid fast staining tests. Moreover, the technology amplifies specific gene sequences of Mycobacterium tuberculosis (eg, regions unique to MTB such as RD1-encoded ESAT-6/CFP-10, as referenced in IGRA design), so it can accurately distinguish Mycobacterium tuberculosis from other non-tuberculous mycobacteria (NTM), effectively avoiding misdiagnosis.[3,17] This is clinically relevant because NTM infections are increasingly common in respiratory specimens, and acid-fast staining cannot differentiate MTB from NTM—leading to potential overdiagnosis of TB.[21] The diagnosis of TB is highly time-dependent. In contrast to the culture time for strain identification, which takes several days or even weeks, TB-PCR technology typically enables the completion of detection within a few hours.[22] This significantly reduces the diagnosis cycle and is beneficial for the timely treatment and disease control of patients. And with the continuous development of the technology, the operation of TB-PCR technology is becoming more and more simple, reducing the influence of human operation on the results.[23] In addition, TB-PCR technology has strong stability and is not easily affected by patients' age, gender, immunity and other factors, so that it can provide effective diagnostic support in the diagnosis of suspected TB patients or in the diagnosis process of TB patients at different stages.[24]

In further work with immunological detection techniques and BALF-based tests, TB-PCR technology still shows better diagnostic results. Compared with T-SPOT detection, TB-PCR has the advantages of good specificity, high sensitivity, and is not easily affected by changes in immune level, while T-SPOT detection depending on immune response may be affected by the immune status of patients.[25] Although TB-Ab testing is simple, antibody production takes time and may not accurately reflect infection in immunocompromised patients.[26] TB-PCR directly detects pathogen genes and is not subject to this limitation. In addition, the detection based on BALF samples, such as BALF-G-XPERT and BALF-TB detection, is easy to be limited by the source of the detection sample and the more cumbersome operation process.[27] In summary, TB-PCR has the advantages of high sensitivity, high specificity, rapidity, simplicity, and strong stability in the diagnosis of pulmonary tuberculosis, which can provide a more effective basis for early diagnosis and treatment of TB.

Although this study confirms the significant advantages of TB-PCR in the early diagnosis of TB, the limitations of its practical application should be noted. First of all, the source of patients and the type of testing instrument in this study are relatively simple, which limits the extrapolation of results to a certain extent. In the future, this study will continue to expand the sample sources and further optimize the detection methods to ensure the stability of the results. Secondly, the relatively high cost of TB-PCR technology may limit its promotion and application in primary medical institutions to a certain extent. Follow-up studies should further explore methods to optimize sample collection and processing processes, reduce detection costs, and improve the accessibility and popularity of TB-PCR technology. Thirdly, although TB-PCR has excellent performance in the diagnosis of TB, it is still necessary to combine other detection methods in some detection scenarios, such as TB drug resistance detection. Therefore, in practical applications, appropriate detection methods should be selected according to the specific conditions of patients and laboratory conditions, and multiple methods can be combined when necessary to improve diagnostic accuracy. Finally, our study utilized samples obtained through various respiratory endoscopic techniques. While these techniques sample different anatomical sites and may have varying diagnostic yields, our study design aimed to reflect real-world clinical practice where the choice of technique is tailored to the patient's specific lesion. The consistent superiority of TB-PCR across the entire cohort, which encompasses this diversity, suggests that its diagnostic advantage is robust. However, we acknowledge that the lack of randomization in sampling technique selection remains a limitation. Nevertheless, the results of this study still found that in the samples taken based on respiratory endoscopy, TB-PCR showed better diagnostic efficacy than other detection methods, and it had better diagnostic accuracy, sensitivity and specificity. The popularization and application of

TB-PCR technology will help to improve the early diagnosis rate and treatment success rate of tuberculosis, and reduce the disease transmission and social burden.

Conclusion

Through retrospective analysis of 729 patients with suspected TB, this study systematically explored the advantages of TB-PCR compared with other detection techniques such as acid-fast staining and strain identification in the early diagnosis of TB. The results of this study showed that TB-PCR had higher accuracy, sensitivity, specificity, PPV and NPV in the diagnosis of TB than acid-fast staining and strain identification tests. In addition, TB-PCR technology can also show a better diagnostic effect than immunological detection, BALF detection, etc. The clinical application of TB-PCR technology in the early diagnosis of tuberculosis not only improves the accuracy and timeliness of diagnosis, but also brings substantial clinical benefits to patients. Early diagnosis means that patients are able to receive standardized treatment earlier, which reduces the risk of disease progression and improves cure rates and quality of life. In conclusion, this study provides strong evidence to support the application of TB-PCR technology in the early diagnosis of TB, and provides an effective diagnostic tool for clinicians. Clinicians should fully recognize the advantages and potential of TB-PCR technology, actively apply it in clinical practice, and select appropriate detection methods according to the specific conditions of patients.

Data Sharing Statement

The datasets generated or analyzed during this study are available from the corresponding author [Dongchun Ma] on reasonable request.

Acknowledgments

We thank all the medical staff in the Interventional Pulmonary Disease & Endoscopic Diagnosis and Treatment Center and Pathology Department of Anhui Chest Hospital for their support and help. Thanks to all patients who participated in this study for their understanding and support. Fei Tang and Xiankui Zha should be considered as co-first authors.

Funding

This study was funded by the Anhui Provincial Medical and Health Key Specialty Construction Project (No. [2021]273).

Disclosure

The authors declare no competing interests.

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