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The Value of Combined Detection Using Four Methods for Early Diagnosis of Active Tuberculosis

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Abstract: ***Objective:*** ***To evaluate the diagnostic value of combining Interferon-gamma Release Assay (IGRA), CD161 flow cytometry, GeneXpert MTB/RIF assay (Xpert), and Tuberculin Skin Test (TST) for early diagnosis of active pulmonary tuberculosis (PTB).*** ***Methods:*** ***A total of 289 patients (139 with active PTB and 150 with other pulmonary diseases) admitted between August 2023 and August 2024 were enrolled. All participants underwent IGRA, Xpert, TST, CD161 flow cytometry, and Erythrocyte Sedimentation Rate (ESR) testing. Chi-square tests were used to compare positive rates between groups. Receiver Operating Characteristic (ROC) curves were generated to analyze the diagnostic performance (Area Under the Curve, AUC) of individual and combined methods.*** ***Results:*** ***IGRA demonstrated the highest sensitivity (91.4%) but lower specificity (61.3%). Xpert exhibited optimal specificity (99.3%) and positive predictive value (98.8%) but limited sensitivity (59.7%). The combined use of four methods achieved an AUC of 0.933 (95% CI: 0.905–0.962), significantly surpassing individual methods (IGRA: 0.764; Xpert: 0.795; TST: 0.638; CD161: 0.626). ESR was excluded from combined analysis due to its inability to differentiate active PTB from other pulmonary diseases.*** ***Conclusion:*** ***While individual methods exhibit limitations, the combination of IGRA, Xpert, TST, and CD161 flow cytometry significantly improves diagnostic accuracy for active PTB, offering critical support for early clinical decision-making.***

Keywords: Active pulmonary tuberculosis, Combined detection, Diagnostic efficacy, Interferon-gamma release assay, GeneXpert MTB/RIF, Tuberculin skin test, CD161 flow cytometry.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis infection. However, in 2022, 1.3 million people worldwide still died from this preventable and generally curable disease, making it the second leading cause of death from a single infectious agent after COVID-19 [1]. Therefore, early diagnosis and initiation of TB treatment are particularly critical. The definitive diagnosis of pulmonary tuberculosis relies on etiological examinations, yet traditional methods such as sputum Mycobacterium tuberculosis smear have low positivity rates and cannot distinguish between different mycobacterial species. Sputum culture remains the gold standard for TB diagnosis but requires prolonged incubation time. In recent years, with advancements in diagnostic testing technologies, interferon-γ release assays (IGRAs) have been widely applied in clinical TB management [2]. especially for detecting latent tuberculosis infection. The Xpert MTB/RIF assay, a fully automated real-time fluorescent nucleic acid amplification technique, enhances detection sensitivity through target gene amplification, provides results within 2 hours, and effectively improves testing efficiency. Additionally, it enables early detection of rifampicin resistance genes and has been recommended by WHO as a first-line diagnostic measure [3]. Studies have also reported that flow cytometry can be used for adjuvant TB diagnosis. This review explores the diagnostic value of interferon-γ release assays, CD161 flow cytometry [4], Xpert MTB/RIF, combined with traditional tests such as tuberculin skin test and erythrocyte sedimentation rate, to provide references for clinical diagnosis of pulmonary tuberculosis.

2. Methods

2.1 General Data

A total of 289 patients were enrolled, including newly diagnosed pulmonary tuberculosis patients admitted to our hospital from August 2023 to August 2024 and other patients with suspected pulmonary tuberculosis but confirmed with other diseases during the same period. Among them, 194 (67.1%) were male and 95 (32.9%) were female, aged 14 to 94 years (median, 53; interquartile range, 70). Active pulmonary tuberculosis was diagnosed according to the Diagnostic Criteria for Pulmonary Tuberculosis (WS 288-2017) and Classification of Tuberculosis (WS 196-2017) [5-6]. The study included 139 (48.1%) active pulmonary tuberculosis patients and 150 (51.9%) patients with other pulmonary diseases, including 54 (18.8%) cases of lung malignancy and 96 (33.2%) cases of other pulmonary infectious diseases.

2.2 Detection Methods

Peripheral blood samples were collected from fasting patients (fasting ≥8 hours) on the second morning after admission and sent to the clinical laboratory of our hospital.

Interferon-gamma release assay (T-SPOT.TB Method)

Collect peripheral venous blood from the patient (typically 6-8 mL) and preserve it in a heparin anticoagulant tube. Seed the isolated peripheral blood mononuclear cells (PBMC) into microplate wells pre-coated with anti-IFN-γ antibodies. Stimulate the cells by adding Mycobacterium tuberculosis-specific antigens (ESAT-6 and CFP-10). Include a positive control (phytohemagglutinin, PHA) and a negative control (no antigen). Incubate at 37°C with 5% CO_2 for 16-20 hours. Quantify the number of spots using an enzyme-linked immunospot (ELISPOT) analyzer.

Interpretation criteria:

Positive: Antigen well spot count minus negative control spot count ≥ 6, and the positive control meets specified criteria.
Negative: Antigen well spot count minus negative control spot count < 6.

Invalid: Positive control fails to meet requirements; repeat testing is required

GeneXpert MTB/RIF

The GeneXpert kit purchased from Cepheid was used and processed according to the operating procedure. Specimens included sputum, bronchoalveolar lavage fluid, pleural effusion, etc., from patients.

Result interpretation:

MTB positive: DNA of Mycobacterium tuberculosis was detected.

MTB negative: DNA of Mycobacterium tuberculosis was not detected.

Tuberculin skin test (using PPD skin test)

Typically, the upper - middle 1/3 of the flexor side of the forearm was selected, avoiding areas with scars, blood vessels, and edema. The skin was disinfected with an alcohol swab and allowed to dry. A 1 - mL syringe was used to draw 0.1 mL of PPD solution. The needle bevel was faced upwards and inserted into the dermis at an angle of 5 - 10 degrees with the skin. 0.1 mL of PPD was slowly injected to form a wheal with a diameter of approximately 6 - 10 mm. After injection, the skin turned white and the pores were clearly visible. The results were read 72 hours later by checking whether there was induration or erythema at the injection site. Induration was the main indicator for judgment, while erythema was not used as a diagnostic basis. A transparent measuring ruler was used to measure the maximum transverse and longitudinal diameters of the skin induration, and the average value was taken. The diameter of the induration was recorded in millimeters.

Interpretation criteria: An induration diameter of < 5 mm was interpreted as negative, and an induration diameter of ≥ 5 mm was defined as positive.

Erythrocyte sedimentation rate (ESR)

An automatic erythrocyte sedimentation rate analyzer purchased from ALIFAX, Italy, was used and operated according to the instruction manual.

Interpretation criteria:

For men ≤ 60 years old, an ESR > 21 (mm/h) was considered positive; for men > 60 years old, an ESR > 43 (mm/h) was considered positive.

For women ≤ 50 years old, an ESR > 26 (mm/h) was considered positive; for women > 50 years old, an ESR > 38 (mm/h) was considered positive.

CD161 flow cytometry

The CD161 detection reagent purchased from Shenzhen United Medical Technology Co., Ltd. was used and operated according to the instruction manual. Finally, the proportion of monocytes (Mon%), the proportion of lymphocytes (L%), and the proportion of $CD161^+$ cells ($CD161^+$%) were obtained. Then, The Retention Factor (RF) of CD161 was calculated through a random forest model (the result was automatically reported by the instrument). In this study, The RF of CD161 was selected as the research indicator.

2.3 Statistical Analysis

Data were analyzed using SPSS 19.0 software. Categorical data were described as "number of cases, rate (%)", and intergroup differences were compared using the chi-square test. The Shapiro-Wilk test was used to assess data normality. Non-normally distributed data were described as "median (interquartile range) [M (Q1, Q3)]". Diagnostic performance of T-SPOT.TB and other methods for microbiologically negative pulmonary tuberculosis was evaluated via receiver operating characteristic (ROC) curve analysis, and the area under the ROC curve (AUC) was calculated. A two-tailed $P < 0.05$ was considered statistically significant.

2.4 Ethics

This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (Ethics No.: 2025-039-01).

3. Results

3.1 Comparative Analysis of Detection Method Positivity Rates between Two Patient Cohorts.

The positive rates of interferon-γ release assay (IGRA) in the active pulmonary tuberculosis group and other pulmonary diseases group were 91.4% and 38.7%, respectively, with a statistically significant difference (χ^2=86.981, $P < 0.01$). The positive rates of Xpert detection were 59.7% and 0.7%, respectively, showing a statistically significant difference (χ^2=121.996, $P < 0.01$). The positive rates of tuberculin skin test (TST) were 59% and 31.3%, respectively, with a statistically significant difference (χ^2=22.335, $P < 0.01$). The positive rates of erythrocyte sedimentation rate (ESR) were 78.4% and 74.7%, respectively, with no statistically significant difference (χ^2=0.564, P=0.453) (Table 1). The Retention Factor (RF) of CD161 in the two groups were 0.73 (0.63–0.75) and 0.69 (0.33–0.74), respectively, with a statistically significant difference (Z=-3.758, $P < 0.01$) (Table 2).

Table 1: Comparison of positive rates between two patient groups across different detection methods

Group	Cases	T-SPOT		X-pert		TST		ESR	
		Pos. (n)	Rate (%)	Pos. (n)	Rate (%)	Pos.(n)	Rate (%)	Pos. (n)	Rate (%)
Active TB	139	127	91.4	83	59.7	82	59	109	78.4
Other Diseases	150	58	38.7	1	0.7	47	31.3	112	74.7
X^2		86.981		121.996		22.335		0.564	
P		＜0.01		＜0.01		＜0.01		0.453	

Table 2: Comparison of CD161 relative migration values (RF) between two patient groups

Group	CD161	Z	P
Active TB	0.73(0.63~0.75)	-3.758	＜0.01
Other Diseases	0.69(0.33~0.74)		

Table 3: Diagnostic Performance of Three Methods for Active Pulmonary Tuberculosis

Test Method	Group		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
	Active TB	Other Disease					
IGRA			91.4	61.3	68.6	88. 5	75.8
Pos.	127(91.4)	58(38.7)					
Neg.	12(8.6)	92(61.3)					
Xpert			59.7	99.3	98.8	72.7	80.3
Pos.	83(59.7)	1(0.7)					
Neg.	56(40.3)	149(99.3)					
TST			59	68.7	63.6	64.4	64.0
Pos.	82(59)	47(31.3)					
Neg.	57(41)	103(68.7)					

Notes: Sensitivity (%) = (True positive cases / (True positive cases + False negative cases)) × 100%;Specificity (%) = (True negative cases / (True negative cases + False positive cases)) × 100%;Positive predictive value (PPV, %) = (True positive cases / (True positive cases + False positive cases)) × 100%;Negative predictive value (NPV, %) = (True negative cases / (True negative cases + False negative cases)) × 100%;Accuracy (%) = ((True positive cases + True negative cases) / Total sample size) × 100%.

3.2 Diagnostic Performance of Three Methods for Active Pulmonary Tuberculosis

Using clinical diagnosis as the reference standard, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of T-SPOT.TB for active pulmonary tuberculosis were 91.4%, 61.3%, 68.6%, 88.5%, and 75.8%, respectively. For GeneXpert, these metrics were 59.7%, 99.3%, 98.8%, 72.7%, and 80.3%, respectively. The tuberculin skin test (TST) yielded sensitivity, specificity, PPV, NPV, and accuracy of 59%, 68.7%, 63.6%, 64.4%, and 64%, respectively. IGRA demonstrated higher sensitivity and NPV for active pulmonary tuberculosis, while GeneXpert showed extremely high specificity and PPV (Table 3).

3.3 Diagnostic Efficacy of Combined Detection

The receiver operating characteristic (ROC) curves of four detection methods individually and in combination are shown in Figure 1. GeneXpert demonstrated an area under the curve (AUC) of 0.795 with a standard error of 0.028 (95% CI: 0.741–0.850), which was higher than the AUC values of the other methods. T-SPOT.TB yielded an AUC of 0.764 (standard error: 0.029; 95% CI: 0.707–0.820), while the tuberculin skin test (TST) showed an AUC of 0.638 (standard error: 0.033; 95% CI: 0.574–0.702). The RF of CD161 had an AUC of 0.626 (standard error: 0.033; 95% CI: 0.562–0.690). These results indicate that GeneXpert exhibits higher diagnostic accuracy for active tuberculosis compared to the other methods. When the four methods were combined, the AUC increased to 0.933 (standard error: 0.014; 95% CI: 0.905–0.962), demonstrating significantly improved diagnostic performance for active tuberculosis through combined testing.

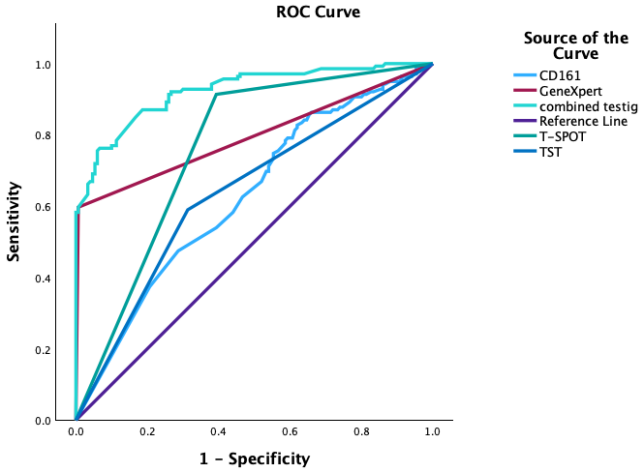

Figure 1: Receiver Operating Characteristic (ROC) Curve of the Combined Four-Method Panel for Active Tuberculosis Diagnosis

4. Discussion

Tuberculosis remains the second-largest global infectious disease affecting humanity, with pulmonary tuberculosis accounting for the majority of cases due to respiratory transmission of its pathogen. Although advancements in diagnostic technologies have enabled earlier detection of pulmonary tuberculosis, the current situation remains challenging. The definitive diagnosis of tuberculosis primarily relies on etiological examinations, including traditional methods such as sputum smear microscopy and mycobacterial culture, as well as rapidly developing molecular biological detection techniques. Sputum smear microscopy, due to its simplicity and low cost, remains the

preferred diagnostic method for pulmonary tuberculosis and is the sole diagnostic approach in underdeveloped regions. However, its sensitivity is limited by its inability to distinguish between tuberculosis and non-tuberculous mycobacteria, as well as its requirement for a certain bacterial load, resulting in potential false positives. While culture remains the gold standard for tuberculosis diagnosis, the slow growth of Mycobacterium tuberculosis-even with liquid medium requiring at least one week for positive results-hinders early diagnosis. Additionally, its accuracy depends on specimen quality and still carries risks of false negatives.

GeneXpert MTB/RIF, a representative of rapidly evolving molecular diagnostic technologies, utilizes automated polymerase chain reaction targeting the rpoB gene to deliver positive results within two hours. It can detect rifampicin-related drug resistance genes and analyze various specimens including sputum, pleural fluid, and bronchoalveolar lavage fluid. Widely adopted clinically, this method received WHO endorsement as early as 2011 for prioritized use in symptomatic populations. In this study, GeneXpert demonstrated exceptionally high specificity and positive predictive value, with a positive result typically confirming active tuberculosis. However, when detecting extremely low mycobacterial loads, it exhibits higher false-positive rates, necessitating comprehensive judgment in conjunction with the patient's clinical manifestations, imaging findings, and immunological results.7

While erythrocyte sedimentation rate (ESR) has historical relevance in monitoring inflammatory states, its non-specific elevation in infections, malignancies, and autoimmune diseases precludes standalone diagnostic value for active TB. Consistent with prior evidence, our study observed overlapping ESR levels between active PTB (78.4%) and other pulmonary conditions (74.7%), prompting its exclusion from diagnostic performance analyses. Nevertheless, serial ESR measurements combined with CT imaging may aid therapeutic monitoring in confirmed TB cases.8

The tuberculin skin test (TST) detects Mycobacterium tuberculosis infection by leveraging the delayed-type hypersensitivity reaction induced by the bacteria. As a long-standing, widely-used, and currently one of the most convenient, rapid, and cost-effective tuberculosis detection methods, the tuberculin skin test remains the primary screening tool in underdeveloped regions and is employed alongside interferon-gamma release assays (IGRAs) to identify latent tuberculosis infection (LTBI). The test involves intradermal injection of tuberculin purified protein derivative (PPD) on the patient's forearm, with infection determined by measuring the induration size. However, since China is a high-burden country for tuberculosis, all newborns receive the Bacillus Calmette-Guérin (BCG) vaccine after birth. The TST cannot differentiate between latent tuberculosis infection and active tuberculosis disease and is susceptible to interference from BCG vaccination, resulting in a high false-positive rate that prevents it from serving as the gold standard for active tuberculosis diagnosis. Additionally, immunocompromised patients or those on immunosuppressive medications may exhibit false-negative results due to weakened immune responses. In this study, the TST demonstrated lower sensitivity and specificity compared to other diagnostic methods, necessitating its use in combination with other testing approaches.

The Interferon-gamma release assay (IGRA) is another immunological method for detecting tuberculosis. It primarily measures IFN-γ produced by sensitized T cells stimulated with Mycobacterium tuberculosis-specific antigens (ESAT-6 and CFP-10). Due to the high specificity of these antigens, which are absent in the Bacillus Calmette-Guérin (BCG) vaccine and most non-tuberculous mycobacteria (NTM), IGRA results are generally unaffected by BCG vaccination or NTM exposure. A systematic review and meta-analysis comparing IGRAs and the tuberculin skin test (TST) demonstrated that IGRAs exhibit superior sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and diagnostic odds ratio compared to TST.9 However, the interferon-gamma release assay can only indicate the presence of Mycobacterium tuberculosis infection in the body and cannot distinguish latent tuberculosis infection (LTBI) from active tuberculosis disease.10 In this study, T-SPOT.TB (a commercial IGRA) showed high sensitivity (91.4%) but relatively low specificity (61.3%), likely due to interference from LTBI cases and the influence of prior tuberculosis history, which is also a risk factor for false-positive results.

CD161+ is a C-type lectin-like receptor widely expressed on various cell types, including CD4+, CD8+, γδ+ T cells, and mucosal-associated invariant T (MAIT) cells. Previous studies have identified CD161+ as a potential biomarker for tuberculosis (TB) diagnosis. For instance, Xu et al. reported that CD161+ demonstrated excellent diagnostic efficacy with an area under the receiver operating characteristic curve (AUC) >0.9 in distinguishing TB patients from healthy individuals.11 However, our current study revealed that flow cytometry-based detection of CD161+ achieved only moderate diagnostic performance for active pulmonary TB, yielding an AUC of 0.626. Given the emerging roles of CD161 in tumorigenesis and autoimmune disorders, reliance on CD161+ as a standalone biomarker may lack specificity for differentiating active pulmonary TB from other pathological conditions. Thus, its clinical utility requires corroboration through combinatorial testing with other diagnostic modalities.

Notably, CD161+ levels have been linked to treatment outcomes in TB. Studies indicate that CD161+ expression normalized in cured patients following Mycobacterium tuberculosis clearance, suggesting its potential as a therapeutic monitoring tool.12

5. Conclusion

No single modality achieves optimal diagnostic performance for active TB. Our multimodal approach combining IGRA, Xpert®, TST, and ESR demonstrated synergistic efficacy (AUC=0.933, 95% CI: 0.905–0.962), significantly outperforming individual methods. This paradigm aligns with WHO recommendations for composite diagnostic strategies, particularly in high-burden settings. Future studies should validate cost-effective algorithm implementations while exploring novel biomarkers like CD161 in therapeutic

monitoring.

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