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Investigation of the Clinical Diagnostic Significance of the T-Cell Test for Tuberculosis combined with Erythrocyte Sedimentation Test in Pulmonary Tuberculosis

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Abstract: *Objective:* To investigate the clinical diagnostic significance of peripheral blood T-cell test (T-spot test) for tuberculosis (TB) infection combined with erythrocyte sedimentation rate (ESR) in pulmonary TB. *Methods:* 41 patients with a clinical diagnosis of TB during hospitalization from January 2020 to April 2023 in our hospital were selected as the experimental group, and 45 patients without TB (bronchopneumonia patients) were selected as the control group. The diagnostic specificity, sensitivity, and accuracy of the T-spot TB test, ESR test, and the combined test of the two were calculated respectively. *Results:* The sensitivity, specificity, and accuracy of the T-spot TB test combined with ESR for the diagnosis of TB in the experimental group were significantly higher than the individual results of the T-spot TB test and ESR test alone ($P < 0.05$). *Conclusion:* The T-spot TB test combined with the ESR test for TB diagnosis has greater clinical value than carrying out the tests individually.

Keywords: Peripheral blood tuberculosis infection T-cell spot test; Erythrocyte sedimentation rate test; Tuberculosis; Clinical diagnosis

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1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. The disease is mainly airborne and produces TB nodules in infected individuals, which destroy lung tissue, leading to respiratory symptoms such as coughing, bloody sputum, and dyspnea [1,2]. In addition to lung symptoms, TB may cause lesions in other organs. For example, bronchial TB may lead to cough, sputum, and shortness of breath; intestinal TB may lead to digestive symptoms such as diarrhea and abdominal pain; and meningeal TB may lead to neurological symptoms such as headache, fever, and nuchal rigidity [3].

The auxiliary diagnosis of pulmonary TB usually includes the following aspects: (1) Symptoms and signs include fever, night sweats, malaise, cough, and blood in sputum [4]. (2) The presence of *Mycobacterium*

tuberculosis can be detected through sputum smear and is one of the key indicators of pulmonary TB. (3) Chest X-ray reveals the location, size, and morphology of lung lesions, which can be used to assist in the diagnosis of pulmonary TB. Some studies have shown that a considerable portion of TB comprises atypical pulmonary TB, which is difficult to distinguish from interstitial pneumonia by imaging [5,6]. (4) *Mycobacterium tuberculosis* culture is a very sensitive and specific test that can be used to detect the presence of *Mycobacterium tuberculosis*. (5) The tuberculin skin test is the intradermal injection of tuberculin where the reaction at the injection site is observed to determine whether or not there has been any previous exposure to *Mycobacterium tuberculosis*. (6) Serologic tests are used to detect the levels of TB antibodies and related immune factors but have low sensitivity and specificity.

2. Materials and methods

2.1. Materials

2.1.1. General information

41 patients clinically diagnosed with pulmonary TB during hospitalization in our hospital from January 2020 to April 2023 were selected as the experimental group, with an age range of 19–84 years old of which 26 were male and 15 were female. 45 non-TB patients who attended the hospital during the same period were selected as the control group (all of them had bronchopneumonia), with an age range of 27–86 years old, of which 28 were male and 17 were female. The erythrocyte sedimentation rate (ESR) and peripheral blood T-cell test for TB (T-spot TB test) of the two groups were retrospectively analyzed, and the diagnostic specificity, sensitivity, and accuracy of the T-spot TB test, ESR, and the combination of the two were calculated respectively. The inclusion criteria of the pulmonary TB group were based on two mandatory health industry standards, namely the WS 196-2017 Classification of Tuberculosis and the WS 288-2017 Diagnosis of Pulmonary Tuberculosis, which were promulgated by the National Health and Sanitation Commission of the People's Republic of China in 2017. The epidemiologic history, clinical manifestations, and symptoms were consistent with this diagnostic standard [7–9].

2.1.2. Instruments and reagents

Saikohid dynamic sedimentation pressure deposition tester, T-cell detection kit for TB infection.

2.2. Methods

The Weiss method of hematocrit is a method for measuring the ESR in blood [10]. This method is suitable for the diagnosis and monitoring of almost all types of blood diseases, especially rheumatic diseases, tumors, and infections, and has an extensive clinical application. The detailed steps involved in the Weiss method are as follows: Firstly, the patient's fresh venous whole blood was obtained and a certain volume of anticoagulant was added to prevent blood coagulation and ensure the mixture was homogeneous. Next, the tube was placed vertically under consistent temperature and left to settle for 2 hours. Finally, the separation height (mm) between the erythrocyte supernatant and the hemoglobin supernatant was measured as the hemosiderosis value. The hematocrit value reflects the speed at which erythrocytes separate from the plasma and settle and varies according to the patient's age, gender, and medical condition, generally ranging from 0–15 mm/h for adult males, and 0–20 mm/h for adult females. Results that fall within the reference range were considered negative, otherwise, they were considered positive. If the ESR value is abnormally high, inflammation, infection, tumor, autoimmune disease, anemia, and other diseases may be present [11,12].

TB infection T-cell test is used to diagnose whether the body is infected with TB and is also known as the T-spot TB test. This is because after the body is infected with TB, the memory T-cells remain for a

long period. Once the body is stimulated by the antigen, the memory T-cells will be activated and rapidly release γ-interferon [13,14]. Although a positive T-spot TB test indicates a positive infection with *Mycobacterium tuberculosis*, it is impossible to determine whether it is a recent or previous infection. Hence, it is necessary to combine the T-spot TB test with other examinations to make a comprehensive judgment. For the T-spot TB test, 5–8 mL of venous blood was collected from the elbow using a heparin anticoagulant tube in the morning upon admission to the hospital, and the blood was separated into peripheral single nucleated cells using the Ficoll-PaqueTM lymphocyte separation solution to make a cell suspension of 2.5×10^6/L. Next, the cell suspension was added to the culture plate and 50 μl of cell culture solution was added to the control wells. Fifty μL of antigen solution A and B were added to the necessary detection wells respectively, and at the same time, 50 μl of cell suspension was added to the positive quality control wells. The plate was then incubated at 5% CO_2, 37°C, for 24 hours. After incubation, the cell culture solution was discarded and washed. Fifty μL of configured antibody reagent was added to the cell culture and incubated for 1 hour. Fifty μL of substrate color development solution was added after washing and incubated for 30 minutes. Finally, the culture plate was washed with distilled water. Under qualified quality control, the number of blue spots in the reaction wells was counted, and ≥ 5 blue spots in the detection wells containing A and B antigens indicated a positive result. It should be noted that this assay cannot distinguish between latent and active TB infection. In the event of a positive test result, further tests should be performed to determine the presence of active TB [15].

2.2.1. Statistical methods

The data were analyzed using the SPSS 26.0 statistical analysis software, and the sensitivity, specificity, and accuracy of ESR and T-spot TB test in the experimental group were calculated respectively; the sensitivity, specificity, and positive and negative predictive value after the joint detection of the two methods were evaluated using the *t*-test. Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. General information

There were a total of 86 cases in this study of which 41 patients with pulmonary TB with an age range of 19–84 years old consisting of 26 males and 15 females made up the observation group; 45 patients with non-pulmonary TB with an age range of 27–86 years old consisting of 28 males and 17 females made up the control group.

3.1.1. Results

As shown in **Table 1**, the T-spot TB test results showed that among 41 cases in the observation group, 39 cases (95.12%) were TB positive and 2 cases (4.88%) were TB negative; in the control group, 16 cases (35.55%) were positive and 29 cases (64.65%) were negative. The ESR tests showed that in the observation group, 78.04% were positive and 21.96% were negative; in the control group, 73.33% were positive and 26.67% were negative.

Table 1. Comparison of T-spot TB test and ESR test results (*n*)

Group	Total cases, *n*	ESR (mm/h)	T-spot TB test
Observation group	41	56.41 ± 44.79	39
Control group	45	40.36 ± 35.55	16
t		1.517	7.515
P		0.004	0.001

Receiver operating characteristic (ROC) curve analysis of ESR versus T-spot TB test was done by considering those diagnosed with TB as the observation group and those without TB as the control group. As shown in **Figure 1**, The ESR test showed that the area under the line (AUC) was 0.537 and had low diagnostic accuracy. In the T-spot TB test, the area under the line (AUC) was 0.805 and had higher diagnostic accuracy.

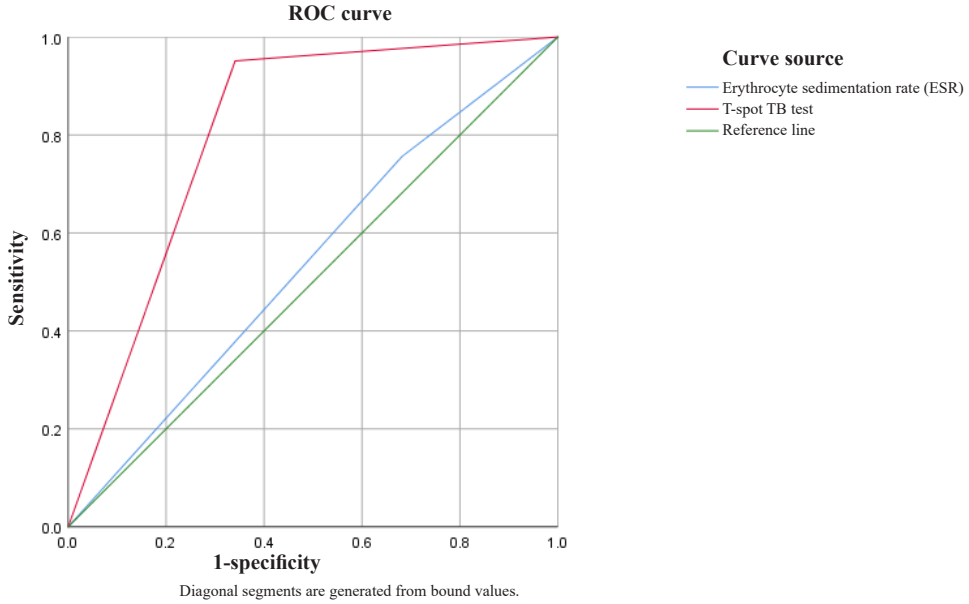

Figure 1. AOC curves for the diagnosis of TB by different methods

3.1.2. Combined test results

As shown in **Table 2**, among 41 cases in the experimental group, the combined test showed 68.29% positive cases and 31.71% negative cases; among 45 cases in the control group, the combined test showed 22.22% positive cases and 77.78% negative cases.

Table 2. Comparison of different diagnostic methods in the clinical diagnosis of TB (*n*)

Group	Gold standards	ESR		T-Spot TB test		ESR and T-spot TB test		Total
	Result	Positive	Negative	Positive	Negative	Positive	Negative	
Observation group	Positive	32	9	39	2	28	0	28
Control group	Negative	31	14	16	29	10	11	21

As shown in **Table 3**, the sensitivity of the combined test was 100% with a specificity of 52.38%. The T-spot TB test had a higher sensitivity and specificity of 95.12% and 33.34% respectively as compared with the ESR test at 78.04% and 26.67%.

Table 3. Comparison of the diagnostic efficacy of different testing protocols [% (*n*/*n*)]

Diagnostic methods	Level of sensitivity	Specificity	Positive predictive value	Negative predictive value
ESR	78.04% (32/41)	26.67% (12/45)	50.79% (32/63)	60.86% (14/23)
T-spot TB test	95.12% (39/41)	33.34% (8/24)	70.90% (39/55)	93.54% (29/31)
ESR and T-spot TB test	100.00% (28/28)	52.38% (11/21)	70.00% (28/40)	45.45% (10/22)

4. Discussion

TB is a severe infectious disease and a global chronic disease, mainly transmitted through the respiratory tract, with a steady increase in global incidences every year. TB patients are often comorbid with lung infections, which, if left untreated, may lead to lesion expansion, thus aggravating the disease progression, or even leading to respiratory failure. With the worsening of the immune system and other factors, the comorbidities of TB patients increase as the disease progresses, thus complicating the diagnosis of TB. The T-spot TB test uses the principle of T-lymphocyte detection to improve diagnostic accuracy by combining specific antigens and stimulating γ-interferon release, followed by spotting technology to analyze and scientifically calculate the frequency of antigen-specific cells. In addition, this test can also detect the reaction of single peripheral cells, demonstrating high sensitivity and accuracy in reflecting the severity of the patient's condition [16]. However, the diagnostic accuracy of the T-spot TB test for TB patients without specific symptoms is low, hence it needs to be combined with other methods to improve its clinical diagnostic accuracy.

5. Conclusion

The T-spot TB test can rapidly diagnose TB, and the ESR test combined with the T-spot TB test demonstrated a high positive rate of TB diagnosis. This can effectively improve the diagnostic rate and is conducive to the early diagnosis of TB and the identification of bronchopneumonia. Furthermore, the combination of multiple indexes can complement each protocol's strengths and weaknesses. For example, the parallel test can improve the diagnostic sensitivity of TB, and the tandem test can increase its specificity.

Disclosure statement

The author declares no conflict of interest.

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