Research on the value of the T cell spot test for tuberculosis for the diagnosis of lung cancer combined with pulmonary tuberculosis

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Abstract

Background: This study was conducted to investigate the value of the T cell spot test for tuberculosis (T-SPOT.TB) for the diagnosis of patients with lung cancer combined with pulmonary tuberculosis (LCTB).

Methods: Thirty-six patients diagnosed with LCTB who received treatment at Shandong Provincial Chest Hospital from September 2014 to 2017 were randomly chosen and enrolled as an observation group; 63 patients diagnosed with LC alone in the same period were included as the control. The T-SPOT.TB results of the two groups were compared.

Results: The positive rate of T-SPOT.TB in 36 patients with LCTB was 88.9% (32/36), and in 63 patients with LC was 23.8% (15/63). The median ESAT-6 result in the LCTB group was 22 SFCs/2.5×10^5 peripheral blood monocytes (PBMC) (interquartile range [IQR] 8–53), which was higher than in the LC group with a median of 1 spot-forming cell (SFC)/2.5×10^5PBMC (IQR 0–5). The median CFP10 result in the LCTB group was 18 SFCs/2.5×10^5PBMC (IQR 7–30), which was significantly higher than in the LC group with a median of 0 SFC/2.5×10^5PBMC (IQR 0–4). The receiver operating characteristic curve of the two groups showed sensitivity of 88.9% and specificity of 84.4% when the positive value of T-SPOT.TB was 11 SFCs/2.5×10^5PBMC.

Conclusions: T-SPOT.TB has comparatively high value for diagnosing LCTB. The referential cutoff value is 11 SFCs/2.5×10^5PBMC, which warrants clinical application.

Introduction

In recent years, the incidence of lung cancer (LC) has significantly increased as a result of factors such as environmental pollution, population aging, and unhealthy living habits. LC has become the leading cause of tumor death in China. There are a substantial number of tuberculosis (TB) patients in China. The World Health Organization reported 10.4 million patients were suffering from TB and 1.7 million died of TB in 2016 worldwide.1 Seven countries account for 64% of new TB cases and China ranks third. Although the pathogenesis of LC is completely different from pulmonary TB, the clinical manifestation and imaging features of coexistent LC and pulmonary TB and single pulmonary TB or LC resemble each other. Therefore, the coexistence of both diseases is often considered a single disease, which causes missed diagnosis or misdiagnosis. The enzyme-linked immunospot T cell spot test for tuberculosis (T-SPOT.TB) is a type of immunoassay technology based on the antigen-specific cellular immune response, which has high sensitivity and specificity for the diagnosis...
of TB infection and has been widely applied in clinical practice. This study investigated the value of T-SPOT.TB for the diagnosis of patients with LC combined with pulmonary TB (LCTB) from a clinical perspective.

Methods

Study subjects

A total of 36 patients diagnosed with LCTB who received treatment at Shandong Provincial Chest Hospital from September 2014 to 2017 were retrospectively chosen as the experimental group, including 31 men and 5 women aged 44–79 years (average age 64.69 ± 9.26); 18 cases were adenocarcinoma and the remaining 18 were squamous carcinoma. Sixty-three patients with LC alone in the same period were enrolled as the control group, including 48 men and 15 women aged 40–78 years (average age 62.35 ± 9.95); 26 patients had adenocarcinoma and 37 had squamous carcinoma. The LC diagnoses in the two groups were all pathologically verified. A comparison of basic clinical information between the groups showed no statistically significant differences (P > 0.05).

Diagnostic criteria

Pulmonary TB is diagnosed when either of the following criteria is met: (i) a histopathologically verified result, or (ii) the detection of mycobacterium TB. The diagnostic basis of negative TB (3 negative sputum smear results and 1 negative culture result) is detailed in the Diagnosis and Treatment Guidelines of Pulmonary Tuberculosis. A diagnosis of LC can be concluded after histologically or cytopathologically locating cancer cells.

The control group did not exhibit any of the criteria for pulmonary TB. Control group criteria were: (i) chest CT typical of LC, pathological tissue was checked for cancer cells, pathological morphology and acid stain did not reveal evidence of mycobacterium TB; or (ii) chest CT, pathological tissue biopsy, phlegm anti acid bacilli culture, smear, and PCR examination did not reveal evidence of mycobacterium TB infection.

Testing

The T-SPOT.TB method was performed as follows: 4–6 mL of venous blood was collected from each patient; after adding heparin/heparin sodium for anticoagulation, peripheral blood monocytes (PBMC) were acquired through centrifugation and were counted after being cleaned. PBMCs and TB specific antigen were then added into the prepackaged antibody reaction holes at the same time and incubated overnight (37°C, 5% CO2). Cytokines released by effective T lymphocytes were combined with the prepackaged antibody. After the plate was washed, secondary antibodies were added and the sample was incubated for an hour. The plate was then washed again and substrate-developing solution was added for another seven minutes of incubation. Distilled water was applied to halt any reaction. One spot represented one effective T lymphocyte. A positive result was concluded when there were: (i) 0–5 spots in the negative control hole, and (spots in antigen A or antigen B holes)–(spots in negative control hole) ≥ 6; or (ii) 6–10 spots in the negative control hole and (spots in antigen A or antigen B holes) ≥ 2 × (spots in negative control hole). If the result did not meet these criteria and positive control holes were normal, the test result was considered negative.

The results of T-SPOT.TB are described by the number of spot-forming cells (SFCs) among every 250 000 PBMCs. After being stimulated by antigens (ESAT-6 or CFP10), if the number of SFCs among every 250 000 PBMC was ≤ 6, the result was negative; if > 6, the result was positive.

Statistical analysis

SPSS version 19.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. A Koimogorov–Smirnov test was used to examine whether the data obeyed normal distribution. Data obeying normal distribution were presented as mean ± standard difference (x ± s); data that did not obey normal distribution were presented as median and interquartile range. An independent sample t test was used for continuous variables that obeyed normal distribution, and a non-parametric rank-sum test for samples that did not obey normal distribution. Comparison of reaction rates between the two groups was performed using a chi-square test. Conformity between the experiment and actual situation was analyzed by Kappa coefficient; the higher the value, the better the conformity.

Results

Analysis of T cell spot test for tuberculosis (T-SPOT.TB) results

The positive rate of T-SPOT.TB in the LCTB group was 88.9% (32/36), which was obviously higher than in the LC group with a positive rate of 23.8% (15/63) (P < 0.001) (Table 1). The ESAT-6 result range of the LCTB group was 0–185 SFCs/2.5 × 10⁵PBMC and the median was 22 SFCs/2.5 × 10⁵PBMC and a median of 1 SFC/2.5 × 10⁵ PBMC (IQR 8–53), which were higher than the LC group with an ESAT-6 result range of 0–38 SFCs/2.5 × 10⁵PBMC and a median of 1 SFC/2.5 × 10⁵ PBMC (IQR 0–5) (P < 0.001). Table 2 shows the ESAT-6 results between the groups. The CFP10 result range of the LCTB group was 0–300 SFCs/2.5 × 10⁵PBMC and the median was 18 SFCs/2.5 × 10⁵PBMC (IQR 7–30), which
was significantly higher than the LC group with a CFP10 result range of 0–34 SFCs/2.5 × 10^5 PBMC and a median of 0 SFC/2.5 × 10^5 PBMC (IQR 0–4) (P < 0.001). Table 3 shows the CFP10 results between the groups.

### Positive cutoff value of T-SPOT.TB

When the positive cutoff value of T-SPOT.TB was increased from 6 SFCs/2.5 × 10^5 PBMC to 11 SFCs/2.5 × 10^5 PBMC, the sensitivity remained the same and the specificity improved. A positive cutoff value of T-SPOT.TB of 11 SFCs/2.5 × 10^5 PBMC achieved the best sensitivity and specificity for diagnosing TB, according to the ROC curve. The Kappa coefficient was used to evaluate reliability: when the positive cutoff value (SFCs/2.5 × 10^5 PBMC) of T-SPOT.TB was 6, the Kappa value was 0.611; when the positive cutoff value (SFCs/2.5 × 10^5 PBMC) of T-SPOT.TB was 11, the Kappa value was 0.705. Therefore, the reliability of the T-SPOT.TB with the new positive cutoff value was higher (Table 4, Fig 1).

### Discussion

Tuberculosis is an infectious respiratory disease that severely threatens human health. LC is a kind of malignant tumor commonly seen in clinical practice, with the highest morbidity and mortality rates in China. The incidence of pulmonary TB combined with LC is reported as 0.22–2.7%; in people aged > 60 years, the incidence rate is 10–15%. The frequency of the coexistence of these two diseases increases each year. As LC and pulmonary TB are both immunocompromising and wasting diseases, they could mutually promote occurrence and development. Firstly, LC cells can damage pulmonary fibrous tissue, which will activate originally static tuberculosis lesions. From an immunological perspective, tumor cells produce an immunosuppressive factor; radiotherapy and chemotherapy cause the decline of the body’s immune function, which further increases the TB infection rate. According to internal medicine statistics, at present, 2–8% of pulmonary TB patients are diagnosed with LCTB. As one of the primary diseases threatening human health, LCTB has become the focus of epidemic disease research. The prognosis of patients with LCTB is generally unsatisfactory, as patients’ nutritional status is poor and body tolerance declines. Meanwhile, the symptoms of pulmonary TB and LC are mutually affected by each other. For example, anemia could cause tumor tissue hypoxia, which in turn decreases a patient’s sensitivity to chemotherapy drugs. The simultaneous application of anti-tuberculosis and antitumor treatments could improve pulmonary TB symptoms and control the progress of malignant tumors, which

### Table 1 T-SPOT.TB results

| Group                                      | Cases | T-SPOT.TB positive | T-SPOT.TB negative |
|--------------------------------------------|-------|--------------------|--------------------|
| Lung cancer combined with pulmonary TB     | 36    | 32 (88.9)          | 4 (11.1)           |
| Lung cancer                                | 63    | 15 (23.8)          | 48 (76.2)          |
|χ²                                          |       |                    | 38.91              |
|P                                           |       |                    | < 0.001            |

T-SPOT.TB, T cell spot test for tuberculosis.

### Table 2 ESAT-6 results of T-SPOT.TB

| Group                                      | Cases | ESAT-6 result (SFCs/2.5 × 10^5 PBMC) |
|--------------------------------------------|-------|--------------------------------------|
|                                            |       | < 6                                 |
|                                            |       | 6–22                                |
|                                            |       | 22–38                               |
|                                            |       | > 38                                |
| Lung cancer combined with pulmonary TB     | 36 (100%) | 7 (19.4%) | 11 (30.5%) | 5 (13.9%) | 13 (36.1%) |
| Lung cancer                                | 63 (100%) | 49 (77.8%) | 13 (20.6%) | 1 (1.6%)  | 0 (0%)     |

T-SPOT.TB, T cell spot test for tuberculosis.

### Table 3 CFP-10 results of T-SPOT.TB

| Group                                      | Cases | CFP-10 result (SFCs/2.5 × 10^5 PBMC) |
|--------------------------------------------|-------|--------------------------------------|
|                                            |       | < 6                                 |
|                                            |       | 6–22                                |
|                                            |       | 22–38                               |
|                                            |       | > 38                                |
| Lung cancer combined with pulmonary TB     | 36 (100%) | 8 (22.2%) | 14 (38.9%) | 7 (19.4%) | 7 (19.4%) |
| Lung cancer                                | 63 (100%) | 52 (82.5%) | 8 (12.7%)  | 3 (4.8%)  | 0 (0%)    |

All percentage values were rounded. T-SPOT.TB, T cell spot test for tuberculosis.

### Table 4 Sensitivity and specificity of T-SPOT.TB with different positive cutoff values

| Positive cutoff value of T-SPOT.TB (SFCs/2.5 × 10^5 PBMC) | Sensitivity (%) | Specificity (%) |
|-----------------------------------------------------------|-----------------|-----------------|
| 6                                                         | 88.9            | 76.2            |
| 11                                                        | 88.9            | 84.1            |
| 15                                                        | 77.8            | 90.5            |

T-SPOT.TB, T cell spot test for tuberculosis.
alleviates the symptoms of pulmonary TB and LC and improves patient quality of life.  

At present, a positive sputum bacteria culture result remains the gold standard for making a definitive diagnosis of pulmonary TB; however, the positive rate is only 30–40%. China’s 13th Five-Year Plan requires that the positive rate of a laboratory sputum TB culture should be > 50%, suggesting that a low positive rate may affect experiment results. For other auxiliary diagnosis experiments, such as the tuberculosis antibody (TB-Ab) test, tuberculin skin test (TST), and nucleic acid amplification test of TB specificity (NAAT), clinical application is limited because of problems such as false negative results caused by the patient’s comprised immune function or Bacille Calmette-Guérin vaccination, the high requirement of the PCR technique, indistinguishable results between live and dead bacteria, and the difficulty collecting samples from patients with extrapulmonary TB. The T-SPOT.TB tests the number of released γ-interferon specific T lymphocytes after stimulation by specific antigens EAST-6 and CFP-10 of mycobacterium TB via an enzyme-linked immunospot technique. The sensitivity and specificity of T-SPOT.TB is higher compared to traditional diagnosis methods. The positive rate of T-SPOT.TB in our LCTB group was 88.9%. Liu et al. conducted a large sample test of 1084 suspected TB patients and reported that T-SPOT. TB sensitivity was 85%. Li et al. tested 120 patients diagnosed with TB and revealed that T-SPOT.TB sensitivity was 88.3%. Our results were consistent with the results of these studies.

In this study, the positive T-SPOT.TB rate and the number of γ-interferon specific T lymphocytes released by EAST-6 and CFP-10 peptide fragments after stimulation in the 63 cases in the LC group were lower than in the LCTB group, illustrating that T-SPOT.TB could be applied to diagnose cases of LC alone or LCTB. After increasing the positive cut-off value of T-SPOT.TB to 11 SFCs/2.5 × 10^5 PBMC, ROC curves revealed sensitivity of 88.9% and specificity of 84.1%, which were the highest for diagnosing TB, suggesting that this is the best method to determine a diagnosis of LCTB.

In conclusion, the value of applying T-SPOT.TB for a diagnosis of LCTB is high. Patient quality of life could be improved by simultaneously combining anti-tuberculosis with anti-tumor treatments, thus promotion of this method in clinical practice is warranted.

**Disclosure**

No authors report any conflict of interest.

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