**The value of T-SPOT.TB in early diagnosis of tracheobronchial tuberculosis**

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**Abstract.** **Objective:** To evaluate the value of T-SPOT.TB in the diagnosis of tracheobronchial tuberculosis.  
**Methods:** This study included in January 2010 to October 2014 in the three gorges university during the first clinical medical college of final 283 diagnosed with tracheal bronchus TB patients (including 273 patients with pathological biopsy diagnosis and 10 called suspected TB patients), at the same time will be 283 active TB patients as a parallel control group included in this study. They were all given traditional detection methods acid fast stain and diseased tissue pathological biopsy and the new detection method T-SPOT.TB.  
**Results:** Sputum smear acid-fast stain sensitivity rate is 39.2% (111/283), typical TB diseased tissue pathology morphology of 221 cases (78.1%), tend to TB 52 cases (18.4%), while T-SPOT. TB testing sensitivity and speciality rate is 93.6% (265/283) and 85.1% (241/283), which is much higher then the former two.  
**Conclusion:** T-SPOT.TB can provide important basis for the diagnosis of tracheobronchial tuberculosis. It is the fastest and most accurate method in the diagnosis of tracheobronchial tuberculosis. *(Sarcoidosis Vasculit. Diffuse Lung Dis 2015; 32:336-341)*

**Key words:** T-SPOT.TB, tracheobronchial tuberculosis, clinical cases, diagnosis

**Abbreviations:**

- TBTB=Tracheobronchial tuberculosis  
- EBTB=endobronchial tuberculosis  
- TB=tuberculosis  
- PBMCs=peripheral blood mononuclear cells  
- ELISPOT=enzyme-linked immunospot assay

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**Introduction**

Tuberculosis (TB) is a serious infectious diseases which affect the safety of people's life all over the world. About a third of the global population is infected with mycobacterium tuberculosis (1). There are nearly 80-100 million people who infected TB newly every year in the developing countries which are highly TB burden (2). Among all the TB, extrapulmonary tuberculosis accounted for nearly 20% (3). But the high risk from different tissues and organs of the body, the symptoms are not typical and the traditional diagnosis methods is limited. Thus makes it Harder to clinical diagnosis (4). Tracheobronchial tuberculosis(TBTB) refers to
tuberculosis such as tracheal and bronchial muco-
sa, submucosa, smooth muscle, cartilage and outer
membrane of TB. Under the bronchoscope tracheal
and bronchial mucosa can be directly observed vi-
lations, combined with bronchial lesions than tra-
cheal pathological changes, clinical so ever more
called endobronchial tuberculosis (EBTB). Such
patients clinical manifestation and imaging exami-
nation lack of specificity, and the misdiagnosis rate
was 68.8%. T-SPOT.TB Is a new method that test
the specific T lymphocytes in the body with TB by
enzyme-linked immune spot testing. When the sen-
sitized T lymphocytes which had been stimulated by
mycobacterium tuberculosis antigen are stimulated
by the same type of antigen such as CFP-10 and
ESA T-6 again, they will produce IFN-γ. T-SPOT.
TB is the new method that detects if the subjects
be infected with mycobacterium tuberculosis by
detecting the concentration of IFN-γ to detect the
number of T cells release IFN-γ and it has the high
sensitivity and specificity (6) which can be applied
to diagnosis of tuberculosis. In this paper, a retro-
spective analysis the of related data of 283 cases of
confirmed tracheobronchial tuberculosis patients
which are selected from department of respiratory
medicine, The first College of Clinical Medicine sci-
cence of Three Gorges University from January 2010
to October 2014 has been done. The following three
detection methods has been done to compare the
value of diagnosis of tracheobronchial tuberculosis,
so as to explore the significance of T-SPOT.TB in
early diagnosis.

Materials and methods

Materials

283 cases of confirmed tracheobronchial tuber-
culosi5 cases which are selected from department
of respiratory medicine, The first College of Clinical
Medicine science of Three Gorges University from
January 2010 to October 2014 (86 male and 197 fe-
male, male to female ratio of 1:2. 29 aged 16 to 49
years old, average age 31.2±2.1 years old). All tu-
berculosis patients were accord with WHO Global
Tuberculosis Control. Geneva: World Health Or-
ganizatio (2). Clinical manifestations and clinical
response to treatment of TB, Sputum smear posi-
tive set the fungus acid fast bacilli positive. It is best
to cultivate the MTB positive. Imaging changed.
PPD test positive. Tracheal and bronchial lesions
under the bronchoscope. Acid fast bacilli positive in
bronchial brush or bronchial washing fluid. TB ve-
neral change by bronchoscopy biopsy samples. All
the cases with no history of virus infection, immune
disease, tumor, etc. Always there is no history of tu-
berculosis. A series of routine examination has been
checked, such as a routine blood, blood biochem-
istry, blood gas analysis, chest CT and so on after
admission. They also accept the three specific detec-
tion of TB: diseased tissue pathological biopsy, acid
fast stainand and T-SPOT.TB of peripheral blood.
The patients that are involved in this study were in-
formed before.

Methods

T-SPOT.TB test of Peripheral blood

T-SPOT.TB reagents imported from Oxford
provided by Shanghai fosun long march medical
science company, LTD. 4~6 mL peripheral blood
was collected in heparin anticoagulant blood ves-
sels. Then peripheral blood mononuclear cells (PB-
MCs) were gotten after centrifuging. Count cells
after washing them. Drip PBMCs and tuberculosis
specific antigen into the reaction hole pre packaged
by antibodies at the same time. Then incubated for
the night (37,5%CO2); the effect of T lymphocytes
release cytokines that combined with antibodies in
the process of the package. Then washing the plate
adding a second antibody and incubating for 1 hour
washing the plate again then adding the substrate
color liquid and incubating for 7 minutes. Finally
terminating the reaction by distilled water. In the
result of this test a spot on behalf of an effect of T
lymphocyte. A positive result with reference to the
following criteria: when the spot number of nega-
tive control hole are 0 to 5 and bore measuring spots
number minus the negative control spot number≥ 6
or when the spot number of negative control hole are
6 to 10 and measuring hole number≥2× (number of
spots in negative control hole). On the other hand,
the test results as a “negative” if don't comply with
the above standard and the number of spot in the
positive control hole is normal.
The diseased tissue pathological biopsy

The typical pathological form of tuberculosis from the inspection organization (Be characterized by lang giant cells epithelioid cells and granulomatous nodules caseous necrosis of tuberculosis); There are epithelioid cell granulomas and caseous necrosis after HE dying in inspection organization and the pathological diagnosis tend to TB. The clinic diagnostic anti-tuberculosis treatment is effection, but there is no specificity is diseased tissue so it cannot confirm TBTB.

The lesion detection acid-fast stain

Diseased tissue was sliced with acid fast stain by phenol fuchsine acid staining method for acid fast stain slice. All measurement data was tested evaluation by more than two intermediate and above intermediate doctors by double blind (independent) method. Method of interpretation of the results: It is positive that find the acid positive mycobacteria (fine superfine slightly bent shaft, long 3 μm, Red dyed, no refraction, focal distribution) at high magnification (×40). Can't find specific acid positive mycobacteria. But to find some like acid positive mycobacteria tiny, red dye, not grain kind material for suspicious positive refraction. Acid positive mycobacteria was not found or granular material is negative.

Statistical methods

Using SPSS 20.0 statistical software for statistical analysis, counting data using $\chi^2$ test for statistical processing, $P < 0.05$ for the difference was statistically significant.

Results

The result of T-SPOT.TB test of Peripheral blood of the 283 patients

The sensitivity of The result of T-SPOT.TB test of Peripheral blood of the 283 cases of tracheobronchial tuberculosis patients is as high as 93.6% (265/283) and the specificity is 85.1% (241/283). The sensitivity of T-SPOT.TB of peripheral blood from patients aged 16 to 35 years old and above 35 old are 92.6% (163/176) and 95.3% (102/107). The male and the female patients with peripheral blood T-Spot, TB sensitivity was 91.8% (79/86 cases) and 95.4% (180/197). There was no statistically significant difference sensitivity of TB in patient’s gender and age ($X^2=0.479, P>0.05$) (Figure 1).

The result of the diseased tissue pathological biopsy

Numbers of patients with typical TB pathological morphology of the 283 cases of tracheobronchial tuberculosis patients is 221, Numbers of patients tend to tuberculosis is 52 (18.4%). There are 10 cases which have no clear pathological characteristics of tuberculosis, but clinical diagnostic anti-tuberculosis treatment to cure (3.5%). There was no statistically significant difference sensitivity of TB in patient’s gender and age ($X^2=0.483, P>0.05$) (Figure 2).

Result of the diseased tissue pathological biopsy

There are 93 cases who were found the acid positive mycobacteria. 18 cases suspected positive and 188 cases of negative at high magnification (×40) in the 283 patients. The total positive rate is 39.2%. There was no statistically significant difference sensitivity of TB in patient’s gender and age ($X^2=0.491, P>0.05$) (Figure 3).

Three detection results analysis (Table 1)

Discussion

Currently, tuberculosis is still one of the infectious diseases that threaten human health seriously all over the world. It has a highest mortality rates in the Infectious diseases which are caused by a single pathogenic bacteria. It is also the severe public health problem which comes under the global attention that become the leading infectious killer in developing countries. In addition to tuberculosis, TBTB patients account for part of extrapulmonary tuberculosis. TBTB disease develops quite slowly clinically and it dose not have obvious clinical manifestations. So it brings a lot of trouble to clinical doctors to diagnose. Bacteriology inspection is the main way to discover the source of infection, the important basis to determine the diagnosis and treatment of TB and the
Reliable standard to assess curative effect and evaluate therapeutic effect plays an important and indispensable role in preventing and controlling TB. But because of the long growth cycle decided by their genetic traits, Mycobacterium tuberculosis usually need a couple of weeks to complete a growth cycle. Thus lead to a low sensitivity, complex operation of bacteriology inspection. On the other hand, as a result of long time, not easy to standardize and a series of other factors, bacteriology inspection can't fully meet the need of clinical diagnosis and it develops slowly. So developing a sensitive, early, rapid, simple laboratory diagnosis that can predict the prognosis is a necessary supplement to diagnosis of TBTB.

In recent years, some high level foreign magazines have published a new technology that apply in the diagnosis of extrapulmonary tuberculosis. Which named enzyme-linked immunospot as-

![Fig. 1. The result of T-SPOT.TB detected T lymphocytes secreting IFN-γ in peripheral blood between the TBTB group and NOT TBTB group. (A) The result of T-SPOT.TB in nor TBTB group is negative (B) The result of T-SPOT.TB in TBTB group is positive.](image)

![Fig. 2. A) Typical TB; B) Tendency to TB; C) No clear TB](image)
say (ELISPOT) and the commercial product name called T-SPOT.TB.

Current literature reports that T-SPOT.TB has important application value in diagnosis of diseases such as tuberculosis (7), breast Tuberculosis (8), spinal tuberculosis (9), et al.

283 patients of this study who were diagnosed with TBTB accepted diseased tissue pathology biopsy, acid fast stain and T-SPOT.TB test of peripheral blood. Then comparing the results, the sensitivity of T-SPOT.TB test’s result is 93.6% (265/283), specificity is 85.1% (241/283), and they are close to the related research (10), and the positive rate was significantly higher than that of histopathologic biopsy acid fast stain (78.1%) and pathological tissues (39.2%) (P < 0.05). A low containing of bacterium in the histopathologic examination samples and a long growth cycle of mycobacterium tuberculosis and other factors make a low positive rate of acid fast stain of histopathologic biopsy and pathological tissues. But other studies have shown that T.SPOT.TB can’t identify active and inactive tuberculosis, so

<table>
<thead>
<tr>
<th>Patients’ characteristics</th>
<th>All patients</th>
<th>Positive rate (%)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Age</td>
<td></td>
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</tr>
<tr>
<td>16-35</td>
<td>176</td>
<td>92.6</td>
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<td>35-49</td>
<td>107</td>
<td>95.3</td>
<td>P&gt;0.05</td>
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<tr>
<td>Gender</td>
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<tr>
<td>male</td>
<td>86</td>
<td>91.8</td>
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<tr>
<td>female</td>
<td>197</td>
<td>95.4</td>
<td>P&gt;0.05</td>
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<td>T-SPOT·TB</td>
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<td></td>
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<tr>
<td>positive</td>
<td>265</td>
<td>93.6</td>
<td>P&lt;0.05</td>
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<tr>
<td>negative</td>
<td>18</td>
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<tr>
<td>Pathological biopsy</td>
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<td></td>
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<tr>
<td>suspicious positive</td>
<td>221</td>
<td>78.1</td>
<td>P&lt;0.05</td>
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<tr>
<td>no specific</td>
<td>52</td>
<td>18.4</td>
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<tr>
<td>Acid fast stain</td>
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<tr>
<td>positive</td>
<td>93</td>
<td>32.9</td>
<td>P&lt;0.05</td>
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<tr>
<td>suspicious positive</td>
<td>18</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>188</td>
<td>32.9</td>
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</tr>
</tbody>
</table>

Use x² test to analysis the data.
P : comparing T-SPOT·TB positive to Pathological biopsy positive
Po : comparing Acid fast stain positive with Pathological biopsy positive
Pc : comparing Acid fast stain positive with Pathological biopsy positive

Fig. 3. A) Positive; B) Suspicious positive; C) Negative

Table 1. Characteristics and test results of the 283 cases of TBTB patients
the specificity of this test for active TBTB is related to the research object (11, 13). And studies show that the sensitivity of T-SPOT.TB test is associated with the length of the illness and pathogenic site (14). In addition, because it takes a lot of time for T lymphocytes to activate after mycobacterium tuberculosis infection and the different of standard of all the cases, the sensitivity of the research could not have reached 100%. In this study T-SPOT.TB positive rate is as high as 93.6%, and the test only need to take a blood test and it only takes two days to get the result. In conclusion, as a result of features such as quick, convenient and accurate, T-SPOT.TB can be used as a kind of important means used in the early diagnosis of tracheal bronchial tuberculosis. It can also bring the gospel and give high tuberculosis burden hope for the majority of TB especially tracheal bronchus TB patients all over the world.

Reference