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 biopsy 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 accu-rate method in the diagnosis of tracheobronchial tuberculosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2015; 32: 336–341)*

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

Abbreviations:

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 mucosa, submucosa, smooth muscle, cartilage and outer membrane of TB. Under the bronchoscope tracheal and bronchial mucosa can be directly observed violations, combined with bronchial lesions than tracheal pathological changes, clinical so ever more called endobronchial tuberculosis (EBTB). Such patients clinical manifestation and imaging examination 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 sensitized 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 retrospective analysisthe 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 science 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 tuberculosis patients 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 female, male to female ratio of 1:2. 29,aged 16 to 49 years old, average age 31.2±2.1 years old). All tuberculosis patients were accord with WHO Global Tuberculosis Control. Geneva: World Health Organizatio (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 venereal change by bronchoscopy biopsy samples. All the cases with no history of virus infection, immune disease, tumor, etc. Always there is no history of tuberculosis. A series of routine examination has been checked, such as a routine blood, blood biochemistry, blood gas analysis, chest CT and so on after admission. They also accept the three specific detection 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 informed 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 vessels. Then peripheral blood mononuclear cells (PBMCs) 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 negative 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 x^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

Nombers of patiens with typical TB pathological morphology of the 283 cases of tracheobronchial tuberculosis patients is 221, Nombers of patiens 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 283patiens. 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 wold. 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
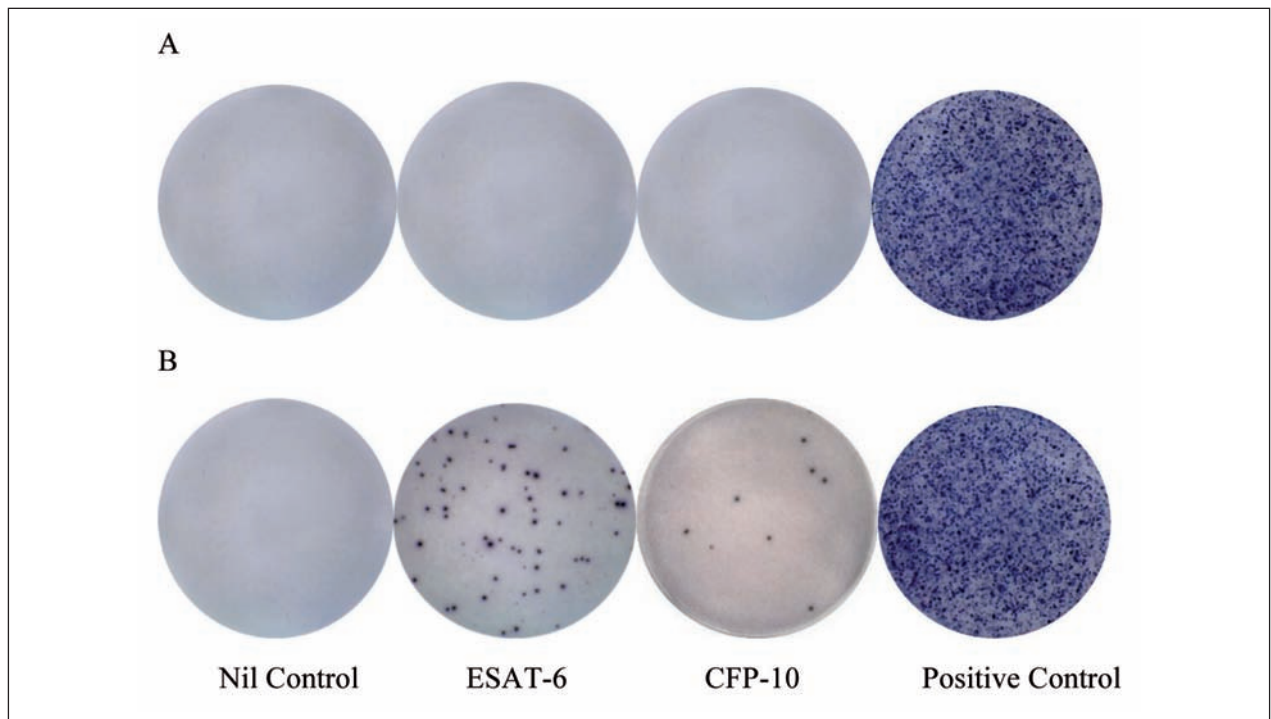
Fig. 1. The result of T-SPOT.TB detected T lymphocytes secreting IFN-γ in peripheral blood between the TBTB group and NOT TBTB group. (A) The result of T-SPOT.TB in not TBTB group is negtive (B) The result of T-SPOT.TB in TBTB group is positive.
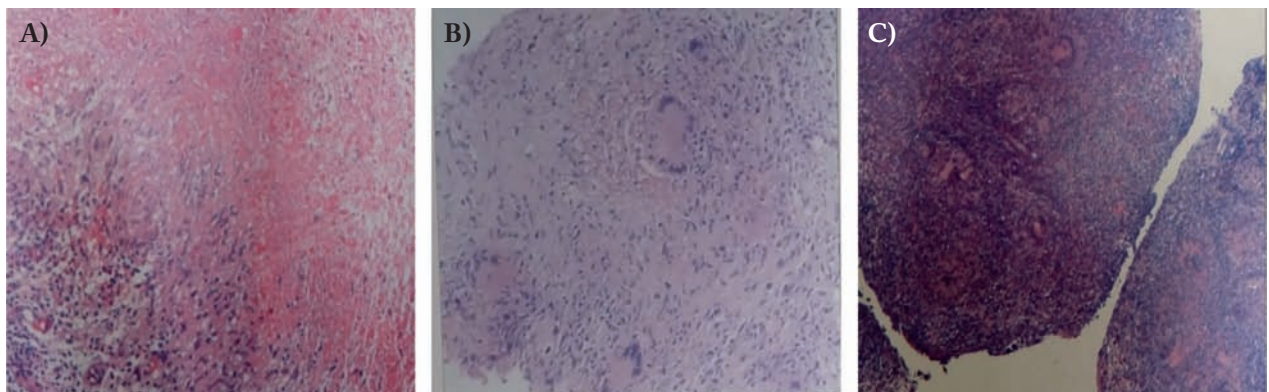

Fig. 2. A) Typical TB; B) Tendency to TB; C) No clear TB

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 there 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 extr-apulmonary tuberculosis Which named enzyme-linked immunospot as-
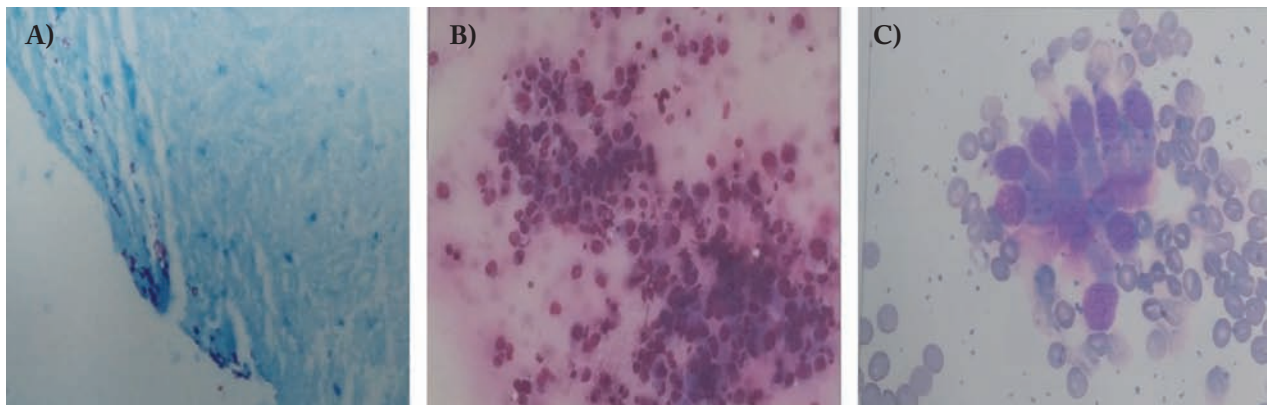
Fig. 3. A) Positive; B) Suspicious positive; C) Negative

Table 1. Characteristics and test results of the 283 cases of TBTB patients

Patients' characteristics	All patients	Positive rate (%)	P value
Age			
16-35	176	92.6	
35-49	107	95.3	P>0.05
Gender			
male	86	91.8	
female	197	95.4	P>0.05
T-SPOT·TB			
positive	265	93.6	P[a]<0.05
negative	18		
Pathological biopsy	221	78.1	P[b]<0.05
suspicious positive	52	18.4	
no specific	10	3.5	
Acid fast stain			
positive	93	32.9	P[c]<0.05
suspicious positive	18	6.3	
negative	188		
positive of all the tree tests	93	32.9	

Use x^2 test to analysis the data.
P[a] : comparing T-SPOT·TB positive to Pathological biopsy positive
P[b] : comparing Acid fast stain positive with Pathological biopsy positive
P[c] : comparing Acid fast stain positive with Pathological biopsy positive

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

the specificity of this test for active TBTB is ralated 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 of 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.

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