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The value of enzyme-linked immunospot assay test in the diagnosis and evaluation of curative effect of tuberculous pleurisy

Wang Lihong, Fu Xiuhua*, Zhang Guizhi, Li Guohua, Gu Yan, Gao Junzhen
Department of Respiration Medicine, the Affiliated Hospital of Inner Mongolian Medical College, Hohhot 010050, (CHINA)
E-mail : fuxiuhua555@sohu.com

ABSTRACT

Objective: To investigate the value of enzyme-linked immunospot assay test (ELISPOT) (T-SPOT.TB) in the diagnosis and therapeutic effect of tuberculous pleurisy. **Methods**: Collected 93 cases of Affiliated Hospital of Inner Mongolia Medical University patients with pleural effusion. The peripheral blood and pleural effusion of the patients with non hemorrhagic exudative pleural effusion were tested by T-SPOT.TB before treatment. The peripheral blood of the patients of clinically diagnosed as tuberculous pleurisy were tested by T-SPOT.TB again after 3 months regular anti tuberculosis. **Result:** There are 63 cases in 93 patients with pleural effusion were diagnosed as tuberculous pleurisy. Among them, the positive rate of T-SPOT.TB test in peripheral blood and pleural effusion was 90.48%(57/63) and 100%(63/63) respectively. There are 30 cases were diagnosed as non tuberculous pleurisy. Among them, the negative rate of T-SPOT.TB test in peripheral blood and pleural effusion was 83.33%(25/30) and 70%(21/30). In our study, the negative predictive value(Specificity) and the positive predictive value of T-SPOT.TB test in peripheral blood was 80.65%(25/31) and 91.94%(57/62). The negative predictive value and the positive predictive value of T-SPOT.TB test in pleural effusion was 100%(21/21) and 87.5%(63/72). The average numbers of spot forming cells(SFCs) in Pleural effusion were 4.69 times that in peripheral blood. The numbers of SFCs in peripheral blood were obviously decreased or loss after 3 months anti tuberculosis therapy. **Conclusion**: T-SPOT TB is a sensitive and specific test in the diagnosis of tuberculous pleurisy, especialy in pleural effusion. T-SPOT TB can be used as evaluation index of tuberculosis treatment.

KEYWORDS

Enzyme-linked immunospot assay test;
Tuberculosis,
Sensitivity;
Specificity.

INTRODUCTION

According to WHO estimates, there are around 8.7 million cases of new tuberculosis patients in 2011, and 1.4 million people died of tuberculosis[1]. Tuberculosis has become the leading cause of death in adults as a global communicable diseases. 2004 WHO report shows that TB diagnosis rate was only 37%, even in

developed countries only 50 % of TB patients was confirmed through bacteriological methods[2]. Tuberculous pleurisy is one of the most common disease of pleura. The accounts for about 54.8% of exudative pleural effusion are tuberculous pleurisy in China[3]. The sensitivity and specificity of the routine methods are poor, and pleural biopsy and thoracoscopy don't suitable for conventional selection because they are invasive examinations. Therefore, seeking a sensitivity and specificity detection method has become an urgent clinical needs. Numerous studies show that tuberculosis -specific antigen induced activation of T lymphocytes which secrete γ interferon, which could be used as a reliable marker of Mycobacterium tuberculosis infection. ELISPOT is considered to be the most sensitive method of detection of T lymphocyte response, and can be applied to vaccine development, clinical diagnostics, and other aspects of basic research[4]. There are 93 patients were detected by T-SPOT TB test in our hospital from July 2011 to April 2012. The objective is to investigate the value of T-SPOT.TB in the diagnosis and evaluation of curative effect of tuberculous pleurisy.

EXPERIMENTAL

Materials and methods

Object

Collected 93 hospitalized patients with pleural effusion of Inner Mongolia Medical University Hospital in July 2011- 2012 April. The patients from the Rheumatology (9 cases), respiratory (84 cases), male 42 cases, female 51 cases, aged 16 to 80 years, mean age (52.50 ± 14.12) years. All patients' pleural effusion were underwent the examinations of routine: biochemical, cellular pathology, carcinoembryonic antigen. T-SOPT TB were tested synchronization in peripheral blood and pleural fluid after eliminated the transudate. The patients were divided according to the final results of the clinical diagnosis into two groups: tuberculous pleurisy group(the experimental group) and non tuberculous pleurisy group (Control group).

The diagnostic criteria of active tuberculosis pleurisy

① Have fever, night sweats, fatigue and other symptoms of Tuberculosis; ② ultrasound suggestive of pleural effusion, lung CT scan with or without tuberculosis; ③pleural effusion laboratory is dominated by monocytes exudate, by enzyme adenosine deaminase (ADA)>45U/L④ investigation of pleural effusion the cancer cells were negative, carcinoembryonic antigen normal; ⑤ pleural effusion is absorpted significantly after anti-TB treatment; pleural biopsy with typical tuberculosis (such as tuberculosis or caseous granulomatous lesions), or mycobacterial sputum smear positive ; meet ① ~ ⑤ or ⑥, and anti-TB treatment for at least two weeks.

Non tuberculous pleurisy group(Control group)

The patients of exudative pleural effusion were excluded tuberculosis pleurisy.

All patients underwent the HIV test were negative.

Exclusion of pregnant women, bloody pleural effusion were excluded, hydrothorax avoid mixed, all pleural effusion were fresh in vivo pleural effusion.

Apparatus and reagents:

Heal Force incubator, T-SPOT.TB kit (Oxford Immunotec Ltd, UK), product standard number YZB / ENG 1702-2010; Fluorescent spot ELISA analyzer (AID, Germany), Thermo scientific Sorva ll ST 40 centrifuge series, lymphocyte separation medium, Gibco AIM-V, Gibco RPMI 1640 cell culture medium provided by Shanghai Foxing chang zheng Medical Science Co Ltd.

The method

Separated peripheral blood lymphocytes

Collection tube with heparin in patients with pretreatment fasting blood 4ml, isolated peripheral blood mononuclear cells (peripheral blood mononuclear cells, PBMCs) with cell culture medium AIM-V hanging from the cells, after counting the cell suspension concentration was adjusted to 1.0 × 106/ml.

Preparation of pleural effusion mononuclear cells

Collected non-bloody pleural effusion 50 ml from patients by sterile syringes of 50 ml, 1250 U heparin anticoagulate. Autoclaved centrifuge tube collected the samples of 10 ml, 20 °C, relative centrifugal force of 1800r / min, centrifuged for 7 min, the supernatant was

FULL PAPER

removed, the cell suspensed from the bottom of the tube, medium washing, add 3 ml culture medium, for dilution, there was slowly added 3.5 ml lymph separated liquid. Made the diluted blood overlap on the separation of the liquid, 20 °C 2300 r / min, centrifuged 22 min. Remove the PBMCs that suspended between the plasma with the separated liquid. washed twice with the medium, 20 °C 1800 r / min, centrifuged 7 min. Hanging the cells with cell culture medium AIM-V, to adjust the concentration of the cell suspension to 1.0×106/ml after counting.

ELISPOT detection

IFN γ has been coated with monoclonal antibody on the 96-well microtiter plates, 50 μl cell culture medium were added to as a negative control, 50 μl phytohemagglutinin as a positive control, 50 μl Mycobacterium tuberculosis hybrid peptides ESAT-6 and 50 μl CFP-10 as a source of stimulation. Then each well was added 100 μl of the cell suspension. Made the 96-well plates in 37 °C incubator for 16 ~ 20 h, then the plates were washed with 200 μl pH 7.4 PBS four times. Then adding alkaline phosphatase -conjugated secondary antibody, and then at 4 °C for 1.5 h. After incubation the plates were washed with 200 μl pH 7.4 PBS four times, finally added to each well 50 μl chromogenic substrate solution, keep in dark place 5 min at room temperature, the reaction was terminated with deionized water.

Result determination:

Experimental valid judgment: blank spots within less than 10 and the number of spots is greater than 20 in the positive control wells. Positive results to determine: a: number of spots of detected wells- number of spots of negative control wells ≥ 6; b: number of spots of blank control wells ≥ 6, the number of spots of detected wells must be> 2 times the number of spots on the blank control wells. The final number of spots : the number of spots of detected wells - number of spots in the negative control well, then × 4, the result is noted as : SFCs / 106, one positive result then the result is considered positive, the threshold value of a positive result is ≥24 SFCs/106.

Statistical methods

SPSS 16.0 software was performed for statistical analysis. The measurement data is non normal distribution that was descripted with the median and four percentile. Comparisons between groups were tested by Wilcoxon rank sum test. Chi-square test was performed for Counting data.

RESULT

There are 63 cases in 93 patients with pleural effusion were diagnosed as tuberculous pleurisy. And the other 30 cases were diagnosed as non tuberculous pleurisy patients. The results of T-SPOT.TB in blood of two groups patients (see TABLE 1). The results of T-SPOT.TB in pleural effusion of two groups patients (see TABLE 2). The number of SFCs in peripheral blood and pleural effusion after different antigen stimulation (see TABLE 3). The average number of SFCs in Pleural effusion was 4.69 times that in peripheral blood. The patients of tuberculous pleurisy were followed up in our study. By the end 42 patients completed the review of the detection of T-SPOT.TB in peripheral blood. Among the 42 patients there were 3o cases negativeÿand the number of SFCs of T-SPOT.TB in

TABLE 1 : Test results of T-SPOT.TB in blood of two groups patients

Group （cases）	Positive （62）	Negative （31）	Total （93）	Positive rate(%)
Tuberculous pleurisy group	57	6	63	90.48
None tuberculous pleurisy group	5	25	30	16.67
positive predictive value （Specificity*） (%)	91.94	80.65*		

TABLE 2 : Test results of T-SPOT.TB in pleural effusion of two groups patients

Group （cases）	Positive （72）	Negative （21）	Total	Positive rate(%)
Tuberculous pleurisy group	63	0	63	100.00
None tuberculous pleurisy group	9	21	30	30.00
positive predictive value （Specificity *） (%)	87.50	100.00*		

TABLE 3 : The numbers of SFCs in peripheral blood and pleural effusion after different antigen stimulation

Specific antigen	cases	The number of spots ($\bar{X}\pm S$)		U	P
		SFCs/10^6 in blood	SFCs/10^6 in pleural effusion		
ESAT-6	93	357.23±406.98	1374.25±770.45	5.51	0.00
CFP-10	93	278.98±327.39	1608.46±835.00	4.88	0.00

peripheral blood of the 12 positive cases were decreased or loss after 3 months anti tuberculosis therapy.

DISCUSS

In recent years, T-SPOT.TB test techniques in the diagnosis of Mycobacterium tuberculosis infection proved to have high sensitivity and specificity, and this technology was certificated by the British NICE in 2006, relevant reports increasing[2, 5]. In our study, The sensitivity (Positive) of T-SPOT.TB test in peripheral blood and in pleural effusion of the tuberculous pleurisy was 90.48%(57/63) and 100%(63/63) respectively. The specificity of T-SPOT.TB test in peripheral blood and in pleural effusion of the tuberculous pleurisy was 80.65%(25/31) and 100%(21/21) respectively. Peng Chunxian[6] reported a similar result.

Wilkinson KA[7] made the first study of mononuclear cell ESAT-6 in pleural effusion detected in the diagnosis of tuberculous pleurisy by ELISOPT in the UK. This study confirmed the feasibility of ELISOPT in the diagnosis of tuberculous pleurisy in 2005, and the study confirmed that the number of pleural IFN γ specific T cells was 15 ± 6 times that in the peripheral blood, while the number of cancerous pleural effusion T cells is lacking or is very low. In our study, The average number of SFCs ESAT-6 and CFP-10 in pleural effusion was 1374.25±770.45 SFCs/106 and 1608.46±835.00 SFCs/106, and that in blood was 357.23±406.98 SFCs/106 and 278.98±327.39 SFCs/106, The average number of SFCs in Pleural effusion were 4.6.9 times that in peripheral blood. These studies confirmed that the sensitivity of T-SPOT.TB in pleural effusion is better compared with that in peripheral blood. T-SPOT.TB test is a good method in the diagnosis of tuberculous pleurisy. Especially when the patient is not suitable for pleural biopsy or biopsy results cannot be diagnosed.

Researches have shown that, effect of T lymphocyte survival time is very short. Generally disappeared after the pathogens are eliminated, so monitor the change of T-SPOT.TB in peripheral blood can be used to assess the efficacy and detect the activity of disease[8]. The patients of tuberculous pleurisy were followed up in our study. By the end 42 patients completed the review of the detection of T-SPOT.TB in peripheral blood. Among the 42 patients there were 3o cases negative and 12 cases were positive of the T-SPOT.TB, the numbers of SFCs in peripheral blood were decreased or loss after 3 months anti tuberculosis therapy. Our esearch conclusion was accord with some reports of overseas[9].

The positive rate of T-SPOT.TB in peripheral blood and pleural effusion of none tuberculous pleurisy group is 16.67% and 30.00%. Although the T-SPOT.TB is a sensitive checking target, there are some false positive rate, the positive results need to be consolidated other clinical manifestations and associated examination to confirm the diagnosis. ELISOPT has more stability compared with the traditional experiments. But in our study found that ELISOPT is still disturbed by some external factors. We found, the number of T cells will be reduced in lower temperature environments, and the number of T lymphocyte will gradually decrease even disappear if the hydrothorax placed for too long time. So the pleural effusion must be collected fresh.

CONCLUSIONS

In short, T-SPOT.TB testing technology has the following advantages:[1] T-SPOT.TB test technique in the diagnosis of Mycobacterium tuberculosis infection proved to have high sensitivity and specificity, especially in pleural effusion and active tuberculosis[10], it is a good way of screening for tuberculosis. u$The result is fast that need only 24 hours.[3] T-SPOT TB can be used as evaluation index of tuberculosis treatment. T-SPOT.TB test has the following problems: The detection step cumbersome, expensive, lack of a large and objective clinical trials. In addition, the relationships of infection with Mycobacterium tuberculosis and tuber-

culosis immune response, development and prognosis has not been fully understood. So the T-SPOT.TB test is not completely identify active, staleness or potential tuberculosis infection. The value of T-SPOT.TB test need further research.

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