

TB-SA ANTIBODY TEST FOR DIAGNOSIS AND MONITORING TREATMENT OUTCOME OF SPUTUM SMEAR NEGATIVE PULMONARY TUBERCULOSIS PATIENTS

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Abstract. The objectives of this study were to evaluate the suitability of the TB-SA antibody test to diagnose tuberculosis in sputum smear negative (SS⁻) pulmonary tuberculosis (TB) patients and its applicability for monitoring treatment outcomes in these patients. This study was conducted in three counties/districts in Chongqing Municipality, Liaoning Province, China between June 2005 and June 2007. A total of 432 SS⁻ suspected pulmonary TB patients were recruited and their blood was collected prior to treatment, at the end of 1 month of treatment, 2 months of treatment and 6 months of treatment (E6MT). The serum samples were analyzed with a TB-SA antibody test kit. Of the 432 SS⁻ suspected pulmonary TB patients, serum samples were obtained at all time points in 316 patients and analyzed. The 316 patients were divided into three groups according to sputum smear and sputum culture results and the chest X-ray results before treatment and at E6MT. Ten point four percent were SS⁻/culture positive (C⁺), 73.1% were SS⁻/culture negative (C⁻) with X-rays abnormalities, and 16.5% were SS⁻/C⁻ without X-rays abnormalities. The positive rates for TB-SA antibody in the three groups were 57.6, 44.6 and 44.2%, respectively, before treatment, and 18.2, 19.1 and 26.9%, respectively, at E6MT. There was a significant decrease in TB-SA antibody positivity with treatment for all 3 groups. The TB-SA antibody test may be a useful adjunct to diagnose tuberculosis in SS⁻ pulmonary TB patients, and may be useful for monitoring treatment outcomes of SS⁻ pulmonary TB patients.

Keywords: pulmonary tuberculosis, sputum smear negative, TB-SA antibody, diagnosis, monitoring, treatment outcome

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INTRODUCTION

The notification rate of sputum smear negative (SS⁻) tuberculosis (TB) cases has increased after the policy of providing free treatment for initial SS⁻ TB cases was implemented (Disease Control

Department, MoH, 2005). The number of registered initial SS⁻ TB cases in China in 2005 was 316,405 with a notification rate of 24.3/100,000, an increase of 19.9% compared to 2004 (Ma *et al*, 2007). The registration rate for SS⁻ TB cases increased by 14.1% from 2002 to 2006; in 2005 there was a 54.1% increase and in 2006 there was a 102.4% increase (Yin *et al*, 2008). Currently in China, physicians diagnose SS⁻ TB based on clinical symptoms, radiographic findings or sputum smear microscopy without confirmation with sputum culture, serology or immunology (Disease Control Department, MoH, 2002). The diagnosis of SS⁻ TB is a major challenge for the current TB control program in China.

The number of SS⁻ TB cases in China is unknown using the current methods of diagnosis. A study by Li *et al* (2003) in Shandong Province found among 160 SS⁻ TB cases, 28 had a positive sputum culture, 77 had a positive PPD test, compared with CT scan results 42 cases were missed by X-rays; the PPD positivity rate was 34.7% and the positive sputum culture rate was 11.1% among new SS⁻ TB cases in Zhejiang Province (Zhang *et al*, 2007).

There is an urgent need to develop simple, accurate, rapid and affordable TB diagnostic tests for developing countries with high TB burdens, particularly in China. In the past several decades, numerous studies have demonstrated detection of antibodies, antigens and immune complexes can be used to diagnose TB (Li *et al*, 2006). Of the various methods, the most popular approach is detection of antibodies against specific antigens of *M. tuberculosis* (TB-SA) (Mazen and Joho, 2000; Perkins *et al*, 2006; Steingart *et al*, 2007). TB-SA antibody testing is based on acid phosphatase secreted by *M. tuberculosis*, an antigen present only in pathogenic mycobacteria not non-pathogenic myco-

bacteria (Mazen and Joho, 2000), which could be an ideal antigen for serological diagnosis of TB.

The TB-SA IgG test kit detects specific antibodies in human serum based on an indirect enzyme-linked immunosorbent (ELISA) assay. In China, numerous studies (Zhao *et al*, 2007; Bi *et al*, 2008; Wang *et al*, 2008; Zhu *et al*, 2008) have found the TB-SA test is a rapid, simple, relatively sensitive and specific method for diagnosing tuberculosis, especially in SS⁻ pulmonary, extra-pulmonary and childhood tuberculosis patients to aid in clinical decision making, where specimens are difficult to obtain. Due to the absence of studies using the TB-SA test to diagnose and follow treatment outcomes of SS⁻ patients, we evaluated the applicability of the TB-SA antibody detection method for diagnosing and monitoring treatment outcomes among SS⁻ pulmonary TB patients.

MATERIALS AND METHODS

Participants and recruitment

From June 2005 to June 2007, individuals working in health care services in three counties/districts in Chongqing Municipality and Liaoning Province of China were examined by an Expert Committee, whose members had extensive clinical experience with TB diagnosis and treatment based on the national protocol for the diagnosis of SS⁻ pulmonary TB cases in China (Disease Control Department, MoH, 2005).

All selected individuals had normal immune function, were over age 15 years and had never previously received anti-TB treatment or had received such treatment for less than 30 days. Once admitted to the study group, all subjects ($n=432$) received standard treatment for 6 months according to the SS⁻ pulmonary

Table 1
 Characteristics of the study groups before treatment.

Variables	SS-/C ⁺		SS-/C ⁻ with X-ray changes		SS-/C ⁻ without X-ray changes		p-value
	n	%	n	%	n	%	
Total	42	-	318	-	72	-	-
Age (M, $\bar{x}\pm S$)	42	34.5, 35.9±12.8	318	34.0, 37.0±15.6	72	34.5, 41.1±18.7	0.3406 ^a
Male	26	61.9	206	64.8	46	63.9	0.9316
Female	16	38.1	112	35.2	26	36.1	
Cough	37	88.1	265	83.3	61	84.7	0.7197
Expectoration	37	88.1	231	72.6	45	62.5	0.0127
Hemoptysis	11	26.2	77	24.2	11	15.3	0.2304
Night sweats	16	38.1	108	34.0	18	25.0	0.2576
Weakness	20	47.6	173	54.4	50	69.4	0.0333
Chest pain	11	26.2	90	28.3	20	28.2	0.9587

^aWilcoxon rank-sum test for nonparametric statistics and analysis.

TB treatment protocol (Disease Control Department, MoH, 2005). Diagnosis by the Expert Committee was based on clinical evaluation, a sputum smear, a sputum culture, and/or a chest X-ray carried out for each individual at specific points during treatment.

Data collection

All individuals were requested to complete a questionnaire, which included age, sex, exposure history, date of initial diagnosis, and symptoms (such as cough and its duration, expectoration and/or emptysis, fever, weakness, anorexia loss of weight, chest pain and night sweats) during the two weeks prior to diagnosis.

TB-SA antibody test

Serum samples were taking from the subjects prior to treatment, after 1, 2 and 6 months of treatment TB-SA ELISA detection kit with lot numbers 060301 and 070301 manufactured by Chengdu Yongan

Pharmaceutical (Chengdu, PR China) were used to evaluate each sample. Multi-skan MK3 microplate readers, Wellwash4 MK2 microplate washers and electrothermal constant temperature water tanks (DKB-8A) were used. The collection of samples, performance of the ELISA assay and analysis of the data were carried out in a double-blinded manner.

Informed consent

All subjects give written informed consent and the study was approved by the Institute Review Board (IRB) of the Chinese CDC.

Data analysis

Databases were created with EpiData. Two persons input the data separately, and the results were compared until consistency between the two databases was achieved. Data analysis was conducted with a χ^2 test and trend χ^2 using SPSS 12.0.

Table 2
Comparison of the rates of positive TB-SA antibody tests among the three groups of subjects.

Phase	SS ⁻ /C ⁺		SS ⁻ /C ⁻ with X-ray changes		SS ⁻ /C ⁻ without X-ray changes		χ^2 value	<i>p</i> -value
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%		
Before treatment	19	57.6	103	44.6	23	44.2	2.03	0.3624
1 month	21	63.6	111	48.1	28	53.9	3.06	0.2162
2 months	18	54.6	87	37.7	24	46.2	4.14	0.1262
6 months	6	18.2	44	19.1	14	26.9	1.73	0.4215

RESULTS

The characteristics of the study groups before treatment are shown in Table 1. Four hundred thirty-two subjects were included in the study. They were divided into three groups according to sputum smear and culture results before treatment and chest X-ray findings before and at the end of 6-months treatment. Smear negative (SS⁻) culture positive (C⁺) results were found in 9.7% (42/432); SS⁻ culture negative (C⁻) results with an abnormal X-ray were found in 73.6% (318/432) and SS⁻, C⁻ and X-rays negative results were found in 16.7% (72/432). The sex and age distributions were the same across the three groups. The percent of patients with expectoration in the first group was slightly higher than in the other two groups ($p=0.0127$) and the percent of patients who experienced weakness in the third group was slightly higher than in the other two groups ($p=0.0333$).

Ninety-six point eight percent (418/432) of the subjects had TB-SA antibody tests carried out prior to treatment, 94.4% (408/432) were tested at the end of 1 month of treatment, 90.7% (392/432) were tested at the end of 2 months, and 84.7% (366/432) were tested at the end

of 6 months; 73.1% (316/432) had all the tests. Of the 316 subjects who had all the tests, 10.4% (33/316) were in the first group, 73.1% (231/316) were in the second group and 16.5% (52/316) were in the third group. Of the 316 subjects, the TB-SA antibody test was positive in 45.9% (145/316) before treatment, 50.6% (160/316), 40.8% (129/316), and 20.3% (64/316) at the end of 1 month, 2 months and 6 months of treatment, respectively.

As shown in Table 2, the rates of positive TB-SA antibody testing for the three groups before treatment were 57.6% (19/33), 44.6% (103/231) and 44.2% (23/52), respectively; 63.6% (21/33), 48.1% (111/231) and 53.9% (28/52) at the end of 1 month of treatment; 54.6% (18/33), 37.7% (87/231) and 46.2% (24/52) at the end of 2 months of treatment; and 18.2% (6/33), 19.1% (44/231) and 26.9% (14/52) at the end of 6 months of treatment. There was no significant differences in the rates of positive TB-SA antibody tests among the three groups before the treatment ($p=0.3624$), at the end of 1 month, 2 months and 6 months of treatment ($p=0.2162$, $p=0.1262$ and $p=0.4215$, respectively).

Table 3 shows the rates of positive TB-SA antibody tests for the 316 subjects

Table 3
Trend for changes in the rates of positive TB-SA antibody tests for the three groups of subjects during treatment.

Group	RR (95% CI) of positive TB-SA antibody			χ^2 value	p-value
	1 month	2 months	6 months		
SS-/C ⁺	1.11 (0.75-1.63)	0.95 (0.62-1.45)	0.32 (0.14-0.69)	10.62	0.0011 ^a
SS-/C ⁻ with X-ray changes	1.08 (0.89-1.31)	0.86 (0.57-1.28)	0.29 (0.13-0.62)	13.61	0.0002 ^b
		0.78 (0.63-0.97)	0.51 (0.37-0.69)	42.69	0.0000 ^b
SS-/C ⁻ without X-ray changes	1.22 (0.82-1.81)	1.04 (0.68-1.59)	0.61 (0.35-1.05)	3.78	0.0526 ^a
		0.86 (0.58-1.26)	0.50 (0.30-0.84)	7.67	0.0056 ^b

^aBefore treatment is reference; ^b1 month is reference

before treatment; the rates at the end of 6 months of treatment were significantly lower for the three groups than the pre-treatment rates; the related RR (95% CI) were 0.32 (0.14-0.69), 0.43 (0.32-0.58), and 0.61 (0.35-1.05), respectively. There were no significant differences between the rates before treatment and at the end of 1 month and 2 months of treatment, or between month 1 and month 2; however, by 6 months the rates were significantly lower in the three groups; and the related RR (95% CI) values were 0.29 (0.13-0.62), 0.51 (0.37-0.69), and 0.50 (0.30-0.84), respectively.

DISCUSSION

In our study, there were no significant differences in the positive TB-SA test rates for the three groups. Our results are in agreement with a previous study in Shandong Province that showed the sensitivity of the TB-SA antibody test for detecting subjects positive and negative for pulmonary TB were 81.7% and 64.1%, respectively (Wang *et al*, 2008). A study in Shanghai showed the sensitivity of the TB-SA antibody test for identifying subjects

positive and negative for pulmonary TB were 72.7% and 66.9%, respectively (Bi *et al*, 2008). A study in Sichuan Province showed the sensitivity of the TB-SA antibody test for identifying subjects positive and negative for pulmonary TB were 77.6% and 72.2%, respectively (Zhao *et al*, 2007). Together, these studies suggest the TB-SA antibody test is reliable for diagnosing sputum smear negative pulmonary TB.

In our study positive TB-SA antibody rates among SS-/C⁺ and SS-/C⁻ with abnormal X-rays were significantly lower at the end of 6 months treatment than before treatment, and there was a decreasing trend in positivity on the TB-SA antibody test with treatment. The decrease in the positive rates with the TB-SA antibody test for the SS-/C⁻ normal X-ray group was less obvious, which is consistent with a lack of clinical improvement with treatment for 6 months in these patients. Studies in Henan Province, Guangdong Province and Zhejiang Province found the number of patients with a positive antibody response decreased during treatment, but in patients with severe TB symptoms the positive antibody response lasted longer

(Zhao *et al*, 2001; Zhang *et al*, 2004; Hu *et al*, 2008), similar to the results of our current study. The TB-SA antibody test may be used to monitor treatment outcomes among sputum smear negative pulmonary TB patients.

Studies in Shandong Province, Shanghai and Sichuan Province found the positive rates for the TB-SA antibody test among healthy individuals were 14.6, 13.4 and 10.0%, respectively (Zhao *et al*, 2007; Bi *et al*, 2008; Wang *et al*, 2008). We found the rates for the first two groups at the end of 6 months of treatment were 18.2% and 19.1%, close to the levels for health people. Zhao *et al* (2001) found no difference between the rate of positive serologic TB antibodies between patients who had recovered for 15 months after treatment and the rate of healthy individuals. Hu *et al* (2008) found the rate for a positive serologic TB antibody test at 9 months following cure was similar to that of healthy people. The rates of the first two groups in our study might approach that of healthy controls if the study was prolonged.

Our data indicate the TB-SA antibody test can provide reliable evidence to diagnose of SS⁻ pulmonary TB, and is useful for monitoring treatment outcome among SS⁻ pulmonary TB patients.

ACKNOWLEDGEMENTS

This study was supported by funding from Operational Research for the China TB Control Project funded by World Bank Loan/ UK Donation, and from National TB Control and Prevention Program in China.

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