Original article

Clinical diagnostic performance of the simultaneous amplification and testing methods for detection of the *Mycobacterium tuberculosis* complex for smear-negative or sputum-scarce pulmonary tuberculosis in China

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Background Early detection of pulmonary tuberculosis (PTB) is a big challenge in smear negative and sputum scarce patients in China. Simultaneous amplification and testing methods for detection of the *Mycobacterium tuberculosis* (MTB) complex (SAT-TB assay) is a novel molecular technique established in our hospital. This method has a high sensitivity and specificity in the lab. In this study, the clinical diagnostic performance of this method in smear-negative or sputum-scarce PTB suspects was investigated and evaluated.

Methods Two hundred smear negative and 80 sputum-scarce patients were recruited in this study. Samples that included sputum or bronchial washing fluid were collected and sent for both bacteria culture and SAT-TB assay. Diagnosis for these patients was based on the comprehensive evaluation of chestX- ray/CT study, histology examination, lab results, and treatment response. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each diagnostic test were investigated and calculated using confirmed tuberculosis (TB) and non-TB cases. The time required for detection of MTB was also measured for each method.

Results Ninety-two patients (33%) were diagnosed as definitive TB, 112 patients (40%) were probable PTB, and 76 (27%) were non-TB. The sensitivity, specificity, PPV, and NPV of SAT-TB in smear-negative PTB suspects were 93% (95% *CI*, 84%–98%), 98% (95% *CI*, 90%–100%), 98% (95% *CI*, 91%–100%), and 93% (95% *CI*, 83%–98%). In sputum scarce PTB suspects, the sensitivity, specificity, PPV, and NPV of the SAT-TB assay on bronchial washing fluids were 90% (95% *CI*, 74%–98%), 100% (95% *CI*, 85%–100%), 100% (95% *CI*, 88%–100%), and 88% (95% *CI*, 69%–97%). The accuracy of the SAT-TB assay is consistent with the bacteria culture assay. The median time required for detecting MTB in the SAT-TB assay was 0.5 day, which was much faster than bacteria culture (28 days).

Conclusions The SAT-TB assay is a fast and accurate method for the detection of MTB. It can be widely applied in the clinic and be an asset in early detection and management of PTB suspects, especially in those patients who are smear negative or sputum scarce.

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Tuberculosis (TB) is a great burden to China. The incidence of pulmonary TB (PTB) is 91.6 per 100 000 each year and mortality of TB is 15% 2–6 years after the most recent TB diagnosis in rural areas in China.[1,2] Ninety percent of TB patients in hospitals in China are smear negative or sputum scarce.[3] These patients have longer waiting time to receive proper chemotherapy than sputum smear positive patients due to the delay in diagnosis. In turn, misdiagnosis and mistreatment rates in these patients are increased, in addition to the rising possibility of transmission of TB. Therefore, early detection is critical to manage smear-negative/sputum-scarce patients to reduce the overall mobility and mortality of TB in China, and would be a great benefit from a public health standpoint.

Bacterial culture is the gold standard for diagnosis of active PTB. However, it is time-consuming and requires a high quality sample. The sputum acid fast bacillus (AFB) smear method is quick but the sensitivity is less than 10% in hospitals in China.[4] In recent years, several molecular diagnostic methods have been developed and applied in clinics in China. DNA amplification techniques such as the Cobas TaqMan *Mycobacterium tuberculosis* (MTB) and real-time PCR assay have low sensitivities. The GenoType Mycobacteria Direct assay has a sensitivity of 97% but a low specificity of 58%.[5] The Xpert MTB/RIF is thought to be as an accurate and valuable tool for the diagnosis of TB, but it is too expensive to be widely used in the clinic.[6,7]

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A novel real-time simultaneous application and testing method for detection of the MTB complex (*SAT-TB assay*) has been established in our hospital.[8,9] This SAT-TB assay takes 1.5 hours to perform. In the lab, the sensitivity of this method reaches 67.6% with a specificity of 100%. Moreover, the cost of this assay is low and the assay can be broadly applied in the clinic. Our previous study[9] had shown that the SAT-TB assay had an excellent diagnostic performance in smear-positive PTB suspects. However, application of this method in the clinic, especially in sputum smear negative or sputum scarce patients, has not been evaluated. In this study, we applied this new assay to 282 sputum smear negative or sputum scarce suspects of PTB. Fiberoptic bronchoscopy is a valuable method for the diagnosis of PTB,[10,11] and we got bronchial washing fluid from sputum scarce suspects while performing bronchoscopy. The sensitivity and specificity of the SAT-TB assay were investigated and its contribution to clinical diagnosis and management of smear negative or sputum scarce TB patients was analyzed. Our data demonstrated that SAT-TB is a valuable and accurate tool for early detection MTB in smear-negative and sputum scarce PTB suspects.

METHODS

Study population

In this study, patients with abnormal chest radiographic findings compatible with active TB were defined as PTB suspects. PTB suspects who had three consecutive negative sputum smear microcopy results were defined as smear negative patients. PTB suspects who were unable to self-expectorate sputum were defined as sputum scarce patients. All PTB suspects in this study were >18 years old. Patients who were sputum smear positive or HIV positive were excluded from this study. All PTB suspects were prospectively enrolled into this study between November 2011 and December 2012 at the Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China. All PTB suspects in this study signed informed consent for this study. The study was performed in compliance with a protocol approved by the Institutional Review Board of the hospital.

Patient evaluation and specimen collection

Detailed patient history, laboratory results, treatment information, as well as follow-up observations were recorded on a standardized case record form. Sputum from smear negative PTB suspects was collected and sent for both bacterial culture and SAT-TB assay. Bronchoscopy was performed in sputum scarce suspects to collect samples. In brief, 40–60 ml volume of sterile saline (0.9%) was instilled into the airway of affected lung segments and 30 ml of bronchoalveolar lavage was collected for bacterial detection or SAT-TB assay. Bronchial brushings and/or trans-bronchial biopsies were performed in patients without contradictions. The double blind principle was applied to this study.

Bacterial culture, SAT-TB assay

Bacterial culture was performed in the BD BACTEC™ MGIT™ 960 Mycobacteria Culture System (Becton Dickinson and Company, USA) according to manufacturer's instructions.

The SAT-TB assay was performed as previously published.[8] Basically, MTB 16s rRNA was isolated from the sample and reverse transcribed to generate a 170-bp DNA fragment. The specific MTB 16S rRNA sense primer, containing the T7 promoter sequence, was 5′-AATTTAATACGACTCACT ATAGGGAGAGTAGGCCGTCACCCCACCA ACAAGCTG-3′, and the antisense primer was 5′-CTGGGAAACTGGGTCTAATAC-3′. The probe sequence was 5′-CCAGCCACGGGAUGCAUGCUGG-3′ and was labeled with 6-carboxyfluorescein (FAM) phosphoramidite at the 5′ end and with 4-(4-(dimethylamino)phenylazo) benzoic acid N-succinimidylester (DABCYL) at the 3′ end. Real-time PCR was performed in a 7 500 real-time PCR system (Applied Biosystems Inc., Foster City, CA, USA).

Diagnostic criteria

Diagnostic criteria for these PTB suspects followed WHO guidelines for treatment of tuberculosis, and are based on the combination of clinical symptoms, radiological evidence, histological observations, bacterial culture results, as well as therapy response.[12] In brief, PTB suspects were classified as definite PTB if they had at least one specimen culture positive, or had pathological evidence from trans-bronchial biopsy that demonstrated the affected lung segments contain caseating or necrotizing granulomatous inflammation compatible with TB. PTB suspects who had culture negative or no histological evidence of TB were defined as probable TB (if initiated on anti-TB treatment based on clinical evaluation and had no improvement in response to a course of broad-spectrum antibiotics) or non-PTB (if not initiated on anti-TB treatment and were diagnosed as other pulmonary diseases).

Follow-up

All patients, including culture negative/SAT-TB positive PTB suspects and culture negative/SAT-TB negative PTB suspects, in this study were followed up for at least six months at out-patient departments. Chest CT or X-ray was taken at follow-up for evaluation.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of SAT-TB were investigated in all smear-negative or sputum scarce patients. Collected data were analyzed by SPSS 18.0 (SPSS Inc., USA). The likelihood ratio for positive test (LR+), and likelihood ratio for negative test (LR−) were presented with 95% confidence intervals. The Mann-Whitney test was used to compare the median time required for detection between different tests. Significance between different detection rates was determined by a chi-square test and a *P* value less than 0.05 was considered statistically significant.

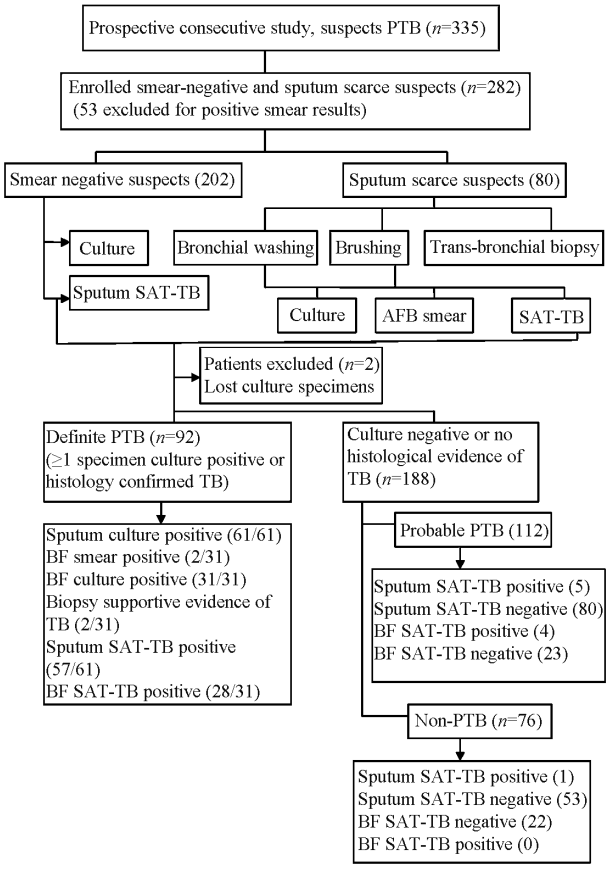

Figure 1. Study flow diagram showing the patients included in the analysis and test results, BF means bronchial washing fluid.

RESULTS

Clinical characterization

Among 335 PTB suspects hospitalized, 282 of them were recruited into this study, fifty-three cases were excluded due to AFB smear positivity. However, two in 282 cases were excluded due to missing culture specimens. Two hundred cases of smear-negative and 80 sputum scarce cases were enrolled into the final analysis. In these patients, 92 patients were finally diagnosed as definite PTB, 112 patients were diagnosed as probable PTB while, 76 patients were diagnosed as non-PTB patients. Forty-two of 76 non-TB patients (55%) were diagnosed as pneumonia, 12 non-TB patients (16%) were diagnosed as bronchiectasis, 13 non-TB patients (17%) were as lung cancer, three non-TB patients (4%) were identified as non-mycobacterium bacterium, three (4%) as lung abscess, one (1%) as cryptogenic organizing pneumonia (COP), one (1%) as SLE, and one (1%) as pneumoconiosis. Our data showed that most PTB suspects had cough and fatigue, which were consistent with the pattern of active PTB (Table 1).[13,14]

Diagnostic performances of SAT-TB assay for smear-negative PTB

In China, a large portion of the TB pool in hospitals is composed of smear negative patients. In this study, the diagnostic performance of the SAT-TB assay was evaluated against bacteria culture. The sensitivity was calculated using culture results as the standard reference, and specificity was calculated as the proportion of patients with non-PTB who test negative. The performance of the SAT-TB assay for smear-negative PTB was evaluated with confirmed PTB vs. non-PTB cases. Among 200 smear-negative suspects, only 61 PTB suspects were culture positive and diagnosed as definite PTB. Fifty-seven of these definite PTB cases were SAT-TB positive with a sensitivity of 93% (57/61, 95% *CI* 84%–98%) indicating consistency with the gold standard diagnostic method. Only six of 139 culture negative PTB suspects were positive in sputum SAT-TB assay with a specificity of 98% (53/54, 95% *CI* 90%–100%). Five of six cases with SAT positive/culture negative results were defined as probable TB, while one was diagnosed as pneumonia, possibly due to contamination. The PPV and NPV of the SAB-TB assay in smear negative PTB suspects were 98% and 93%, respectively (Table 2).

Diagnostic performance of SAT-TB assay for sputum scarce PTB

In patients without sputum, obtaining TB samples from a bronchoscope is an important tool for diagnosis. In this study, 31 of 80 sputum scarce PTB cases were culture positive using bronchial washing fluid, 28 of them were SAT-TB positive with a sensitivity of 90% (28/31, 95% *CI* 74%–98%). In 49 culture negative cases, only four were positive in the SAT-TB assay of bronchial washing fluid.

Table 1. Demographic and clinical characteristics (*n*=280)

Characteristics	Values
Age (mean±SD)	43±18
Female (*n* (%))	54 (25)
Body mass index (kg/m^2)	21.6 (19.1–25.9)
Cough (*n* (%))	149 (69)
Fever (*n* (%))	38 (18)
Hemoptysis (*n* (%)))	18 (8)
Chest pain (*n* (%))	11 (5)
Asymptomatic (*n* (%))	28 (13)
Fatigue (*n* (%))	81 (38)
Prior tuberculosis treatment (*n* (%))	4 (2)
Accompanied by COPD (*n* (%))	7 (3)
Accompanied by diabetes (*n* (%))	9 (4)

Table 2. Accuracy of SAT-TB for the detection of culture positive PTB

Variables	SAT-TB (Sputum) Estimate (95% *CI*)	SAT-TB (Bronchial washing fluid, BF) Estimate (95% *CI*)	SAT-TB (Sputum & BF) Estimate (95% *CI*)
Sensitivity (%)	93 (84–98)	90 (74–98)	92 (85–97)
Specificity (%)	98 (90–100)	100 (85–100)	99 (93–100)
PPV (%)	98 (91–100)	100 (88–100)	99 (94–100)
NPV (%)	93 (83–98)	88 (69–97)	91 (83–96)
Likelihood ratio (+)	50.46 (7.23–352.14)	40.97 (2.63–637.16)	70.22 (10.01–492.51)
Likelihood ratio (−)	0.07 (0.03– 0.17)	0.11 (0.04–0.30)	0.08 (0.04–0.16)

BF: bronchial washing fluid.

These four patients were finally diagnosed as possible PTB. The performance of the SAT-TB assay for sputum scarce PTB was evaluated with confirmed PTB vs. non-PTB. The specificity of SAT-TB for bronchial washing fluid in sputum scarce PTB suspects was 100% (0/22, 95% *CI* 85%–100%), and the PPV and NPV were 100% and 88%, respectively. Our data demonstrate that the SAT-TB assay is consistent with results from bacteria culture with high specificity in both sputum and bronchial washing fluid.

The time required for detection of TB in PTB suspects between conventional methods and SAT-TB assay

The time required for detecting TB using different tests in PTB suspects was also measured in this study. We recorded the detection rate of different methods, and for 61 culture confirmed PTB patients the detection rate by culture was 100%, but the median time of detection (IQR) using sputum bacteria culture was 28.2 days (15–50 days), while the median time was 0.5 day for SAT-TB assay of sputum samples. There was a significant difference between the two groups, P <0.001. The IQR for the bronchial washing fluid SAT-TB assay was also 0.5 day, which is significantly faster than that of bacteria culture (29.8 days), P <0.001. The time of culture for bronchial washing fluid was a little bit longer than in sputum and might result from there being fewer bacteria in bronchial washing fluid. Our study demonstrated that the SAT-TB assay can significantly reduce the time required for detection TB in PTB suspects compared with conventional culture method.

Diagnostic performance of SAT compared with other methods referred by final PTB diagnosis

Bacteriological evidence, such as a sputum smear or culture, is the gold standard for TB diagnosis and chemotherapy initiation. However, they are time consuming and have low sensitivity. Histological evidence is an affirmative tool for diagnosis, but it is contraindicated in many patients and the sensitivity is also disappointing. In this study, the final clinical diagnosis of PTB was arrived at by the combination of lab results, X-ray film, histological study, and treatment response. When compared to the final diagnosis, the sensitivity and specificity of SAT-TB for smear-negative and sputum scarce PTB were 46% (94/204) and 99% (75/76). The result is comparable to bacterial culture which has a sensitivity of 45% (92/204) with 100% (76/76) specificity. In contrast, the sensitivity of a biopsy was only 3% (2/58) although the specificity was high (100%) (22/22). The sensitivity of a biopsy was significantly lower than that of SAT-TB, P <0.000 1. The data indicate that the SAT-TB assay is as valuable as bacterial culture.

DISCUSSION

The SAT-TB assay is a fast, novel diagnostic method by directly detecting RNA fragments of MTB in sputum that we previously reported.[8,9] In the SAT-TB assay, *M. tuberculosis* 16S rRNA is reverse transcribed using a pair of primers and detected by a specific probe. The SAT-TB assay has several advantages over other methods for the diagnosis of TB. First, the SAT-TB assay targets thousands of copies of rRNA in the bacteria, thus has a high sensitivity. It is a powerful tool for detecting bacteria in limited samples such as bronchial washing fluid and trans-bronchial biopsies. Second, the SAT-TB assay has a high sensitivity due to the specific primer and probe design. It can accurately distinguish non-tuberculosis Mycobacterium (NTM) from MTB. As demonstrated in this study, the SAT-TB assay is as reliable as bacterial culture in smear-negative or sputum scarce patient. As an instant detection method (it takes only 1.5 hours), it is much more valuable than bacterial culture in early detection of TB patients, especially in smear negative or sputum scarce PTB suspects,

In this study, we report the diagnostic performance of the SAT-TB assay in smear negative and sputum scarce PTB suspects. Our study showed that it can detect 93% of culture confirmed smear-negative and 90% of culture confirmed sputum scarce PTB cases in less than two hours. We demonstrated that the SAT-TB assay had a specificity of 98% in the diagnosis of smear-negative and a specificity of 100% in sputum scarce PTB cases. Compared to other fast molecular tools, the sensitivity (93%) of SAT-TB in sputum was similar to that of loop-mediated isothermal amplification (LAMP) (89.6%–92.8%) and in-house developed sdaA PCR (95.9%), and higher than that of Cobas TaqMan MTB real-time PCR (82.7%) and IS6110 PCR (83.1%).[15-18] The sensitivity of SAT-TB is significantly higher than that of in-house PCR (75%) and of routine PCR (39%) as reported.[19,20] The Xpert MTB/RIF is generally recognized as a fast and excellent molecular method of diagnosis for TB and its sensitivity for PTB varies from 86.0% to 90.3%.[6,21,22] Our study demonstrated that the SAT-TB assay has similar sensitivity to Xpert MTB/RIF in the diagnosis of smear-negative PTB in both sputum samples and bronchial washing fluids or transbronchial biopsies. The sensitivity of the SAT-TB assay for bronchial washings fluid was 90% which is similar to that of Xpert MTB/RIF, which varies from 81.6% to 93.0% in reports.[23,24] The specificity of the SAT-TB assay in detecting TB in bronchial washing fluid was 100%, as high as that in Xpert MTB/RIF. In addition, the most impressive advantage of SAT-TB assay is that SAT-TB can produce as accurate a result as conventional culture in two hours while culture takes 4–8 weeks. Taken together, the SAT-TB assay is a valuable and quick asset for diagnosis of smear negative and sputum scarce patients in the clinic.

Although the SAT-TB assay has high specificity, the sensitivity is just slightly higher than bacteria culture, and some specimens were SAT-TB negative but culture positive, and the accordance rate of SAT-TB and culture was lower than the result we reported in the lab. We speculate that sample handing and transportation during a large scale clinical application for long time and a peripheral localized lesion, may all contribute to the unexpected lower sensitivity. The clinically diagnosed PTB cases that are SAT-TB positive and culture negative we attribute to the high sensitivity of the SAT-TB assay in clinical samples.

Our data indicated that 46% of cases that were SAT-TB positive can be given initial anti-TB treatment, although there was a lack of smear and pathological evidence, instead of empirical treatment

In summary, the SAT-TB assay is a fast, accurate method with high specificity for diagnosis of TB. It is a valuable method for early detection, prevention, and managing smear negative/sputum scarce PTB suspects, and can be widely applied in the clinic, especially for fast diagnosis of smear-negative or sputum-scarce PTB suspects.

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