Adjusted T-SPOT.TB criteria can increase the specificity of diagnosis when differentiating spinal tuberculosis from other spinal infections

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ABSTRACT

Background: The ability of T-SPOT.TB to differentiate *Mycobacterium tuberculosis* infection of the spine from other infections is little known. This study quantified the efficiency, sensitivity, and specificity of the T-SPOT.TB assay to distinguish between spinal tuberculosis (STB) caused by *M. tuberculosis* and other infections of the spine and evaluated whether diagnostic performance was improved by adjusting the T-SPOT.TB assay criteria.

Methods: From January 2010 to May 2020, 147 patients with spinal infections were recruited. Peripheral blood mononuclear cells were collected, and the number of spot-forming cells was observed. Patients’ white blood cell (WBC) counts, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin (PCT), and TB antibodies were recorded. Specimen/tissue bacteriological culture was the reference standard for sensitivity and specificity.

Results: There were 77 (52.4%) participants with confirmed TB and 70 (47.6%) with other infections. The groups were comparable in T-SPOT.TB assay results, age, sex, lesions in the segments, WBC count, CRP, procalcitonin, ESR, and TB antibodies. The sensitivity and specificity of the T-SPOT.TB assay for identifying STB was 88.3%-and 40.0%, respectively. On the basis of Relative operating characteristic curve (ROC) analysis and the Youden index, when we adjusted the T-SPOT.TB assay’s diagnostic criteria, ESAT-6>12 or CFP-10>19, the sensitivity and specificity of the T-SPOT.TB assay for identifying STB was 83.1%-and 64.3%, respectively.

Conclusion: The T-SPOT.TB assay has great sensitivity to distinguish STB from other spinal infections; however, the specificity is extremely low. Specificity can be significantly improved while sensitivity is guaranteed by adjusting the diagnostic criteria.
Keywords: Tuberculosis, T-SPOT.TB, Spinal infection, Diagnosis

INTRODUCTION

Tuberculosis (TB) is a communicable disease caused by infection with the bacterium *Mycobacterium tuberculosis*, and TB is a major cause of ill health worldwide. Most TB cases in 2018 occurred in Southeast Asia (44%), including China (9%) [1]. Extrapulmonary TB accounts for 10% of cases, of which half involve the musculoskeletal system. The spine is the most common musculoskeletal site involved in extrapulmonary TB (1 to 2% of cases) [2]. Early treatment can reduce the incidence of physical disability and injury in STB, but diagnosis is extremely challenging. There are scarce bacteria in the articular effusion of lesion sites, and specimens are not easy to obtain, which reduces the positive rate of puncture fluid or joint surgical specimens [3].

The T-SPOT.TB is an assay of interferon (IFN)-γ release from *M. tuberculosis*-specific effector T-cells stimulated by the *Mycobacterium*-specific antigens ESAT-6 (early-secreted antigenic target 6) and CFP-10 (culture filtrate protein 10). Thus, the antigens ESAT-6 and CFP-10 have been successfully utilized to determine the presence of *M. tuberculosis* infection. The T-SPOT.TB assay is an economical, efficient, rapid, and simple laboratory technique with high sensitivity and specificity for the diagnosis of TB in patients and healthy individuals [4]. However, the effectiveness of the standard T-SPOT.TB assay for distinguishing spinal infection is unclear.

Causative agents of spinal infection can be pyogenic (e.g., *Staphylococcus* or *Brucella*) or granulomatous (fungal); STB is the latter [5]. However, the imaging and clinical features of STB are similar to those of other spinal infections, and there is no specific test to differentiate them. This complicates the clinicians’ task of developing
treatment strategies. Many studies of TB diagnostic methods have compared the results of infected and healthy individuals, but in clinical practice, patients present with spinal infections from a variety of causes. A method to distinguish them is important.

In particular, data regarding the ability of the T-SPOT.TB assay to differentiate STB from other spinal infections are extremely limited; few studies have reported the application of the T-SPOT.TB assay to pinpoint the cause of spinal infection. The present study analyzed the diagnostic efficacy, sensitivity, and specificity of the T-SPOT.TB assay to distinguish STB from non-STB spinal infections. In addition, T-SPOT.TB assay diagnostic criteria were adjusted to improve its diagnostic efficacy in spinal infection.

**METHODS**

The ethics committee of Xiangya Hospital, South Centre University approved this prospective study and the number of 201303232. All patients provided signed informed consent to the study.

**Research participants**

Patients with suspected spinal infection (n = 147) were enrolled from January 2010 to May 2020 at Xiangya Hospital, South Centre University. Data on demographics, symptoms, and the results of each test were collected.

Patients meeting the following were included: tissues or specimens were obtained from the affected region via culture or polymerase chain reaction (PCR); pathological
examination suggested spinal infection; no severe underlying disease or human immunodeficiency virus infection was present; and the patient was followed up for at least three months.

The confirmation of STB was based on the identification of *M. tuberculosis* in tissues or specimens by culture or by PCR in addition to clinical, radiographic, or other supporting evidence and medical history suggestive of TB.

Probable (rather than confirmed) STB was considered when the results of *M. tuberculosis* culture were unclear, but pathological examination indicated TB infection, and clinical, radiographic, and other supporting tests and medical history suggested TB.

The diagnosis of other infection was made when the results of culture or PCR in tissues or specimens indicated infection other than *M. tuberculosis*, anti-TB treatment prior to surgery was ineffective, and the pulmonary and bacteriological culture of sputum was negative.

Finally, 147 patients were apportioned to three groups as follows. The confirmed STB group comprised 35 patients for whom the culture or PCR results indicated *M. tuberculosis*. The group with probable STB consisted of 42 patients in whom the pathological examination indicated TB infection. The confirmed STB and probable STB groups both received usual treatment for STB. The other infection group had culture or PCR results that suggested a cause other than *M. tuberculosis* and included 70 patients.

**T-SPOT.TB assay**

The T-SPOT.TB diagnosis kit was provided by Shanghai Fosun Long March Medical Science (Oxford Immunotec, United Kingdom). The T-SPOT.TB tests were
performed in accordance with the manufacturer’s instructions. Briefly, a peripheral venous blood sample was collected from each patient for the T-SPOT.TB assay to determine an IFNγ-producing T-cell response. Peripheral blood mononuclear cells (PBMCs) were separated from peripheral venous blood, and $2.5 \times 10^5$ PBMCs were plated per well in wells precoated with anti-human IFNγ antibody. The PBMCs were cultured at 37 °C for 18 hours, and spots were counted with an automated microscope. The results were recorded and interpreted as recommended by the kit manufacturer, i.e., ESAT-6 or CFP-10, with ≥6 spot-forming cells regarded as T-SPOT.TB-positive. The T-SPOT.TB result was considered negative if both ESAT-6 and CFP-10 showed <6 spot-forming cells. The test results were uncertain if there were >10 spot-forming cells in the blank control well or <20 spot-forming cells in the positive control well. When the results were uncertain, blood samples were retaken for another test.

Statistical analysis

The Wilcoxon signed-rank test incorporated C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) in the analysis. The statistical method used for procalcitonin (PCT), age and white blood cell (WBC) count, were independent-sample t-test. The chi-squared test was used to investigate associations between the diagnosis of spinal infection by specimen/tissue bacteriological culture or PCR results and the following: T-SPOT.TB assay result, sex, PCT, TB antibodies, and lesions in the segments. The following were analyzed by logistic regression and the odds ratio (OR): sex, T-SPOT.TB assay, PCT, TB antibodies, CRP, ESR and WBC count. A $P$-value < 0.05 was considered significant. All statistics were analyzed using SPSS 25.0 software (IBM, Armonk, NY, USA).

RESULTS
Patient characteristics

Overall, 147 patients with spinal infection were registered in this study (Table 1). According to the specified classification standard, 35 (23.8%), 42 (28.6%), and 70 (47.6%) patients were classified as having confirmed STB, probable STB, and other infection, respectively. For most statistical analyses, the confirmed STB and probable STB groups were combined and considered to have STB (Figure 1). The 70 cases of other infections consisted of the following: 4 of brucellosis; 9 Escherichia coli; 10 Staphylococcus aureus; 5 Salmonella; 6 fungal; 8 bacteria other than M. tuberculosis; and 28 chronic infections, with TB eliminated (Figure 2).

General indicators

Among the diagnostic groups (confirmed STB, probable STB, and other infection), CRP and ESR were statistically significant (P=0.03, P=0.01, respectively), but there were no significant differences in age, sex, lesions in the segments, WBC count, PCT (Table 2 and Table 3). Based on the chi-squared test, TB antibodies was significant differences (P = 0.003, Table 3), but no differences were shown in CRP, ESR, PCT, TB antibodies, WBC count, sex by logistic regression (Table 4).

T-SPOT.TB assay

Among 147 patients with valid T-SPOT.TB assay results, the sensitivity of the T-SPOT.TB assay was 88.3% (68/77), and the specificity was 40.0% (28/70). The sensitivity of the T-SPOT.TB assay to confirm STB was 94.3% (33/35) in the confirmed STB group and 83.3% (35/42) in the probable STB group. The false-positive rate of other infections was 60% (42/70). The positive predictive value (PPV) and positive likelihood ratio (PLR) were 61.8% and 1.5, respectively. There was a significant difference
between the results of the T-SPOT.TB assay and the diagnosis of spinal infection and in the results of the T-SPOT.TB assay among the confirmed STB, probable STB, and other infection groups (P < 0.01; Tables 3 and 4). The binary logistic regression analysis showed that for the diagnosis of spinal infection, sex, PCT, CRP, ESR, WBC count were not significant factors, but the T-SPOT.TB assay (OR 5.838, 95% CI 1.686-20.208; P 0.005) was significant (Table 4).

**Adjusted T-SPOT.TB assay**

A total of 147 patients were tested using the standard T-SPOT.TB assay (positive was ESAT-6 or CFP-10 >6). The adjusted values for the T-SPOT.TB assay were calculated by the ROC curve and Youden index, and the area under curve for ESAT-6 and CFP-10 was 0.775 and 0.785, respectively (P<0.001), and the Youden indexes were ESAT-6=12.5 and CFP-10=19.5. By using the adjusted-T-SPOT.TB criteria, the sensitivity of the adjusted-T-SPOT.TB assay was 83.1% (64/77), and the specificity was 64.3% (45/70).

The sensitivity of the T-SPOT.TB assay to confirm STB was 91.4% (32/35) for the confirmed STB group and 76.2% (32/42) for the probable STB group. The false-positive rate of other infections was 35.7% (25/70). The positive predictive value (PPV) and positive likelihood ratio (PLR) were 71.9% and 2.3, respectively (Table 5). Binary logistic regression analysis showed that the adjusted T-SPOT.TB assay was significant (OR 44.425, 95% CI 7.828-252.123; P <0.001) (Table 4). When we focused only on ESAT-6>12, the sensitivity was 76.6% (59/77), the specificity was 68.6% (48/70), and the PPV and PLR were 72.8% and 2.4, respectively; when we focused only on CFP-10>19, the sensitivity was 72.7% (56/77), the specificity was 84.3% (59/70), and the PPV and PLR were 83.6% and 4.6, respectively; and when we focused only on ESAT-6>12 and CFP-10>19, the sensitivity was 66.2% (51/77), the specificity was 88.6% (62/70), and the PPV and PLR were 86.4% and 5.8, respectively (Table 5).
DISCUSSION

There have been many studies of the mechanisms, diagnosis, and treatment of pulmonary TB, but until recently, extrapulmonary TB has been relatively neglected [6]. Few studies have focused on the diagnosis of spinal infections, which include but are not limited to TB, brucellosis, pyogenic spondylitis of common bacterial infection (e.g., *Staphylococcus*), and others. Bone and joint TB can cause limb deformity, limited mobility, and even paraplegia if improperly treated [7]. The T-SPOT.TB assay is a great laboratory technique with high sensitivity and specificity for distinguishing TB and healthy individuals [4]. In the present study, we focus on the T-SPOT.TB assay for distinguishing spinal infection. Bone destruction is clinically common, but the cause of the disease is uncertain by radiographic and medical history. The identification of the causative agent has a great influence on the next treatment decision. We explored the T-SPOT.TB assay’s diagnostic efficiency in spinal infections and whether it could be improved by adjusting the diagnostic criteria.

In the present study, the 147 patients were apportioned to 3 groups according to the results of culture or PCR: confirmed STB, probable STB, and other infection, with 35, 42, and 70 cases, respectively. In the diagnosis of spinal infection, the T-SPOT.TB test results were compared with traditional serological test results (e.g., ESR, CRP). The traditional serological parameters include WBC count, ESR, CRP, PCT, and TB antibodies. However, WBC count, ESR, CRP, PCT and TB antibodies had no significant specificity for the differential diagnosis of spinal infections, while the results of the T-SPOT.TB tests were statistically significant ($P < 0.01$) for the differential diagnosis of spinal infections (Tables 2, 3 and 4).
Many reports have shown that the T-SPOT.TB assay has great sensitivity and specificity. The reported sensitivity of the T-SPOT.TB test ranges from 75.3% to 93.6%, and its specificity ranges from 63.4% to 85.2% [9-15]. In this study, the sensitivity of the T-SPOT.TB assay was 88.3%, which was consistent with other literature reports; nevertheless, the specificity was 40%, which was much lower than that in other reports. A false-positive rate of 60% makes it difficult to use this assay to determine the cause of spinal infection. Recently, some studies reported that the number of spots on the T-SPOT.TB assay may be helpful for detecting CFP-10 and ESAT-6 antigens in the diagnosis of pulmonary or extrapulmonary TB [8]. In this study, the standard T-SPOT.TB criteria were ESAT-6>6 or CFP-10>6 among 147 patients, and the ESAT-6 and CFP-10 spot numbers are shown in Figure 3. In addition, the ROC curves of ESAT-6 and CFP-10 show great diagnostic performance, as shown in Figure 4. We obtained Youden indexes of ESAT-6=12.5 and CFP-10=19.5 when we adopted the adjusted T-SPOT.TB criteria of ESAT-6>12 or CFP-10>19; the sensitivity was 83.1%, and the specificity was 64.3% (Table 4). The result shows that specificity increased to more than 60% with almost constant sensitivity guaranteed by adjusting the diagnostic criteria of the T-SPOT.TB assay. Some studies have suggested that the diagnostic efficiency of ESAT-6 and CFP-10 may not be exactly the same. CFP-10 was responsible for significantly more positive T-SPOT.TB tests [16], and studies have shown the dominance of the CFP-10 antigen in causing T-cells to release IFN-γ in The Netherlands and India [17,18]. Therefore, we divided the patients into three groups where we focused on ESAT-6 and CFP-10 separately and together: only ESAT-6>12, only CFP-10>19, and ESAT-6>12 and CFP-10>19. The specificity of the group where we focused only on CFP-10 was higher than that where we focused only on ESAT-6 (84.3% and 68.6%, respectively), but the sensitivity was lower than that of the group where we focused only on ESAT-6 (72.7% and 76.6%, respectively) (Table 5). This result shows that the false-positive rate of the T-SPOT.TB
assay was as low as 15.7% with CFP-10\textgreater{}19, indicating that ESAT-6 and CFP-10 should possibly be evaluated clinically to guide the differential diagnosis of spinal infections.

The adjusted T-SPOT.TB assay still had a 35.7% false-positive rate, and the effect of ESAT-6 and CFP-10 homologs from nontuberculosis mycobacterium (NTM) on IGRAs could be a potential source of false positives; however, this has not been widely studied. ESAT-6 from *Mycobacterium leprae* has been shown to elicit a T-cell response from confirmed TB patients [19]. According to a study from Denmark, a higher T-SPOT positivity rate was found among individuals with NTM disease with RD1 than individuals with NTM believed to not have RD1 antigens, showing that the presence of ESAT-6 and CFP-10 in NTM affects the rates of IGRA positivity [20]. Individuals exposed to NTM may potentially have false-positive T-SPOT.TB tests if the NTM has homologous ESAT-6 or CFP-10 genes. T-SPOT.TB assays in areas with endemic NTM will likely have a lower assay specificity [16]. This conclusion is consistent with our results. Another possible reason for the false-positive rate is the effects of extraspinal tuberculosis. Prior to enrollment, medical histories were rigorously examined, and chest X-rays were compared for all participants; however, there may be patients who do not know they have TB, and some TB symptoms are not obvious and difficult to detect and diagnose.

In conclusion, the standard T-SPOT.TB assay has great sensitivity but very low specificity, and the specificity was significantly improved with almost constant sensitivity guaranteed by adjusting the diagnostic criteria of the T-SPOT assay. The Youden index calculated in our study is not necessarily the best diagnostic threshold, and a larger sample size and a closer approach to the clinical threshold are needed to use the adjusted T-SPOT.TB assay for clinical identification of spinal infections.

**DECLARATIONS**
Ethics approval and consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Xiangya Hospital, South Centre University and the number of 201303232. Written informed consent was obtained from individual or guardian participants. The ethics committee of Xiangya Hospital, South Centre University approved this prospective study. All patients provided signed informed consent to the study.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

Jiang wrote the main manuscript text and prepared figures and tables. Gao, Tang, Zhang provided important ideas and suggestions. Li provided the collated data. Xu, Guo, Liu collected and analyzed the data. All authors reviewed the manuscript.

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205 Table 1. Demographic characteristics of the patients in the different categories

|                  | All          | Confirmed STB | Probable STB | Other infection |
|------------------|--------------|---------------|--------------|----------------|
| Subjects, n      | 147 (100%)   | 35 (23.8%)    | 42 (28.6%)   | 70 (47.6%)     |
| Age, y           | 50.9 ± 15.4  | 55.4 ± 13.1   | 43.6 ± 17.9  | 53.0 ± 13.5    |

|                  |              |               |              |                |
|------------------|--------------|---------------|--------------|----------------|
| Sex              | Male         | Female        |              |                |
| Subjects, n      | 82 (55.8%)   | 65 (44.2%)    |              |                |
| Age, y           | 21 (14.3%)   | 14 (9.5%)     | 24 (16.3%)   | 27 (18.4%)     |

Lesion location
| Region         | Cervical (n) | Other infection (n) | Pearson's | P      |
|----------------|--------------|---------------------|-----------|--------|
| Cervical       | 2 (1.4%)     | 0                   |           |        |
| Cervicothoracic| 13 (8.8%)    | 3 (2.0%)            | 16 (10.9%)| 2 (1.4%)|
| Thoracic       | 42 (28.6%)   | 13 (8.8%)           | 16 (10.9%)| 13 (8.8%)|
| Thoracolumbar  | 7 (4.8%)     | 2 (1.4%)            | 2 (1.4%)  | 3 (2.0%)|
| Lumbar         | 70 (47.6%)   | 15 (10.2%)          | 19 (12.9%)| 36 (24.5%)|
| Lumbosacral    | 13 (8.8%)    | 2 (1.4%)            | 3 (2.0%)  | 8 (5.4%)|

Table 2. Statistical methods and results of general indicators

| Indicator  | Wilcoxon signed-rank test (P-value) | Independent-sample t-test (P-value) |
|------------|----------------------------------|----------------------------------|
| CRP        | 0.03                             |                                  |
| ESR        | 0.01                             |                                  |
| Age        |                                  | 0.109                            |
| PCT        |                                  | 0.415                            |
| WBC        |                                  | 0.423                            |

Table 3. Characteristics of the STB and other infection groups, n (%) *

| Indicator       | STB(n) | Other infection(n) | Pearson's | P     |
|-----------------|--------|--------------------|-----------|-------|
| T-SPOT.TB       | Positive | 68 (46.3%) | 42 (28.6%) | 15.604 | <0.001 |
|                 | Negative | 9 (6.1%) | 28 (19.0%) |       |       |
|                | Female | Male | Sex | OR (95% CI) | P     | OR (95% CI) | P     |
|----------------|--------|------|-----|-------------|-------|-------------|-------|
| **Sex**        |        |      |     |             |       |             |       |
| Female         | 38 (25.9%) | 27 (18.4%) | 1.727 | 0.189       |       |             |       |
| Male           | 39 (26.5%) | 43 (29.3%) |       |             |       |             |       |
| **PCT**        |        |      |     |             |       |             |       |
| <0.05          | 31 (34.1%) | 31 (34.1%) | 1.922 | 0.166       |       |             |       |
| >0.05          | 10 (11.0%) | 19 (20.9%) |       |             |       |             |       |
| **TB antibodies** |      |      |     |             |       |             |       |
| IgG (+)/IgM (+)| 15 (11.1%) | 3 (2.2%) | 9.027 | 0.003       |       |             |       |
| IgG (-) and IgM (-) | 53 (39.3%) | 64 (47.4%) |       |             |       |             |       |
| **Lesion location** |     |      |     |             |       |             |       |
| Cervical       | 0 |      |     | 2 (1.4%) | 9.368 | 0.095       |       |
| Cervicothoracic | 5 (3.4%) | 8 (5.4%) |       |             |       |             |       |
| Thoracic       | 29 (19.7%) | 13 (8.8%) |       |             |       |             |       |
| Thoracolumbar  | 4 (2.7%) | 3 (2.0%) |       |             |       |             |       |
| Lumbar         | 34 (23.1%) | 36 (24.5%) |       |             |       |             |       |
| Lumbosacral    | 5 (3.4%) | 8 (5.4%) |       |             |       |             |       |

* The STB group included both confirmed and probable STB cases.

**Table 4.** Binary logistic regression analysis of patients

|                | T-SPOT.TB | Adjusted T-SPOT.TB |
|----------------|-----------|--------------------|
| OR (95% CI)    | P         | OR (95% CI)        | P     |

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|                   | T-SPOT.TB                          | Adjusted T-SPOT.TB                   | ESAT-6>12<sup>Only</sup> | CFP-10>19<sup>Only</sup> | ESAT-6>12 and CFP-10>19 |
|-------------------|------------------------------------|--------------------------------------|---------------------------|---------------------------|--------------------------|
|                   | 5.838 (1.686-20.208)               | 0.005 (0.766-1.325)                  | 0.005                     | 0.005                     | 0.005                    |
| Sex               | 0.713 (0.256-1.987)                | 0.518 (0.766-1.325)                  | 0.674 (0.196-2.312)       | 0.674 (0.196-2.312)       | 0.674 (0.196-2.312)      |
| CRP               | 1.003 (0.986-1.019)                | 0.762 (0.766-1.325)                  | 1.002 (0.982-1.022)       | 1.002 (0.982-1.022)       | 1.002 (0.982-1.022)      |
| PCT               | 1.257 (0.411-3.847)                | 0.689 (0.766-1.325)                  | 5.675 (0.937-34.354)      | 5.675 (0.937-34.354)      | 5.675 (0.937-34.354)     |
| ESR               | 0.999 (0.980-1.019)                | 0.943 (0.766-1.325)                  | 1.002 (0.980-1.026)       | 1.002 (0.980-1.026)       | 1.002 (0.980-1.026)      |
| Antibody          | 0.112 (0.012-1.001)                | 0.050 (0.766-1.325)                  | 0.074 (0.005-1.114)       | 0.074 (0.005-1.114)       | 0.074 (0.005-1.114)      |
| WBC count         | 1.007 (0.766-1.325)                | 0.959 (0.766-1.325)                  | 0.887 (0.636-1.237)       | 0.887 (0.636-1.237)       | 0.887 (0.636-1.237)      |

**Table 5.** Characteristics of the standard T-SPOT.TB and the adjusted-T-SPOT.TB assays
| Other infections | n  | 42 | 28 | 25 | 45 | 22 | 48 | 11 | 59 | 8  | 62 |
|------------------|----|----|----|----|----|----|----|----|----|----|----|
| False-positive (%) |    | 60.0 | 35.7 | 31.4 | 15.7 |    | 11.4 |
| Specificity (%)   |    | 40.0 | 64.3 | 68.6 | 84.3 | 88.6 |
| PPV(%)            |    | 61.8 | 71.9 | 72.8 | 83.6 | 86.4 |
| PLR               |    | 1.5 | 2.3 | 2.4 | 4.6 | 5.8 |

T-SPOT.TB, ESAT-6>6 or CFP-10>6; Adjusted T-SPOT.TB, ESAT-6>12 or CFP-10>19; ESAT-6>9<sup>day</sup>, only ESAT-6>12; CFP-10>19<sup>day</sup>, only CFP-10>19; PPV, positive predictive value; PLR, positive likelihood ratio.

**Figure 1** Flow diagram summarizing patient recruitment
Patients with bone destruction in spine (n=147)

All patients were analyzed (n=147)

The condition of T-SPOT.TB

Positive (n=110)
  - STB (n=68)
    - confirmed (n=33)
    - Probable (n=35)
  - Not STB (n=42)
    - confirmed (n=42)

Negative (n=37)
  - STB (n=9)
    - confirmed (n=2)
    - Probable (n=7)
  - Not STB (n=28)
    - confirmed (n=28)
**Figure 2** Illustration of nontuberculous spinal infections

**Figure 3** ESAT-6 and CFP-10 spot numbers

**Figure 4** The ROC curve of ESAT-6 and CFP-10
