The utility of T-SPOT.TB for the diagnosis unconventional pleural tuberculosis is superior to ADA in high prevalence: A perspective analysis of 601 cases

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Abstract

BACKGROUND Recently, Interferon Gamma Release Assay (IGRA) is still controversial in differentiating tuberculous pleural effusion (TPE), through recommended by World Health Organization (WHO) for identification of latent tuberculosis infection. OBJECTIVES Aim to qualify the diagnostic efficacy for TPE by IGRA in comparison to Adenosine deaminase (ADA), to clarify its appropriate scene in clinical diagnosis.

METHODS A prospective, single-centre study including all suspected pleural effusion patients consecutively from June 2015 to October 2018. Through receiver operating characteristic (ROC) curves, all enrolled participants were determined technical cut-off and the utility of IGRA (T-SPOT) for pleural fluid (PF). Obtain the independent risk factors by logistic regression analysis for TPE, and evaluate the performance of T-SPOT stratified by risk factors, in comparison to ADA.

RESULTS A total of 601 individuals were consecutively recruited. The maximum of early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) in PF T-SPOT had the best diagnostic efficiency in our study, with a sensitivity of 83.0% and a specificity of 83.1%, corresponding cut-off value is 466, which was equal to ADA (0.885 vs 0.887, P=0.957) and superior than in PB. Among the TPE patients with low ADA (<40 IU/L), the sensitivity and specificity of PF T-SPOT was still 87.9%, 90.5% respectively. The utility of ADA was negative related to age ascents, but PF T-SPOT had steady performance at any age-stage. The age (<45 yrs; odds ratio (OR) 5.61), gender (male; OR 2.7) and body mass index (BMI)<22; OR 1.93) was independently associated with the risk of TB by multivariate logistic regression analysis. Stratified by risk factors, notably the PF T-SPOT had superior sensitivity (76.5% vs. 23.5%, P=0.016) than ADA meanwhile had the non-inferior specificity (84.4% vs. 96.9%, P=0.370).

CONCLUSIONS In conclusion, the overall potency of PF T-SPOT assay is equal to ADA for diagnosing TPE. In addition, PF T-SPOT can effectively discriminate the TPE patients whose ADA lower than 40IU/L, and extremely superior to ADA in unconventional TPE patients (age>45 yrs, female or BMI<22). PF T-SPOT assay is an extremely good choice to supplement ADA to diagnose TPE.

Background

Tuberculous pleural effusion (TPE), one of the commonest forms of extrapulmonary TB, entity with a spectrum of presentations from fully absorbed benign to complicated pleural thickening or even serious complications such as empyema and fibrothorax, which may have a perpetual effect on lung function. Early and effective diagnosis could minimize hospital days and maximize quality of life. At present, the most direct evidence for Mycobacterium tuberculosis (MTB) infection is etiology[1], but showing suboptimal sensitivity. Therefore, the patients whose pleural effusion characterized by lymphocytic exudates[2] combined with high ADA level were frequently deemed as TPE by clinicians in high prevalence.

Adenosine deaminase (ADA), an enzyme produced from lymphocytes and involved in purine metabolism, long-term dominating in diagnosing TPE since its matchless sensitivity and specificity[3 – 4]. Its advantage is that high level as a diagnosis indicator and low value could term as a proof of excluding TPE simultaneously. However, recent studies have shown that the patients with empyema, malignancy, or rheumatoid pleurisy also could be observed high ADA levels[5], negative result in older TPE patients[6], and fluctuating obviously affected by the patient profile and local tuberculosis (TB) prevalence[7].

Interferon Gamma release assay (IGRA), a commercially available cost-effective indirect assay for detecting Interferon Gamma (IFN-γ). The WHO guidelines[8] rejected the recommendation of IGRA for differentiation active TB, especially in high burden countries, but another published the European Centre for Disease Prevention and Control (ECDC) guideline[9] proposed that IGRA could contribute supplementary information on those who test negative for acid-fast bacilli (AFB) and MTB culture in spumt. In recent years, a growing number of studies have researched the utility of IGRA to diagnosing TPE, a recent meta-analysis[10] assessing the performance of IGRA for TPE exhibited satisfied outcome (PPV = 82%, NPV = 87%), but results are heterogeneous (I² = 92.0%-82.5%), suggesting still controversial and polarized. Theoretically, tuberculosis antigen-specific responses by IGRA should provide superior discriminatory value than non-specific inflammatory biomarkers (e.g. unstimulated IFN-γ or ADA), but it's not the case. We boldly presume that its superiority could be concealed by discrepancy in population characteristics, and causing inconsistency between studies.

In order to further to clarify the diagnostic role of IGRA for TPE in high TB burden, this prospective study was conducted to investigate the utility of IGRA (T-SPOT.TB) applied for discrimination of TPE, and comparing the difference of potency with ADA in TPE subjects of different characteristics.

Material And Methods

Participants population and study procedure

A prospective study was performed at Beijing chest Hospital, Capital Medical University from June 2015 to October 2018, in which all suspected pleural effusion (PE) patients were enrolled consecutively. The patients enrolled should meet the following criteria: (1) age ≥ 14 years; (2) presenting with PE through chest ultrasonic examinations; (3) tolerable to thoracic puncture and providing more than 100 ml pleural effusion. We used no exclusion criteria.

Clinical samples (including sputum, peripheral blood (PB) and pleural fluid) from all participants were processed for diagnostic purposes after obtaining informed consent, composed of routine clinical biochemical testing for each PF sample, which contains total protein, glucose, lactate dehydrogenase (LDH) and ADA, and smear microscopy, culture and Gene-Xpert for each sputum and PF sample. All participants’ clinical data were extracted by the
investigators, and tracked the treatment process and discharge diagnoses. All participants had followed up for 12 months to verify the final diagnosis, and the patients with negative outcome of anti-TB treatment at the last 12 months were deemed as indeterminate diagnosis.

**Clinical categories of pleurisy**

Patients were divided into three groups according to the composite reference standard (CRS), which was composed of clinical, laboratory, and radiological examinations and follow-up data of diagnostic treatment. (1) Bacteriologically confirmed TPE was defined as any positive TB etiology testing from PE or sputum; (2) Probable TPE were lacking bacteriological confirmation but all were treated empirically for TB based on clinical suspicion (e.g. typical clinical symptoms, remarkable radiological imaging and positive outcome of anti-TB treatment during follow-up); (3) Non TPE indicated cases diagnosed definitely as other diseases, such as malignant, empyema (Non-tuberculous disease) etc.

**T-SPOT.TB in PF and PB**

The samples of PB (4 mL) and PF (45 mL) collected from each participant were tested within 6 hours. PB samples were diluted 1 fold and centrifuged at 900 g for 20 min and PF sample centrifuged at 500 g for 10 min respectively, both discarding plasma supernatant for T-SPOT.TB testing.

T-SPOT.TB was conducted following the manufacturer's instructions (Oxford Immunotec Ltd, Oxford, UK), which is identical for PB and PF samples. The pellets were resuspended in 8 mL AIM-V medium (GIBCO, Rockville, MD, USA) and the cell suspension was separated to obtain mononuclear cells. Setting an empty wells as negative controls, the T lymphocyte mitogen phytohaemagglutinin as positive control, and ESAT-6 and CFP-10 peptides in separate wells, respectively. Isolated peripheral blood mononuclear cells (PBMCs) and pleural fluid mononuclear cell (PFMCs) were added to the wells (2.5 x 10^5 cells per well) that were pre-coated with a monoclonal antibody against IFN-γ, and incubated at 37 °C for 16 to 20 hours. The spot-forming cells (SFCs) were read from an automated enzyme-linked immunosorbent spot (ELISPOT) reader (CTL-ImmunoSpotS5 Versa Analyzer).

**Diagnosis**

**Smear, Acid fast bacilli(AFB) and Mycobacterial culture**

Specimens including the sputum and PF (5 mL) was prepared for direct smear and stained with auramine, examined by light-emitting diode microscopy. The smear was read and interpreted in accordance with the WHO guidelines [11]. The sputum and PF (5 mL) were preprocessed by N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH), centrifugated and discarding supernatant, the resuspended pellet was transferred to solid Lowenstein-Jensen medium (Encode Medical Engineering Co., Ltd, China) and liquid medium, and subjected to cultivating in mycobacteria growth indicator tube (MGIT) 960 system (Becton, Dickinson and Company, USA). The presence of Mtb complex in any medium represented the positive of MPT64 antigen testing. Recording the positive events and time.

**Gene-Xpert**

The Gene-Xpert were performed as the manufacturer's instructions (Cepheid, Sunnyvale, CA, USA). Briefly, fully premixing the specimen (including sputum and concentrated PF) and sample reagent at the room temperature. Final 2 ml mixture was collected and transferred into the cartridge and loaded into the automatic GeneXpert instrument. Duplicated testing was performed on the sample with an invalid result.

**Statistical analysis**

Data were analyzed using IBM SPSS 25.0 (SPSS Inc. Chicago, IL, USA) GraphPad Prism 8.2.1 (GraphPad Software, Inc. La Jolla, USA). Quantitative variables were presented as mean ± standard deviation (SD) or median (interquartile ranges (IQR)), and categorical variables were presented as frequencies (percentages). To identify differences between two independent groups, Chi-square test was used to detect differences between categorical variables, Mann-Whitney U test and unpaired t-test was applied for continuous data in non-normal or normal distribution, respectively. A result was considered statistically significant when the P-value was < 0.05.

Receiver operating characteristic (ROC) curves were plotted to evaluate the diagnostic performance of ADA and T-SPOT.TB, respectively, obtaining optimal cut-off value and calculating corresponding areas under the ROC curve (AUCs), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+ LR), negative likelihood ratio (LR), diagnostic odds ratio (DOR) and accuracy were calculated.

Extracting From the clinical record, physical examination and PF biochemical data (Including age, gender, body-mass-index (BMI), onset-time, history of previous anti-TB, symptoms, PF total protein, glucose and LDH), independently risky factors of TB were obtained from regression models combining with multicolinearity assessment. The final bioclinical scores for each factor were assigned through odds ratio (OR) in the final multivariate model. Overall research were completed in keeping with the Standards for Reporting of Diagnostic Accuracy (STARD) template [12].

**Results**

**Participants characteristics**

From June 2015 to October 2018, a total of 601 suspected PE patients were enrolled, patients with indeterminate diagnosis (n = 16), with unverifiable patients detail (n = 9), lost to follow-up (n = 4) and age < 14 years old (n = 1) were excluded. According to the CRS, 145 cases with confirmed TPE, 252 cases with clinically diagnosed probable TPE and 174 cases non TPE (Fig. 1). Among the 174 non TPE patients, 117 (67.2%) were diagnosed as
malignant pleural effusion, 32(18.4%) as parapneumonia or empyema (Non-tuberculous), 8(4.6%) as exudation effusion(Non-tuberculous), 4(2.3%) as transudate effusion and 13(7.5%) as other(Fig. 1), which was in line with the current epidemiology of PE.

Among confirm TPE group, the sputum smear.AFB, culture, Gene-xpert were positive in 27.6%, 60.5% and 64.6%, respectively; while for PF corresponding positive in only 2.2%,37.4% and 25.4%, respectively, and higher total detection rate in sputum (68.3% vs. 46.9%) convinced that obtaining the direct proof of MTB infection from PF is more difficult than from sputum.

Clinic, demographic and biochemical data

The demographic and clinical characteristics of all participants were summarized in Table 1. The patients in TPE group is more younger than that in non TPE group(42.15 ± 19.78 vs. 57.59 ± 15.36, p < 0.001), and there were more male subjects in TPE group (75.3%,299/397 vs. 59.2%,103/174, p < 0.001), and predominant in unilateral PE(83.9%, 333/397), all features were in accordance with a case series from Qatar[13]. The TPE group had significantly thinner than the non TPE group (21.70 ± 4.24 vs. 23.23 ± 3.37, p < 0.001). Patients with TPE more frequently presented with fever (74.8% vs. 39.7%, p < 0.001), but had less chest tightness (58.6% vs. 71.8%, p = 0.013). the probable TPE that inferred by clinicians had more obvious clinical symptoms relating to TB infection, for instance more night sweat (21.4% vs. 10.3%, p = 0.009), more wight lose (34.1% vs. 23.6%, p = 0.042)and less hemoptysis(1.2% vs. 7.5%, p < 0.001). However, there were no significant differences in various characteristics between the confirm and probable TPE(P > 0.05).
| Table 1 | Descriptive characteristics of participants (N = 571) |
|---------|--------------------------------------------------|
|         | Non TPE (n = 174) | TPE group | *P value | Probable TPE (n = 252) | §P value |
|         | Confirm TPE (n = 145) (n = 141) | Probable TPE (n = 252) | *P value | Probable TPE (n = 252) | §P value |
| Age, ys (mean ± sd) sd (SD) | 57.59 ± 15.36 | 45.00 ± 20.71 | < 0.001# | 40.5 ± 19.07 | < 0.001# | < 0.001# |
| Gender (%) male | 103 (59.2) | 119 (82.1) | < 0.001 | 180 (71.4) | 0.009 | < 0.001 |
| BMI | 23.23 ± 3.37 | 21.40 ± 7.43 | 0.004¶ | 21.88 ± 3.30 | < 0.001¶ | < 0.001¶ |
| Onset time (day) | 35 (20–90) | 30 (14–60) | 0.033# | 21.50 (13.65–45.71) | < 0.001# | < 0.001# |
| Smoking | | | | | |
| none | 107 (61.5) | 91 (62.8) | 0.817 | 164 (65.1) | 0.450 | 0.532 |
| years of smoking | 30.79 (20.25–41.73) | 21.67 (7.67–33.33) | 0.259# | 13.67 (8.53–28.64) | 0.038# | 0.050# |
| Previous anti-TB treatment | 31 (17.8) | 44 (30.3) | 0.009# | 36 (14.3) | 0.325 | 0.516# |
| Symptoms | | | | | |
| Fever | 69 (39.7) | 117 (80.7) | < 0.001 | 180 (71.4) | < 0.001 | < 0.001 |
| Cough | 131 (75.3) | 120 (82.8) | 0.105 | 191 (75.8) | 0.905 | 0.422 |
| Chest pain | 76 (43.7) | 73 (50.3) | 0.235 | 126 (50.0) | 0.199 | 0.156 |
| Chest tightness | 125 (71.8) | 85 (58.6) | 0.013 | 190 (75.4) | 0.411 | 0.537 |
| Night sweat | 18 (10.3) | 22 (15.2) | 0.195 | 54 (21.4) | 0.003 | 0.009 |
| Weight loss | 41 (23.6) | 41 (28.3) | 0.338 | 86 (34.1) | 0.019 | 0.042 |
| Hemoptysis | 13 (7.5) | 3 (2.1) | 0.057 | 3 (1.2) | 0.002 | < 0.001 |
| Effusion site | | | | | |
| left | 66 (37.9) | 56 (38.6) | 0.900 | 93 (36.9) | 0.830 | 0.928 |
| right | 69 (39.7) | 65 (44.8) | 0.351 | 119 (47.2) | 0.122 | 0.138 |
| bilateral | 39 (22.4) | 24 (16.6) | 0.190 | 40 (15.9) | 0.088 | 0.072 |
| Pleural effusion volume | | | | | |
| small | 72 (41.6) | 59 (40.7) | 0.867 | 106 (42.1) | 0.927 | 0.990 |

TPE, tuberculous pleural effusion; BMI, body mass index; TB, tuberculosis; LDH, lactate dehydrogenase; ADA, adenosine deaminase;

*Comparisons were performed between non- and TPE group using chi-square test and #Mann–Whitney U test and ¶unpaired t-test.

§Comparisons were performed between non- and probable- TPE group using chi-square test and ¶Mann–Whitney U test and ¶unpaired t-test.

*Comparisons were performed between non TPE and TPE group using chi-square test and #Mann–Whitney U test and ¶unpaired t-test.

Data are presented as mean ± standard deviation (SD), median (interquartile ranges) or frequency (percentage).
| Non TPE (n = 174) | TPE group | *P value | §P value |
|------------------|-----------|----------|----------|
|                  | Confirm TPE (n = 145) | (n = 141) |                  |
| moderate         | 52(30.1)  | 42(29.0) | 0.832     | 66(28.0) | 0.382 | 0.486 |
| massive          | 49(28.3)  | 44(30.3) | 0.693     | 81(32.1) | 0.410 | 0.451 |
| total protein    | 34.36 ± 18.68 | 37.63 ± 19.57 | 0.003# | 43.47 ± 14.56 | < 0.001# | < 0.001# |
| Glucose          | 5.81 ± 2.92 | 4.50 ± 2.36 | < 0.001# | 5.04 ± 2.17 | < 0.001# | < 0.001# |
| LDH              | 256(158.25–482) | 425(276–704) | < 0.001# | 377.5(240.5–580.75) | < 0.001# | < 0.001# |
| ADA              | 11.8(8.25–18.65) | 50(37.85–62.15) | < 0.001# | 45(31.875–57.9) | < 0.001# | < 0.001# |

TPE, tuberculous pleural effusion; BMI, body mass index; TB, tuberculosis; LDH, lactate dehydrogenase; ADA, adenosine deaminase.

§Comparisons were performed between non- and probable- TPE group using chi-square test and *Mann–Whitney U test and ¶unpaired t-test.

*Comparisons were performed between non TPE and TPE group using chi-square test and #Mann–Whitney U test and ¶unpaired t-test.

Data are presented as mean ± standard deviation (SD), median (interquartile ranges) or frequency (percentage).

Through multivariate logistic regression analysis, there were age (<45 yrs; odds ratio (OR) 5.61, 95% confidence interval (CI) 3.59–8.78; p < 0.001), gender (male; OR 2.7, 95% CI 1.75–2.88; p < 0.001) and BMI (<22; OR 1.93, 95% CI 1.30–2.88; p = 0.001) including in the final model, intended that independently associated with the risk of TB (Table 2).

### Table 2
Logistic regression analysis of risk factors for TPE

| Risk Factor | OR (95% CI) | P value |
|-------------|-------------|---------|
| Gender      |             |         |
| male        | 2.68(1.75–4.11) | < 0.001 |
| female      | 1.00        |         |
| BMI         |             |         |
| < 22        | 1.93(1.30–2.88) | 0.001   |
| > 22        | 1.00        |         |
| Age         |             |         |
| < 45y       | 5.61(3.59–8.78) | < 0.001 |
| > 45y       | 1.00        |         |

**Diagnostic utility of T-SPOT.TB assay for PB and PF**

As shown in Fig. 2, the final ESAT-6, CFP-10, and Max SFCs (represents the larger of ESAT-6 and CFP-10) for PB and PF respectively were affirmed to discriminate TPE from non TPE, and no significant differences were observed between confirm- and probable- TPE. In Table 3, We exhibited that cut-off value of PB derived from receiver operating characteristic curve (ROC) analysis between confirm TPE group and non TPE group (Fig. 2), which is extremely close to the positive cutoff value (24 SFCs/10⁶ mononuclear cells) provided by the manufacturer. Overall, when taken the same cut-off value = 22 SFCs/10⁶ mononuclear cells, the performance of ESAT-6 was slightly better than CFP-10 in PB, with AUC of 0.840 vs 0.796 (P = 0.055), as well as a sensitivity of 82.1% vs 75.2% (P = 0.123) and a specificity of 75.3% vs 77.9% (P = 0.847); However, when considering Max SFCs (cut-off value = 22
SFCs/10⁶ mononuclear cells), of AUC, sensitivity and specificity was 0.83 (95% CI 0.794–0.884), 90.3% and 67.2% respectively, no better than ESAT-6 (P = 0.954). (Table 3).

Table 3
Diagnostic utility of T-SPOT.TB (PF & PB) and ADA for diagnosing TPE.

| variables         | cut-off value | sensitivity% | specificity% | PPV%  | NPV%  | +LR   | -LR   | DOR   | AUC  | 95%CI      |
|-------------------|---------------|--------------|--------------|-------|-------|-------|-------|-------|------|------------|
| peripheral blood(PB) |               |              |              |       |       |       |       |       |      |            |
| ESAT-6            | 24            | 77.9         | 77.0         | 73.9  | 80.7  | 3.39  | 0.29  | 11.80 |      |            |
|                   | 22            | 82.1         | 75.3         | 73.5  | 83.4  | 3.32  | 0.24  | 13.98 | 0.840| 0.794–0.878|
| CFP-10            | 24            | 72.4         | 74.7         | 70.5  | 76.5  | 2.86  | 0.37  | 7.75  |      |            |
|                   | 22            | 75.2         | 74.7         | 71.2  | 78.3  | 2.97  | 0.33  | 8.95  | 0.796| 0.747–0.839|
| Max SFCs          | 24            | 86.9         | 69.0         | 70.0  | 86.3  | 2.80  | 0.19  | 14.77 |      |            |
|                   | 22            | 90.3         | 67.2         | 69.7  | 89.3  | 2.75  | 0.14  | 19.07 | 0.839| 0.794–0.884|
| pleural fluid(PF) |               |              |              |       |       |       |       |       |      |            |
| ESAT-6            | 170           | 86.9         | 78.2         | 76.8  | 87.7  | 3.99  | 0.17  | 23.80 | 0.874| 0.833–0.909|
|                   | 142           | 85.5         | 73.6         | 72.9  | 85.9  | 3.24  | 0.20  | 16.44 | 0.857| 0.813–0.894|
| CFP-10            | 146           | 81.4         | 82.8         | 79.7  | 84.2  | 4.73  | 0.22  | 21.07 | 0.885| 0.846–0.923|
| Max SFCs          | 466           | 89.0         | 83.9         | 82.2  | 90.1  | 5.53  | 0.13  | 42.16 | 0.887| 0.847–0.920|
| ADA               | 22            | 71.0         | 93.1         | 89.6  | 79.4  | 10.29 | 0.31  | 33.03 |      |            |

ADA, adenosine deaminase; TPE, tuberculous pleural effusion; NPV, negative predictive value; PPV, positive predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio; DOR, diagnostic odds ratio

SFCs, spot-forming cells; Max, the larger of ESAT-6 and CFP-10; AUC, the area under curve;

*: manufacturer derived cut-off point for PB; ¶: AUC derived cut-off point.

a Values are expressed as U/L for ADA, and as SFCs/10⁶ mononuclear cells for T-SPOT.
Table 4
Performance outcomes of a bioclinical score for ADA and T.SPOT

| Score (non:TPE) | Assay (cut-off value) | sensitivity | specificity | PPV | NPV | accuracy | \( \chi^2 \) | \( \chi^2 \) | \( \chi^2 \) | \( \chi^2 \) |
|-----------------|-----------------------|-------------|-------------|-----|-----|----------|-----------|-----------|-----------|-----------|
| 0 (32:17)       | ADA(40)               | 23.5        | 96.9        | 80  | 70.5| 71.4     | 5.82      | 0.016     | 0.80      | 0.370     |
|                 | PB T.SPOT(24)         | 76.5        | 62.5        | 52  | 83.3| 67.3     |           |           |           |           |
|                 | PF T.SPOT(466)        | 76.5        | 84.4        | 72.2| 87.1| 81.6     |           |           |           |           |
| 2/3/5 (107:155) | ADA(40)               | 51.0        | 94.4        | 92.9| 57.1| 68.7     | 21.42     | < 0.001   | 7.26      | 0.006     |
|                 | PB T.SPOT(24)         | 79.4        | 64.5        | 76.4| 68.3| 73.3     |           |           |           |           |
|                 | PF T.SPOT(466)        | 76.1        | 80.4        | 84.9| 69.9| 77.9     |           |           |           |           |
| 6/8/9 (30:127)  | ADA(40)               | 73.2        | 86.7        | 95.9| 43.3| 75.8     | 10.26     | 0.001     | 0.14      | 0.705     |
|                 | PB T.SPOT(24)         | 93.7        | 93.3        | 98.3| 77.8| 93.6     |           |           |           |           |
|                 | PF T.SPOT(466)        | 89.8        | 90.0        | 97.4| 67.5| 89.8     |           |           |           |           |
| 11 (5:98)       | ADA(40)               | 81.6        | 80.0        | 98.8| 18.2| 81.6     | 0.04      | 0.840     | 0         | > 0.999   |
|                 | PB T.SPOT(24)         | 85.7        | 60.0        | 97.7| 17.6| 84.5     |           |           |           |           |
|                 | PF T.SPOT(466)        | 80.6        | 80.0        | 98.8| 17.4| 79.0     |           |           |           |           |

ADA, adenosine deaminase; BMI, body mass index; TPE, tuberculous pleural effusion; NPV, negative predictive value; PPV, positive predictive value;

Age < 45 yrs, male and BMI < 22 scored 6,3,2 points, respectively;

§Comparisons were performed for sensitivity between ADA and PF T.SPOT using chi-square test.

¶ Comparisons were performed for specificity between ADA and PF T.SPOT using chi-square test.

Values are expressed as IU/L for ADA, and as SFCs/10⁶ mononuclear cells for T-SPOT. Data are presented as percentage.

As expected, the performance of PF T-SPOT was distinctly improved in contrast to PB T-SPOT. ESAT-6 and CFP-10 specific cells were more highly concentrated in PF than in PB by median ratio of 12.13(IQR 3.29) and 9.30(IQR 1.23–30.15) in confirm TPE group, and median ratio of 11.87(IQR 3.96–35.15) and 10.60(IQR 2.63–32.21) in probable TPE group, and no significance were observed between any groups. Based on the ROC analysis, the optimal cut-off point was 170 for PF ESAT-6, which produced a sensitivity of 86.9% and specificity of 78.2%; and 142 for PF CFP-10, which produced a sensitivity of 85.5% and specificity of 73.6%. While, of that Max SFCs in PF exhibited the best diagnostic efficiency which was equal to ADA(0.885 vs 0.887, \( P = 0.957 \)), with a sensitivity of 83.0% and a specificity of 83.1%, corresponding cut-off value is 466 SFCs/10⁶ mononuclear cells(Fig. 2, Table 3).

Comparison of diagnostic utility of ADA and T-SPOT.TB assay stratified by bioclinical score

The median of ADA levels in non-confirm- and probable- TPE group were 11.8 U/L(IQR 8.25–18.65), 50 U/L(IQR 37.85–62.15), and 45 U/L(IQR 31.875–57.9), respectively(Table 1), confirming that low ADA level was satisfactory in excluding the TPE, but the ADA level in probable- TPE group was slighter lower than that in confirm- TPE group(\( P = 0.055 \)).
Each participant was grouped by scoring of logistic regression coefficient [14]. As shown in Table 5, when score = 11, meeting three risky factors(< 45 yrs, male and BMI < 22), the performance of PF T-SPOT was non-inferior to ADA; comparing to the stable utility of PF T-SPOT, the sensitivity of ADA was positive related to score decline, while specificity negatively, suggesting that PF T-SPOT performed better than ADA in the unconventional patients, especially when score = 0 (represents the female patients whose age more than 45 yrs and BMI ≥ 22), the PF T-SPOT had the non-inferior specificity (84.4% vs. 96.9%, P = 0.370) meanwhile had superior sensitivity (76.5% vs. 23.5%, P = 0.016) than ADA.

### Comparison of diagnostic utility of ADA and T-SPOT.TB stratified by age

In Fig. 3A, the scatter plot showed that the distribution of ADA levels in the TPE group had shifted downwards (P < 0.05) from age of 40+, especially the median ADA level in patients over than 60 years old were lower than clinical diagnostic point (40 U/L), intended that ADA activity level had significantly negatively correlated with age-ascent (P < 0.001); notably, the performance of PF T-SPOT at different age stage is steady, which had no significant differences among all groups (P = 0.604) (Fig. 3B). The above results showed that the performance of T-SPOT.TB was superior to ADA in older patients.

### Diagnostic utility of T-SPOT.TB assay in patients with ADA indeterminate (ranging from 20 to 40)

In our study, the cut-off value of ADA derived from ROC analysis was 22.4 U/L, which had higher sensitivity (89.0%); conversely, when ADA more than 40 U/L recognized as positive (International Recommends), it produced higher specificity (93.1%). Therefore, we defined 112 patients (19.6%) with ADA value ranging from 20 to 40 (21 in non TPE, 26 in confirm TPE, 65 in probable TPE) as the ADA indeterminate. In Fig. 4, the scatter plot exhibited the diagnostic utility of PF T-SPOT in the indeterminate, the sensitivity, specificity, PPV and NPV was 87.9%, 90.5%, 97.6% and 63.3%, respectively. Youden index was 0.784; and the sensitivity, specificity, PPV and NPV of PB T-SPOT was 83.5%, 76.2%, 93.8% and 51.6%, respectively. Youden index was 0.597. These data intended that T-SPOT.TB assay could discriminate the TPE patients with ADA ranging from 20 to 40, and the utility of T-SPOT in PF was superior to that in PB.

### Discussion

According to our grasping, through there were some publications on evaluating the utility of T-SPOT for diagnosis of TPE [9–10], the majority of subjects recruited small sample population (n < 100) for evaluation and the results was conflicting. Our study provided the largest cohort (TPE: non TPE = 397:145) so far, confirmed that the value of T-SPOT assay for diagnosis of TPE with high confidence, and providing specific reference suggestions for clinical application of T-SPOT.

In this study, all suspected PE participants had been consecutively, unselectedly enrolled, and all TPE patients had the definite diagnosis by bacteriological confirmation or positive outcome for anti-TB therapy, which highly reflecting the demographic epidemiological characteristics of tuberculosis-prone areas. The recruited samples covered from adolescents to elderly patients aging 90+, confirmed again that the TPE patients had the clinical characteristics of younger male, combining with higher ADA activity and higher T-SPOT response for PF and PB. It's similar to other studies when IGRA are applied to PB, the unsatisfactory specificity was common as a result of inefficiency to discriminating active TB from latent TB infection, reversely the specificity of T-SPOT for PF is much higher with approximate sensitivity, thus the diagnostic cutoff obtained from PB is not available for PF; in our study the cut-off value derived from ROC analysis was higher than that in low prevalence areas, majority of which had taken as equal as PB cutoff in Europe [15], and some subjects obtained the cutoff by ROC analysis also is very low, such as 30SFCs/10⁶ mononuclear cells in London [16] and 300 SFCs/10⁶ mononuclear cells in Korea [17], respectively, which was in line with the expect of high-burden settings low threshold would compromise specificity, therefore it's crucial for tuberculosis-prone areas that to chose a proper threshold to apply PF T-SPOT.

Another biomarker ADA, a value of more than 40 IU/L in lymphocyte dominate PE carries a PPV of 98% in high TB endemic region [3–4]; while an retrospective analysis of assessing ADA in 1637 subjects confirmed less than 15 IU/L can get a NPV of 100% [18]. As same as in our study, we found that recognizing > 40 IU/L as the solo indicator of TPE maybe not the most suitable, as it got the high specificity meanwhile sacrificing sensitivity, 35.5% (141/397) TPE patients’ ADA level was lower than 40 IU/L in this study, 29.8% of which had definite etiological basis. In addition, the utility of cutoff 22.4 IU/L derived from ROC analysis was better than > 40 IU/L (Youden index 0.729 vs. 0.641). However, comprehensively considering to the non-specific elevation of ADA level caused by the non tuberculosis inflammatory PE [18] (particularly complicated parapneumonic effusions and empyemas) and lymphomas, the patients whose ADA more than 20 IU/L and less than 40 IU/L could be classified as ADA indeterminate appreciatively. Reversely, PF T-SPOT showed the excellent diagnostic utility between ADA indeterminate groups, whose accuracy is higher to 90.2%, predicted that the result of PF T-SPOT could be a considerable indicator for the highly-suspected TPE patients with indeterminate ADA.

Many previous studies indicated that [19–20], the performance of diagnosing TPE for patients aging more than 45yrs is still an open question, a recent study about only 4.65% of TPE subjects that main compose of elderly primarily had levels over 40 IU/L [21], our research fully demonstrated this phenomenon simultaneously. In addition, we observed that, the fuzzy boundary influenced by age on ADA is opportunely concentrated in ADA indeterminate groups among the patients of ADA indeterminate, there were 85.7% (18/21), 73.7% (19/26) and 47.7% (31/65) patients aging more than 45yrs in non-, confirm- and probable- TPE group, respectively. While the superior performance of PF T-SPOT between ADA indeterminate groups may be explained by its steady at all age stages, besides the interference for ADA by other inflammatory etiologies. Still, ADA is a widely-use biomarker for screening TPE due to its simplicity, rapidity, and low finical costs, but above results proved that over-reliance on ADA differentiation may lead to missed diagnosis/misdiagnosis in clinical diagnosis, especially in indeterminate range. Meanwhile, PF T-SPOT as luck would fill this blank.
Besides age, we screened another two high risk factors that were significantly related to TPE, gender and BMI. There were barely 5 non TPE patients scoring 11 (simultaneously fulfill three high risk factors: age < 45 yrs, male and BMI < 22), it has directly demonstrated these clinical characters could be the effective reference index for discrim TPE from other PEs. We often defined these patients as the high incidence population to TPE, also called as typical population. However, it’s notably that the utility of ADA fluctuated distinctly by stratified analysis, and if not satisfied any one condition (defined as the unconventional population) it is inferior to that in T-SPOT assay. The unconventional population frequently are the focal points and have difficulties in diagnosing, then PF T-SPOT can provide powerful identification evidence for those.

There are several limitations on current study. First, The whole study were performed in a single center which specialized in TB. The geography, relative single control composition and etiological attribution error and/or bias was incalculable. Second, we got the optimal cut-off of PF T-SPOT.TB from ROC analysis in this training cohort, and its definite accuracy would need the further validation. Finally, 38.1% of the clinical diagnosis patients lack the etiological basis due to the objective factors such as no sputum or sputum unsatisfied with the detection standard, which may bias sputum detection rate.

Conclusion

In conclusion, whether ADA or IGRA, they both can’t be defined as the definite proof of ruling in or out, but as a powerful reference tool for TPE diagnosis. The overall potency of PF T-SPOT assay is equal to ADA for diagnosing TPE in our study. In addition, PF T-SPOT can effectively discriminate the TPE patients with low ADA, and extremely superior to ADA in unconventional TPE patients (age > 45 yrs, female or BMI < 22), intending applied population are wider than ADA. PF T-SPOT assay is an extremely good choice to supplement ADA to diagnose TPE.

Abbreviations

AFB: acid-fast bacilli; ADA: Adenosine deaminase; AUC: areas under the curve; BMI: body-mass-index; CRS: composite reference standard; CI: confidence interval; CFP-10: culture filtrate protein-10; DOR: diagnostic odds ratio; ESAT-6: early secretory antigenic target-6; ELISPOT: enzyme-linked immunosorbent spot; ECDC: European Centre for Disease Prevention and Control; IGRA: Interferon Gamma Release Assay; IFN-γ: Interferon Gamma; IQR: interquartile range; LDH: lactate dehydrogenase; +LR: positive likelihood ratio; -LR: negative likelihood ratio; MTB: Mycobacterium tuberculosis; NPV: negative predictive value; OR: odds ratio; PB: peripheral blood; PBMCs: peripheral blood mononuclear cells; PE: pleural effusion; PFMCs: pleural fluid mononuclear cell; PF: pleural fluid; PPV: positive predictive value; ROC: receiver operating characteristic; SFCs: spot-forming cells; TB: tuberculosis; TPE: tuberculous pleural effusion; WHO: World Health Organization.

Declarations

Ethical approval and consent to participate

We have complied with the World Medical Association Declaration of Helsinki regarding ethical guideline involving human subjects. The study protocol was approved by the Ethics Committee of the Beijing chest Hospital affiliated to Capital Medical University, China. We have obtained informed consent from all the participants.

Consent for publication

We have obtained consent to publish from all the participants.

Authors’ contributions

Conception and design of the study: XTY, XYC, ZDZ; Sample and data collection, reviewing manuscript: QTL, LPHFD, YY, HL, CG, JZ; Experiments performing: HYJ, BPD, RRW, AYX; Data management and statistical analysis: JZ. Drafting the manuscript: JZ, XTY. All authors were involved in preparation and review of the manuscript and approved the final version to be submitted.

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Availability of data and materials

The datasets analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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All suspected pleurisy patient from June 2015 to October 2018 were enrolled: pleurisy volume ≥ 100 ml (n=601)

Exclusions:
- With indeterminate diagnosis (n=16)
- With unverifiable patients detail (n=9)
- Lost to follow-up (n=4)
- Age < 14 years old (n=1)

Pathogenic basis

TPE group (n=397)  Non TPE group (n=174)

N

Y

Control group including:
- Malignant (n=117)
- Parapneumonia & Empyema (n=32)
- Nontuberculous exudation (n=8)
- Transudative (n=4)
- Other (n=13)

Probable TPE group (n=252)

Confirm TPE group (n=145)

319 included in intention to treat analysis

Figure 1
Trial profile.

Figure 2
The receiver operating characteristic (ROC) curves of adenosine deaminase (ADA) and T-SPOT assay on the pleural fluid (PF) or peripheral blood (PB) for diagnosing tuberculous pleural effusion (TPE). Samples were obtained from enrolled participants that included 145 with confirm TPE and 174 with non
Figure 3

Comparison of performance of adenosine deaminase (ADA) and T-SPOT in pleural fluid (PF) stratified by age. (A) Comparison of median ADA level between different age stage groups. (B) Comparison of median spot-forming cells (SFCs) in PF T-SPOT between different age stage groups. For group comparison by the Kruskal-Wallis test. ns, not significant. *p<0.05, **p<0.01, ***p<0.005, & represents internationally recognized cut-off (40IU/L), # represents the cut-off (22.4IU/L for ADA, 466 SFCs/10^6 mononuclear cells for PF T-SPOT) deprived from receiver operating characteristic (ROC) curves.
Figure 4

Diagnostic accuracy of T-SPOT assay distinguishing tuberculous pleural effusion (TPE) patients with low adenosine deaminase (ADA) [ranging from 20 to 40]. (a) the T-SPOT assay in pleural fluid (PF) showed high sensitivity of 87.5% and specificity of 90.5%. (b) the T-SPOT assay in peripheral blood (PB) showed relatively high sensitivity of 83.5% and specificity of 76.2%. Comparison of spot-forming cells (SFCs) using the T-SPOT assay in PF and in PB, between the confirm-, probable- and non- TPE groups. For group comparison by the Mann-Whitney test. ns, not significant. ****p<0.001, # represents the cut-off (22 SFCs/10^6 mononuclear cells for PB T-SPOT, 466 SFCs/10^6 mononuclear cells for PF T-SPOT) deprived from receiver operating characteristic (ROC) curves.