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The role of in vitro interferonγ-release assay in differentiating intestinal tuberculosis from Crohn's disease in China

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KEYWORDS
Crohn's disease;
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T-SPOT.TB;
ELISPOT

Abstract

Aim: Intestinal tuberculosis (ITB) and Crohn's disease (CD) have overlapping clinical, radiographic, endoscopic and histologic features, which makes the distinction between these two disease entities a great challenge in tuberculosis-endemic countries. The aim of the study was to investigate the value of in vitro interferonγ release assay (T-SPOT.TB) in differentiating ITB from CD.
Methods: From June 2008 to February 2010, a total of 93 consecutive patients with undetermined ITB or CD were prospectively recruited. Clinical, endoscopic, histologic and therapeutic

Abbreviations: AFB, acid-fast bacilli; ASCA, anti-*Saccharomyces cerevisiae* antibody; BCG, Bacille de Calmette Guerin; CD, Crohn's Disease; CFP-10, culture filtrate protein; CI, confidence interval; CRP, C-reactive protein; ELISPOT, enzyme-linked immunospot assay; ELISA, enzyme-linked immunosorbent assay; ESAT-6, early secreted antigenic target; ESR, erythrocyte sedimentation; HIV, human immunodeficiency virus; IBD, inflammatory bowel disease; IFN, interferon; IGRAs, interferon-gamma (IFNγ) release assays; IU, international units; IQR, interquartile range; ITB, intestinal tuberculosis; NPV, negative predictive value; OR, odds ratio; p-ANCA, peri-nuclear anti-neutrophil cytoplasmic antibody; PBMC, Peripheral blood mononuclear cells; PHA, phytohaemagglutinin; PPD, purified protein derivative; PPV, positive predictive value; PUMC, peking union medical college; QFT-G-IT, QuantiFERON-TB Gold In-Tube; RD1, region of difference 1; SD, standard deviation; SFCs, spot-forming cells; TNFα, tumor necrosis factor α; TST, tuberculin skin test.

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responses were longitudinally monitored at follow-up evaluation until the final definite diagnosis has been reached.
Results: After a median of 6 months' follow-up (interquartile range [IQR], 3.0 to 7.5 months), definitive diagnosis was achieved in 84 of the 93 patients (90%), with 19 having ITB and 65 having CD. On univariate analysis, a long duration of illness, chronic diarrhea, and anemia were significantly more common in CD ($P<0.05$). While night sweat, ascites, pulmonary lesions, circumferential ulcer on endoscopy, ileo-cecal valve involvement and epithelioid granulomas were significantly more common in ITB ($P<0.05$). On multivariate analysis, T-SPOT.TB (Hazard ratio 7.0, 95% confidence interval [CI] 1.9–25.7) was found to be a good predictor for ITB diagnosis. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of T-SPOT.TB were 84.2%, 75.4%, 50.0%, and 94.2%, respectively.
Conclusions: When differentiating ITB and CD in tuberculosis-endemic regions, T-SPOT.TB blood test may be a helpful and practical diagnostic tool for its high NPV to rule out ITB.
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1. Introduction

Intestinal tuberculosis (ITB) and Crohn's disease (CD) are both chronic granulomatous disorders[1] with overlapping clinical, radiographic and endoscopic features, which makes their differential diagnosis difficult. With an increasing incidence of CD in some developing countries,[2,3] distinguishing CD from ITB is important, especially in TB-endemic regions like China.

Misdiagnoses of TB and CD were common in China. In one Chinese study, which reviewed all pathologically diagnosed CD patients from January 1989 to December 2003, 69.4% (2357/3397) had initially been diagnosed with non-IBD gastrointestinal diseases. Among the misdiagnosed patients, 32.2% (759/2357) were initially diagnosed with ITB. [4] In the same study, 53.5% (208/389) of patients who were initially misdiagnosed with CD were eventually diagnosed with ITB. Distinction between ITB and CD, which is critical for proper treatment, can be difficult even in tertiary-care settings and in experienced hands. A delay in diagnosis of both ITB and CD may affect patients' quality of life as well as exert negative socioeconomic impact.[5] Due to high prevalence of TB in China and difficulty in differential diagnosis, initial diagnostic/therapeutic trial of anti-TB agents has been listed as part of diagnosis and treatment algorithm for newly diagnosed CD.[6]

In recent years, T-cell based interferon-gamma (IFNγ) release assays (IGRAs) have increasingly been used to replace traditional tuberculin skin test (TST) as a diagnostic tool for TB. The assay has been shown to have a superior sensitivity and specificity.[7,8,9] There are two commercially available methods for IGRAs: QuantiFERON-TB Gold In-Tube (QFT-G-IT) method (Cellestis, Carnegie, Australia) and T-SPOT.TB method (Oxford Immunotec, Oxford, United Kingdom). QFT-G-IT uses an enzyme-linked immunosorbent assay (ELISA) to measure antigen-specific production of IFNγ by circulating T cells in whole blood being challenged with *Mycobacterium tuberculosis*-specific antigens. T-SPOT.TB uses an enzyme-linked immunospot assay (ELISPOT) to measure peripheral blood mononuclear cells that produce IFNγ. The *M. tuberculosis*-specific antigens in these assays, early secreted antigenic target (EAST-6, 6 kD) and culture filtrate protein (CFP-10, 10 kD), are encoded by the genes found in the region of difference 1 (RD1) of the *M. tuberculosis* genome, which is absent from the genome of *Mycobacterium bovis*, BCG (Bacille de Calmette Guerin) and certain nontuberculous mycobacteria like *Mycobacterium avium*. QFT-G-IT also incorporates a third RD11 antigen, TB 7.7.

There were scant data on clinical utility of IGRAs in distinguishing ITB from CD,[10,11] one being a case report and the other being from Korea which published in Korean. We hypothesized that T-SPOT.TB may be of value in differential diagnosis between newly diagnosed TB and CD. The study aimed to investigate the accuracy of diagnosis and differential diagnosis of T-SPOT.TB and to evaluate risk factor associated with ITB in longitudinally monitored cohort.

2. Patients and methods

2.1. Participants

From June 2008 to February 2010, a prospective clinical study was undertaken at Division of Gastroenterology at Peking Union Medical College Hospital. In collaboration with Division of Infectious Disease, 93 consecutive patients for whom with unclear diagnosis of ITB and CD were included in the study population. Fig. 1 illustrated the patients' enrollment and the brief study protocol. All patients were BCG-vaccinated after being born, as a part of health maintenance program in China. Informed consent was obtained from all subjects, and the study was approved by the Ethical Committee of PUMC Hospital.

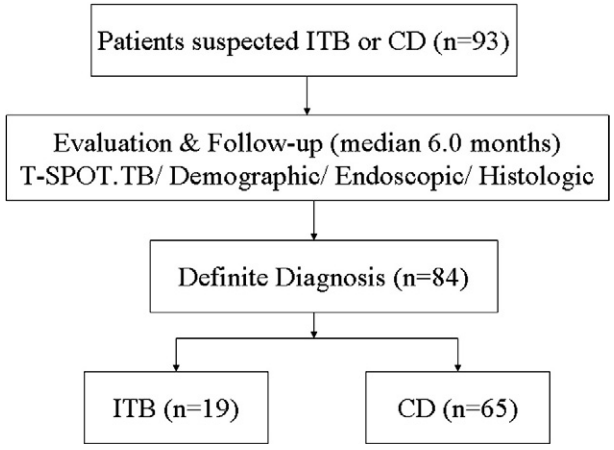

Figure 1 Flowchart of the study protocol. (ITB, intestinal tuberculosis; CD, Crohn's disease).

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2.2. Inclusion and exclusion criteria

Inclusion criteria included patients with an age between 14 and 80 years, and suspected of having either CD or ITB based on clinical presentations, radiographic, endoscopic and histologic findings. Exclusion criteria were patients who were not able to give consent, those with human immunodeficiency virus (HIV) infection, inherited or acquired known immunologic defects, and those with a history of clearly diagnosed ITB or CD.

2.3. Study protocol

After enrolled in the study, all patients' peripheral venous blood samples were collected for T-SPOT.TB assay. Tuberculin skin test (TST) was performed by trained hospital staff through intradermal inoculation (Mantoux method) of 5 international units (IU) of purified protein derivative (PPD, ChengDu Institute of Biological Products). The results of TST were recorded at 72 h measuring the number of millimeters (mm) of the main transverse diameter of skin induration. The cut-off value of 5 mm was considered to be positive.[12]

2.4. Clinical evaluation

During the study period, all enrolled patients with suspected diagnosis of CD or ITB were prospectively evaluated. Baseline demographic, clinical, laboratory, radiographic, endosocpic and histologic data as well as therapeutic response were entered into the database. Follow-up evaluation for next 3 to 12 months was conducted until the final definite diagnosis was reached.

Demographic (age and gender), clinical (duration of symptoms, abdominal pain, chronic diarrhea, hematochezia, abdominal mass, intestinal obstruction, ascites, fever, night sweat, history of appendectomy, oral ulcers) and laboratory features (hemoglobulin,platelet count, erythrocyte sedimentation [ESR] and C-reactive protein [CRP]) were documented in all patients.

Colonoscopic features, supplemented by CT enterography in some cases, included longitudinal ulcer, circumferential ulcer, cobble-stone appearance, pseudo-polyps appearance, stricture and location of disease. Histologic features of biopsy specimens, which were paraffin-embedded and stained with hematoxylin and eosin, were evaluated by experienced gastrointestinal pathologists.

Histologic features were evaluated for the presence of epithelioid granulomas and characteristics of granulomas (caseating or non-caseating). All of the biopsy specimens were subjected to Ziehl-Neelsen staining for acid-fast bacilli (AFB).

Serological tests, including p-ANCA (peri-nuclear antineutrophil cytoplasmic antibody) and the IgA and IgG subtypes of ASCA (anti-*Saccharomyces cerevisiae* antibody), were performed in all enrolled patients.

2.5. In vitro interferon-γ ELISPOT Assays

The RD1 antigen specific T cells were detected by T-SPOT.TB (Oxford Immunotec, Abingdon, UK). AIM-V (GIBCO™ AIM V Medium liquid, Invitrogen, US) was used as the negative control; mitogen (phytohaemagglutinin [PHA]) as positive control; and ESAT-6 and CFP-10 as specific antigens. ESAT-6 and CFP-10 peptide-pools comprise serial 15-mer peptides, overlapping by 10 amino acid residues that span the complete amino acid sequence of each antigen.

Peripheral blood mononuclear cells (PBMC) were separated by centrifugation from an 8 ml sample of heparinized peripheral venous blood within six hours from being drawn and plated (2.5×10^5 per well) on a plate precoated with antibody against IFNγ. The plates were incubated for 16–18 h at 37°C in 5% carbon dioxide. After incubation, the conjugate against the antibody and enzyme substrate were added into the wells. Spot-forming cells (SFCs) were counted by an automated ELISPOT reader (KS Elispot version 4.9, ZEISS, Germany). Each SFC represents an antigen-specific T cell that secrets IFNγ.

According to the protocol provided by the manufacturer of T-SPOT.TB, when the negative control well contains 5 SFCs or less, the result (the number of SFCs counted in the antigen-well subtracted by that in the negative control well) is interpreted as the following: positive, if subtracted SFCs $\geq$6 in either of the antigen wells, irrespective of the number of SFCs in the positive-control well; negative, if subtracted SFCs $<$6 in both antigen wells and SFCs $\geq$20 in the positive-control well; indeterminate, if subtracted SFCs $<$6 in both antigen wells and SFCs $<$20 in the positive-control well. If the negative-control well contains 6 SFCs or more, the result of the test is interpreted as positive if the number of SFCs in either of the antigen wells is more than twice that in the negative control well, irrespective of that in the positive-control well.

2.6. Diagnostic criteria

A diagnosis of ITB was made when any of the following criteria was met: (1) demonstration of AFB on histological examination of Ziehl-Neelsen stained sections; (2) positive *M. tuberculosis* culture; (3) radiological, colonoscopic, and/or operative evidence of ITB with proven TB elsewhere; and (4) response to anti-TB therapy without subsequent recurrence in patients with radiological, colonoscopic, and/or operative evidence of ITB.[13]

Diagnosis of CD was based comprehensively on both morphological and pathological features. Morphological features were depicted by radiological, endoscopic or surgical findings as following: (1) discontinuous/segmental and asymmetrical mucosal involvement; (2) deep mucosal longitudinal fissures/ulcers; (3) transmural inflammation; (4) rigid and strictured intestinal wall; and (5) presence of enterocutaneous or entero-enteric fistula and/or chronic perianal disease. Pathological features include: (1) normal mucus content in the goblet cells of the inflamed region; (2) lymphocyte aggregation in the mocosa and submucosa; (3) non-caseating granulomas; (4) longitudinal ulcers/fissures; and (5) transmural inflammation or inflammation beyond mucosa. For definite diagnosis of CD, the following criteria were used: presence of at least 3 different histologic features; or presence of non-caseating granulomas on histology with at least one other feature; or resolution of symptoms and morphologic (endoscopic and radiographic) features after 3 to 12 months' treatment of

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Table 1 Demographic and clinical features.

Variables	Intestinal tuberculosis (n = 19)	Crohn's disease (n = 65)	*P* value
Demographic features			
Mean age ± SD (range), yr	31.1 ± 15.3(16-66)	35.1 ± 15.7(14-67)	0.334
Gender (male: female)	11:8	43:22	0.509
Clinical features			
Mean duration of symptoms ± SD (range), mo	10.2 ± 8.8(0.5-36)	46.5 ± 51.5(2-300)	*0.003**
Abdominal pain, *n* (%)	15(78.9)	59(90.8)	0.319
Chronic diarrhea, *n* (%)	6(31.6)	40(61.5)	*0.021**
Hematochezia, *n* (%)	6(31.6)	16(24.6)	0.756
Fever, *n* (%)	13(68.4)	37(56.9)	0.369
Night sweat, *n* (%)	9(47.4)	12(18.5)	*0.024**
Abdominal mass, *n* (%)	4(21.1)	16(24.6)	0.988
Intestinal obstruction, *n* (%)	2(10.5)	12(18.5)	0.641
Ascites, *n* (%)	2(10.5)	0(0)	*0.049**
History of appendectomy, *n* (%)	6(31.6)	10(15.4)	0.212
Oral ulcers, *n* (%)	5(26.3)	19(29.2)	0.805

* Factors identified in univariate analysis, $p<0.05$.

corticosteroid and 5-ASA preparations or anti-TNFα biologics.[14]

2.7. Outcome measurement

The primary endpoint of the study was the diagnostic accuracy of the in vitro assay, along with serology tests, for ITB, in distinction from CD. The secondary endpoint was the preferred factors of ITB diagnosis.

2.8. Statistical analysis

The mean and standard deviation were calculated for continuous variables. Percentage was used for comparison of continuous variables. Student's *t*-test was used for comparison of continuous variables. The chi-square test or Fisher's exact test was used to explore associations of categorical data in two independent groups. A *P* value <0.05 was considered statistically significant. Selective variables showing significant association ($P<0.05$) were taken as candidates for multivariable Cox regression. Data were collected in a Microsoft Excel database (Microsoft Excel 2003; Microsoft Corp., Seattle, WA) and analyzed with SPSS software for Windows, release 10.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Demographic and clinical features of patients with ITB and CD

After a median of 6 months' follow-up (interquartile range [IQR], 3.0 to 7.5 months), 84 (90.3%) out of a total of 93 cases for whom with difficulty in differential diagnosis between ITB and CD were eventually reached definite diagnosis, with 19 cases being diagnosed ITB and 65 cases being diagnosed CD (Fig. 1). The other 9 (9.7%, 9/93) cases were diagnosed Behcet's disease (n = 3), non-Hodgkin's lymphoma (n = 2), ulcerative colitis (n = 1) and unconfirmed diagnosis (n = 3). Among patients diagnosed ITB, 11 (57.9%, 11/19) were confirmed by culture and histopathological findings and 8 (42.1%, 8/19) were confirmed by response to anti-TB treatment.

Demographic and clinical features of both ITB and CD patients are demonstrated in Table 1. There was no significant difference in either the age or the gender ratio between ITB and CD patients. CD patients tended to have much longer duration of symptoms than ITB ($P=0.003$). All clinical features listed in Table 1 did not show any difference between ITB and CD patients except for chronic diarrhea and night sweat ($P=0.021$, 0.024 respectively).

3.2. Laboratory, serological, morphological and histological features

Laboratory, serological, morphological features including endoscopic features and site of involvement as well as histological features of biopsy specimens are described in Table 2. For routine laboratory tests, CD patients' hemoglobin was lower than ITB patients whose were within normal limit ($P=0.034$). There is no significant difference between CD and ITB patients in platelet count, ESR, CRP, p-ANCA as well as ASCA. Furthermore, about 15.8% (3/19) of ITB patients were ASCA positive.

Other variables found to be prominently significant for differentiating ITB from CD were as following: 1) pulmonary involvement; 2) circumferential ulcer; 3) ileo-cecal valve involvement; 4) epithelioid granuloma found in biopsy specimens. From colonoscopy biopsy specimens, only 1 out of 19 (5.3%) ITB patients were found to be AFB positive by Ziehl-Neelsen stain. Neither caseating granuloma nor non-caseating granuloma was found in biopsy specimens of all the patients.

Results of T-SPOT.TB to different specific antigens including ESAT-6 (384 ± 620 vs. 23 ± 59, $P=0.021$) and CFP-10 (441 ± 816 vs. 20 ± 65, $P=0.037$) between ITB and CD patients

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Table 2 Laboratory, morphological, serological, histological features and T-SPOT.TB results.

Variables	Intestinal tuberculosis (n = 19)	Crohn's disease (n = 65)	*P* value
Laboratory/radiological features			
Mean Hemoglobulin ± SD , mg/dL	122.1 ± 19.1	108.7 ± 24.6	*0.034**
Mean Platelet count ± SD, $\times 10^9$/L	291.2 ± 136.7	321.2 ± 139.0	0.422
Mean ESR ± SD , mm at 1 h	32.6 ± 26.2	35.4 ± 28.1	0.701
Mean CRP ± SD, mg/dL	28.1 ± 30.2	31.6 ± 29.8	0.675
Presence of Pulmonary infiltration /fibrosis / nodule on Chest X ray/CT, *n* (%)	13(68.4)	12(18.5)	*0.001**
Abdominal lymphadenopathy on CT, *n* (%)	7(36.8)	32(49.2)	0.341
Serological features			
p-ANCA, *n* (%)	0(0)	5(7.7)	0.213
IgG or IgA ASCA, *n* (%)	3(15.8)	14(21.5)	0.583
Endoscopic features			
Longitudinal ulcer, *n* (%)	2(10.5)	20(30.8)	0.077
Circumferential ulcer, *n* (%)	12(63.2)	11(16.9)	*0.000**
Cobble-stone appearance, *n* (%)	7(36.8)	31(47.7)	0.403
Pseudo-polyps, *n* (%)	2(10.5)	17(26.2)	0.152
Stricture, *n* (%)	8(42.1)	32(49.2)	0.584
Site of involvement (By endoscopy and CT enterography)			
Stomach and duodenum, *n* (%)	2(10.5)	7(10.8)	0.976
Jejunum, *n* (%)	0(0)	6(9.2)	0.169
Ileum, *n* (%)	7(36.8)	30(46.2)	0.472
Ileo-cecal valve, *n* (%)	17(89.5)	41(63.1)	*0.029**
Right colon, *n* (%)	11(57.9)	22(33.8)	0.059
Left colon, *n* (%)	3(15.8)	18(27.7)	0.292
Rectum, *n* (%)	1(5.3)	14(21.5)	0.103
Perianal region, *n* (%)	3(15.8)	12(18.5)	0.789
Histological features of biopsy specimens			
Epithelioid granulomas, *n* (%)	6(31.6)	8(12.3)	*0.047**
Caseating granulomas, *n* (%)	0(0)	0(0)	NS
Non-caseating granulomas, *n* (%)	0(0)	0(0)	NS
AFB positive by Ziehl-Neelsen stain, *n* (%)	1(5.3)	0(0)	0.226
TST, *n* (%)	15(78.9)	27(41.5)	*0.004**
T-SPOT.TB			
ESAT-6, Mean ± SD, SFCs/10^6 PBMC	384 ± 620	23 ± 59	*0.021**
CFP-10, Mean ± SD, SFCs/10^6 PBMC	441 ± 816	20 ± 65	*0.037**

SD, standard deviation; AFB, acid-fast bacilli; ESAT-6, early secreted antigenic target; CFP-10, culture filtrate protein; SFC, spot-forming cells; TST, tuberculin skin test.
* Factors identified in univariate analysis, $p<0.05$.

were found with significant difference. ITB patients had much higher T-SPOT.TB results than CD patients.

3.3. Results of multivariate analysis

On multivariate analysis showed in Table 3, T-SPOT.TB (HR 7.0, 95%CI 1.9–25.7) was found to be a stronger predictor of an ITB diagnosis rather than pulmonary involvement (HR 2.8, 95%CI 0.9–9.1), circumferential ulcer on colonoscopy (HR 2.0, 95%CI 0.6–6.2) and epithelioid granuloma (HR 1.4, 95%CI 0.5–3.9).

3.4. Diagnostic value analysis of T-SPOT.TB

Of the 84 patients who reached definite diagnosis of either ITB or CD, TST was found positive in 78.9% (15/19) of ITB patients and 41.5% (27/65) of CD patients. T-SPOT.TB was positive in 84.2% (16/19) of ITB patients and 24.6% (16/65) of CD patients. The overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of TST and T-SPOT.TB are listed in Table 4. T-SPOT.TB had much higher NPV and Accuracy (94.2% and 76.5%, respectively) than TST (90.5%, 63.1%).

4. Discussion

Tuberculosis still remains to be a major health problem in China. The prevalence of pulmonary tuberculosis was 367/100,000 estimated in 2002,[15] making China rank second for tuberculosis prevalent around the world. In the past

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Table 3 Predictors of intestinal tuberculosis diagnosis on multivariate analysis.

Variables	HR	95% CI
T-SPOT.TB	7.0	1.9–25.7
Pulmonary involvement	2.8	0.9–9.1
Circumferential ulcer	2.0	0.6–6.2
Epithelioid granuloma	1.4	0.5–3.9

HR, hazard ratio; CI, confidence interval.

55 years, the extrapolated disease incidence and prevalence rates of CD in China are 0.848/100,000 and 2.29/100,000 respectively,[16] and are found to be growing rapidly every year.[17] Under this circumstance, Chinese clinical practitioners are particularly in struggling in differentiating ITB and CD, for both of these two diseases have marked overlap in demographic and clinical findings[18] and misdiagnosis usually carries grave consequence.

There are many proposed distinguishing clinical, radiological, endoscopic and histological features between ITB and CD, which are as follows: prolonged duration of illness, diarrhea, hematochezia, weight loss, extra-intestinal manifestations, fever, ascites, deep linear ulcers, cobble-stone appearance, involvement of the sigmoid colon, co-existing pulmonary lesions, abdominal lymphadenopathy and so on.[14,19,20] The present study confirmed some of these distinguishing features. Prolonged duration of illness, chronic diarrhea and anemia were more common in CD patients; while night sweat, ascites, pulmonary lesions, circumferential ulcer, ileo-cecal valve involvement and epithelioid granuloma were more common in ITB patients (Tables 1, 2). By multivariate analysis, T-SPOT.TB (HR 7.0 [95%CI 1.9–25.7]) was found to be a stronger predictor for ITB diagnosis.

Careful interpretation and combination of these features are necessary for differential diagnosis between ITB and CD, but still not very practical for clinical practice. The current study further investigated the diagnostic value of T-SPOT.TB, which had much higher NPV and Accuracy (94.2% and 76.5%, respectively) than TST (90.5%, 63.1%).

Novel diagnostic modalities may supplement current methods for distinguishing between ITB and CD. T-SPOT.TB is one of the most promising methods. The present study confirmed T-SPOT.TB is superior to TST both in sensitivity and specificity for diagnosing TB, which is in agreement with published studies.[8,9] Pai et al. confirmed that IGRAs have excellent specificity for latent tuberculosis infection that is unaffected by BCG vaccination in a systemic review published in 2008.[8] A most recent metaanalysis by Diel et al. showed that IGRAs are superior to the TST for detecting confirmed active TB disease, especially when performed in developed countries. The pooled sensitivity and specificity of T-SPOT.TB is 87.5% and 86.0%, respectively.[21] In the present study, the specificity (75.4%) is not optimal enough compared with western data which is considered from non-TB endemic area. So far, all review and metaanalysis studies of IGRAs were mostly conducted in settings with a low incidence rate of tuberculosis. Although, it is generally assumed that IGRAs do not discriminate between latent and active TB. In some literature, IGRAs are reported to be able to indicate mycobacterial burden and disease activity.[22] While, in settings with high tuberculosis incidence like China, where latent infection is widespread, a positive T-SPOT.TB result may not necessarily discriminate active tuberculosis from latent.[23,24] In this situation, a negative T-SPOT.TB result in TB endemic regions could be very helpful to rule out intestinal tuberculosis when distinguishing from CD, for its high NPV (94.2%) documented in the present study.

In the present study, we also found a high NPV (90.5%) of TST when discriminating between ITB and CD. While, TST could be false negative in immunocompromised patients such as HIV-infection, malignancy, chronic inflammatory disease, and on steroids or immunosuppressive treatment. IGRAs are also reported to be more sensitive than TST in detecting TB infection in chronic inflammatory diseases.[25,26,27] Considering T-SPOT.TB has higher sensitivity and specificity than TST in the present study, the higher NPV of T-SPOT.TB is of more clinical value than that of TST.

To our knowledge, this is the first cohort study conducted in tuberculosis endemic country to investigate the diagnostic value of T-SPOT.TB in distinguishing ITB and CD. However, there are still several limitations to the current study, i.e. limited sample size, being conducted in one tertiary medical center and without comparison of the other IGRAs. This present study's data rests on well-established diagnostic standards achieved by combination of histopathology, microbiology and long-term follow-up prospectively. CD, which shares many similarities with ITB, was choused as control group when evaluating T-SPOT.TB diagnostic values. The well-established diagnostic standards, consecutive patient recruitment and control selection may significantly increase the reliability of the present results besides the limitation of sample size especially in ITB group. All patients were from Peking Union Medical College Hospital, which is a major tertiary referral center in China. The findings from this patient population may not be extrapolated to other setting such as non-TB endemic regions or

Table 4 The diagnostic values of tuberculin skin test and T-SPOT.TB in differentiating intestinal tuberculosis and Crohn's disease.

	Sensitivity(%) (95% CI)	Specificity(%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	Accuracy (%) (95% CI)
Tuberculin skin test	78.9 (70.2–87.6)	58.5 (48.0–69.0)	35.7 (25.5–45.9)	90.5 (84.2–96.8)	63.1 (52.8–73.4)
T-SPOT.TB	84.2 (76.4–92.0)	75.4 (66.2–84.6)	50.0 (39.3–60.7)	94.2 (89.2–99.2)	76.5 (67.4–85.6)

CI, confidence interval.

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smaller Chinese cities and countryside for lower CD prevalence. The other IGRA, i.e. QFT-G-IT, is not currently commercially available in the area where the study is conducted. Hopefully, further studies using both IGRAs may add more information on discriminating ITB and CD.

In conclusion, although ITB and CD are diseases sharing many similarities, they still could be differentiated on the basis of a combination of clinical manifestations, radiological, endoscopic and histological features. T-SPOT.TB may contribute important supplementary information in excluding ITB, but could not be taken as a single method.

Conflict of interest

Guarantor of the article: Yue Li, MD.

Specific author contributions: Concept, design, data collection, analysis, manuscript writing: Yue Li; data collection, analysis: Zhang Lifan; Concept, design: Jiaming Qian, Xiaoqing Liu; Data collection: Xi Wang, Jian Wang; Data analysis: Li Wang.

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