Diagnostic Performance of a 5-Marker Predictive Model for Differential Diagnosis Between Intestinal Tuberculosis and Crohn's Disease

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Background: The differentiation between intestinal tuberculosis (ITB) and Crohn's disease (CD) is a challenge. The aim of this study was to investigate a predictive model for differential diagnosis between ITB and CD.

Methods: A total of 268 patients who were suspected of having ITB or CD were prospectively recruited between January 2013 and September 2016. The clinical, laboratory, radiological, endoscopic, and histological features were investigated and subjected to univariate and multivariate analyses. The final predictive model was developed based on the regression coefficients of multivariate logistic regression. To validate the model, the same regression equation was tested on the other group.

Results: A total of 239 patients had a final diagnosis, including 86 ITB and 153 CD. Five variables (perianal disease, pulmonary involvement, longitudinal ulcer, left colon, and ratio of tuberculosis-specific antigen to phytohaemagglutinin) were selected for the predictive model to discriminate between ITB and CD. In the predictive model of the training data set, the area under the receiver operating characteristic (ROC) curve, sensitivity, specificity, and accuracy, with a cutoff level of 0.29, were 0.975 (95% confidence interval [CI], 0.939–0.993), 96.7%, 90.7%, and 92.8%, respectively. Application of the predictive model to the validation data set showed similar performance in distinguishing ITB from CD. The area under the ROC curve, sensitivity, specificity, and accuracy were 0.950 (95% CI, 0.871–0.987), 88.5%, 93.5%, and 91.7%, respectively.

Conclusions: This 5-marker predictive model could be conveniently used by clinicians to draw a reliable differential diagnosis between ITB and CD in clinical practice.

Key Words: intestinal tuberculosis, Crohn's disease, differential diagnosis, predictive model, TBAg/PHA ratio

INTRODUCTION

Crohn's disease (CD) is a chronic idiopathic inflammatory bowel disease characterized by the segmental, transmural involvement of the gastrointestinal tract.[1, 2] Although CD is generally considered to be caused by the interactions of genetic and environmental factors, its etiology is still not fully understood.[3] The incidence and prevalence of CD have increased worldwide.[4] In China, the estimated incidence rate of CD was found to have increased from 0.28/100,000 in 1950–2002 to 0.848/100,000 in 1950–2007.[5] On the other hand, intestinal tuberculosis (ITB) is a common form of extrapulmonary tuberculosis in developing countries. Also, it is being increasingly encountered in Western countries due to the AIDS epidemic and transnational migration.[6, 7]

The differential diagnosis between ITB and CD remains a challenge because the 2 diseases share confusingly similar clinical, radiological, endoscopic, and pathological features.[8–10] Owing to these characteristics, it is difficult to differentiate ITB and CD, and misdiagnoses of them are common in clinical practice. It was reported that the rate of misdiagnosis between these 2 diseases reaches 50%–70%.[11] Although the diagnosis of ITB can be made when acid fast bacilli (AFB) and granulomas with caseous necrosis are identified in histopathological examination, these findings are positive in less than 50% of patients.[12, 13] More seriously, there is currently no definitive diagnostic test for CD. Furthermore, the treatments for ITB and CD are quite different, and a misdiagnosis could produce serious consequences. If ITB is misdiagnosed as CD, immunosuppressive agents would be used, which can accelerate the development of TB. Likewise, if CD patients are treated with anti-TB drugs, this could carry a risk of drug toxicity and delay the treatment of CD.[14] In all, with the increasing incidence of

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CD in some developing countries, especially in TB-endemic regions like China, differential diagnosis between ITB and CD becomes more important than ever.

Although patients with ITB or CD have similar clinical symptoms and test results, a comprehensive analysis of some discriminative clinical and endoscopic features can help to distinguish these 2 conditions. For instance, ascites, night sweat, fever, transverse ulcers, and patulous ileocecal valve are found more frequently in patients with ITB, whereas blood stools, chronic diarrhea, perianal disease, longitudinal ulcers, and cobblestone appearance are more frequent in CD patients.[15–20] Furthermore, T-SPOT.TB, as a commercially available interferon-gamma release assay, has been shown to have better sensitivity and specificity than traditional tuberculin skin test (TST) in the diagnosis of ITB.[21] However, the great limitation of this assay is its inability to discriminate between active tuberculosis (ATB) and latent tuberculosis infection (LTBI).[22] As China is a high–TB burden country, the CD patients associated with LTBI are very common, which indicates the low specificity of using T-SPOT.TB to distinguish ITB from CD. Our group has previously shown that a further calculation of the ratio of TB-specific antigen (TBAg) to phytohaemagglutinin (PHA; TBAg/PHA ratio) in T-SPOT.TB could increase the specificity for distinguishing active TB from latent infection,[23, 24] which suggests that the TBAg/PHA ratio might have some potential in ITB diagnosis.

Therefore, the aim of this study was to evaluate the TBAg/PHA ratio and the clinical, radiological, endoscopic, and histological features of patients with ITB and CD, and to establish a predictive model for differential diagnosis between ITB and CD.

METHODS

Participants

This study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. All subjects provided written informed consent.

A total of 268 consecutive patients who were suspected of having ITB or CD were prospectively enrolled between January 2013 and September 2016 at Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China. The inclusion criteria were as follows: (1) patients older than 18 years of age; (2) patients presented with symptoms such as abdominal pain, diarrhea, constipation, and hematochezia; and (3) patients were suspected of having either ITB or CD based on endoscopic and histologic findings. Patients with HIV infection, undergoing anti-TB or immunosuppressive therapy, with uncertain diagnosis or with other definite diagnosis, or lost to follow-up were excluded.

After patients were enrolled, routine laboratory tests, TST, and T-SPOT.TB assay were performed for all subjects. Before therapy, chest x-ray, computed tomography (CT), and colonoscopic examination were also carried out. In some cases of uncertain diagnosis, additional examinations such as abdomen-pelvis CT scan, capsule endoscopy, and barium study were undertaken for differential diagnosis. Abdominal surgery was performed in more severe cases with bowel obstruction or perforation. Biopsy specimens from colonoscopy or surgical resection were used for hematoxylin and eosin staining, Ziehl-Neelsen smear for AFB, and AFB culture.

Clinical Evaluation

Data regarding the patients' demographics (age, sex, duration of disease, history of appendectomy, and history of tuberculosis), clinical features (abdominal pain, chronic diarrhea, constipation, hematochezia, fever, night sweat, weight loss, abdominal mass, intestinal obstruction, perianal disease, and extraintestinal manifestations), and therapeutic response were collected. Laboratory results (white blood cells [WBCs], hemoglobin, platelets, total protein, albumin, erythrocyte sedimentation rate [ESR], hypersensitive C-reactive protein [hs-CRP], cytoplasmic antineutrophil cytoplasmic antibody [c-ANCA], perinuclear antineutrophil cytoplasmic antibody [p-ANCA], TST, and T-SPOT.TB assay), radiological findings (abdominal lymphadenopathy, ascites, segmental bowel wall thickening, intestinal wall edema, and pulmonary involvement such as infiltration, fibrosis, nodule, and cavity), and colonoscopic features (longitudinal ulcers, ring-shaped ulcers, cobblestone appearance, pseudopolyps, scar changes, stricture, mucosal bridge, patulous ileocecal valve, fistula, and location of lesion involvement) were documented in all patients. Histological features such as characteristics of granulomas, AFB smear, ulcers, and chronic inflammation were evaluated.

Diagnostic Criteria for ITB and CD

The diagnosis of ITB was established when at least 1 of the following criteria was met: (1) presence of caseating granuloma on histological investigation; (2) demonstration of AFB on smears or histological sections; (3) positive culture for AFB; or (4) strong suspicion of tuberculosis by the combination of clinical, endoscopic, and histological characteristics, together with a good response to anti-TB treatment without recurrence. A good response to anti-TB treatment was determined by loss of symptoms and disappearance of ulcerations on endoscopic examination.[25] In addition, patients with confirmed active extraintestinal tuberculosis were considered tentative ITB and were administered anti-TB therapy. The diagnosis of CD was defined on the basis of the European Crohn's and Colitis Organization guidelines, a combination of clinical evaluation, endoscopic, histological, and radiological features and/or biochemical investigations.[26, 27]

When the initial diagnosis was uncertain, empirical anti-TB therapy was started. If there was no good response to anti-TB therapy, the patients would be given therapy for CD. The final diagnosis was confirmed after a period of at least

6 months of follow-up based on successful response to anti-TB or anti-CD therapy.

The TBAg/PHA Ratio of T-SPOT.TB Assay

Samples of heparinized peripheral blood were collected from all patients and were analyzed using the T-SPOT.TB ELISpot assay (Oxford Immunotec, Oxford, UK) according to the manufacturer's instructions. There are 4 parameters in the results of the T-SPOT.TB assay: negative control spot-forming cells (sfc), TBAg sfc including early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), and positive control PHA sfc. Based on the results of ESAT-6, CFP-10, and PHA sfc in the T-SPOT.TB assay, we further calculated the ratios of (1) ESAT-6 sfc to PHA sfc and (2) CFP-10 sfc to PHA sfc. The larger of the above 2 values was defined as the TBAg/PHA ratio of 1 individual.

Statistical Analysis

Differences between the 2 groups were compared using the Student's *t*-test, Pearson's chi-square test, or the Fisher's exact test. The Student's *t*-test was used for comparison of continuous variables. The chi-square test or Fisher's exact test was used for categorical data. Statistical significance was determined as a P value of less than 0.05. To differentiate ITB from CD, patients were randomly assigned to the training set (70% of study participants) or validation set (30% of study participants), after stratification according to disease type. Data from the training set were used to develop a predictive model, and the remaining data were used for validation. All variables with statistical significance ($P < 0.05$) were taken as candidates for further multivariable logistic regression analyses, and then the regression equation (predictive model) was obtained. The regression coefficients of the predictive model were regarded as the weights for the respective variables, and a score for each patient was calculated. Receiver operating characteristic (ROC) curve analysis was performed on these scores to assess the ability and the optimal cutoff value for discriminating ITB from CD. Area under the ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), and accuracy, together with their 95% confidence intervals (CIs), were calculated. Statistical analyses were performed using SPSS 19.0 (SPSS, Chicago, IL, USA).

RESULTS

Study Subjects

Of the 268 enrolled patients, 239 (89.2%) patients had final definite diagnosis after a median follow-up period (range) of 12 (6–29) months, including 86 ITB and 153 CD. The remaining 29 (10.8%) patients were excluded from analysis: 9 patients were lost to follow-up; 11 patients received a diagnosis other than ITB or CD (3 with Behçet's disease, 3 with ulcerative colitis, 2 with nonspecific enterocolitis, 2 with non-Hodgkin's lymphoma, 1 with pseudomembranous enteritis); and 9 patients had no definitive diagnosis during the follow-up period. Among patients diagnosed with ITB, 37 (43.0%) were confirmed by 1 or more of the following methods: caseating granulomas in 9 patients, positive AFB smear in 8 patients, growth of Mtb on tissue culture in 5 patients, and proven extraintestinal tuberculosis in 15 patients who had clinical and endoscopic response to anti-TB treatment. The remaining 49 (57.0%) patients were confirmed after a period of follow-up based on good responses to anti-TB treatment and the absence of subsequent disease recurrence. All patients with ITB had been followed up and had completed anti-TB treatment with relief of symptoms and without recurrence. Of the patients with CD, 112 (73.2%) patients were diagnosed according to the diagnostic criteria of CD at the initial work-up. The other 41 (26.8%) patients were given empirical anti-TB treatment first; however, there was no clinical or endoscopic response to this therapy, and these patients were then given therapy for CD and were finally diagnosed as having CD. In all 153 patients, the diagnosis of CD was confirmed after a period of follow-up based on their positive response to therapy for CD.

Univariate Analysis for Differentiation of ITB and CD

Demographic and clinical features

The demographic and clinical features of patients with ITB and CD are summarized in Table 1. There was no significant difference in age, sex, or appendectomy history between patients with ITB and CD. The duration of the disease in CD patients was much longer than in ITB patients ($P < 0.001$), whereas TB history was more prevalent in ITB patients than in CD patients ($P = 0.019$). The clinical features of chronic diarrhea ($P = 0.004$), hematochezia ($P < 0.001$), weight loss ($P = 0.003$), and perianal disease ($P < 0.001$) were significantly more common in patients with CD than in those with ITB. However, night sweat ($P = 0.008$) was found more often in patients with ITB than in those with CD.

Laboratory and radiological features

The laboratory and radiological features of patients with ITB and CD are shown in Table 2. Routine laboratory test results did not show any significant difference between ITB and CD except for hemoglobin ($P = 0.044$). For TST and T-SPOT.TB results, ITB patients showed a significantly higher positive rate than CD patients. Likewise, the result of the TBAg/PHA ratio ($P < 0.001$) in ITB patients was significantly higher than in CD patients. The radiological findings of pulmonary involvement ($P < 0.001$) and ascites ($P < 0.001$) were more frequently identified in patients with ITB than in those with CD, whereas segmental bowel wall thickening ($P = 0.001$) was more common in CD patients than ITB patients.

TABLE 1. Demographic and Clinical Features of Patients with ITB and CD

Variables	ITB (n = 86)	CD (n = 153)	*P*
Demographic features			
Age, mean ± SD, y	35.4 ± 15.4	33.9 ± 12.0	0.437
Sex, male:female	55:31	94:59	0.700
Duration of disease, mean ± SD, mo	9.3 ± 18.3	25.9 ± 39.2	<0.001
Appendectomy history, n (%)	6 (7.3)	9 (5.9)	0.738
Tuberculosis history, n (%)	17 (19.8)	14 (9.2)	0.019
Clinical features, n (%)			
Abdominal pain	66 (76.7)	114 (74.5)	0.701
Chronic diarrhea	24 (27.9)	72 (47.1)	0.004
Constipation	7 (8.1)	9 (5.9)	0.503
Hematochezia	13 (15.1)	57 (37.3)	<0.001
Fever	33 (38.4)	43 (28.1)	0.102
Night sweat	15 (17.4)	10 (6.5)	0.008
Weight loss	43 (50.0)	106 (69.3)	0.003
Abdominal mass	6 (7.0)	5 (3.3)	0.321
Intestinal obstruction	19 (22.1)	21 (13.7)	0.096
Perianal disease	5 (5.8)	48 (31.4)	<0.001
Extraintestinal manifestations	14 (16.3)	39 (25.5)	0.100

ITB = intestinal tuberculosis; CD = Crohn's disease; SD = standard deviation.

Endoscopic and histological features

The endoscopic and histological findings of patients with ITB and CD are shown in Table 3. The typical endoscopic features of these 2 diseases were different. Longitudinal ulcer ($P < 0.001$), cobblestone appearance ($P = 0.008$), stricture ($P < 0.001$), and fistula ($P = 0.022$) were seen more frequently in CD patients compared with ITB patients (Fig. 1). In contrast, ring-shaped ulcer ($P = 0.004$) and patulous ileocecal valve ($P = 0.007$) were seen more often in patients with ITB than in those with CD (Fig. 2). Furthermore, the involvement of left colon ($P < 0.001$), rectum ($P = 0.032$), and perianal region ($P = 0.001$) were significantly more common in patients with CD than in those with ITB. From colonoscopy biopsy specimens, caseous necrosis and positive AFB staining were seen in 9 (10.5%) and 8 (9.3%) patients with ITB, respectively. However, the histological findings of granuloma ($P = 0.273$), ulcers ($P = 0.093$), and chronic inflammation ($P = 0.281$) were not significantly different between the 2 groups.

Development of a Predictive Model for Differentiating Between ITB and CD

There were 239 participants in this study, including 86 ITB patients and 153 CD patients. To establish a predictive model, we divided these patients randomly into a training set (70%) and a validation set (30%) after stratifying them according to disease type. The training set comprised 167 cases (60 ITB patients and 107 CD patients), and the validation set comprised 72 cases (26 ITB patients and 46 CD patients). The mean age (34.5 ± 12.7 and 34.8 ± 14.9 years, respectively; $P = 0.858$) and distribution of males and females (M:F, 103:64 and 46:26, respectively; $P = 0.746$) were not significantly different between the training and validation data sets. All variables with statistical significance ($P < 0.05$) selected by univariate analysis were taken as candidates for further multivariable logistic regression analyses. It is noted that our group has previously shown that calculation of the TBAg/PHA ratio in T-SPOT.TB is better than directly using T-SPOT.TB results in the diagnosis of active TB.[23, 24] Moreover, there was significant correlation between the TBAg/PHA ratio and T-SPOT.TB results ($P < 0.001$). Thus, we selected the TBAg/PHA ratio but not T-SPOT.TB results as a candidate marker.

On multivariable binary logistic regression analysis, perianal disease, pulmonary involvement, longitudinal ulcer, left colon, and the TBAg/PHA ratio were chosen as predictive model markers in the training set. Among these markers, pulmonary involvement and the TBAg/PHA ratio were valuable for ITB diagnosis, whereas perianal disease, longitudinal ulcer, and left colon were valuable for CD diagnosis. Based on regression coefficients, we established a mathematical equation as follows to predict the sensitivity and specificity of selected

TABLE 2. Laboratory and Radiological Features of Patients with ITB and CD

Variables	ITB (n = 86)	CD (n = 153)	*P*
Laboratory findings			
WBCs, mean ± SD, $\times 10^9$/L	6.89 ± 3.04	7.02 ± 2.86	0.749
Hemoglobin, mean ± SD, g/L	111.6 ± 20.9	105.6 ± 23.1	0.044
Platelet, mean ± SD, $\times 10^9$/L	330.4 ± 115.5	312.2 ± 123.8	0.265
Total protein, mean ± SD, g/L	68.1 ± 9.1	67.1 ± 11.0	0.507
Albumin, mean ± SD, g/L	35.4 ± 6.7	34.7 ± 7.4	0.484
ESR, mean ± SD, mm/H	29.1 ± 24.8	25.6 ± 20.4	0.245
hs-CRP, mean ± SD, mg/L	42.3 ± 38.5	45.3 ± 30.1	0.531
c-ANCA, n (%)	3 (3.5)	9 (5.9)	0.614
p-ANCA, n (%)	2 (2.3)	13 (8.5)	0.059
TST positive, n (%)	55 (64.0)	51 (33.3)	<0.001
T-SPOT.TB positive, n (%)	72 (83.7)	39 (25.5)	<0.001
TBAg/PHA ratio, mean ± SD	0.723 ± 1.280	0.036 ± 0.103	<0.001
Radiological findings, n (%)			
Abdominal lymphadenopathy	58 (67.4)	112 (73.2)	0.346
Ascites	24 (27.9)	8 (5.2)	<0.001
Segmental bowel wall thickening	14 (16.3)	56 (36.6)	0.001
Intestinal wall edema	24 (27.9)	37 (24.2)	0.526
Pulmonary involvement (infiltration/fibrosis/no dule/cavity)	64 (74.4)	41 (26.8)	<0.001

ITB = intestinal tuberculosis; CD = Crohn's disease; WBCs = white blood cells; SD = standard deviation; ESR = erythrocyte sedimentation rate; hs-CRP = hypersensitive C-reactive protein; c-ANCA = cytoplasmic classical antineutrophil cytoplasmic antibody; p-ANCA = perinuclear antineutrophil cytoplasmic antibody; TST = tuberculin skin test; TBAg/PHA ratio = the ratio of TB-specific antigen to phytohaemagglutinin.

TABLE 3. Endoscopic and Histological Features of Patients with ITB and CD

Variables	ITB (n = 86)	CD (n = 153)	*P*
Endoscopic features, n (%)			
Longitudinal ulcer	5 (5.8)	55 (35.9)	<0.001
Ring-shaped ulcer	25 (29.1)	21 (13.7)	0.004
Cobblestone appearance	4 (4.7)	25 (16.3)	0.008
Pseudopolyps	16 (18.6)	39 (25.5)	0.225
Scar changes	15 (17.4)	14 (9.2)	0.060
Stricture	11 (12.8)	53 (34.6)	<0.001
Mucosal bridge	3 (3.5)	8 (5.2)	0.768
Patulous ileocecal valve	24 (27.9)	21 (13.7)	0.007
Fistula	2 (2.3)	16 (10.5)	0.022
Site of involvement, n (%)			
Stomach and duodenum	1 (1.2)	5 (3.3)	0.570
Jejunum	2 (2.3)	11 (7.2)	0.196
Terminal ileum	30 (34.9)	68 (44.4)	0.149
Cecum	17 (19.8)	20 (13.1)	0.170
Ileocecal valve	54 (62.8)	88 (57.5)	0.425
Right colon	32 (37.2)	50 (32.7)	0.479
Transverse colon	18 (20.9)	29 (19.0)	0.712
Left colon	22 (25.6)	78 (51.0)	<0.001
Rectum	16 (18.6)	48 (31.4)	0.032
Perianal region	3 (3.5)	28 (18.3)	0.001
Histological features, n (%)			
Granuloma	44 (51.2)	67 (43.8)	0.273
Caseous necrosis	9 (10.5)	0 (0)	<0.001
Noncaseous nercosis	7 (8.1)	37 (24.2)	0.002
Positive AFB stain	8 (9.3)	0 (0)	0.001
Ulcers	54 (62.8)	112 (73.2)	0.093
Chronic inflammation	77 (89.5)	143 (93.5)	0.281

ITB = intestinal tuberculosis; CD = Crohn's disease; AFB = acid fast bacilli.

markers in the differentiation of ITB and CD. The score of each patient was calculated, and a higher score would predict greater likelihood of ITB. For the mathematical equation, the markers of perianal disease, pulmonary involvement, longitudinal ulcer, and left colon were given a score of 1 if present and 0 if absent. ROC analysis showed that the AUC of the predictive model was 0.975 (95% CI, 0.939–0.993) (Fig. 3A). When the cutoff value was set at 0.29, the sensitivity, specificity, and accuracy were 96.7%, 90.7%, and 92.8%, respectively (Table 4).

$$P = 1/[1 + e^{-(-1.950 - 2.372 \times \text{perianal disease} + 2.746 \times \text{pulmonary involvement} - 3.284 \times \text{longitudinal ulcer} - 1.738 \times \text{left colon} + 7.477 \times \text{TBAg/PHA ratio})}]$$

P, predictive value; e, natural logarithm; TBAg/PHA ratio, the ratio of TB-specific antigen to phytohaemagglutinin.

We have also done multivariable binary logistic regression analysis of the demographic and clinical features, laboratory and radiological features, and endoscopic features to establish 3 other mathematic models (demographic-clinical model, laboratory-radiological model, endoscopic model, respectively) for differentiating between ITB and CD (Supplementary Fig. 1A–C). Furthermore, we analyzed the performance of using the TBAg/PHA ratio in distinguishing these 2 conditions (Supplementary Fig. 1D). As expected, the AUCs of the 3 other models and the TBAg/PHA ratio were all lower than the AUC of the above 5-marker model (Supplementary Fig. 1E, F).

Validation of the Predictive Model

The risk score of the 5-marker predictive model was validated using the validation data set. ROC analysis showed that the AUC of the predictive model was 0.950 (95% CI, 0.871–0.987) (Fig. 3B). If using 0.29 as the cutoff value of the predictive model in the validation set, the sensitivity, specificity, PPV, NPV, PLR, NLR, and accuracy were 88.5%, 93.5%, 88.5%, 93.5%, 13.6, 0.12, and 91.7% in differentiating ITB from

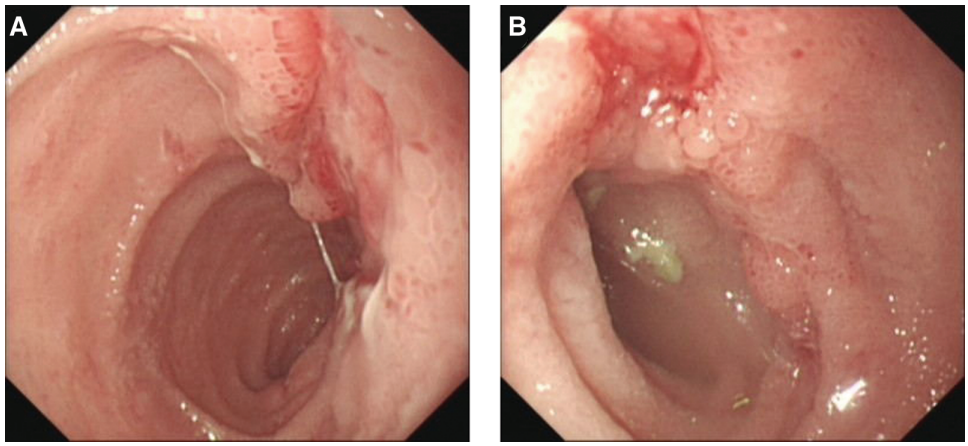

FIGURE 1. Colonoscipic images showing (A) longitudinal ulcers and (B) cobblestone appearance in a patient with CD.

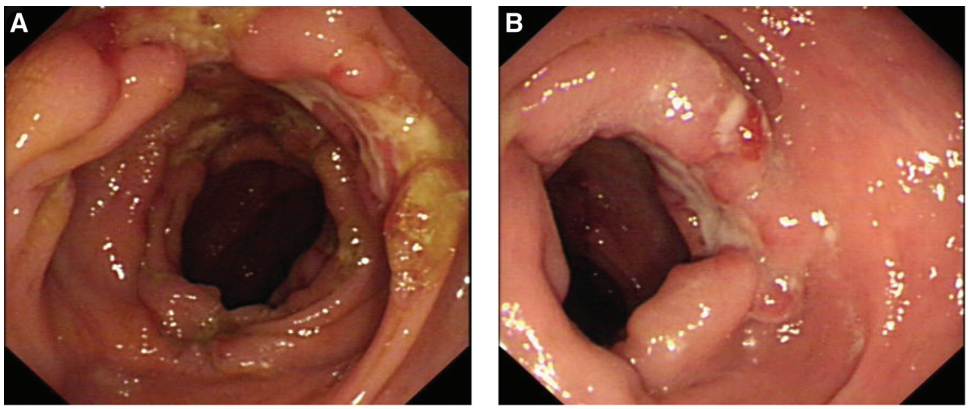

FIGURE 2. Colonoscipic images showing (A) transverse (ring-shaped) ulcer and (B) patulous ileocecal valve in a patient with ITB.

CD, respectively (Table 4). Almost all the validity indexes listed in Table 4 were similar, and the predictive model showed good discriminative power in both data sets. As shown in Figure 4, when the cutoff point is 0.29, the proportions of false-negative ITB patients and false-positive CD patients were 2/60, 10/107 in the training set, and were 3/26, 3/46 in the validation set, respectively.

DISCUSSION

The incidence and prevalence of CD have increased rapidly in the past few decades in Asia, where TB burden is also heavy.[4] Differential diagnosis between ITB and CD is very difficult because of the confusing similarities in clinical, laboratory, endoscopic, and histological findings of the 2 diseases. More importantly, misdiagnosis or delayed diagnosis of ITB or CD can result in grave consequences, such as tubercle bacillus diffusion, drug toxicity, and increasing medical costs.[14] Even now, the differentiation between ITB and CD is still a challenge, especially in China where both ITB and CD remain prevalent. In this study, we have identified a 5-marker model for differentiating between ITB and CD, and this predictive model might serve as a diagnostic tool for inflammatory bowel diseases.

Some clinical, laboratory, radiological, and endoscopic features are helpful in distinguishing ITB from CD. On univariate analysis of variables, a longer disease duration, chronic diarrhea, hematochezia, weight loss, and perianal disease were more common in patients with CD, whereas TB history and night sweats were suggestive of ITB. Although both ITB and CD patients had a decreased hemoglobin level, anemia was likely to be more severe in patients with CD. This indicated that poor nutrition absorption and chronic nutrient consumption were more severe in patients with CD than in patients with ITB, which is consistent with previous reports.[19, 28] Our results also showed that both the sensitivity and specificity of T-SPOT.TB are better than TST in the diagnosis of ITB, which is in agreement with previous studies.[21, 29] Ferrara et al. reported that the T-SPOT.TB assay has superior sensitivity and specificity in the diagnosis of ITB

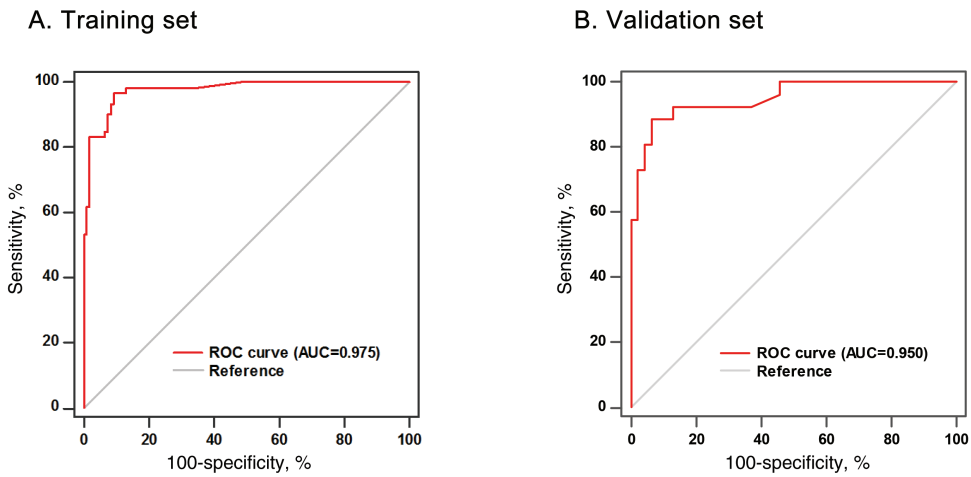

FIGURE 3. ROC curve showing the predicting ability of the predictive model in the training set and the validation set.

TABLE 4. The Diagnostic Values of the 5-Marker Predictive Model in Differentiating ITB from CD in the Training and Validation Sets

Variable	Value (95% CI)	
	Training Set (n = 167)	Validation Set (n = 72)
AUC	0.975 (0.939–0.993)	0.950 (0.871–0.987)
Cutoff score	0.29	0.29
Sensitivity, %	96.7 (87.5–99.4)	88.5 (68.7–97.0)
Specificity, %	90.7 (83.1–95.2)	93.5 (81.1–98.3)
PPV, %	85.3 (74.2–92.3)	88.5 (68.7–97.0)
NPV, %	98.0 (92.2–99.6)	93.5 (81.1–98.3)
PLR	10.3 (5.7–18.7)	13.6 (4.5–40.9)
NLR	0.04 (0.01–0.14)	0.12 (0.04–0.36)
Accuracy, %	92.8 (88.9–96.7)	91.7 (85.3–98.1)

AUC = area under the curve; PPV = positive predictive value; NPV = negative predictive value; PLR = positive likelihood ratio; NLR = negative likelihood ratio.

compared with TST because it is not affected by previous Bacille de Calmette Guerin (BCG) vaccination and most nontuberculous mycobacteria infections.[29] Extraintestinal tuberculosis is usually considered a strong indicator for ITB diagnosis. It was reported that 21% of gastrointestinal tuberculosis cases had extraluminal tuberculosis.[25] Li et al.[21] found that 68.4% of patients with ITB had a suspicion of pulmonary tuberculosis, which is similar to our study, showing that 74.4% of ITB patients have pulmonary involvement,

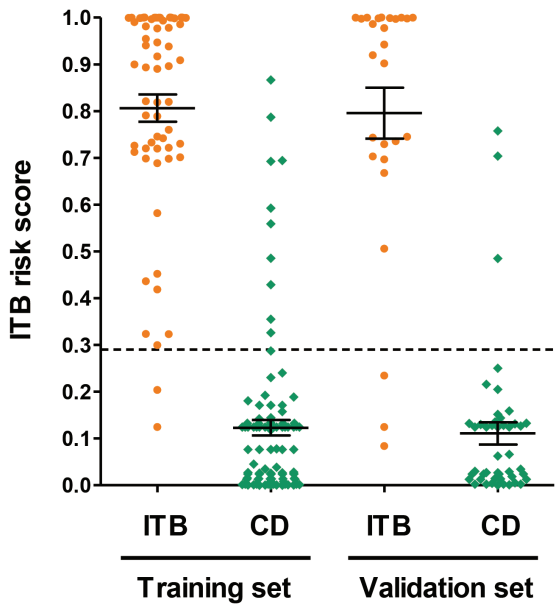

FIGURE 4. Dot plots of the 5-marker ITB risk score in differentiating ITB and CD by data sets. Error bars indicate the mean and standard error of mean (SEM). The dotted line represents the cutoff value for predicting ITB at 0.29.

such as infiltration, fibrosis, nodule, and cavity. In addition, ascites is also a valuable marker for ITB diagnosis, whereas segmental bowel wall thickening is a characteristic for CD in our study, which is also consistent with previous studies in China.[18, 30]

Endoscopy is an important tool for the differential diagnosis of ITB and CD, and it also can be used for treatment evaluation and follow-up of the 2 diseases. The endoscopic features of longitudinal ulcers, cobblestone appearance, stricture, and fistula, favored the diagnosis of CD, whereas ring-shaped ulcers and patulous ileocecal valve favored the diagnosis of ITB in our study, which is consistent with previous reports.[10, 17, 31] Our study also illustrated that the involvement of the left colon, rectum, and perianal region were common in CD patients. However, no characteristic involvement of the colon was found in ITB patients. A possible explanation is that ITB can occur in any area of the bowel, although the ileocecal valve is the most commonly involved segment in both ITB and CD, which is similar to previous findings.[31, 32] Furthermore, pathological examination is the key to distinguishing ITB from CD. The diagnosis of ITB can be confirmed on the basis of caseous necrosis and being positive for AFB. However, these typical findings were present in a small proportion of patients, which limits their use in clinical practice.[13, 33] As expected, only 22 (25.6%) ITB patients were confirmed by histopathological or bacteriological examination in our study. Over half of the patients had a final diagnosis based on the clinical and endoscopic response to empirical anti-TB therapy. In this condition, empirical anti-TB therapy may not only be used for the diagnosis of ITB, but also for the diagnosis of CD.[8]

Although many parameters discussed above were different between ITB and CD, the value of using a single parameter in distinguishing these 2 conditions is very limited in clinical practice because of low sensitivity or specificity. Thus, combination of the selected valuable parameters to establish a mathematical model may help to solve this problem. Recently, several researchers have made attempts to develop scoring systems for the discrimination of ITB and CD. Makharia et al.[14] reported a scoring model including blood in the stool, weight loss, histological focally enhanced colitis, and involvement of the sigmoid colon in an Indian population, and the AUC, sensitivity, and specificity were 0.910, 83.0%, and 79.2% in the original data set, and 0.892, 90.0%, and 60.0% in the validation data set, respectively. Also, Jung et al.[31] have formulated a predictive model including age, sex, ring-shaped ulcers, suspicion of radiological pulmonary tuberculosis, longitudinal ulcers, diarrhea, and sigmoid colon involvement in differentiating between ITB and CD in a Korean population. This predictive model had a better performance, with a sensitivity of 95.9% and a specificity of 94.9%, and the AUC was 0.979. We also validated this predictive model by using our data sets, and the AUC, sensitivity, and specificity were 0.838, 80.0%,

and 79.4% in training data set, and 0.772, 80.8%, and 65.2% in validation data set, respectively. Except for age and sex, all other markers in the predictive model reported by Jung et al. were significantly different between ITB and CD in our data sets. The relatively low diagnostic accuracy of this model in our data sets may be due to the different prevalence rates of ITB or CD in different populations.

Thus, it is necessary to reassess the predictive models if they are used in a different population. On multivariate analysis, we established a 5-marker predictive score model including perianal disease, pulmonary involvement, longitudinal ulcer, left colon, and the TBAg/PHA ratio for differential diagnosis of ITB and CD. ROC analysis showed a good diagnostic accuracy of this predictive model to discriminate ITB and CD. The AUC, sensitivity, and specificity of this model were 0.975, 96.7%, and 90.7% in the training set and 0.950, 88.5%, and 93.5% in the validation set, respectively. The regression coefficients were regarded as the weights for the variables in the predictive model. Based on multivariable logistic regression analysis, longitudinal ulcer (odds ratio [OR] for ITB, 0.037; 95% CI, 0.001–0.975) was the strongest predictor for CD diagnosis, with a regression coefficient value of −3.284, and the TBAg/PHA ratio (OR for ITB, 1767.3; 95% CI, 105.1–29,710.4) was the strongest predictor for ITB diagnosis, with the largest regression coefficient value of 7.477. As described in our previous study,[23, 24] the T-SPOT.TB results are affected by individual immune status. LTBI individuals with a robust immune system may have a relatively high level of TBAg results, whereas the active TB patients with immunosuppression may have a low level of TBAg results. However, PHA response in the positive control well of T-SPOT.TB will correspondingly increase or decrease according to different immune statuses. Thus, calculation of the TBAg/PHA ratio is better than directly using T-SPOT.TB results in distinguishing TB disease from LTBI because this ratio can eliminate the impact of individual immune variation on T-SPOT.TB assay.

Several limitations of this study should be noted. First, some serology markers for CD diagnosis, such as anti–*Saccharomyces cerevisiae* antibody (ASCA), were not evaluated in our study, as only some included patients underwent that test. Nevertheless, previous studies have shown that ASCA showed no significant difference between ITB and CD.[21, 34] Second, not all ITB patients were confirmed by microbiology, and over half of ITB patients were diagnosed by clinical and endoscopic response to anti-TB treatment. However, the presence or absence of endoscopic and clinical response to anti-TB therapy has been credible for the diagnosis of ITB or CD.[8, 35] Third, this is a single-center study with a limited number of patients. Therefore, further studies with a larger sample size from multiple centers are needed to validate this predictive model.

In conclusion, we have identified a promising 5-marker predictive model for differential diagnosis between ITB and CD. This predictive model might serve as a useful diagnostic tool to distinguish ITB from CD in clinical practice.

SUPPLEMENTARY DATA

Supplementary data are available at *Inflammatory Bowel Diseases* online.

REFERENCES

1. Nikolaus S, Schreiber S. Diagnostics of inflammatory bowel disease. *Gastroenterology*. 2007;133:1670–1689.
2. Cosnes J. Crohn's disease phenotype, prognosis, and long-term complications: what to expect? *Acta Gastroenterol Belg*. 2008;71:303–307.
3. Almadi MA, Ghosh S, Aljebreen AM. Differentiating intestinal tuberculosis from Crohn's disease: a diagnostic challenge. *Am J Gastroenterol*. 2009;104:1003–1012.
4. Ng SC, Bernstein CN, Vatn MH, et al; Epidemiology and Natural History Task Force of the International Organization of Inflammatory Bowel Disease (IOIBD). Geographical variability and environmental risk factors in inflammatory bowel disease. *Gut*. 2013;62:630–649.
5. Zheng JJ, Zhu XS, Huangfu Z, et al. Prevalence and incidence rates of Crohn's disease in mainland China: a meta-analysis of 55 years of research. *J Dig Dis*. 2010;11:161–166.
6. Debi U, Ravisankar V, Prasad KK, et al. Abdominal tuberculosis of the gastrointestinal tract: revisited. *World J Gastroenterol*. 2014;20:14831–14840.
7. Fee MJ, Oo MM, Gabayan AE, et al. Abdominal tuberculosis in patients infected with the human immunodeficiency virus. *Clin Infect Dis*. 1995;20:938–944.
8. Epstein D, Watermeyer G, Kirsch R. Review article: the diagnosis and management of Crohn's disease in populations with high-risk rates for tuberculosis. *Aliment Pharmacol Ther*. 2007;25:1373–1388.
9. Onal IK, Kekilli M, Tanoglu A, et al. Tuberculosis and Crohn's disease revisited. *J Coll Physicians Surg Pak*. 2015;25:443–448.
10. Ma JY, Tong JL, Ran ZH. Intestinal tuberculosis and Crohn's disease: challenging differential diagnosis. *J Dig Dis*. 2016;17:155–161.
11. Ng SC, Hirai HW, Tsoi KK, et al. Systematic review with meta-analysis: accuracy of interferon-gamma releasing assay and anti-*Saccharomyces cerevisiae* antibody in differentiating intestinal tuberculosis from Crohn's disease in Asians. *J Gastroenterol Hepatol*. 2014;29:1664–1670.
12. Lee YJ, Yang SK, Myung SJ, et al. The usefulness of colonoscopic biopsy in the diagnosis of intestinal tuberculosis and pattern of concomitant extra-intestinal tuberculosis. *Korean J Gastroenterol*. 2004;44:153–159.
13. Pulimood AB, Peter S, Ramakrishna B, et al. Segmental colonoscopic biopsies in the differentiation of ileocolic tuberculosis from Crohn's disease. *J Gastroenterol Hepatol*. 2005;20:688–696.
14. Makharia GK, Srivastava S, Das P, et al. Clinical, endoscopic, and histological differentiations between Crohn's disease and intestinal tuberculosis. *Am J Gastroenterol*. 2010;105:642–651.
15. Limsrivilai J, Shreiner AB, Pongpaibul A, et al. Meta-analytic Bayesian model for differentiating intestinal tuberculosis from Crohn's disease. *Am J Gastroenterol*. 2017;112:415–427.
16. Huang X, Liao WD, Yu C, et al. Differences in clinical features of Crohn's disease and intestinal tuberculosis. *World J Gastroenterol*. 2015;21:3650–3656.
17. Lee YJ, Yang SK, Byeon JS, et al. Analysis of colonoscopic findings in the differential diagnosis between intestinal tuberculosis and Crohn's disease. *Endoscopy*. 2006;38:592–597.
18. Yu H, Liu Y, Wang Y, et al. Clinical, endoscopic and histological differentiations between Crohn's disease and intestinal tuberculosis. *Digestion*. 2012;85:202–209.
19. Li X, Liu X, Zou Y, et al. Predictors of clinical and endoscopic findings in differentiating Crohn's disease from intestinal tuberculosis. *Dig Dis Sci*. 2011;56:188–196.
20. Zhou ZY, Luo HS. Differential diagnosis between Crohn's disease and intestinal tuberculosis in China. *Int J Clin Pract*. 2006;60:212–214.
21. Li Y, Zhang LF, Liu XQ, et al. The role of in vitro interferonγ-release assay in differentiating intestinal tuberculosis from Crohn's disease in China. *J Crohns Colitis*. 2012;6:317–323.
22. Pulimood AB, Peter S, Rook GW, Donoghue HD. In situ PCR for *Mycobacterium tuberculosis* in endoscopic mucosal biopsy specimens of intestinal tuberculosis and Crohn disease. *Am J Clin Pathol*. 2008;129:846–851.
23. Wang F, Hou HY, Wu SJ, et al. Using the TBAg/PHA ratio in the T-SPOT(®).TB assay to distinguish TB disease from LTBI in an endemic area. *Int J Tuberc Lung Dis*. 2016;20:487–493.
24. Bosco MJ, Hou H, Mao L, et al. The performance of the TBAg/PHA ratio in the diagnosis of active TB disease in immunocompromised patients. *Int J Infect Dis*. 2017;59:55–60.
25. Patel N, Amarapurkar D, Agal S, et al. Gastrointestinal luminal tuberculosis: establishing the diagnosis. *J Gastroenterol Hepatol*. 2004;19:1240–1246.

26. Ouyang Q, Tandon R, Goh KL, et al. Management consensus of inflammatory bowel disease for the Asia-Pacific region. *J Gastroenterol Hepatol*. 2006;21:1772–1782.
27. Stange EF, Travis SP, Vermeire S, et al; European Crohn's and Colitis Organisation. European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. *Gut*. 2006;55:i1–i15.
28. Zhao XS, Wang ZT, Wu ZY, et al. Differentiation of Crohn's disease from intestinal tuberculosis by clinical and CT enterographic models. *Inflamm Bowel Dis*. 2014;20:916–925.
29. Ferrara G, Losi M, D'Amico R, et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. *Lancet*. 2006;367:1328–1334.
30. Mao R, Liao WD, He Y, et al. Computed tomographic enterography adds value to colonoscopy in differentiating Crohn's disease from intestinal tuberculosis: a potential diagnostic algorithm. *Endoscopy*. 2015;47:322–329.
31. Jung Y, Hwangbo Y, Yoon SM, et al. Predictive factors for differentiating between Crohn's disease and intestinal tuberculosis in Koreans. *Am J Gastroenterol*. 2016;111:1156–1164.
32. Goh K, Xiao SD. Inflammatory bowel disease: a survey of the epidemiology in Asia. *J Dig Dis*. 2009;10:1–6.
33. Kirsch R, Pentecost M, Hall P de M, et al. Role of colonoscopic biopsy in distinguishing between Crohn's disease and intestinal tuberculosis. *J Clin Pathol*. 2006;59:840–844.
34. Makharia GK, Sachdev V, Gupta R, et al. Anti-*Saccharomyces cerevisiae* antibody does not differentiate between Crohn's disease and intestinal tuberculosis. *Dig Dis Sci*. 2007;52:33–39.
35. Inflammatory Bowel Disease Co-operation Group, Chinese Society of Gastroenterology. Chinese consensus on diagnosis and treatment standard of inflammatory bowel disease. *Chin J Gastroenterol*. 2007;12:545–550.