The T-SPOT.TB assay used for screening and monitoring of latent tuberculosis infection in patients with Behçet's disease pre- and post-anti-TNF treatment: A retrospective study

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Abstract

Background: Patients undergoing anti-tumor necrosis factor (TNF) treatment are more susceptible to latent tuberculosis infection (LTBI). The aim of the current study was to determine the rate of active tuberculosis (TB) in patients with Behçet's disease (BD) pre- and post-anti-TNF treatment and to evaluate the long-term efficacy of LTBI screening as primary prophylaxis in China.

Methods: This retrospective study included BD patients eligible for anti-TNF therapy at a single institution in Fudan University, China. On the basis of the results of T-SPOT.TB assay, chest radiograph, and history of exposure to TB, patients were screened and regularly followed up at 3-months interval.

Results: Eighty-nine BD patients with mean disease duration of 87.5 ± 86.1 months were included. Their median duration of anti-TNF therapy was 10.6 months; 51 patients were treated with Infliximab, 38 with Etanercept, and four with Adalimumab. While 84 patients received a consecutive single anti-TNF drug therapy, five patients switched to a second drug. Twelve patients demonstrated positive results in LTBI screening: three had history of TB exposure and nine were solely T-SPOT.TB-positive patients. Before anti-TNF treatment, LTBI treatment was initiated in 11 patients, and one patient refused treatment. With a median follow-up period of 27.9 months, we observed only one case (1.1%) of intestinal TB during Infliximab treatment.

Conclusion: Regardless of anti-TNF treatment, long-term screening via T-SPOT.TB assay might represent a more sensitive approach to identify BD patients with LTBI. As a secondary prophylaxis, the LTBI treatment is effective in a country with high risk of TB.

Keywords: Anti-TNF therapy; Behçet's disease; Latent tuberculosis infection; T-SPOT.TB assay; Tuberculosis

1. INTRODUCTION

Behçet's disease (BD) is a multisystem inflammatory disorder characterized by recurrent oral and genital ulcers, relapsing uveitis and epididymitis, with mucocutaneous, articular, gastrointestinal, neurologic, and vascular manifestations.[1] In China, anti-tumor necrosis factor (TNF)-α agents have been widely used in the therapy of inflammatory disorders. These agents include recombinant human TNF-α receptor II: IgG Fc Fusion Protein (such as Etanercept) and anti-TNF-α monoclonal antibodies (such as Infliximab and Adalimumab). Anti-TNF-α agents were first approved for the treatment of BD-associated refractory retinitis/uveitis.[2] Over the last decade, a considerable amount of literature has been accumulated, demonstrating the broad use of anti-TNF-α agents for treating BD.[2–7]

Suppressing the action of TNF-α helps relieve the symptoms of BD by reducing inflammation. However, anti-TNF-α agents simultaneously weaken the immune response to microbes such as tubercle bacilli, which maybe the reason why patients undergoing anti-TNF-α therapy are at a much higher risk of developing tuberculosis (TB).[8] Rheumatic disease itself is associated with an increasing risk of TB without the use of anti-TNF-α agents (approximately two to four times).[9] Therefore, patients who are eligible for anti-TNF-α therapy require careful evaluation to exclude possible reactivation of latent tuberculosis infection (LTBI) resulting from earlier exposure to *Mycobacterium tuberculosis* (MTB), especially in countries where the risk of TB exposure is high.

In recent years, whole-blood interferon-γ release assays (IGRAs), such as the T-SPOT.TB assay, were introduced for the diagnosis of LTBI.[10] Accumulating studies have confirmed that IGRAs specifically screen for LTBI, especially in patients undergoing immunosuppressive treatments.[11–14] In particular, the sensitivity and specificity of T-SPOT.TB assay were very high and it performs well for the detection of MTB infection. Nevertheless, data on the performance of the T-SPOT.TB assay in BD patients are limited. To evaluate the long-term efficacy of the T-SPOT.TB assay in LTBI screening and primary prophylaxis in BD patients undergoing anti-TNF-α agent therapy, patients with LTBI and without LTBI were retrospectively studied to assess the role of this assay.

2. METHODS

2.1. Study subjects

This was a retrospective cohort study that included 231 BD patients recruited at the Department of Rheumatology of Huadong Hospital, Fudan University, China between October

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2012 and July 2017. All patients were hospitalized. All subjects provided written, informed consent. This study was approved by the institutional review board of Huadong Hospital, Fudan University.

2.2. Exclusion criteria

Subjects were excluded if they had the following: (1) active pulmonary TB (n = 1), (2) active lymphatic TB (n = 2), and (3) no available T-SPOT.TB assay results (n = 3). The final screening revealed that 89 BD patients were eligible for anti-TNF therapy (Fig. 1). Three anti-TNF agents (Infliximab, Adalimumab, and Etanercept) were used for therapy. Patients received an intravenous (IV) infusion of Infliximab or subcutaneous injections of Adalimumab/Etanercept, according to our standard treatment protocols (Infliximab, 200 mg IV at 0, 2, and 6 weeks, followed by every 6 weeks thereafter; Adalimumab, 40 mg biweekly; Etanercept, 25 mg twice weekly subcutaneous injection).

2.3. Diagnosis of BD

All patients were diagnosed according to the international criteria for Behcet's disease (ICBD).[15] Based on the training set data and agreed upon by the research teams, ICBD defines a scoring system covering oral aphthosis, genital aphthosis, ocular lesions, neurological manifestations, skin lesions (pseudo folliculitis, skin aphthosis, erythema nodosum), and vascular manifestations (arterial thrombosis, large vein thrombosis, phlebitis, or superficial phlebitis). Following ICBD, oral aphthosis, genital aphthosis, and ocular lesions were each attributed 2 points, whereas 1 point was assigned to each of skin lesions, vascular manifestations, neurological manifestations, and pathergy test. Patients scoring 4 or more points were classified as having BD.

2.4. Definitions of BD subtypes

Clinical evidence of BD was obtained in all cases. Subjects with only oral, genital aphthosis, and skin lesions were defined as mucosa BD patients; subjects with intestinal lesion were defined as intestinal BD patients; subjects with eye involvement were defined as ocular BD patients; subjects with mucosa lesion and arthritis were defined as joint BD patients; subjects with vascular lesions, central nervous system (CNS) lesions, or multisystem involvement were defined as vascular BD patients, neural BD patients, and multisystem BD patients, respectively. The following demographic, clinical, and serologic data were collected at the time of the T-SPOT.TB assays: sex, age, duration of BD, genital ulcer incidence, oral ulcers, nodular erythema, pseudo folliculitis, gastrointestinal ulcers, eye inflammation, arthritis, CNS involvement, and vascular injury. Laboratory tests included assays measuring erythrocyte sedimentation rate and C-reactive protein (CRP) levels.

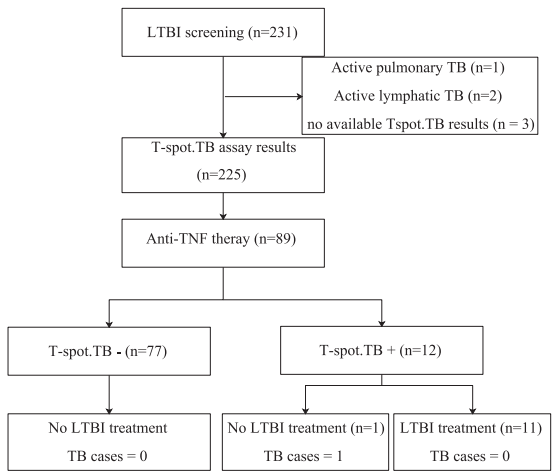

Fig. 1 Flow diagram of screening, selection, treatment, and follow up of the study population based on the T-SPOT.TB assay. TB, tuberculosis; LTBI, latent tuberculosis infection.

2.5. The T-SPOT.TB assay

For the T-SPOT.TB assay, approximately 8 mL of peripheral blood was collected from each patient and healthy control into lithium heparin anticoagulant tubes, as previously described.[16] Samples were transported to our laboratory within 6 hours of collection and the T-SPOT.TB tests were carried out according to the manufacturer's instructions (Oxford Immunotec Ltd., Oxford, UK). The T-SPOT.TB assay measures the number of IFN-γ-secreting spot-forming T cells (SFCs) after stimulation with the MTB-specific antigens ESAT-6 and CFP-10. Results were presented as the number of SFCs by ELISPOT read plate counts. Positive and negative outcomes were defined according to the criteria recommended by the manufacturer: (1) ≥6 SFCs/250 000 PBMCs to ESAT-6 or CFP-10 antigen, adjusted from the negative control (<6 SFCs); (2) if the negative control was ≥ 6 SFCs, the number of SFCs ≥ twice of the negative control was deemed as positive).

2.6. LTBI screening, diagnosis, and treatment

Systematic LTBI testing and treatment were performed on at-risk populations, including patients who had undergone initial anti-TNF treatment, according to the latest WHO guidelines.[17] Before anti-TNF-α therapy, all patients reported clinical medical history and underwent physical examination, standard laboratory tests, chest radiograph, and T-SPOT.TB assay. Patients with positive T-SPOT.TB assays were considered affected by LTBI; LTBI treatment was administered as necessary prophylaxis, based on the positive T-SPOT.TB assay results. The treatment regimen was 300 mg/d Isoniazid for 6 or 9 months, 450 mg/d Rifampin for 3 to 4 months, or a combination of Isoniazid and Rifampin for 3 to 4 months, according to the guidelines for the management of latent MTB infection.[18] Over the course of anti-TNF therapy, the occurrence of pulmonary and extra-pulmonary active TB infection was monitored clinically, radiologically, and microbiologically for every 3 months. All subjects were regularly followed up at 3-month intervals, with the possibility of unscheduled visits when necessary.

2.7. Statistical analysis

All statistical analyses were performed by using SPSS software (version 19.0, SPSS Inc., Chicago, USA). Categorical variables were compared using Fisher's exact test or the χ^2 test. Differences between the median values of defined patient groups were assessed using the nonparametric Mann–Whitney U test. $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Characteristics of the study population

This retrospective study included 89 eligible BD patients (51 males and 38 females) hospitalized at Huadong Hospital between October 2012 and July 2017. The mean age of 89 BD patients was 35.3 years. While the average duration of anti-TNF therapy was 10.6 (range 1-64) months, the follow-up duration since initial anti-TNF treatment was 27.9 (range 3-64) months (Table 1). The clinical manifestations of BD were diverse, but recurrent mucocutaneous lesions were usually the only symptoms at the onset of the disease. Thus, we divided the BD patients according to organ involvement in disease development. As shown in Table 1, mucosa BD (n = 12, 13.5%), intestinal BD (n = 31, 34.8%), eye BD (n = 15, 16.9%), joint BD (n = 10, 11.2%), vascular BD (n = 6, 6.7%), neural BD (n = 3, 3.4%), polyorgan BD (n = 12, 13.5%) were observed in our study population.

All patients received anti-TNF therapy at standard doses. The distribution of patients receiving immunosuppressive drugs was as follows: thalidomide (n = 80, 89.9%), glucocorticoids (n = 77, 86.5%), cyclosporine (n = 42, 47.2%), mycophenolate

Table 1

Baseline demographic and clinical characteristics of BD patients before anti-TNF therapy

Variables	BD patients (n = 89), n, %
BD subtypes	
Mucosa BD	12 (13.5)
Intestinal BD	31 (34.8)
Ocular BD	15 (16.9)
Joint BD	10 (11.2)
Vascular BD	6 (6.7)
Neural BD	3 (3.4)
Multisystem BD	12 (13.5)
Demographic data	
Male gender	51 (57.3)
Age of BD diagnosis, y	35.3 ± 16.9
Age at anti-TNF start, y	36.4 ± 12.9
Duration of anti-TNF therapy, mo	10.6 (1-64)
Follow-up duration since anti-TNF start, mo	27.9 (3-58)
ESR, mm/1st h	35.7 ± 29.6
CRP, mg/L	23.5 ± 25.6
Treatment	
Prednisone	77 (86.5)
Thalidomide	80 (89.9)
Cyclosporine	42 (47.2)
Mycophenolate mofetil	18 (20.2)
Colchicine	15(16.9)
Azathioprine	6(6.7)

BD = Behçet's disease; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; TNF = tumor necrosis factor.

mofetil (n = 18, 20.2%), colchicine (n = 15, 16.9%), and azathioprine (n = 6, 6.7%). Some patients simultaneously received more than two types of drugs. The vast majority of patients (n = 85, 95.5%) were treated with a single anti-TNF agent, whereas only five (4.5%) patients switched to a second anti-TNF agent during therapy. Calculated by the end of our follow up, the TNF-α antagonists Etanercept, Infliximab, and Adalimumab were administered to 36 (40.4%), 47 (52.8%), and 2 (2.2%) patients, respectively (Table 2).

3.2. Clinical parameters did not differ significantly between the LTBI and non-LTBI groups

On the basis of T-SPOT.TB assay screening, 12 patients (13.5%) were diagnosed with LTBI (9 males and 3 females, mean age 41.3 ± 13.7 years). Among them, three reported previous TB exposure and nine solely exhibited T-SPOT.TB-positivity. Seventy-seven BD patients (86.5%) had negative T-SPOT.TB results (42 males and 35 females, with the mean age of 35.6 ± 12.8 years) and were defined as the non-LTBI group. Baseline demographic and clinical characteristics for both groups were presented in Table 3.

The following demographic and clinical characteristics did not differ between groups: sex, age, course of the disease, other clinical manifestations including oral ulcer, genital ulcer, erythema nodosum, pseudo folliculitis, gastrointestinal ulcers, incidence of eye involvement, arthritis, CNS, and vascular involvement. The results of laboratory tests indexes such as erythrocyte sedimentation rate and CRP levels also did not differ significantly between these two groups. There was no difference between groups in using TNF antagonists and immunosuppressive drugs (Table 3).

Table 2

Clinical characteristic of BD patients who received anti-TNF therapy

Variables	BD patients (n = 89) values
Anti-TNF agent	n, %
Infliximab	47 (52.8)
Etanercept	36 (40.4)
Adalimumab	2 (2.2)
Etanercept→Infliximab	1 (1.1)
Adalimumab→Infliximab	2 (2.2)
Infliximab→Adalimumab	1 (1.1)
Duration of anti-TNF exposure, mo	Median (range)
Infliximab	11.8 (1-56)
Etanercept	9.1 (2-64)
Adalimumab	7.5 (5-10)

BD = Behçet's disease; TNF = tumor necrosis factor.

3.3. Diagnosis and treatment of LTBI

Our T-SPOT.TB assay indicated that 12 patients (13.5%) were positive for LTBI. One BD patient diagnosed as LTBI-positive refused to receive LTBI treatment but the remaining 11 patients completed a full course of LTBI treatment (91.7%). Six (54.5%) subjects received combinational therapy with Isoniazid and Rifampin for 3 months, while five patients received Isoniazid alone or Rifampin alone due to strong side effects. Three patients received Rifampin alone for 4 months, and the other two received Isoniazid alone for 9 months.

3.4. Follow-up study

During a median of 27.9 (range 3-58) months of follow up, only one patient (1.1%) developed active TB 3 months later after initiation of anti-TNF treatment (Fig. 2). This patient was a 44-year-old female who received three doses of Infliximab treatment. As evidenced by colonoscopic and histopathologic findings (positive for acid-fast staining, Fig. 2), intestinal TB was confirmed in this patient. During the follow-up period, five BD patients had second T-SPOT.TB assays 3 months after the LTBI treatment (Table 4). Only one (8.3%, number 5) patient showed conversion to T-SPOT.TB-negative after the TNF-α antagonist therapy. For the LTBI-negative BD patient (number 6) with higher negative control background (SFC > 6), it was found that she was still negative for LTBI by the second T-SPOT.TB assay even after immunosuppressant treatment for 3 months (Table 4).

4. DISCUSSION

The present study reports our institutional experience of TB screening in BD patients. Our results demonstrate the critical role of the T-SPOT.TB assay and subsequent primary prophylaxis in minimizing the long-term risk of LTBI in BD patients after anti-TNF therapy. Only 13.5% (12/89) of BD patients were found to require primary prophylaxis for positive LTBI test, a frequency that is consistent with the prevalence of latent TB in rural populations in China (13%-20%).[19] However, this incidence of LTBI in BD patients is lower than that observed in other rheumatic diseases (30%).[20] During a median follow-up period of 27.9 months, one patient (1.1%) developed active TB after initiation of anti-TNF treatment. Notably, this patient did not receive prophylaxis treatment and had a positive T-SPOT.TB result. A study undertaken in China reported that 3.5% (6/172) of ankylosing spondylitis patients developed active TB during long-term follow up.[21] Recently, Borekci et al.[22] studied BD patients in Turkey, and found that 3.6% (3/83) developed active TB. However, there were no such data describing the incidence of TB in BD patients in China. The lower frequency of active TB development we observed may indicate that the T-SPOT.TB assay is a sensitive predictor of LTBI allowing its consequences to be minimized in BD patients who are eligible to receive anti-TNF therapy.

BD is a diverse and rare disease involving multiple systems, and its etiopathogenesis remains largely unclear. Although BD is a rare disease, it can eventually cause life threatening inflammation damaging ocular, vascular, central nervous and gastrointestinal systems. Unfortunately, many patients are resistant to conventional therapies but recently biologics provide effective

Table 3

Comparison of other clinical characteristics between T-SPOT.TB-positive and T-SPOT.TB-negative patients

	LTBI group(n = 12)	non-LTBI group(n = 77)	
Characteristics	**No., %**	**No., %**	***p***
Age, y, mean ± SD	41.3 ± 13.7	35.6 ± 12.8	0.504
Male gender	9 (75.0)	42 (54.5)	0.223
Disease duration, mo, mean ± SD	92.1 ± 75.7	86.8 ± 88.1	0.996
Oral ulcer	12 (100)	76 (98.7)	1.000
Genital ulcer	9 (75.0)	45 (58.4)	0.352
Erythema nodosum	6 (50.0)	72 (45.3)	0.367
Pseudofolliculitis barbae	4 (33.3)	46 (28.9)	0.495
Intestinal ulcers	3 (25.0)	22 (13.8)	0.193
Eye involvement	3 (25.0)	12 (15.6)	0.418
Arthritis	3 (25.0)	23 (29.9)	1.000
Vascular involvement	0 (0)	10 (13.0)	0.346
CNS involvement	0 (0)	4 (5.2)	1.000
Biologic assessment			
ESR, mm/1st h	38.1 ± 35.4	38.6 ± 27.6	0.485
CRP, mg/L	23.2 ± 30.5	23.7 ± 25.1	0.763
TNF antagonist, n, %			
Infliximab	8 (66.7)	39 (50.6)	0.363
Etanercept	3 (25.0)	33 (42.9)	0.347
Adalimumab	1 (8.3)	1 (13.0)	0.253
Two TNF antagonists	1 (8.3)	3 (3.9)	0.446
Immunosuppressive drugs, n, %			
Steroid	11 (91.7)	66 (85.7)	1.000
Thalidomide	11 (91.7)	69 (89.6)	1.000
Cyclosporine	8 (66.7)	34 (44.2)	0.215
Mycophenolate mofetil	2 (16.7)	16 (20.8)	1.000
Colchicine	2 (16.7)	13 (16.9)	1.000
Azathioprine	0 (0)	6 (7.8)	1.000

CNS = central nervous system; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; LTBI = latent tuberculosis infection; TNF = tumor necrosis factor.

alternative treatments. European League against Rheumatism recommendations and several retrospective studies have suggested that TNF-α blockers are the first major options, especially as a rescue therapy.[23,24] TNF-α is a cytokine that plays an important role in the mediation of immune regulation in inflammation. Cytokines are required for the inflammatory response against intracellular organisms, particularly during MTB infection. TNF-α is involved in the pathological changes typical of latent tuberculous infection, especially in the formation and function of the granuloma, which prevents mycobacteria from disseminating into the blood.[25] Screening for LTBI in BD patients who receive anti-TNF treatment is crucial because such patients are at increased risk of LTBI reactivation and also more susceptible to new TB infections. Therefore, it is recommended that any patient diagnosed with BD, who is likely to require treatments by immunosuppressive drugs and/or high dose corticosteroids, should undergo screening for LTBI and be managed according to the local guidelines.[26]

To date, there is no gold standard for LTBI diagnosis. Tuberculin skin testing (TST) has long been an important and commonly used method for detecting LTBI. However, this technique has acknowledged operational and biological limitations.[27] The TST method may produce false-positive results due to prior Bacillus Calmette-Guérin (BCG) vaccination or nontuberculous mycobacterial infection. As a result, the poor specificity of TST might lead to unnecessary treatment and thus the risk of drug toxicity.[28] In China, the guidelines for TB treatment published in 2017[19] recommend an interferon-γ releasing assay rather than TST for screening of LTBI among high-risk populations in rural China, especially for individuals at a high risk of disease reactivation from previous TB. Jung et al.[29] Compared two approaches, IGRAs, QuantiFERON-TB Gold InTube

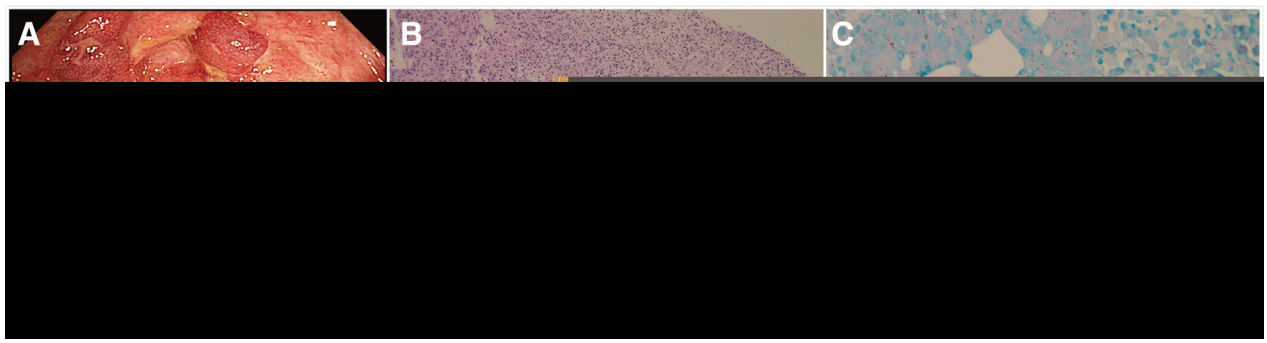

Fig. 2 Colonoscopic findings and histopathologic examinations of one patient with BD who developed active TB after anti-TNF treatment. **A**, Colonoscopic image of the patient shows multiple ulcers and hyperplastic polyp in the ileocecus. **B**, Ileocecus histopathology shows the erosion exudate and necrosis, disordered gland structure, decreased goblet cells, lymphocyte and neutrophil infiltration, and granulomatous formation in the mesenchyma. **C**, Positive acid-fast staining of histologic colonoscopy biopsy (black arrow) of this patient. BD, Behçet's disease; TB, tuberculosis.

Table 4

The results of the T-SPOT.TB assays for six BD patients before and after LTBI treatment during the follow up

LTBI patients	Sex	Negative control	ESAT-6	CFP-10	LTBI treatment
1	Male	0	30	>30	I + R 3 mo
1	Male	0	17	>30	
2	Male	0	>30	0	I + R 3 mo
2	Male	4	>50	50	
3	Female	0	>30	>30	R 4 mo
3	Female	1	24	10	
4	Female	4	6	10	I + R 3 mo
4	Female	3	7	11	
5	Male	1	16	12	I + R 3 mo
5	Male	0	0	1	
6	Female	9	14	15	N
6	Female	7	13	10	

I = isoniazid; LTBI = latent tuberculosis infection; mo = month; N = no antituberculosis medication; R = rifampicin.

(QFT-GIT) and the T-SPOT.TB assay, for diagnosis of LTBI in patients pre- and post-TNF-α antagonist therapy. Similar to our conclusions, they also suggested that the T-SPOT.TB test should be included in the initial evaluation as well as in the follow-up protocols for patients with rheumatic diseases and during TNF-α antagonist therapy.

During follow up, some patients had a second T-SPOT.TB assay after LTBI treatment, and our results demonstrated a reversion rate of 8.3% (1/12). This reversion rate is lower than the reversion rate observed in other rheumatic diseases (12.1%), which used the QFT-GIT method.[29] This might be partially attributed to LTBI treatment.[30,31] However, differences in patient groups and test methods might also explain this discrepancy. Exposure to MTB[32] is considered a crucial environmental trigger of BD. In the current study, we compared environmental factors between LTBI-positive and LTBI-negative patients before anti-TNF treatment. However, we found no statistically significant difference between two groups. Further studies of more patients may provide further insight. Moreover, our study had several limitations. First, patients were enrolled from only a single, tertiary center, thus it was subject to referral bias. Therefore, it might not be appropriate to arbitrarily apply the results of our study to patients in other areas. Second, we used only one of the two available IGRAs, ie, the T-SPOT.TB assay, and application of other IGRAs may not yield similar results. Third, the sample size of T-SPOT.TB-positive patients was small, and we will need to enroll more patients to better discriminate the differences between LTB-positive and LTBI-negative patients.

In conclusion, the T-SPOT.TB assay combined with primary prophylaxis appears to be an effective screening mechanism for preventing TB reactivation in BD patients before anti-TNF treatment. Although more studies with multiple centers, large cohorts, and longer follow-up periods are necessary to strengthen these findings, our preliminary investigation has important implications for advancing the effective management of BD patients in a country with high risk of TB.

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