RESEARCH ARTICLE

T-SPOT.TB for Detection of Tuberculosis Infection among Hematological Malignancy Patients and Hematopoietic Stem Cell Transplant Recipients

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Abstract

The diagnosis of latent *Mycobacterium tuberculosis* infection (LTBI) is recommended in hematological malignancy patients and before hematopoietic stem cell transplantation (Guidelines for the prevention and management of infectious complications of solid organ transplantation, 2004). Compared to traditional methods such as tuberculin skin test (TST), T-SPOT.TB has been shown to be more specific. In the present study we enrolled 536 patients for whom T-SPOT.TB was performed, among which 295 patients also received the TST test. The agreement (79%) between T-SPOT.TB and TST was poor (κ=0.274, P<0.001). The patients with positive T-SPOT.TB results numbered 62 (11.6%), in which only 20 (48.8%) of the 41 receiving the TST test had positive results. A majority of the patients with T-SPOT.TB positive results had some other evidence ofTB, such as TB history, clinical symptoms and an abnormal chest CT scan. Active TB was found in 9 patients, in which 2 had negative TST results. We followed up the patients and no one developed active TB. Our study suggested that the T-SPOT.TB may be more useful for screening LTBI and active TB in hematological malignancy patients and hematopoietic stem cell transplant recipients than the TST test.

Keywords: T-SPOT.TB - tuberculosis - tuberculin skin test - hematological malignancy - stem cell transplant cases

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Introduction

Hematological malignancies such as leukemia and lymphoma are the types of cancer that affect blood, bone marrow, and lymph nodes. As the three are intimately connected through the immune system, hematological malignancies are often associated with immune deficiency. The more aggressive form of disease requires treatment with chemotherapy, radiotherapy, immunotherapy and in some cases a bone marrow transplant. In fact, hematopoietic stem cell transplantation is a curative therapy to hematological malignancy. Hematological malignancy patients and hematopoietic stem cell transplant recipients are high-risk patients who are prone to getting causative agent infection, especially, increasing the risk from latent to active TB (Lee et al., 2011). Nearly one third of the world's population is infected with mycobacterium tuberculosis (Dolin et al., 1994), and the rate continues to increase. Accurate diagnosis of tuberculosis (TB) infection is very significant to prevent the progress from LTBI to active TB, so that the overall burden of tuberculosis disease is diminished.

The most widely used test for diagnosis of infection is the tuberculin skin test (TST), which has been an important and traditional way for detecting LTBI for a long time. However, the limitations of TST have been acknowledged: operational and biological (Benito et al., 2002; Muñoz et al., 2005). With all of operational steps, errors or problems can occur that will affect the accuracy of the test. Biological limitations of the TST are related to the antigens used to stimulate an immune response and to the state of the tested patient's immune response. Taken together, the operational and biological limitations of the TST indicate the need for an improved approach to the diagnosis of TB infection.

New in vitro T cell–based interferon-γ release assays (IGRAs), which utilize specific Mycobacterium tuberculosis antigens, have been introduced into routine clinical practice in recent years (Pai et al., 2004; Pai et al., 2008). T-SPOT.TB is one of the IGRAS which improves the specificity for the diagnosis of LTBI and it is not affected by the BCG and other mycobacterial

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Table 1. 536 Patients' Detailed Clinical Characteristics

Characteristics	n=536(%)
Median age	37(3-81)
Male/Famale	327(61.01)/209(38.99)
Hematologic malignancis	
Acute myelogenous leukemia	
M0	7 (1.31)
M1	32 (5.97)
M2	66 (12.31)
M3	12 (2.24)
M4	37 (6.90)
M5	44 (8.21)
M6	10 (1.87)
M7	1 (0.19)
unclassified	10 (1.87)
MAL	14 (2.61)
Acute lymphoblastic leukemia	
T-ALL	21 (3.92)
B-ALL	87 (16.23)
unclassified	2 (0.37)
Chronic myelogenous leukemia	43 (8.02)
Chronic lymphoblastic leukemia	1 (0.19)
CMML	3 (0.56)
Myelodysplastic syndrome	56 (10.45)
Non-Hodgkin's lymphoma	47 (8.77)
Hodgkin's lymphoma	6 (1.12)
Multiple myeloma	24 (4.48)
Waldenstrom's macroglobulinemia	2 (0.37)
Eosinophilic leukemia	1 (0.19)
Plasma cell leukemia	1 (0.19)
Others	9 (1.68)

M0, minimally differentiated AML; M1, AML without maturation; M2, AML with maturation; M3, acute promyelocytic leukemia; M4, acute myelomonocytic leukemia; M5, acute monocytic leukemia; M6, erythroleukemia; M7, acute megakaryoblastic leukemia; MAL, mixed lineage acute leukemia; CMML, chronic myelomonocytic leukemia

species (Mazurek et al., 2010). The aim of this study was to compare the performance of the TST and the T-SPOT.TB in detecting latent TB infection in hematological malignancy patients and hematopoietic stem cell transplant recipients.

Materials and Methods

Study population

All patients with hematological disorders admitted for T-SPOT.TB between May 2011 and January 2013 at the first affiliated hospital of Soochow university, Suzhou, China, were prospectively enrolled, including hematological malignancy patients and hematopoietic stem cell transplant recipients and patients with iron deficient anemia or hemolytic anemia were excluded from this study. The followed-up of these patients was until June 2013. The ethics committee of the first affiliated hospital of Soochow university approved the study and all participants signed the informed consent before the study.

Tuberculin skin test (TST)

The TST was carried out by the Mantoux method. 2-TU of PPD RT23 was injected into the patient's forearm. The diameter of induration was measured after 48-72 hours. If the induration was ≥5 mm, we considered it as a positive TST result. The TST and the result was administered and read by experienced trained nurses and doctors.

T-SPOT.TB

The T-SPOT.TB test was carried out according to the manufacturer's instructions (Oxford Immunotec Ltd., Oxford, UK). A fresh peripheral venus blood sample about 6ml was collected and isolated the mononuclear cells (PBMCs). The PBMCs were washed twice with 1640 medium by centrifugation, then, resuspended with AIM-V medium. Viable cells were counted using a microscope. Seed cells in each of four wells of the test plate and add antibodies, positive and negative control in each well. The cells were incubated for 16-20 hours under 5% carbon dioxide at 37°C. Finally, use ELISPOT technology to detect IFN-γ released by stimulated T-cells. Results were presented by counting the number of spots. We used the criteria for positive and negative outcomes recommended by the manufacturer. The lab technicians did not know the results of TST.

Statistical analysis

Statistical analysis was performed with SPSS 17.0 (SPSS, Inc., Chicago, IL). The concordance between T-SPOT.TB and TST was assessed using Cohen's $\varkappa$ coefficient. The values of $\varkappa \geq 0.75$ represented excellent agreement, 0.4-0.75 represented moderate agreement, and ≤ 0.4 represented poor agreement. *P* values of <0.05 were considered statistically significant.

Results

Patient characteristics

536 patients with hematological malignancy were enrolled in the study from May 2011 to January 2013. The patients included 327 males (61.01%) and 209 females and the median age was 37 (3-81). All 536 patients were performed T-SPOT.TB on, but only 295 patients were received the TST test for some reasons. The patients' detailed clinical characteristics were shown in Table 1.

T-SPOT.TB assay results

A total of 62 patients in the study cohort were T-SPOT.TB positive (11.57%). In fact, 6 patients had repeated twice T-SPOT.TB, and the result was unified. So that, 56 patients (male 40 (71.43%), median age 41±14 (12-70)) remained in our study. The T-SPOT.TB positive patients' characteristics were listed in Table 2. Among those patients with positive T-SPOT.TB, 41 (73.21%) were received TST test and the positive result was 20 (48.78%). Active TB was found in 9 patients, in which, 2 patients had negative TST results and 1 patient didn't receive TST test. Among the 56 patents, 15 patients had TB history, 22 patients had clinical symptoms, such as fever and cough and almost all the patients had multifarious abnormal chest CT scan. Considering of the result of T-SPOT.TB and TST, TB history, clinical symptoms, and CXR findings, 28 patients were started on LTBI or TB treatment. Of the 56 patents, 32 were received stem cell transplantation, in which 15

Table 2. The T-SPOT.TB Positive Patients' Characteristics

No.	Sex	Age	Diagnosis	Transplantation	Treatment	Fever	Cough	Chest CT scan	TB history	TST
1	M	34	M5	YES	NO	NO	NO	pneumonia	-	-
2	M	59	MM	NO	NO	NO	NO	Old tuberculosis	-	-
5	M	33	B-ALL	YES	NO	NO	NO	normal	-	Pos.
6	M	34	MDS	YES	NO	NO	NO	normal	-	-
7	M	62	MM	NO	NO	YES	YES	pneumonia	-	Neg.
8	M	58	M1	YES	NO	NO	NO	-	-	Pos.
9	F	29	B-ALL	NO	YES	NO	NO	calcification	YES	-
10	M	45	MDS	YES	NO	NO	YES	normal	YES	Pos.
11	M	31	MAL	YES	YES	YES	NO	Old tuberculosis	-	Neg.
12	M	68	NHL	YES	NO	NO	NO	-	-	-
13	M	43	M4	NO	NO	NO	NO	other	YES	Pos.
14	M	61	other	NO	NO	NO	NO	-	-	-
15	M	58	M5	YES	YES	NO	NO	Old tuberculosis	-	Pos.
16	F	36	M6	YES	YES	NO	NO	normal	-	Pos.
17	F	32	MDS	YES	NO	NO	NO	-	-	Neg.
18	M	43	CML	YES	NO	NO	NO	normal	-	-
19	F	58	MM	NO	YES	YES	NO	pneumonia	-	-
20	M	70	MDS	NO	NO	YES	YES	pneumonia	YES	Pos.
21	M	15	B-ALL	NO	YES	NO	NO	pneumonia	-	Pos.
22	M	49	M1	YES	NO	NO	NO	-	-	Neg.
23	M	41	B-ALL	YES	NO	NO	NO	-	-	Neg.
24	M	50	M4	NO	NO	YES	NO	Old tuberculosis	YES	Pos.
25	F	46	MAL	YES	YES	YES	YES	pneumonia	YES	Neg.
26	F	36	ALL	YES	YES	NO	NO	normal	-	Neg.
27	M	29	B-ALL	NO	NO	NO	NO	Old tuberculosis	YES	Pos.
28	M	42	B-ALL	NO	YES	NO	NO	normal	-	Pos.
29	M	44	CML	NO	YES	NO	NO	pneumonia	-	Pos.
30	F	44	MM	YES	YES	NO	NO	pneumonia	-	Neg.
31	M	57	M4	YES	YES	NO	NO	Old tuberculosis	YES	Neg.
32	M	16	CML	YES	NO	YES	NO	normal	-	-
33	M	31	NHL	NO	NO	NO	YES	pneumonia	-	Neg.
34	F	25	B-ALL	YES	NO	NO	NO	normal	-	Neg.
35	F	12	CML	YES	NO	NO	NO	normal	-	-
36	M	17	T-ALL	YES	YES	NO	YES	other	-	Pos.
37	F	43	MDS	NO	YES	NO	NO	normal	YES	Pos.
38	F	44	M4	YES	YES	NO	NO	Old tuberculosis	YES	Neg.
39	M	45	M1	YES	YES	NO	YES	pneumonia	-	Neg.
40	M	46	M1	YES	YES	YES	YES	other	-	Neg.
41	M	40	M2	NO	YES	NO	NO	Old tuberculosis	YES	Pos.
42	M	48	MM	YES	YES	NO	NO	normal	-	Neg.
43	M	38	CML	YES	NO	NO	NO	normal	-	Pos.
44	F	45	M2	YES	NO	NO	NO	normal	-	Neg.
45	F	49	MM	YES	YES	YES	NO	Old tuberculosis	YES	Pos.
46	M	29	CML	YES	NO	NO	NO	normal	-	-
47	M	26	MAL	NO	YES	YES	YES	pneumonia	-	-
48	F	40	MAL	NO	YES	NO	YES	normal	-	-
49	M	21	CML	YES	NO	NO	NO	normal	-	Neg.
51	F	45	M1	YES	YES	YES	NO	other	-	Pos.
52	M	50	M3	NO	YES	YES	YES	cavity	-	-
53	M	70	M4	NO	NO	YES	YES	other	-	-
54	F	19	NHL	NO	NO	YES	NO	calcification	-	Neg.
55	M	47	MDS	NO	NO	NO	NO	pneumonia	-	Neg.
56	M	47	MDS	NO	YES	YES	YES	Tuberculosis?	YES	Pos.
57	M	26	B-ALL	YES	YES	NO	YES	cavity	-	Neg.
58	M	17	ALL	NO	YES	NO	NO	pneumonia	YES	Pos.
59	M	56	M4	NO	YES	YES	YES	Old tuberculosis	YES	Neg.

Patient 28, 31, 37, 40, 41, 47, 51, 56, 58 had active TB

patients received the treatment, 9 patients had the TST positive results and only 5 patients had TB history.

Agreement between T-SPOT.TB and TST

Among 536 participants, 295 patients had complete results for T-SPOT.TB and TST. We analyzed the agreement of the two tests (Table 3). 44 (14.92%) subjects had the positive T-SPOT.TB result and 61 (20.68%) subjects had the positive TST result. 21 (7.12%) subjects had both the T-SPOT.TB and the TST positive results. The agreement (79%) between T-SPOT.TB and TST was poor (κ=0.274, P<0.001).

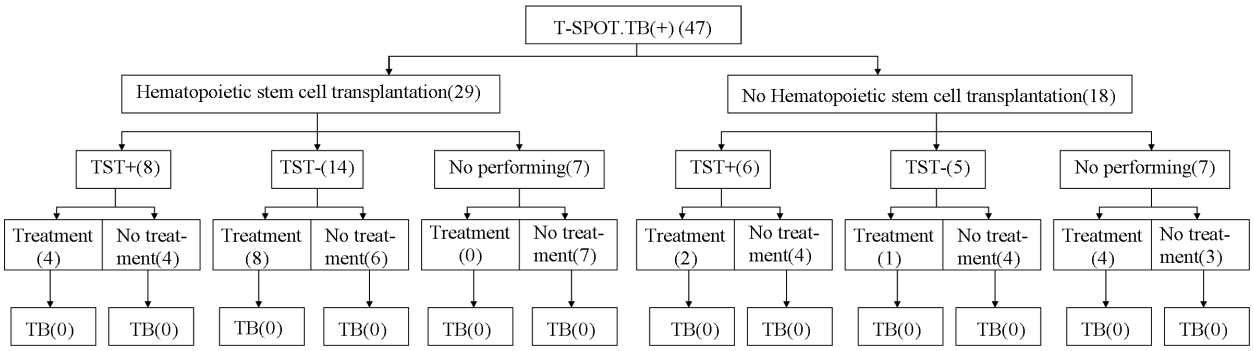

Figure 1. Following up. Excluding the 9 patients with active tuberculosis, no one developed active TB

Table 3. The Agreement Between T-SPOT.TB and TST

	TST		
T-SPOT.TB	Positive	Negative	Total
All patients(n=295)			
Positive	21(7)	23(8)	44(15)
Negative	40(14)	211(71)	251(85)
Total	61(21)	234(79)	295(100)
Agreement	79%		
$\varkappa$ coefficients	0.274		

44(14.92%) subjects had the positive T-SPOT.TB result and 61(20.68%) subjects had the positive TST result. 21(7.12%) subjects had both the T-SPOT.TB and the TST positive results. The agreement (79%) between T-SPOT.TB and TST was poor ($\varkappa$=0.274, P<0.001)

Following up

We performed chest computed tomography, sputum analysis and culture for tuberculosis to get the signs of TB development. The median follow-up duration after receiving T-SPOT.TB was 14.2 mouths. Of the 47 patients, no one developed active TB (Figure 1).

Discussion

536 patients were enrolled in our study. To our knowledge, this is the largest study about the utility of the T-SPOT.TB among hematological malignancy patients and hematopoietic stem cell transplant recipients for detection of tuberculosis infection (Piana et al., 2006; Moon et al., 2013). Patients with hematological malignancy and receiving hematopoietic stem cell transplantation are both high-risk patients to get tuberculosis infection. The TST or IGRAs is recommended for screening TB in these people as they have high opportunity to active TB. Timely treatment may reduce the risk of progression. 295 patients had complete results for T-SPOT.TB and TST. The agreement (79%) between T-SPOT.TB and TST was poor ($\varkappa$=0.274, P<0.001). In our study, among the 56 T-SPOT.TB positive patients, only 20 patients had a positive TST result. The phenomenon is consistent with previous findings. According to previous study and Meta-analysis, tuberculin skin test result was high False-Negative and False-Positive (Huebner et al., 1993). As the false negative results, TST results are always not exact on immunocompromised people. Other reasons, such as TB history, BCG vaccination, immune status, and using of immunosuppressor, may affect the TST result. The T-SPOT.TB using on the immunocompromised people will improve specificity, as it uses negative and positive controls and not affected by the BCG vaccination (Andersen et al., 2000). Among the 56 patents, 15 patients had TB history, 22 patients had clinical symptoms, such as fever and cough and almost all the patients had multifarious abnormal chest CT scan. On the other hands, all the patients with T-SPOT.TB positive result had some symptoms associated with TB. In our study, active TB was found in 9 patients, in which, 2 patients had negative TST results and 1 patient didn't receive TST test. The T-SPOT.TB seemed to be more useful than TST for diagnosing active TB. The result consists with previous findings (Menzies et al., 2007; Pai et al., 2008; Thijsen et al., 2009; Santín Cerezales et al., 2011).

Our study had many limitations. Firstly, although we got the conclusion that the accordance between TST and T-SPOT.TB was poor, for some reasons only a small number of patients completed the TST test. Secondly, clinical symptoms and abnormal CXR findings can be discovered in other lung diseases, such as pneumonia, lung cancer and pleural effusion. The patients who had TB history were not the equivalent of the patients with LTBI. For these reasons, the deficiency of criterion of LTBI was the major limitation in our study. Thirdly, we didn't investigate the patients' BCG vaccination history and the contact history with active TB, therefore, it may influence the result of this study. Finally, a longer time of follow-up on hematological malignancy patients and hematopoietic stem cell transplant recipients may reflect a true impact of T-SPOT.TB screening incidence of the progression from LTBI to active TB.

Although the study had some limitations, our study suggested that the T-SPOT.TB may be more useful for screening LTBI and active TB on hematological malignancy patients and hematopoietic stem cell transplant recipients, as the higher specificity of T-SPOT.TB on immunocompromised people and the false negative results of TST. In our view, clinicians should combine the T-SPOT.TB, TST, TB history, clinical symptoms, and CXR findings to diagnose LTBI or active TB and the patients can receive a timely treatment as soon as possible. Our destination is minimizing the progression from LTBI to active TB and reducing the tuberculosis related mortality rate.

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