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Original Article

Diagnostic efficacy of the TSPOT assay for tuberculosis infection and drug resistance in mycobacterium tuberculosis in patients with malignant tumors

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Abstract: Immunosuppression in cancer patients clearly elevates the risk of acquiring tuberculosis (TB) infection, and traditional diagnostic methods are characterized by low sensitivity (Sen) and long turnaround times. Although the interferon-gamma release assay (IGRA) is widely used, its ability to distinguish active tuberculosis (ATB) from latent tuberculosis infection (LTBI) in cancer patients, as well as its relationship with drug resistance (DR), remains unclear. This study aimed to evaluate the diagnostic efficacy of T-SPOT for active tuberculosis and its ability to distinguish active tuberculosis from latent tuberculosis infection in patients with malignant tumors. A retrospective analysis was conducted on data from 634 patients with malignant tumors, and patients were grouped: active tuberculosis (ATB) group (AG, n = 76), LTBI group (BG, n = 184), and non-TB group (CG, n = 374). The interferon-gamma (IFN-γ) response induced by Early Secreted Antigenic Target 6 (ESAT-6)/Culture Filtrate Protein 10 (CFP-10) was detected by TSPOT, and the relationship between TSPOT and DR was analyzed. Subgroup analysis and multivariate regression were used to explore the immune-related factors affecting the positive rate of TSPOT. The results showed Sen for ATB was 86.8%, and specificity (Spe) was 73.3%. The Sen for screening TB infections was 80.4%, with a Spe of 98.4% and a positive predictive value (PPV) of 97.2%. The Spe for differentiating ATB from LTBI was only 22.3%. The overall DR rate in the AG was 59.2% (45/76), including rifampicin (RIF) resistance (36.8%), multidrug-resistant TB (MDR-TB) (21.1%), and pre-extensively drug-resistant TB (Pre-XDR-TB) (7.9%). The median total spot count of TSPOT in the drug-resistant group was significantly lower than that in the drug-sensitive group [52 (IQR 25-85) vs. 120 (IQR 85-188), $P < 0.001$], and the spot count showed a decreasing trend as the number of drug-resistant types increased ($P < 0.001$). Multivariate regression showed that low lymphocyte count (< 0.8×10^9/L) and high-dose steroid use (> 40 mg/d) were independent factors associated with a reduced positive rate of TSPOT. In summary, TSPOT is suitable for screening TB infection in patients with malignant tumors but has limited capacity to differentiate ATB from LTBI. The significant reduction in TSPOT spot counts was closely associated with MTB drug resistance and host immunosuppressive status, suggesting that this indicator may serve as an auxiliary early warning tool for drug-resistant tuberculosis.

Keywords: TSPOT, malignant tumors, ATB, LTBI, drug-resistant TB, diagnostic efficacy, immune response

Introduction

The immunocompromised status resulting from cancer-related pathophysiology and its treatment modalities (e.g., radiotherapy and chemotherapy) clearly elevates TB infection likelihood in patients with malignant tumors. A study in 2022 suggested that there were 115,478 new TB cases associated with cancer (95% CI: 110,482-123,007), with a global TB incidence rate of 1.85% among cancer patients [1]. This risk further increases after treatment with immune checkpoint inhibitors (ICIs) [2]. However, the clinical diagnosis of cancer combined with TB is highly challenging. The traditional tuberculin skin test (TST) has a high rate of false negatives in immunocompromised populations [3]; microbial culture takes up to 2-8 weeks, which can delay treatment [4]; imaging findings are often nonspecific and can be con-

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fused with tumor progression or pulmonary infections [5].

The interferon-gamma release assay (IGRA) provides a new diagnostic strategy for immunosuppressed populations by detecting T-cell immune responses induced by the Mycobacterium tuberculosis (MTB)-specific antigens Early Secreted Antigenic Target 6 (ESAT-6) and Culture Filtrate Protein 10 (CFP-10) [6]. The TSPOT, based on enzyme-linked immunospot (ELISPOT) technology, can directly count interferon-gamma (IFN-γ)-producing T cells, with high Sen, unaffected by Bacillus Calmette-Guérin (BCG) vaccination, and superior diagnostic performance to other IGRAs in immunosuppressed states [7, 8]. Multiple studies have indicated that in high TB-endemic areas, TSPOT has high diagnostic efficacy for ATB and latent tuberculosis infection (LTBI), especially in immunocompromised patient groups [9]. However, existing studies have mainly focused on the general population, and there is a lack of systematic evidence on the diagnostic performance of TSPOT in patients with malignant tumors, especially in differentiating ATB from LTBI and its clinical value in detecting drug-resistant TB.

More complexly, even if diagnosed with TB, the anti-TB treatment of cancer patients still faces many difficulties. Multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) make the treatment period longer, drug side effects more severe, treatment costs higher, and success rates lower [10, 11]. Immunosuppression and the use of broad-spectrum antibiotics further increase the risk of drug resistance (DR). A study based on a nationwide database in South Korea suggested that the risk of MDR-TB in users of anti-TNF-α biologics was 3.26 times that of ordinary TB patients (95% CI: 1.69-6.28) [12]. Currently, traditional phenotypic drug susceptibility testing (DST) takes a long time, and although molecular DST (such as Xpert MTB/including rifampicin (RIF) Ultra) can quickly detect RIF resistance, its Sen and Spe in complex samples (such as blood, serous fluid, or tumor background tissue) still need to be verified [13]. The early prediction methods for drug-resistant TB based on TSPOT or combined with other platforms are still a research gap in the clinical application of immunosuppressed populations.

In recent years, accumulating evidence has suggested a potential link between the intensity of the host immune response and MTB drug resistance. Drug-resistant strains may affect T cell recognition and activation by altering antigen expression or inducing immune evasion, resulting in decreased spot counts in TSPOT assays. However, research exploring the relationship between TSPOT spot counts and drug resistance specifically in an immunocompromised population, such as those with malignant tumors, is currently lacking. Therefore, to address the clinical challenge of cancer combined with TB, it is of great significance to conduct systematic research to verify the diagnostic efficacy of TSPOT in this population for cancer-related TB infection and to identify LTBI and ATB, as well as to explore its potential application in TB DR assessment. It aims to promote the development of individualized diagnosis and treatment strategies.

Research methods

Sample size estimation

The sensitivity (Sen) and specificity (Spe) of TSPOT for diagnosing ATB in patients with malignant tumors were evaluated, adopting a cross-sectional diagnostic study design. Referring to previous literature, the Sen of TSPOT for ATB in the general population is approximately 85%, with a Spe ranging from 75% to 85% [7, 8]. Considering that immunosuppression is common in cancer patients and may affect the accuracy of immunological tests, to ensure the statistical power of the estimated diagnostic Sen, this article used the one-sided precision method for sample size calculation. The Sen was set at 0.85, with an allowable error (d) of 0.08 and a confidence level of 95% ($Z = 1.96$). This study selected 0.08 (i.e., ±8%) based on the following clinical considerations: in diagnostic research, the allowable error reflected the maximum acceptable margin of error for sensitivity estimation, which was typically controlled within the range of 5% to 10%, and the researcher set it according to the required confidence interval width and clinically acceptable precision [14]. Based on the formula $n = Z^2 * Sen (1-Sen)/d^2$, the estimated minimum number of ATB cases required for diagnostic sensitivity was approximately 75. Combined with historical data from our hospital showing that the proportion of clinically diagnosed active tuberculosis (ATB) among malignant tumor patients was about 12%, to obtain at least 75 ATB patients, the required total sample size was at

least 75/0.12 ≈ 625 cases. Therefore, collecting at least 625 tumor patients who underwent T-SPOT testing was sufficient to meet the estimation requirements for diagnostic sensitivity.

Subjects

Through collaboration with multiple clinical departments such as the Department of Respiratory and Critical Care Medicine, Infectious Diseases, Medical Oncology, and Hematology at First Hospital of Shanxi Medical University, this study retrospectively collected clinical data from 634 patients with malignant tumors who were hospitalized or visited the outpatient clinics of the aforementioned departments between January 2019 and January 2026. The study protocol was approved by the ethics committee of First Hospital of Shanxi Medical University, with a waiver for patient informed consent.

Inclusion criteria: (1) Patients with malignant tumors confirmed by histopathology (covering both solid tumors and hematological malignancies); (2) Completion of TSPOT testing before, during, or within 6 months after anti-tumor treatment (chemotherapy, radiotherapy, targeted therapy, immunotherapy); (3) Complete medical records (microbiological results, pathological reports, etc.); (4) Age ≥ 18 years.

Exclusion criteria: (1) Severe immunodeficiency (e.g., human immunodeficiency virus (HIV) infection); (2) Current or previous receipt of anti-TB treatment for ≥ 1 month; (3) Indeterminate TSPOT results; (4) History of ATB within 5 years prior to enrollment. Patients with other definite active infections (e.g., fungi) that could not confirm MTB as the causative pathogen.

TB infection grouping and criteria for determination

Based on clinical symptoms, imaging, microbiological findings, and follow-up data, patients were grouped.

AG: Positive MTB culture in clinical samples (such as sputum, bronchoalveolar lavage fluid, pleural fluid, tissue, blood).

BG: TST induration ≥ 10 mm and positive IGRA, and no ATB lesions found on chest imaging or other examinations, determined to have LTBI.

CG: Without TB-related clinical symptoms or imaging findings, no history of high-risk contact with TB, all negative microbiological and pathological test results, and no TB-related symptoms or confirmed TB diagnosis during at least 12 months of follow-up.

Data collection

Baseline data of all enrolled patients were collected, including age, gender, type of malignant tumor (such as lung cancer, gastrointestinal tumor, breast cancer, hematological tumor), clinical stage [solid tumors based on the tumor-node-metastasis (TNM) staging system] [15], hematological tumors using the Ann Arbor staging system [16], time of initial diagnosis, Eastern Cooperative Oncology Group performance status (ECOG) score [17], and major comorbidities (such as diabetes, hypertension, chronic liver and kidney diseases).

To assess the immune function of the patients, complete blood count data within 7 days before TSPOT testing were further collected, with a focus on recording peripheral blood lymphocyte and neutrophil counts; serum albumin levels were also collected as an indirect indicator of nutritional and immune status.

Records were kept of whether immunosuppressants had been used within the past month, especially the dosage of corticosteroids (calculated as methylprednisolone equivalents), and the use of other immunomodulatory drugs (such as anti-tumor necrosis factor-alpha (TNF-α) antibodies, ICIs). In addition, the anti-tumor treatment regimens currently being received by the patients were meticulously registered to comprehensively evaluate their potential impact on the immune system.

TSPOT testing

TSPOT testing was performed using a commercial kit produced by Oxford Immunotec Ltd. (UK), strictly following the instructions. 4-6 mL of heparinized peripheral blood was collected from the antecubital vein of the subject and processed within 4 hours. Peripheral blood mononuclear cells (PBMC) were separated using Ficoll-Paque PLUS density gradient centrifugation (GE Healthcare, Sweden) and adjusted to 2.5×10^6/mL with RPMI 1640 medium (Gibco, USA). 2.5×10^5 PBMCs were seeded per

well into a 96-well ELISPOT plate containing ESAT-6, CFP-10, negative control, and positive control wells, and incubated (37°C, 5% CO_2, 20-24 hours) to induce IFN-γ release. After incubation, 3,3',5,5'-tetramethylbenzidine (TMB) color development was performed, and the AID ELISPOT Reader System (Germany) was employed. The interpretation criteria were as follows: ≥ 6 spots in the antigen wells compared to the negative control were considered positive, < 6 spots and ≤ 10 spots in the negative control were considered negative, and > 10 spots in the negative control or no response in the positive control were considered invalid. Positive and negative controls were set throughout the test, with strict quality control. All operators were professionally trained to ensure the standardization of the experiment and the reliability of the results. To minimize observer bias, the evaluation of T-SPOT results was performed by trained laboratory technicians who were blinded to the patients' clinical grouping information, including tuberculosis infection status, drug resistance results, and treatment history.

DR analysis (for AG)

All DST reports for first- and second-line anti-TB drugs from patients with microbiologically confirmed ATB were collected as the basis for DR analysis.

DR was defined as follows: mono-drug resistant (Mono-DR) refers to resistance to either isoniazid (INH) or RIF alone; poly-drug resistant (Poly-DR) refers to resistance to one or both of INH and RIF, in addition to resistance to at least one other first-line drug [ethambutol (EMB), pyrazinamide (PZA)], but not meeting the definition of MDR; MDR-TB is resistance to both INH and RIF; pre-extensively drug-resistant (Pre-XDR-TB) is MDR with additional resistance to any fluoroquinolone or at least one second-line injectable drug; XDR-TB is MDR with resistance to both fluoroquinolones and second-line injectable drugs; RIF-resistant TB (RR-TB) is defined as resistance to RIF, regardless of resistance to other drugs. The study statistically analyzed the rates and patterns of various types of DR and obtained TSPOT test results for both drug-sensitive and drug-resistant individuals to analyze the relationship between DR and TSPOT test results.

Subgroup analysis

To further investigate the immune-related factors influencing the TSPOT positivity rate, the study stratified patients based on variables including lymphocyte count (cutoff at 0.8×10^9/L), corticosteroid use (cutoff at a methylprednisolone equivalent dose of 40 mg/d), tumor type (solid tumor vs. hematologic malignancy), and treatment phase (initial treatment vs. multi-line therapy). The TSPOT positivity rates were compared within each stratum. Subsequently, using TSPOT positivity as the dependent variable, an analysis of influencing factors was conducted by incorporating independent variables such as age, sex, tumor type, clinical stage, lymphocyte count (continuous variable), neutrophil count, albumin level, corticosteroid use (binary: > 40 mg/d vs. ≤ 40 mg/d or none), immunosuppressant use, and lines of treatment.

In-depth analysis of spot counts and drug resistance

To further clarify the relationship between drug resistance and TSPOT spot counts, the drug-resistant group was subdivided based on resistance patterns: isoniazid-monoresistant, rifampicin-monoresistant, Poly-DR, multidrug-resistant (MDR-TB), and re-XDR-TB. The total spot counts were compared among these subgroups. For each drug-resistant patient, the number of drugs to which their MTB isolate was resistant was calculated, and the correlation between this number and the total spot count (ESAT-6 + CFP-10) was analyzed.

Statistical methods

SPSS 26.0 software was employed. Categorical variables were represented as frequencies and percentages, and χ^2 test or Fisher's exact test was adopted for contrast. Normally distributed measurement data were represented as mean ± SD, while non-normally distributed data were represented as median [interquartile range (IQR)], with contrast made adopting t-test/Mann-Whitney U-test. Sen, Spe, positive predictive value (PPV), negative predictive value (NPV), and their 95% CI of TSPOT diagnosis were computed adopting the gold standard in the grouping method. Receiver operating characteristic (ROC) curves were plotted to assess the ability of TSPOT to differentiate between

Table 1. Contrast of baseline characteristics of subjects

Characteristics	AG (n = 76)	BG (n = 184)	CG (n = 374)	*P*
Age (years)	62 (53-70)	60 (50-68)	61 (52-69)	0.845
Male [n (%)]	48 (63.2)	102 (55.4)	206 (55.1)	0.732
Distribution of tumor types [n (%)]				0.566
Lung cancer	25 (32.9)	51 (27.7)	86 (23.0)	
Gastrointestinal tumor	19 (25.0)	44 (23.9)	92 (24.6)	
Breast cancer	13 (17.1)	22 (12.0)	44 (11.8)	
Hematological tumor	10 (13.2)	29 (15.8)	67 (17.9)	
Others	9 (11.8)	38 (20.6)	85 (22.7)	
TNM/Ann Arbor III-IV [n (%)]	57 (75.0)	127 (69.0)	250 (66.8)	0.702
ECOG ≥ 2 [n (%)]	32 (42.1)	63 (34.2)	136 (36.4)	0.799
Lymphocyte count ($\times 10^9$/L)	1.19±0.47	1.21±0.51	1.14±0.46	0.531
Neutrophil count ($\times 10^9$/L)	4.33±1.21	4.18±1.29	4.10±1.55	0.621
Serum albumin (g/L)	36.3±6.2	36.1±5.6	36.3±5.3	0.307
Use of immune suppressants [n (%)]	29 (38.2)	51 (27.7)	89 (23.8)	0.398
Daily dose of corticosteroids > 40 mg [n (%)]	19 (25.0)	25 (13.6)	105 (28.1)	0.076
Receiving ≥ 2 types of cancer treatments simultaneously [n (%)]	44 (57.9)	111 (60.3)	209 (55.9)	0.839

Note: TNM: tumor-node-metastasis; ECOG: Eastern Cooperative Oncology Group performance status.

ATB and LTBI, using the total spot count of ESAT-6 and CFP-10 as the test variable. A multivariate logistic regression model was employed to identify immune-related factors influencing the TSPOT positivity rate, calculating adjusted odds ratios (OR) and their 95% confidence intervals (CI). Model goodness-of-fit was evaluated using the Hosmer-Lemeshow test. Spearman's rank correlation was used to analyze the relationship between the number of resistant drugs and the total spot count. $P < 0.05$ was considered statistically meaningful. Corrections were made for multiple contrasts adopting the Bonferroni method. Simultaneously, the Bootstrap resampling method (1000 resamples) was used for internal validation of the primary diagnostic performance indicators (sensitivity, specificity, positive predictive value, negative predictive value) and the ORs of independent influencing factors identified in the multivariate logistic regression.

Results

Grouping and contrast of baseline characteristics

A total of 634 patients with malignant tumors were included, and according to the TB infection grouping criteria, 76 patients (12.0%) were classified into the AG, 184 (29.0%) into the BG, and 374 (59.0%) into the CG. The baseline characteristics and immune status indicators are presented in **Table 1**. No visible distinctions were noted in age, gender, tumor type (including the proportion of lung cancer), advanced stage (stage III-IV), ECOG score ≥ 2, peripheral lymphocyte count, neutrophil count, and serum albumin level ($P > 0.05$). Although the use of immune suppressants and multiple courses of treatment were slightly higher in the AG, no statistical distinctions were noted ($P > 0.05$), ensuring no visible confounding bias in the subsequent comparison analysis of TSPOT test performance.

Results of TSPOT testing

In the AG, the positive rate of TSPOT testing was 86.8% (66/76), in the BG it was 77.7% (143/184), and in the CG, 6 cases (1.6%) were positive, indicating the presence of false positives (**Table 2**).

Diagnostic efficacy of TSPOT testing

(1) Diagnostic efficacy of TSPOT for ATB (ATB vs. Non-TB + LTBI): The Sen of TSPOT for ATB was calculated to be 86.8% (77.1-93.5%), 73.3% (69.4-77.0%), PPV 30.7% (24.5-37.5%), and NPV 97.6% (95.5-98.9%) (**Table 3**).

(2) Diagnostic efficacy of TSPOT for TB infection (including latent) (ATB + LTBI vs. Non-TB): The Sen of TSPOT for TB infection was calculated to

Table 2. Distribution of TSPOT results corresponding to different TB infection status

TB infection status	TSPOT positive	TSPOT negative	Total	Positive rate (%)
AG (n = 76)	66	10	76	86.8
BG (n = 184)	143	41	184	77.7
CG (n = 374)	6	368	374	1.6
Total	215	419	634	33.9

Note: TB: tuberculosis.

Table 3. Diagnosis of TSPOT for ATB

	TSPOT positive	TSPOT negative	Total
AG (n = 76)	66 (TP)	10 (FN)	76
Non-TB + LTBI (n = 558)	149 (FP)	409 (TN)	558
Total	215	419	634

Note: TP: True Positive; FP: False Positive; FN: False Negative; TN: True Negative; ATB: active tuberculosis; LTBI: latent tuberculosis infection; TB: tuberculosis.

Table 4. Diagnosis of TSPOT for TB infection

	TSPOT positive	TSPOT negative	Total
ATB + LTBI (n = 260)	209 (TP)	51 (FN)	260
CG (n = 374)	6 (FP)	368 (TN)	374
Total	215	419	634

Note: TP: True Positive; FP: False Positive; FN: False Negative; TN: True Negative; ATB: active tuberculosis; LTBI: latent tuberculosis infection; TB: tuberculosis.

Table 5. Diagnosis of TSPOT for TB infection

	TSPOT positive	TSPOT negative	Total
AG (n = 76)	66 (TP)	10 (FN)	76
BG (n = 184)	143 (FP)	41 (TN)	184
Total	209	51	260

Note: TP: True Positive; FP: False Positive; FN: False Negative; TN: True Negative; TB: tuberculosis.

be 80.4% (74.9-85.2%), Spe 98.4% (96.6-99.4%), PPV 97.2% (94.0-98.9%), and NPV 87.7% (84.3-90.6%) (**Table 4**).

(3) Ability of TSPOT to differentiate ATB from latent infection (ATB vs. LTBI): The Sen of TSPOT for differentiating ATB from latent infection was calculated to be 86.8% (77.1-93.5%), Spe 22.3% (16.4-29.2%), PPV 31.6% (25.2-38.5%), and NPV 80.4% (66.1-90.6%) (**Table 5**).

The Sen of TSPOT for ATB was relatively high (86.8%), but its Spe markedly decreased when differentiating ATB from LTBI (22.3%), indicating its limited capacity in distinguishing between active and latent infections. When the diagnostic target was expanded to all TB infections (ATB + LTBI), TSPOT testing demonstrated extremely high Spe (98.3%) and PPV (97.1%), and could serve as a screening tool for TB infection (**Figure 1**).

ROC curve and cut-off value optimization: TSPOT for distinguishing ATB from LTBI

Among 260 patients with tuberculosis infection (AG + BG), using the total TSPOT spot count (ESAT-6 + CFP-10) as the test variable and ATB (1) vs. LTBI (0) as the state variable, an ROC curve was plotted (**Figure 2**). The area under the curve (AUC) was 0.612 (95% CI: 0.540-0.684), indicating a low efficacy of TSPOT in distinguishing ATB from LTBI. Based on the Youden index, the optimal cut-off value was 15.5 spots, yielding a sensitivity of 71.1% (95% CI: 59.5-81.0%), specificity of 52.2% (95% CI: 44.8-59.5%), positive likelihood ratio of 1.49, negative likelihood ratio of 0.55, and diagnostic accuracy of 58.1%. Compared to the manufacturer-recommended cut-off of 6 spots (sensitivity 86.8%, specificity 22.3%), the new cut-off improved specificity but at the cost of some sensitivity, and the overall discriminatory ability remained suboptimal.

Subgroup analysis: the influence of immune status on the positive rate of TSPOT

Subgroup analysis results (**Table 6**) showed that the TSPOT positivity rate was significantly lower in patients with a lymphocyte count < 0.8×10^9/L compared to those with a count ≥ 0.8×10^9/L (24.1% vs. 36.8%, P = 0.012). Patients receiving a daily methylprednisolone equivalent dose > 40 mg also had a significantly lower TSPOT positivity rate than non-users or low-dose users (21.3% vs. 35.7%, P = 0.008). The TSPOT positivity rate in patients with hematologic malignancies was slightly lower than in those with solid tumors, but the difference was not statistically significant (30.2% vs. 34.7%, P = 0.232). No significant difference was found between patients receiving multi-line therapy (≥ 2 lines) and those initially treated (33.1% vs. 34.5%, P = 0.718).

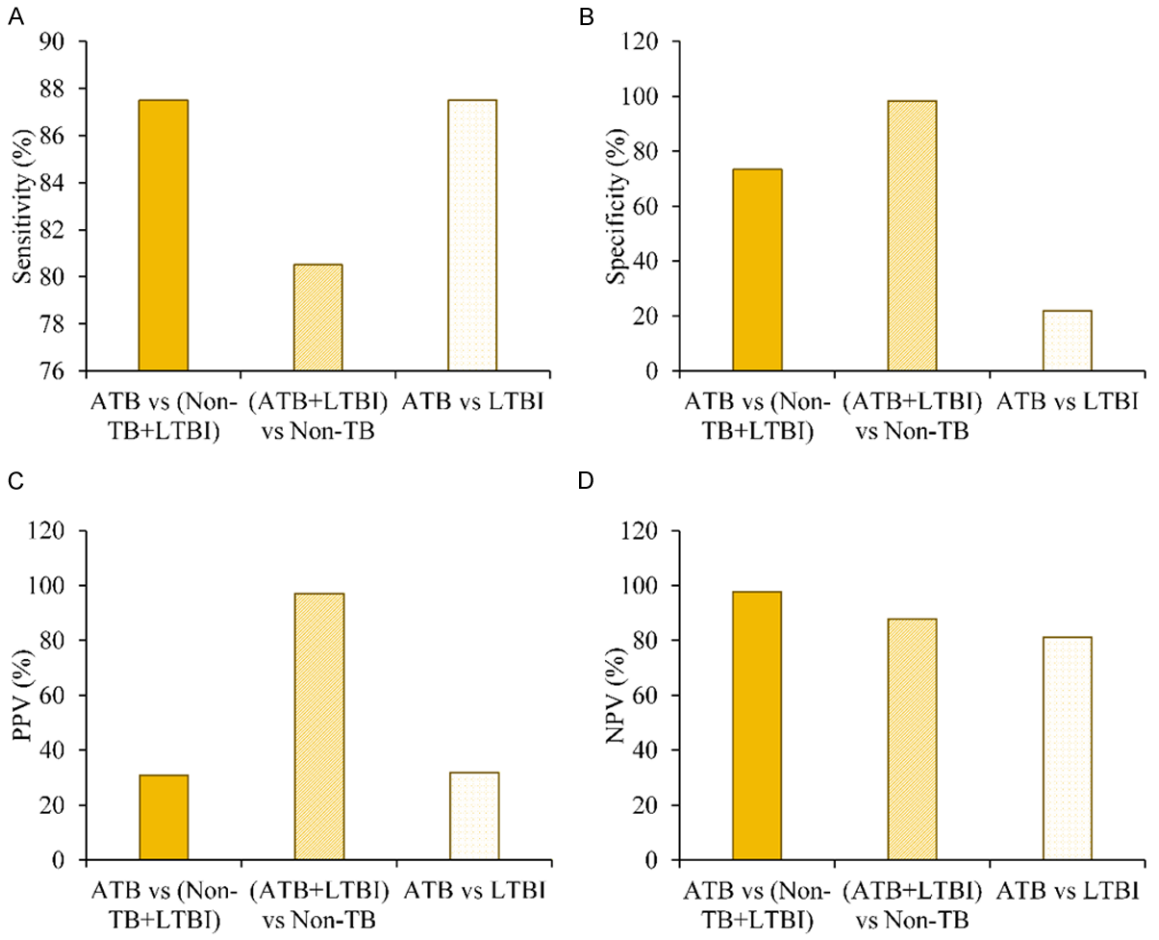

Figure 1. Contrast of diagnostic efficacy of TSPOT for different tuberculosis (TB) infection status. A: Sensitivity (Sen); B: Specificity (Spe); C: Positive predictive value (PPV); D: Negative predictive value (NPV). ATB: active tuberculosis; LTBI: latent tuberculosis infection; TB: tuberculosis.

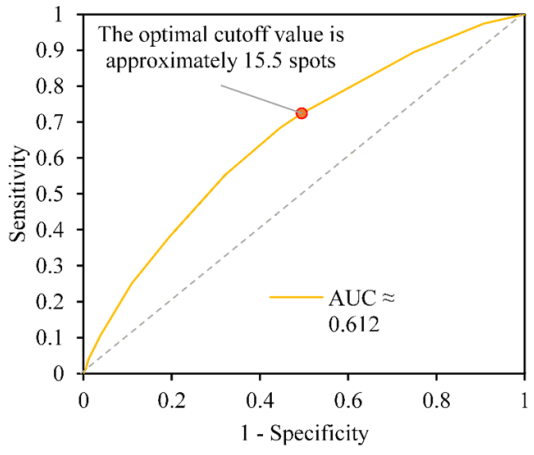

Figure 2. ROC curve of TSPOT for distinguishing active tuberculosis (ATB) from latent tuberculosis infection (LTBI). AUC: area under the curve.

Multivariate logistic regression analysis of factors influencing TSPOT positivity

The results of the multivariate logistic regression analysis are shown in **Table 7**. The final model retained lymphocyte count and corticosteroid use > 40 mg/d as variables. The results indicated that lymphocyte count (per 0.1×10^9/L increase) was an independent protective factor for TSPOT positivity (OR = 1.82, 95% CI: 1.23-2.69, P = 0.003), meaning that for every 0.1×10^9/L increase in lymphocytes, the odds of a positive TSPOT increased by approximately 82%. Corticosteroid use > 40 mg/d was an independent risk factor for TSPOT positivity (OR = 0.45, 95% CI: 0.28-0.73, P = 0.001); individuals using high-dose corticosteroids had an approximately 55% reduction in

Table 6. Comparison of TSPOT positivity rates across different patient subgroups

Subgroup variable	Grouping	N	TSPOT positive [n (%)]	*P*
Lymphocyte count	< 0.8×10^9/L	87	21 (24.1)	0.012
	≥ 0.8×10^9/L	547	201 (36.8)	
Corticosteroid use (methylprednisolone)	> 40 mg/d	150	32 (21.3)	0.008
	≤ 40 mg/d or none	484	173 (35.7)	
Tumor type	Solid tumor	528	183 (34.7)	0.232
	Hematologic malignancy	106	32 (30.2)	
Treatment phase	Initial treatment	290	96 (33.1)	0.718
	Multi-line therapy (≥ 2 lines)	344	119 (34.6)	

Table 7. Multivariate logistic regression analysis of factors influencing TSPOT positivity

Variable	Univariate analysis		Multivariate analysis (final model)*	
Lymphocyte count (per 0.1×10^9/L increase)	OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Corticosteroid use > 40 mg/d (yes vs. no)	1.75 (1.20-2.55)	0.004	1.82 (1.23-2.69)	0.003
Age (per 1-year increase)	0.48 (0.31-0.74)	0.001	0.45 (0.28-0.73)	0.001
Sex (male vs. female)	1.01 (0.99-1.03)	0.312	-	-
Tumor type (hematologic vs. solid)	0.92 (0.68-1.24)	0.584	-	-
Clinical stage (III-IV vs. I-II)	0.82 (0.52-1.28)	0.376	-	-
Neutrophil count (per 1×10^9/L increase)	1.15 (0.84-1.58)	0.387	-	-
Albumin (per 1 g/L increase)	0.96 (0.85-1.09)	0.524	-	-
Immunosuppressant use (yes vs. no)	1.03 (0.98-1.08)	0.219	-	-
Lines of treatment (multi-line vs. initial)	0.73 (0.50-1.07)	0.106	-	-
Constant	1.07 (0.78-1.47)	0.673	-	-
Lymphocyte count (per 0.1×10^9/L increase)	-	-	0.21	< 0.001

Note: *Model Construction Method: Backward stepwise regression (Likelihood Ratio test, removal criterion $P > 0.10$) was used, initially including all 10 variables listed, finally retaining lymphocyte count and corticosteroid use > 40 mg/d. Hosmer-Lemeshow goodness-of-fit test $P = 0.562$, indicating good fit. OR: odds ratios.

Table 8. Distribution of MTB DR profiles

DR category	Number of cases (n = 76)	Proportion (%)	Representative resistance patterns
Fully sensitive	31	40.8	
Resistance status	45	59.2*	
Mono-DR	12	15.8	INH: 6; RIF: 6
Poly-DR	10	13.2	INH + EMB: 3; RIF + PZA: 7
MDR-TB	16	21.1	INH + RIF: 10; INH + RIF + EMB: 6
Pre-XDR-TB	6	7.9	MDR + levofloxacin: 6
XDR-TB	0	0	--
RR-TB	28	36.8	Containing Mono-DR (6) + Poly-DR (6) + MDR (16)

Note: "*": As against the fully sensitive, $P < 0.05$; TB: tuberculosis; DR: drug resistance; Pre-XDR: pre-extensively drug-resistant; MDR: multidrug-resistant; RR-TB: RIF-resistant; INH: isoniazid; RIF: rifampicin; EMB: ethambutol; PZA: pyrazinamide.

the odds of a positive TSPOT. Other factors, such as tumor type and lines of treatment, were not retained in the final model (all $P > 0.05$).

Analysis of DR in MTB

Among the 76 patients with ATB, the overall DR rate was 59.2% (45/76), which was markedly higher than that of the fully drug-sensitive group (40.8%, $P = 0.009$) (**Table 8**). The proportion of RR-TB was as high as 36.8% (28/76), among which 6 cases were Mono-DR (only RIF resistance), and 6 cases were Poly-DR (RIF combined with other drugs). The rate of MDR-TB was 21.1% (16/76), and Pre-XDR-TB was detected in 6 cases (37.5% of MDR-TB), both of

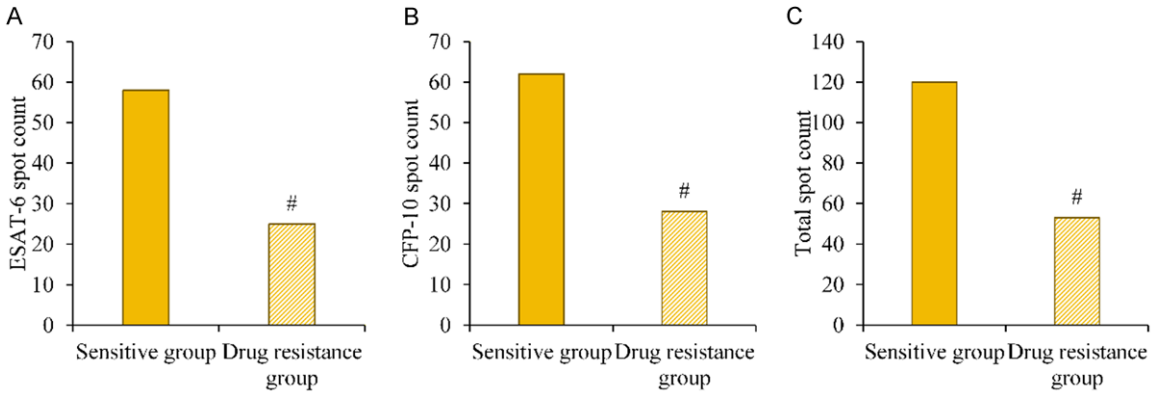

Figure 3. Contrast of TSPOT spot counts between drug-resistant and drug-sensitive groups (median). A: Early Secreted Antigenic Target 6 (ESAT-6) spot count; B: Culture Filtrate Protein 10 (CFP-10) spot count; C: Total spot count. "#": As against the drug-sensitive group, $P < 0.05$.

Table 9. Comparison of total TSPOT spot counts across different drug resistance types

Resistance type	N	Median total spot count (IQR)	*P* (vs. sensitive group)*
Drug-sensitive	31	120 (85-188)	Reference
Isoniazid-monoresistant	6	89 (70-112)	0.214
Rifampicin-monoresistant	6	76 (58-95)	0.086
Poly-Drug resistant (Poly-DR)	10	58 (40-72)	0.003
Multidrug-Resistant (MDR)	16	46 (28-63)	< 0.001
Pre-Extensively Drug-Resistant (Pre-XDR)	6	30 (18-41)	< 0.001

Note: *Dunn's test with Bonferroni correction was used.

which were resistant to fluoroquinolones (levofloxacin) in addition to MDR. Further statistics suggested that the order of resistance rates was INH (51.3%) > RIF (36.8%) > EMB (21.1%) > PZA (17.1%).

In-depth analysis of spot counts and drug resistance

(1) Overall comparison of spot counts between drug-resistant and drug-sensitive groups: The study first compared TSPOT spot counts between 31 drug-sensitive patients and 45 drug-resistant patients (**Figure 3**). Upon analysis, it was found that patients with drug-resistant TB exhibited a markedly weakened cellular immune response. The median total spot count in the drug-resistant group was only 52 (IQR: 25-85) [ESAT-6 spot count: 24 (10-40) + CFP-10 spot count: 28 (14-45)], which was 56.7% lower than that in the drug-sensitive group (120, IQR: 85-188) [ESAT-6 spot count: 58 (40-92) + CFP-10 spot count: 62 (44-96)] ($P < 0.001$).

(2) Stratified comparison of spot counts by different resistance patterns: The 45 drug-resistant patients were grouped according to their resistance pattern and compared with the 31 drug-sensitive patients regarding total spot counts (ESAT-6 + CFP-10). The Kruskal-Wallis test revealed a significant difference among the groups ($P < 0.001$). Further pairwise comparisons (**Table 9**) showed that while the isoniazid-monoresistant (median 89) and rifampicin-monoresistant (median 76) groups had lower median counts than the drug-sensitive group (median 120), these differences were not statistically significant (adjusted $P > 0.05$). However, the total spot counts in the poly-drug resistant (median 58), multidrug-resistant (median 46), and pre-extensively drug-resistant (median 30) groups were all significantly lower than that of the drug-sensitive group (all adjusted $P < 0.01$), showing a decreasing trend with increasing severity of resistance.

(3) Correlation analysis between the number of resistant drugs and spot counts: Among the 45 drug-resistant patients, the number of drugs to which resistance was observed ranged from 1 to 5 (median 2). Spearman's correlation analysis revealed a significant negative correlation between the number of resistant drugs and the

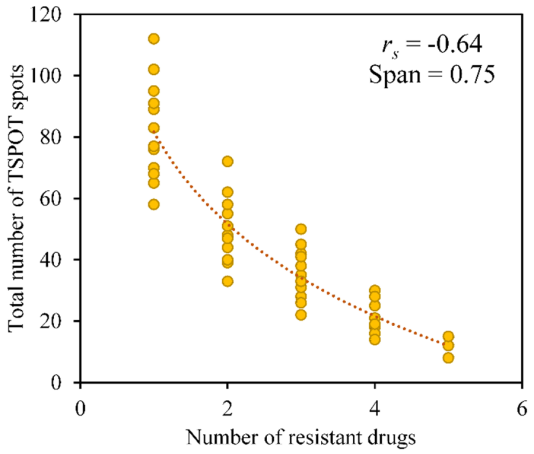

Figure 4. Correlation between number of resistant drugs and total TSPOT spot count (scatter plot + locally weighted scatterplot smoothing (LOWESS) curve).

total TSPOT spot count (rs = -0.64, $P < 0.001$). This indicated that a higher number of resistant drugs was associated with lower TSPOT spot counts. A scatter plot with a locally weighted scatterplot smoothing (LOWESS) fitting curve (**Figure 4**) visually confirmed this negative trend.

Robustness validation

The Bootstrap resampling method (1000 resamples) was used for internal validation of the primary diagnostic performance indicators and the ORs from the multivariate logistic regression model. The results demonstrated that the Bootstrap 95% confidence intervals for sensitivity in diagnosing active tuberculosis (original 86.8%, Bootstrap 95% CI 77.5%-93.8%), specificity (original 73.3%, Bootstrap 95% CI 69.8%-77.2%), sensitivity for diagnosing TB infection (original 80.4%, Bootstrap 95% CI 75.2%-85.6%), specificity (original 98.4%, Bootstrap 95% CI 97.0%-99.4%), as well as the ORs for lymphocyte count (original OR = 1.82, Bootstrap OR = 1.80, 95% CI 1.20-2.71) and corticosteroid use > 40 mg/d (original OR = 0.45, Bootstrap OR = 0.46, 95% CI 0.26-0.74) from the multivariate regression, were all highly consistent with the original point estimates. This indicated that the main findings of this study possessed good robustness.

Discussion

Through this retrospective analysis of 634 patients with malignant tumors, incorporating multivariate regression, ROC curve optimization, drug resistance stratification comparisons, and correlation analyses, this study confirmed the high value of TSPOT in screening for TB infection and its limitations in distinguishing ATB from LTBI. It was the first to quantify and reveal the independent associations of decreased TSPOT spot counts with host immunosuppressive status (low lymphocyte count, high-dose corticosteroid use) and MTB drug resistance (resistance pattern, number of resistant drugs). This suggested that the TSPOT spot count could serve as a quantitative indicator reflecting the host's T-cell immune response intensity against MTB, providing new clinical clues and a quantitative basis for immune-based early warning of drug-resistant tuberculosis.

The study found that the Sen of T-SPOT.TB in patients with ATB was 86.8%, comparable to the pooled Sen values for ATB diagnosis in previous studies (85%-90%). For instance, a systematic review of 20 studies in 2023 suggested that the pooled Sen of T-SPOT.TB for detecting ATB was 0.89 (89%, 95% CI: 0.85-0.92) [18]. Another meta-analysis focusing on immunocompromised populations, such as those with solid tumors or organ transplants, reported a Sen of 82.3% (95% CI: 10.7%-99.5%) [19]. However, a systematic review and Bayesian network meta-analysis clearly pointed out that commercial IGRA "can't effectively distinguish" between ATB and LTBI [20]. In addition, prospective studies and multiple reviews have repeatedly emphasized that neither TST nor IGRA can accurately determine the activity of the infection; they only reflect the T cells' historical exposure to the infection and cannot make a qualitative distinction [21]. This result corroborates the finding of the study that the Spe of T-SPOT.TB was only 22.3%, meaning that despite its high Sen for ATB, it can't differentiate latent infection. On the other hand, when the detection target was expanded to all TB infections (ATB + LTBI), T-SPOT.TB demonstrated excellent Spe (98.4%) and PPV (97.2%), which is in line with the US Preventive Services Task Force (USPSTF) and several studies indicating that T-SPOT.TB has high Spe for diagnosing latent infection (95%-97%) [22]. Among the immunosuppressed population of patients with malignant tumors, T-SPOT.TB still maintains excellent discriminatory ability and is particularly suitable as a screening tool for TB infection (including latent infection). The study

further confirmed through ROC analysis that even with an adjusted cut-off value, the AUC of TSPOT for distinguishing ATB from LTBI was only 0.612, and the specificity at the optimal cut-off (15.5 spots) was only 52.2%, far from a clinically acceptable level. The AUC value was close to the critical level for random classification (0.5), indicating that the diagnostic ability of T-SPOT in distinguishing infection activity was extremely limited and could not serve as an independent tool for clinical decision-making. Specifically, at an AUC of 0.612, even after optimizing diagnostic performance by adjusting the cutoff value, it remained impossible to simultaneously achieve adequate sensitivity and specificity, leading to a high risk of misclassification: when high sensitivity was targeted, a large number of individuals with latent infection would be misclassified as having active tuberculosis, potentially resulting in unnecessary anti-tuberculosis treatment and associated drug toxicities; when high specificity was targeted, a considerable proportion of patients with active tuberculosis would be missed, delaying critical treatment opportunities. Therefore, this low AUC value clinically implied that the guidance value of T-SPOT results for determining infection status was severely limited [23]. This again emphasized that, within the current technical framework, relying solely on TSPOT cannot differentiate infection activity, and a comprehensive judgment integrating clinical, imaging, and microbiological findings is essential.

The study suggested a markedly higher DR rate in patients with malignant tumors complicated by ATB, indicating that this particular population faces a severe challenge in drug tolerance. The overall DR rate reached 59.2%, with a proportion of RR-TB of 36.8% and MDR-TB accounting for 21.1%, markedly higher than the incidence of RR/MDR-TB in the general population (about 3.2-3.3%) [24]. In addition, the study found that the resistance rates were 51.3% for INH, 36.8% for RIF, 21.1% for EMB, and 17.1% for PZA. This distribution has been confirmed to some extent in recent studies. According to a systematic review and meta-analysis, the resistance rates for INH and RIF are approximately 17.2% and 7.3%, respectively, with higher resistance rates in the Asia region. The main resistance mutation for INH is the S315T mutation in the KatG gene, accounting for 23.7%; the main resistance mutation for RIF is the S531L mutation in the RpoB gene, accounting for 13.5% [25]. Moreover, studies have found that the rate of RIF resistance is on an upward trend [26].

The study also first reported the phenomenon of a visible decrease in the number of TSPOT spots in drug-resistant patients with malignant tumors complicated by ATB. The specific results suggested that the median total spot count in the drug-resistant group was only 43.3% of that in the drug-sensitive group (52 vs. 120, $P < 0.001$). To further explore the mechanisms underlying this phenomenon, the present study performed a stratified analysis based on drug resistance patterns. It was found that T-SPOT spot counts decreased stepwise with increasing severity of drug resistance and showed a significant negative correlation with the number of drug types to which the strain was resistant. This trend suggested that the development of drug resistance might be closely associated with the attenuation of host T-cell immune responses. From the pathogen perspective, specific drug resistance mutations (e.g., those affecting genes involved in antigen processing or presentation) might alter the immunogenicity of Mycobacterium tuberculosis, thereby impairing T-cell recognition and activation and ultimately leading to decreased spot counts. From the host perspective, drug resistance in active tuberculosis might itself be associated with weakened immune responses, and the immunosuppression caused by cancer itself or by treatments such as radiotherapy and chemotherapy further exacerbated this immune dysfunction [27]. Notably, in patients receiving radiotherapy or immune checkpoint inhibitor therapy, the distribution and functional status of T-cell subsets might be significantly altered, rendering them less responsive to drug-resistant strains and further reducing T-SPOT spot counts. Therefore, T-SPOT spot counts not only reflected the overall intensity of host T-cell immune responses against Mycobacterium tuberculosis, but might also indirectly indicate the drug resistance burden of the strain, providing new quantitative clues for exploring the role of the “host-pathogen immune dialogue” in the development and progression of drug-resistant tuberculosis. Although causality cannot be established, this provides clues for exploring the role of the “host-

pathogen immune dialogue" in the development of drug-resistant tuberculosis.

In addition, this study further revealed the complex impact of the immunosuppressive background in cancer patients on the results of the TSPOT assay. During treatments such as chemotherapy, radiotherapy, and immune checkpoint inhibitors, the immune system of patients with malignant tumors was often significantly suppressed. This suppression was reflected not only by a reduction in the number of peripheral blood lymphocytes but also by alterations in T cell function (14) (Monti et al., 2024). Chemotherapeutic agents directly weakened the body's immune response to Mycobacterium tuberculosis-specific antigens by inducing lymphocyte apoptosis and inhibiting T cell activation and proliferation, thereby reducing the positivity rate of TSPOT. Radiotherapy further interfered with the release of IFN-γ by disrupting local and systemic immune cell homeostasis. Although immune checkpoint inhibitors enhanced T cell activity, their role in the context of tuberculosis infection was bidirectional: on one hand, they might increase the sensitivity of TSPOT; on the other hand, they might elevate the risk of immune-related adverse events and even trigger active tuberculosis. The subgroup analysis and multivariate regression results of this study also supported the above views, showing that low lymphocyte count (< 0.8×10^9/L) and high-dose glucocorticoid use (> 40 mg/d) were independent influencing factors for the TSPOT positivity rate, further confirming the significant regulatory effect of the host immune status on the test results. Therefore, when interpreting TSPOT results clinically, the type and intensity of the anti-tumor therapy received by the patient, as well as their current immune status, must be fully considered to avoid relying solely on the test value for diagnostic judgment.

However, this study has several limitations. First, it adopted a retrospective design, and data collection relied on clinical records, which may introduce information bias. Moreover, the lack of standardized follow-up across patients limited the reliability of causal inferences. Second, this was a single-center study, with all enrolled cases from the same tertiary hospital. The patient population, treatment protocols, and laboratory procedures exhibited considerable homogeneity, and the generalizability of the findings requires further validation. Third, despite a relatively large overall sample size, the number of cases in drug-resistant subgroups (e.g., MDR-TB, Pre-XDR-TB) was limited, which reduced statistical power for detailed comparisons among resistance types. Although some subgroup differences showed trends, they did not reach statistical significance. Fourth, the T-SPOT assay only reflected T-cell immune responses in peripheral blood at a single time point. The study did not incorporate dynamic monitoring, cytokine profiling, or T-cell subset analysis, limiting the depth of exploration into immunosuppressive mechanisms. Fifth, analyses of diagnostic performance and influencing factors were based solely on internal data without an independent external validation cohort, so the generalizability of the models requires confirmation through future multicenter prospective studies. Sixth, complete genotyping and drug susceptibility testing were not available for all patients, and drug-resistant cases may have been underestimated or underdiagnosed. Furthermore, although a dose-response relationship was observed between T-SPOT results and drug resistance, the causal direction of this association could not be determined in the present study, and further basic research is needed to elucidate the underlying mechanisms. Nevertheless, internal validation was performed using bootstrap resampling (1,000 iterations) for the core diagnostic indicators and multivariate regression results, and the point estimates were highly consistent with the original findings, indicating good robustness of the main conclusions. Future multicenter, prospective studies incorporating dynamic monitoring of host immune status and molecular drug susceptibility testing are warranted to further validate and extend the findings of this study.

Conclusion

TSPOT is an efficient screening tool for TB infection in patients with malignant tumors (with Spe > 98% when excluding non-infection), but it can't reliably distinguish between active and latent infections. This study found that a significant reduction in TSPOT spot count was closely related to MTB drug resistance and host immunosuppressive status, with low lymphocyte count and high-dose corticosteroid use being

independent factors associated with TSPOT positivity. Among drug-resistant patients, spot counts decreased as the drug resistance burden increased, suggesting that the TSPOT spot count may serve as a potential indicator to aid in assessing drug resistance risk and host immune status. Future prospective studies are needed to explore the stratified diagnostic strategy of "TSPOT combined with molecular testing" and the value of dynamic changes in spot counts for monitoring treatment efficacy.

Disclosure of conflict of interest

None.

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