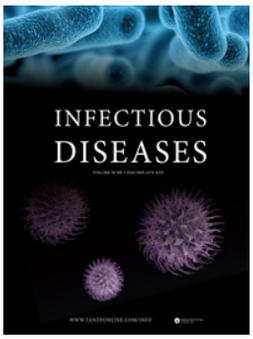

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RESEARCH ARTICLE

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CD8$^+$ T cell response in QuantiFERON-TB Gold Plus testing was associated with tuberculosis recurrence: a 2-year prospective study

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ABSTRACT

Background: Recurrence posed an important challenge to pulmonary tuberculosis (PTB) control in China. The prospective study aimed to identify potential risk factors and to explore the value of QuantiFERON-TB Gold Plus (QFT-Plus) in identifying at-risk individuals with treated prior PTB history.

Methods: All eligible individuals aged ≥18 years who had been diagnosed with PTB before 2016 in Zhongmu County, where with an average level of TB prevalence in China, were included and received baseline survey including chest radiography, QuantiFERON-TB Gold In-Tube (QFT-GIT) and QFT-Plus, then PTB recurrence was tracked through a 2-year follow-up.

Results: Half of 1068 (52.34%, 559/1068) included eligible participants were QFT-Plus positive at baseline and 21 of them recurred active TB in 2-year follow-up. Individuals aged ≥ 60 years, who had a recent history of TB and smokers were associated with increased risk of TB recurrence with an adjusted odds ratio (aOR) of 3.97 (95% confidence interval (CI): 1.29–12.24), 7.71 (95% CI: 1.74–34.25) and 4.56 (95% CI: 1.62–12.83), respectively. Compared to QFT-Plus negatives, those who were TB2+/TB1- (aOR = 15.34) exhibited stronger association with the risk of TB recurrence than those who were TB1+/TB2+ (aOR = 6.06). A dose response relationship was also found between the risk of TB recurrence with the baseline level of TB2-TB1 (p for trend < 0.001).

Conclusions: High burden of TB infection and high risk of PTB recurrence were observed in the study population. Those with recent onset of prior TB, elderly smokers and QFT-Plus positives especially with TB2 single positive deserved further attention in active TB surveillance.

KEYWORDS
Tuberculosis recurrence
history of treated prior PTB
risk factors
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Introduction

Despite the reported treatment success rate for new tuberculosis (TB) cases has reached 89%, recurrence remains an important challenge to TB control. A recently published meta-analysis reported the pooled recurrent TB incidence rate ranged from 1.47 per 100 person years in low TB incidence settings to 4.10 per 100 person years for studies conducted in high TB incidence settings [1]. Varied recurrence rates, ranging from 2.0% to 15.2%, have also been reported in different regions of China [2–6]. Our previous population-based prospective study consistently observed that only 7.7% (567/7388) interferon gamma release assay (IGRA) positives reported a history of prior TB but they contributed 45.3% (53/117) TB recurrence cases in 5-year follow-up [7]. A report based on mathematical model also revealed that restraining reactivation would be an effective tool for reducing TB burden in China [8]. In addition, according to World Health Organisation (WHO) report [9], the proportion of drug-resistant TB cases among recurrence patients were remarkably higher than those among new TB cases. Therefore, it is crucial to enhance active surveillance and mitigate the risk of recurrence on TB control in China for individuals with prior TB history. Clarifying the epidemiological character of the population and identifying the subgroups under high risk of TB recurrence are key step before implementation of active surveillance.

There are many factors have been reported to influence TB recurrence including inadequate treatment of prior TB, clinical characteristics of patients, genotypes of strains, and HIV or diabetes coinfections and so on [10]. On one hand, tracking TB recurrence by means of prospective design would facilitate the determination of risk factors related with TB development. On the other hand, exploring potential TB biomarkers capable of identifying subgroups under high risk of progressing to active TB is another approach. Our previous study, which conducted in individuals with latent tuberculosis infection (LTBI) identified by IGRA, revealed that those with higher baseline IFN-γ levels were more likely to develop TB and acquired greater protective efficacy from preventative treatment [11]. In addition, the latest generation of IGRA, QuantiFERON-TB Gold Plus (QFT-Plus), was hypothesised to play an important function in reflecting recent TB infection due to it contains additional set of peptides that characterise TB-specific $CD8^+$ T-cell responses [12–14]. In consideration of recent infection was an important indicator for TB development [15], the potential value of $CD8^+$ T-cell responses of the commercially available QFT-Plus as biomarkers in predicting TB recurrence had been rarely studied and need further exploration.

In China, majority of individuals with active TB live in rural areas, the standardisation of treatment and patient management are relatively weak in rural areas which might aggravate the risk of TB recurrence. It is of great significance to evaluate the recurrence risk among rural residents with a history of prior TB and to identify related factors for improving TB control. Thus, the present study recruited individuals with treated prior pulmonary TB (PTB) history from rural areas and investigated their baseline IGRA positivity. Then, a 2-year follow-up tracking PTB recurrence was conducted to evaluate potential risk factors and to determine the clinical value of QFT-Plus in identifying individuals at-risk of TB recurrence.

Methods

Study design and populations

The present prospective study, conducted in Zhongmu County in Henan province with an average level of national TB prevalence, aimed to investigate the current IGRA positivity and PTB recurrence in next 2 years among individuals with a record of treated prior PTB. The recruitment was based on Tuberculosis information management system (TBIMS) and manually recorded PTB registration information (MRTRI), which systematically recorded the information of incident PTB cases including diagnosis, treatment regimens and outcomes. The inclusion criteria of study participants were: 1) had been registered as PTB patients, self-reported cured or completed the anti-TB treatment at least two years before the start of the study (June 30, 2016); 2) aged $\geq$ 18 years old; 3) with household registration or residence permit in Zhongmu County. Exclusion criteria were: 1) with current active TB; 2) being pregnant or expectant women; 3) not willing to provide signed informed consent and to complete the necessary investigations.

The ethics committees of the Institute of Pathogen Biology and the Chinese Academy of Medical Sciences approved the study protocol (IPB-2018-1).

Procedures

Baseline survey

The baseline survey was conducted from July to October 2018. We first retrospectively extracted information (demographic, diagnostic and treatment information) of

PTB patients who had completed anti-TB treatment before June 30, 2016, then, we re-checked their recorded information through the door-to-door survey. For those who still lived in the local village and were willing to participate in the current study, a standard questionnaire, chest radiography examination and IGRA testing were performed for each subject. First, a standard questionnaire mainly containing information on the date of birth, gender, smoking and drinking habit, history of diabetes, history of autoimmune disease or immunodeficiency diseases, history of anti-TB treatment and current suspected symptoms of active PTB was administered face by face by trained staff. Then, two radiologists with experienced clinical practice, interpreted each participant's radiographs using standard criteria for reading results, adapted from the People's Republic of China Health Industry Standard (Classification of tuberculosis (WS196—2017)) [16]. Finally, QuantiFERON-TB Gold In-Tube (QFT-GIT) and QFT-Plus (Qiagen; Valencia, CA, USA) were used in parallel to detect mycobacterium tuberculosis infection (MTB) infection according to manufacturer's recommendation with a cut-off value of 0.35 IU/mL [17]. Based on above detection, any participants suspected of having PTB because of clinical symptoms or radiographic abnormalities consistent with suspected active PTB would be referred to local institution. Further diagnostic examination would be conducted to exclude active PTB according to the national guidelines [18]. Briefly, individuals with positive results for any of the three kinds of bacteriological evidence (sputum smear with acid-fast bacillus microscopy, culture, and GeneXpert MTB/RIF assay were defined as bacteriologically confirmed cases. If patients were negative for all three tests, 2-week diagnostic anti-inflammatory therapy using anti-infectious agent but not including anti-tuberculosis drug would be given firstly. If the individual showed no response to the anti-inflammatory treatment, diagnostic anti-TB treatment would be subsequently provided. A diagnosis of clinically diagnosed PTB would be given if a positive response was reported during the anti-TB treatment.

Follow-up survey

In 2019–2020, individuals with baseline positive QFT-GIT results and without suspect active PTB were followed for 24 months. Active case finding was conducted by an annual survey through questionnaire mainly containing information on current suspected symptoms of active PTB and chest radiography screening to identify participants suspected of having PTB. Any participants suspected of having PTB because of clinical symptoms or radiographic abnormalities consistent with active TB would be referred to further diagnosis. The diagnosis procedures were same as baseline survey.

For individuals with baseline QFT-GIT negative results, regular passive case finding was adopted and PTB cases were acquired through TBIMS. All PTB cases registered in the TBIMS during 2019-2020 in the study site were exported and matched with our study participants by identification number.

Statistical analyses

The data were analysed with SAS 9.4. For included individuals, their diagnostic and treatment information came from previous records and for parts of the participants with missing information, the corresponding variables were treated with null values during analyses. Pearson's χ^2 test and Fisher's exact tests were used to compare the different distributions of the categorical variables. Agreement between the QFT-GIT and QFT-plus was calculated and presented with concordance and Cohen's kappa coefficient. Variables with $p < 0.05$ in the univariate analysis were included into the unconditional multivariable logistic regression analyses and the significant associations were assessed with odds ratio (OR) and 95% confidence interval (CI). Tests for linear trend were conducted by Cochran–Armitage (chi-square) tests in the univariate analysis and by entering the categorical variable or continuous variable as an ordinal variable in the multivariable logistic regression analyses. $p < 0.05$ was considered reaching statistical significance.

Results

Major characteristics of the study population

A total of 1713 eligible subjects extracted from TBMIS and MRTRI were still lived in the study site after door-to-door re-checking, 1343 subjects finally participated in the baseline study after excluding 370 subjects who were not willing to provide signed informed consent. Among 1343 participants, 207 participants without QFT-plus testing and 5 participants with indeterminate results were excluded from current analyses. In addition, 58 participants with baseline chest radiography abnormalities suggestive of suspect PTB (11 of them were finally diagnosed with active PTB) and 5 participants with baseline chest radiography abnormalities suggestive of other pulmonary disease were no longer included in 2-year follow-up. After excluding these 275

Table 1. Major characteristics of the study participants.

Variables	N^a (%)
Total	1068
Sex	
Female	339 (31.74)
Male	729 (68.26)
Age	
18–49 years	245 (22.94)
50–59 years	227 (21.25)
60–69 years	282 (26.40)
≥70 years	314 (29.40)
Smoke status	
Never smoked	647 (60.64)
Ever smoked	420 (39.36)
Alcohol drinking	
No	683 (64.07)
Yes	383 (35.93)
With self-reported type 2 diabetes	
No	53 (4.97)
Yes	1013 (95.03)
With self-reported autoimmune disease or immunodeficiency diseases[b]	
No	1024 (95.88)
Yes	44 (4.12)
QFT-GIT results at baseline	
Negative	533 (49.91)
Positive	535 (50.09)
QFT-Plus results at baseline	
Negative	509 (47.66)
Positive	559 (52.34)

QFT-GIT: QuantiFERON-TB Gold In-Tube; QFT-Plus: QuantiFERON-TB Gold Plus.
[a]Sum might not equal to total because of missing data.
bAutoimmune system disease mainly included rheumatoid arthritis, systematics lupus erythematosus, ankylosing spondylitis. Immune deficiency diseases referred to HIV infection.

Table 2. Results of QFT-GIT and QFT-plus among the study participants.

QFT-GIT results	QFT-Plus results			Cohen's κ	Concordance
	Negative	Positive	Total		
Negative	468 (43.82)	65 (6.09)	533 (49.91)	0.80	90.07%
Positive	41 (3.84)	494 (46.25)	535 (50.09)		
Total	509 (47.66)	559 (52.34)	1068 (100.00)		

QFT-GIT: QuantiFERON-TB Gold In-Tube; QFT-Plus: QuantiFERON-TB Gold Plus.

participants, 1068 participants were included in final analyses. Demographic distribution between individuals included in the final analysis and those excluded from the final analysis were showed in Supplementary Table 1. Apart from age, no significant differences were found between the two groups.

As shown in Table 1, nearly 70% (68.26%, 729/1068) of the final included subjects were males with median age of 61 years old (interquartile range: 51 years old-71 years old). The average period between QFT-GIT testing since last episode of PTB was 8 years. There were 39.36% (420/1068) and 35.93% (383/1068) participants had smoking and drinking habit, respectively. Forty-three participants reported a history of autoimmune system diseases. One reported HIV infection. The prevalence of positive QFT-GIT and positive QFT-Plus was 50.09% (535/1068) and 52.34% (559/1068), respectively. The agreement between the results of QFT-GIT and QFT-Plus was shown in Table 2. The concordance of QFT-GIT and QFT-Plus was 90.07% with a Kappa coefficient of 0.80.

IGRA positivity and associated factors

The association analyses with respect to QFT-GIT positivity were shown in Table 3. A higher QFT-GIT positivity was found for those with smoking and drinking habit and those with smear positive results at the last episode of PTB as compared with those without smoking and drinking habit and those with smear negative results with adjusted OR of 1.50 (95% CI: 1.09–2.08), 1.69 (95% CI: 1.23–2.33) and 1.80 (95%CI: 1.33–2.45), respectively. In addition, a reverse dose-response relation was found between increasing age and QFT-GIT positivity with p for trend <0.001. The relation was still statistically significant in multivariable analysis with an adjusted OR of 0.84 (95% CI: 0.74–0.95) for each age group.

PTB recurrence in 2-year follow-up and associated factors

After 2-year follow-up, 71 participants with chest radiographic abnormalities accepted further microbiological test in QFT-GIT positive group. Ten of them were defined as bacteriologically confirmed PTB. The rest 61 participants accepted anti-TB treatment after ineffective anti-inflammatory therapy and 7 of them with improved conditions were clinically diagnosed with active PTB. In addition, after matched with TBIMS by identification number, 4 active PTB cases were identified in QFT-GIT negative group. A total of 21 PTB cases were finally diagnosed with 11 of them were bacteriologically confirmed. Among 11 bacteriologically confirmed cases, 7 cases had drug susceptibility testing results and none of them was multidrug resistant. Detailed information on the 21 PTB cases was shown in Supplementary Table 2.

As displayed in Table 4, in the univariate analyses, the elderly (≥ 60 years), smokers, and those being IGRA positive at baseline were more likely to have PTB recurrence. In addition, a reverse trend was observed between PTB recurrence and diagnosis time of last onset (p for trend <0.001). After adjusting for the co-variables, the elderly (≥60 years old vs 18–59 years old) and smokers showed increased risk of developing active PTB with adjusted OR of 3.97 (95% CI: 1.29–12.24) and 4.56 (95% CI:1.62–12.83), respectively. Participants diagnosed between 2012 and 2015 at last onset were more likely to recurrence than those diagnosed before 2007, with an

Table 3. Association analyses with respect to IGRA positivity among the study participants.

Variables	IGRA positivity n[b]/N[a] (%)	*p* for $\chi 2$ test	Adjusted OR (95% CI)*
Total	600/1068 (56.18)		
Sex			
Female	162/339 (47.79)	**<.001**	Ref.
Male	438/729 (60.08)		0.97 (0.67–1.39)
Age			
18–49 years	160/245 (65.31)	**<.001**	Ref.
50–59 years	139/227 (61.23)		0.88 (0.59–1.33)
60–69 years	147/282 (52.13)		**0.63 (0.43–0.92)**
≥70 years	154/314 (49.04)		**0.62 (0.42–0.90)**
***p* for trend**		**<.001**	**0.84 (0.74–0.95)[c]**
Diagnostic time in last onset			
Before 2007	199/330 (60.30)	0.192	
Between 2007–2011	195/360 (54.17)		
Between 2012–2015	206/378 (54.50)		
Smear positive at last onset			
No	358/693 (51.66)	**<.001**	Ref.
Yes	170/256 (66.41)		**1.80 (1.33–2.45)**
With a history of relapse TB			
No	522/917 (56.92)	0.428	
Yes	78/146 (53.42)		
Smoke status			
Never smoked	329/647 (50.85)	**<.001**	Ref.
Ever smoked	270/420 (64.29)		**1.50 (1.09–2.08)**
Alcohol drinking			
No	344/683 (50.37)	**<.001**	Ref.
Yes	254/383 (66.32)		**1.69 (1.23–2.33)**
With self-reported type 2 diabetes			
No	568/1013 (56.07)	0.538	
Yes	32/53 (60.38)		
With self-reported autoimmune disease or immunodeficiency diseases[d]			
No	581/1024 (56.74)	0.076	
Yes	19/44 (43.18)		

CI: confidence interval; IGRA: interferon gamma release assay; OR: odds ratio; TB: tuberculosis.
[a]The sum of the sample sizes might not equal the total because of missing data.
[b]Individuals with either QFT-GIT positive or QFT-Plus positive were defined as IGRA positive.
[c]Variable entered into the model as ordinal variable.
[d]Autoimmune system disease mainly included rheumatoid arthritis, systematics lupus erythematosus, ankylosing spondylitis. Immune deficiency diseases referred to HIV infection.
*Variables with $p < 0.05$ in the univariate analysis were all entered into multivariable logistic regression.

adjusted OR of 7.71 (95% CI: 1.74–34.25). Compared with baseline IGRA negatives, baseline IGRA positives showed a 9.74-fold (95%CI: 2.20–43.11) risk of developing PTB recurrence (Table 4).

Sensitivity analyses including only PTB cases diagnosed bacteriologically were shown in Supplementary Table 3, major findings were not significantly changed.

Subgroups analysis on the relation of QFT-GIT and QFT-plus with PTB recurrence

The risk of PTB recurrence under different combinations of QFT-GIT and QFT-plus was described in Table 5. The overall risk of developing PTB recurrence increased 5.63 and 6.78 times for individuals with QFT-GIT positives results and QFT-Plus positive results, respectively. Baseline IFN-γ level of QFT-GIT results were divided into four subgroups according to quartiles. A positive dose-response relation between the recurrence risk and IFN-γ level with adjusted OR of 1.41 (95% CI: 1.05–1.88) for each level was found. Compared with QFT-Plus negatives, individuals with antigen tube 2 minus nil (TB2) single positive were observed at a much higher risk of PTB recurrence with an adjusted OR of 15.34 (95% CI: 3.11-75.60) than those with antigen tube 1 minus nil (TB1) and TB2 double positive with an adjusted OR of 6.06 (95% CI: 1.68-21.94). The proportion of TB2 single positive was much higher than that double positive among the elderly and individuals with a recent onset of PTB (Supplementary Table 4). The baseline IFN-γ level of TB2 − TB1 were divided into four subgroups, a positive dose response relation was found between the risk of PTB recurrence and the level of TB2-TB1 with adjusted OR of 1.89 (95% CI: 1.30–2.75) for each level.

Discussions

Though prospectively tracking PTB recurrence and identifying associated risk factors among 1068 individuals with a history of treated prior PTB, we found half of them (56.18%, 600/1068) were IGRA positive at baseline and 1.97% (21/1068) of them developed active PTB in the 2-year follow-up. Elderly people aged ≥60 years, baseline IGRA positive, the last onset of PTB was recent

Table 4. Association analyses with respect to TB recurrence in 2-year follow-up among the study participants.

Variables	TB incidence n/N[a](%)	*p* for $\chi 2$ test	Adjusted OR (95% CI)*
Total	21/1068 (1.97)		
Sex			
Female	3/339 (0.88)	0.083	
Male	18/729 (2.47)		
Age			
18–59 years	4/472 (0.85)	**0.019**	Ref.
≥60 years	17/596 (2.85)		**3.97 (1.29–12.24)**
Diagnostic time in last onset			
Before 2007	2/330 (0.61)	**<.001**	Ref.
Between 2007–2011	2/360 (0.56)		0.88 (0.12–6.34)
Between 2012–2015	17/378 (4.50)		**7.71 (1.74–34.25)**
p for trend		**<.001**	**3.91 (1.75–8.76)[b]**
Smear positive in last onset			
No	12/693 (1.73)	0.097	
Yes	9/256 (3.52)		
With a history of relapse TB			
No	18/917 (1.96)	0.941	
Yes	3/146 (2.05)		
IGRA results at baseline			
Negative	2/468 (0.43)	**0.001**	Ref.
Positive	19/600 (3.17)		**9.74 (2.20–43.11)**
Smoke status			
Never smoked	5/647 (0.77)	**<0.001**	Ref.
Ever smoked	16/420 (3.81)		**4.56 (1.62–12.83)**
Alcohol drinking			
No	11/683 (1.61)	0.259	
Yes	10/383 (2.61)		
With self-reported type 2 diabetes			
No	1/53 (1.89)	1.000	
Yes	20/1013 (1.97)		
With self-reported autoimmune disease or immunodeficiency diseases [c]			
No	20/1024 (1.95)	0.590	
Yes	1/44 (2.27)		

CI:, confidence interval; IGRA: interferon gamma release assay; OR: odds ratio; TB: tuberculosis.
[a]The sum of the sample sizes might not equal the total because of missing data.
[b]Variable entered into the model as ordinal variable.
[c]Autoimmune system disease mainly included rheumatoid arthritis, systematics lupus erythematosus, ankylosing spondylitis. Immune deficiency diseases referred to HIV infection.
*Variables with $p < 0.05$ in the univariate analysis were all entered into multivariable logistic regression.

(2012-2015) and smokers were found to be associated with increased risk of PTB recurrence. Additionally, with respect to baseline results of QFT-Plus, subjects with TB2 single positive were found at a higher risk of PTB recurrence than those with TB1 and TB2 double positive. A dose response relation was also found between the risk of PTB recurrence with the baseline level of TB2-TB1 (p for trend < 0.001). It might suggest the clinical value of the TB2 tube in indicating recent infection and predicting disease occurrence, but such speculation needs further verification by mechanism research and longitudinal studies with large sample size.

Few studies investigated the results of IGRAs in individuals with healed TB previously. A report conducted in 2009 evaluated the response to the QuantiFERON TB-2G (QFT-2G) test in 208 patients with healed pulmonary TB and found 34% of them were positive [19]. In our study population, around half of them were QFT-GIT positive. In addition, a negative relationship between increasing age and IGRA positivity was found, which were contrary to our previous study conducted in rural residents [20]. In the present study, all included participants were regarded with MTB infection at the last onset of TB, the criteria of cure are not the elimination of infection, but the improvement of symptoms and the absence of bacteria in sputum. Therefore, the chance of early detection of the disease, the severity of the disease, and the response to treatment of TB patients of different ages may influence infection status after treatment as well. Furthermore, the attenuated test sensitivity among the elderly might be another potential underlying explanation for the reverse relation between age and IGRA positivity [21,22]. Our previous and current prospective study also identified incident cases among subgroups with negative QFT-GIT results [7]. Therefore, it is noteworthy that the limited sensitivity of LTBI testing in the elderly needs to be kept in mind, the incident cases that occurred from baseline IGRA negatives might not completely attribute to exogenous reinfection.

Due to QFT-Plus had not been approved in China until 2021, data on the performance of QFT-plus was very few especially among individuals with prior TB. Comparing QFT-GIT and QFT-plus in different populations has important practical guidance significance. A

Table 5. Subgroup analysis between QFT-GIT and QFT-plus results with the risk of TB recurrence in 2-year follow-up.

Variables	TB incidence n/N (%)	*p* for $\chi 2$ test	Adjusted OR (95% CI)*
Total	21/1068 (1.80)		
QFT-GIT			
Negative	4/533 (0.75)	**0.004**	Ref.
Positive	17/535 (3.18)		**5.63 (1.82–17.42)**
QFT-Plus			
Negative	3/509 (0.59)	**0.002**	Ref.
Positive	18/559 (3.22)		**6.78 (1.93–23.85)**
TBAg-Nil (IU/ml)			
<0.35	4/533 (0.75)	**0.039**	
0.35-0.95	6/133 (4.51)		
0.95-2.43	4/134 (2.99)		
2.43-6.19	4/133 (3.01)		
≥6.19	3/135 (2.22)		
P for trend		0.083	**1.41 (1.05–1.88)[a]**
QFT-GIT/QFT-plus results			
Double negative	2/468 (0.43)	0.016	Ref.
QFT-plus single positive	2/65 (3.08)		**7.76 (1.03–58.41)**
QFT-GIT- single positive	1/41 (2.44)		9.86 (0.81-120.63)
Double positive	16/494 (3.24)		**10.08 (2.24–45.35)**
TB 1 and TB 2 results			
QFT-Plus negative	3/509 (0.59)	<0.001	Ref.
TB2 single positive	4/43 (9.30)		**15.34 (3.11–75.60)**
TB1 single positive	0/16 (0.00)		NA
TB1 and TB2 double positive	14/500 (2.80)		**6.06 (1.68–21.94)**
TB2-TB1 results			
QFT-Plus negative	3/509 (0.59)	0.006	Ref.
<-0.02 IU/ml	3/164 (1.83)		
−0.02-0.02 IU/ml	3/105(2.86)		
≥0.02 IU/ml	12/290 (4.14)		
P for trend		<0.001	**1.89 (1.30–2.75)[a]**

CI: confidence interval; NA: not available; QFT-GIT: QuantiFERON-TB Gold In-Tube; QFT-Plus: QuantiFERON-TB Gold Plus; OR: odds ratio; TB: tuberculosis. TB1: antigen tube 1 minus nil; TB2: antigen tube 2 minus nil.
*Age, diagnostic time in last onset and smoke status were entered into multivariable logistic regression.
[a]Variable entered into the model as ordinal variable.

good agreement between QFT-GIT and QFT-Plus were found in our study population and the new QFT-Plus test demonstrated a higher sensitivity than QFT-GIT as the TB2 tube contains an additional set of peptides targeted for a cell-mediated immune response from $CD8^+$ cytotoxic T lymphocytes. Our results consistently confirmed the extra value of TB2 tube. When QFT-plus results were divided into four classes according to TB1 and TB 2 qualitative results. Subjects with baseline TB2 single positive showed the highest risk of recurrence with adjusted OR as high as 15. Then the baseline level of TB2-TB1 were classified into four groups, a positive dose response relationship was observed between TB recurrence and the difference. From both qualitative and quantitative perspective, TB2 single positive showed a higher predicting value. Whether the $CD8^+$ T-cell response reflected by TB2 tube could be served as a marker of recent TB infection, there are still no consistent conclusion. In previous studies [12,13,23], $CD8^+$T-cell activity as measured with TB2-TB1 showed a significant association with recent exposure, exposure time and positive sputum smear of the index case. While another study got a completely opposite result, no differences were found between the distribution of TB2 − TB1 values with sputum smear microscopy result of the index case or extent of exposure [24]. For cross-sectional study design, it was easy to understand the inconsistence conclusion due to the difference of study population, the definition of contact, exposure time and frequency. Thus, cohort study is needed to track TB development from contacts with different baseline TB2-TB1 values to explore the possible causal relationship. Our study indeed based on a prospective design, but unfortunately, information on close contact was not collected. Thus, whether the TB2 tube, had the clinical value for predicting TB recurrence in patients with a history of treated prior TB needs further exploration.

Previous studies mainly investigated TB recurrence in a short time after treatment, but we did not limit the time since the completion of treatment in order to observe the contribution of exogenous reinfection to TB recurrence. As well, it permits us to estimate a reversed relation between the time from the last onset of TB and the risk of TB recurrence. The closer to the last diagnosis, the higher risk of relapse rather than exogenous re-infection. Compared with relapse which occurred relatively more concentrated, recurrence caused by re-infection usually occurred separately over the subsequent years [25]. Based on the above

evidence, individuals with a history of treated prior TB should be attached more importance for preventing re-exposure and monitoring TB recurrence, especially among those concomitants of other risk factors, such as with TB2 single positive result, recent onset and smokers. As endogenous relapse was significantly more common than exogenous reinfection [5], how to avoid the occurrence of drug-resistant TB in preventing TB recurrence caused by last insufficient treatment need consideration. Immune therapy instead of chemoprophylaxis, such as using Vaccae vaccine, might be an alternative tool for preventive treatment. Of course, evidence from clinical trials are needed to support our results.

When interpreting our results, several limitations should be kept in mind. First, the study participants might not completely represent the population with a history of treated prior TB in rural China. The severity of the last onset PTB and the concern about privacy might influence their current health status and their willingness to participate in the study, this will inevitably result in selection bias. Second, active PTB case finding was conducted only for baseline QFT-GIT positives, which might make the diagnosis of relapse/re-infection more probable or earlier. While the information on PTB cases among QFT-GIT negatives were only collected from TBIMS through passive case finding. In addition, individuals with prior PTB diagnosed between 2016 and 2017 were more likely to develop recurrent PTB, but they were not included in the study in order to avoid the potential influence of insufficient treatment on recurrence. Thus, the recurrence rate observed in the current study might not reflect the true recurrence rate in the study population, and the exploration of related risk factors might be limited by the insufficient number of events. Third, around half of recurrence PTB cases were clinically diagnosed in the present study, we could not completely exclude the potential bias caused by a misdiagnosis although we took strict diagnosis criteria. As a sensitivity analysis, our major findings were not significantly changed when only addressing bacteriologically confirmed cases. Fourth, the treatment and management of patients with PTB are unified by the country, 6-month regimen composed of four first line TB medicines – isoniazid, rifampicin, ethambutol and pyrazinamide (2HRZE/4HR) was generally recommended to patients with drug susceptibility testing (DST) sensitive or unknown. Although the included participants were self-reported cured or registered with completed regular regimen mentioned above, as the treatment were not under fully directly observed therapy, the possibility of inaccurate treatment outcome caused by memory bias or non-standard recording cannot be ruled out. Fifth, other known recurrence risk factors including BMI, underlying lung disease, cavitary disease, and new exposure to PTB causing re-infection during follow up period were absent, risk factors identified in current study could not fully explain the risk of PTB recurrence. In addition, as we did not limit the participants' diagnostic time of their last PTB, it is difficult to determine the timing of IGRA conversion, since those IGRA positives at baseline might have already been positive even before the first PTB diagnosis, which could undermine the value of the association between post treatment IGRA status and recurrence risk. Sixth, only 7 of 11 bacteriologically confirmed TB cases' drug susceptibility testing were available and none of them was multidrug resistant, we can't speculate whether there was more resistance among those treated TB cases due to the limited data.

In summary, MTB infection control and surveillance of active PTB should be further strengthened for individuals with treated prior PTB, especially for those with recent onset of active disease. The result of QFT-Plus, especially of the TB2 tube, showed a potential predictive value for PTB recurrence. Further studies involved more study sites with varied TB burdens and with larger sample size are needed to verify our findings.

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Author Contributors

LG and QJ designed the study. WD, SP, ZL, DW and BZ organised the implement of the study. HX, LG, FS and JD did epidemiological investigation and quality control. BF, XC, LS, YH, YD and TG did IGRA test. XG and ZZ interpreted radiographs. HX and LG did data management and data analyses. LG and HX wrote the report. All authors contributed to review and revision and have seen and approved the final version of manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary material.

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