EXTENDED REPORT

Biphasic emergence of active tuberculosis in rheumatoid arthritis patients receiving TNFα inhibitors: the utility of IFNγ assay

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ABSTRACT

Objectives The risk of active tuberculosis increases in rheumatoid arthritis (RA) patients receiving antitumour necrosis factor alpha (TNFα) therapy. Longitudinal data concerning serial interferon γ (IFNγ) assays for detecting tuberculosis have been limited. This study investigated the time course of the development of active tuberculosis, and evaluated the utility of serial QuantiFERON-TB Gold (QFT-G) assays for detecting its emergence in RA patients undergoing long-term anti-TNFα therapy.

Methods 242 RA patients who received anti-TNFα therapy and serial QFT-G assays were prospectively evaluated. QFT-G was performed by measuring IFNγ levels in whole blood treated with tuberculosis-specific antigens.

Results Among 242 RA patients, 75 (31.0%) had a positive tuberculin skin test (TST) and 45 (18.6%) had positive QFT-G results, with another nine (3.7%) showing indeterminate QFT-G assay. Isoniazid prophylaxis was given to 37 patients with TST+/QFT-G+ results and 24 TST+/QFT-G− patients with TST induration diameter ≧10 mm. Four patients (three with baseline QFT-G+ results) developed tuberculosis within the first 3 months of anti-TNFα therapy, whereas five patients with baseline TST−/QFT-G− results developed active tuberculosis after 20–24 months' anti-TNFα therapy. Progressively rising levels of released IFNγ (2.17±0.98 vs 5.93±2.92 IU/ml in early secretory antigenic target-6-stimulated well; 1.12±0.84 vs 2.96±1.02 IU/ml in culture filtrate protein-10-stimulated well) were observed in those who developed tuberculosis early in anti-TNFα therapy. QFT-G conversion was found in baseline QFT-G-negative patients who developed tuberculosis late in treatment.

Conclusion The emergence of active tuberculosis follows a biphasic pattern. Persistently high levels of released IFNγ or QFT-G conversion strongly indicate the development of active tuberculosis in patients undergoing long-term anti-TNFα therapy.

Despite the extensive implementation of bacillus Calmette–Guérin (BCG) vaccination for infants with a booster for 7–10-year-old children in Taiwan, tuberculosis is prevalent at a notification rate, ranging from 74/100 000 people in 2004 to 85/100 000 people in 2007.[1 2] An increased prevalence of active tuberculous disease has been reported in rheumatoid arthritis (RA) patients,[3] and the risk of active tuberculosis increased further in those receiving antitumour necrosis factor alpha (TNFα) therapy.[4 5] Guidelines have recommended that effective tuberculosis screening should be routinely carried out and prophylactic therapy be initiated before starting anti-TNFα therapy if latent tuberculosis infection (LTBI) exists.[6 7]

The tuberculin skin test (TST) has several drawbacks including variability in test application, and low specificity due to the presence of purified protein derivative in non-tuberculous mycobacteria as well as in BCG strains.[8 9] Moreover, the application of TST for detecting LTBI is limited in RA patients by the frequent presence of anergy.[10] Therefore, the clinical utility of TST is not reliable in BCG-vaccinated RA patients.

Recent studies showed that QuantiFERON-TB Gold (QFT-G) assays, which detect interferon gamma (IFNγ) secreted by T cells stimulated with *Mycobacterium tuberculosis*-specific antigens, offer higher specificity than TST in detecting LTBI (96% vs 59%) or active tuberculous disease (99% vs 81%) for a BCG-vaccinated population.[11–13] The validity of the QFT-G assay has been documented in LTBI screening for RA patients.[14–17] However, the QFT-G assay has limitations of sensitivity similar to those of TST and has an increased rate of indeterminate results in immunocompromised hosts.[18 19]

According to the updated guidelines for using IFNγ release assays,[20] RA patients who are receiving anti-TNFα therapy are a high-risk population for the progression of LTBI to active tuberculous disease. It is clinically important to determine a test's ability to detect the emergence of active tuberculosis in RA patients undergoing anti-TNFα therapy. Recently, Diel *et al*[21] reported that the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay was superior to TST in predicting progression to tuberculosis in close contacts with active tuberculosis patients. However, there are scant longitudinal data concerning the clinical utility of serial QFT-G assays in detecting the emergence of active tuberculosis in RA patients undergoing anti-TNFα therapy.

The aim of this study was to investigate the dynamic change in the released IFNγ levels by serial QFT-G assays and the emergence of active tuberculosis in RA patients undergoing long-term anti-TNFα therapy in an intermediate tuberculosis burden area.

METHODS

Study population

Two hundred and forty-two consecutive patients with RA[22] scheduled for anti-TNFα therapy were enrolled between 2006 and 2009. All patients had persistently active disease after having been treated with disease-modifying antirheumatic drugs (DMARD), so that anti-TNFα therapy was initiated based on the British Society for Rheumatology guidelines.[23] RA patients were excluded if they had clinically active tuberculosis infection or characteristic radiological findings suggesting healed pulmonary tuberculosis. Disease activity was assessed by the 28-joint disease activity score (DAS28).[24] Active tuberculous disease was proved by positive culture or pathological findings of tissue biopsy. The Clinical Research Ethics Committee at our hospital approved this study and each participant's written consent was obtained.

TST and QFT-G assay for RA patients

All patients were evaluated at baseline using a standardised interview, chest radiographs, TST and QFT-G assays before anti-TNFα therapy. Blood was drawn for QFT-G assay, followed by TST using the Mantoux method that was done by the intradermal injection of two tuberculin units of purified protein derivative RT-23 (Staten Serum Institute, Copenhagen, Denmark). If the induration diameter was 5 mm or greater, the result was considered positive TST.[25] Because the QFT-GIT assay was not available until August 2008 in Taiwan, the second-generation QFT-G assay was performed according to the manufacturer's instructions (Cellestis Ltd, Carnegie, Australia). The results of the QFT-G assay were considered positive if the IFNγ level was 0.35 IU/ml or greater in the antigens (early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10))-stimulated wells after subtracting the level of the nil well.[11] All TST+/QFT-G+ patients and 24 patients who had TST+/QFT-G− results with TST induration diameter 10 mm or greater received isoniazid prophylaxis (INHP) 1 month before and 8 months into the anti-TNFα therapy. All patients received serial QFT-G assays including at the ninth month for evaluating the possible effects of INHP on QFT-G results, and at the 18th and 24th month for evaluating the effects of TNFα inhibitors on QFT-G results. To assess the utility of the QFT-G assay for detecting the emergence of tuberculosis, we also performed the QFT-G assay at the first time of any sign or symptom suggestive of active tuberculosis in patients undergoing anti-TNFα therapy (figure 1). QFT-G conversions were defined as baseline IFNγ less than 0.35 IU/ml and follow-up IFNγ of 0.35 IU/ml. QFT-G reversions were defined as baseline IFNγ of 0.35 IU/ml and follow-up IFNγ of less than 0.35 IU/ml.[26]

Statistical analysis

Results are presented as mean±SD unless specified otherwise. The non-parametric Kruskal–Wallis test was used for between-group comparison. Only when this test showed significant differences were the exact p values determined using the Mann–Whitney U test. For the comparison of IFNγ release levels before and after anti-TNFα therapy or anti-tuberculosis therapy during follow-ups, the Wilcoxon signed rank test was employed. p Values less than 0.05 were considered statistically significant.

RESULTS

Demographic data, clinical characteristics and results of TST and QFT-G assay

Among 242 RA patients, 75 (31.0%) patients had positive TST and 45 (18.6%) had positive QFT-G results, with another nine

Table 1 Demographic data and laboratory findings of four subgroups of RA patients according to the results of TST and QFT assay†

	RA patients (n=233)			
	TST (−)	TST (−)	TST (+)	TST (+)
	QFT (−)	QFT (+)	QFT (−)	QFT (+)
	(n=150)	(n=8)	(n=38)	(n=37)
Mean age, years	54.9 ± 14.3	58.3 ± 14.1	49.0 ± 12.4	59.0 ± 12.3*
Female proportion	127 (84.7%)	7 (87.5%)	33 (86.8%)	25 (67.6%)
Disease duration, years	9.2 ± 3.1	10.5 ± 2.5	8.3 ± 3.7	8.6 ± 2.9
Radiographic stage (III + IV)	130 (86.7%)	7 (87.5%)	29 (76.3%)	31 (83.4%)
RF positivity	106 (70.7%)	6 (75.0%)	26 (68.4%)	27 (73.0%)
Anti-CCP positivity	90 (60.0%)	4 (50.0%)	22 (57.9%)	27 (73.0%)
BCG vaccination	147 (98.0%)	8 (100%)	37 (97.4%)	36 (97.3%)
Baseline DAS28	7.13 ± 0.68	6.97 ± 0.88	7.21 ± 0.66	6.98 ± 0.75
Daily steroid dose (mg)	5.5 ± 1.2	5.3 ± 0.9	5.8 ± 1.3	5.4 ± 0.9
Used DMARD				
Methotrexate	146 (97.3%)	8 (100%)	38 (100%)	35 (94.6%)
Sulfasalazine	140 (93.3%)	7 (87.5%)	37 (97.4%)	36 (97.3%)
Hydroxychloroquine	142 (94.7%)	8 (100%)	37 (97.4%)	35 (94.6%)
Ciclosporine	42 (28.0%)	2 (25.0%)	10 (26.3%)	8 (21.6%)
TNFα inhibitors				
Etanercept	101 (67.3%)	3 (37.5%)	15 (39.5%)	22 (59.5%)
Adalimumab	49 (32.7%)	5 (62.5%)	23 (60.5%)	15 (40.5%)
Frequency of comorbidities				
Diabetes mellitus	11 (7.3%)	0 (0.0%)	2 (5.3%)	3 (8.1%)
Anaemia (<9.0 g/dl)	16 (10.7 %)	1 (12.5%)	4 (10.5%)	5 (13.5%)

*p<0.05, versus RA patients with TST+/QFT− results.
†Values are mean ± SD or the number (%) of patients.
Anti-CCP, anticyclic citrullinated peptide antibodies; DAS28, disease activity score for 28 joints; DMARD, disease-modifying antirheumatic drug; QFT, quantiFERON; RA, rheumatoid arthritis; RF, rheumatoid factor; TNFα, tumour necrosis factor alpha; TST, tuberculin skin test.

Table 2 Demographic data and clinical characteristics of active tuberculosis in nine patients with RA undergoing anti-TNFα therapy

	Age/sex	Duration of RA (years)	Baseline TST/QFT	INHP	Used TNFα inhibitors	Duration of anti-TNF before tuberculosis (months)	Concomitant medications	Location of active tuberculosis	Anti-tuberculosis drug sensitivity
1	66/F	8.5	TST+/QFT+	+	Adalim.	2	MTX 15 mg/week PSL 5 mg/day	Pulmonary	INH-R RIF-S
2	54/F	10.6	TST+/QFT+	+	Adalim.	3	MTX 12.5 mg/week PSL 7.5 mg/day	Pulmonary	INH-S
3	62/F	10.2	TST−/QFT+	−	Adalim.	3	MTX 15 mg/week PSL 7.5 mg/day	Miliary	INH-S
4	72/F	8.5	TST−/QFT−	−	Adalim.	3	MTX 10 mg/week PSL 5 mg/day	Pleura (Lt)	INH-S
5	68/F	8.3	TST−/QFT−	−	Etaner.	20	MTX 15 mg/week PSL 5 mg/day	Miliary	INH-S
6	44/F	9.2	TST−/QFT−	−	Etaner.	22	MTX 12.5 mg/week PSL 5 mg/day	Pulmonary	INH-S
7	55/M	8.4	TST−/QFT−	−	Adalim.	23	MTX 15 mg/week PSL 7.5 mg/day	Pleura (Lt)	INH-S
8	40/F	12.2	TST−/QFT−	−	Etaner.	23	MTX 15 mg/week PSL 5 mg/day	Joint (5th MTP)	NA
9	61/F	10.8	TST−/QFT−	−	Adalim.	24	MTX 15 mg/week PSL 5 mg/day	Pulmonary	INH-S

Adalim, adalimumab; Etaner, etanercept; F, female; INHP, isoniazid prophylaxis; INH-R, resistant to isoniazid; INH-S, sensitive to isoniazid; Lt, left side; M, male; MTP, metatarsophalangeal joint; MTX, methotrexate; NA, not applicable; PSL, prednisolone; QFT, QuantiFERON-G assay; RA, rheumatoid arthritis; RIF-S, sensitive to rifampicin; TNFα, tumour necrosis factor alpha; TST, tuberculin skin test.

(3.7%) showing indeterminate QFT-G assay. After the exclusion of those with indeterminate QFT-G assay, the remaining 233 patients were divided into four groups: 37 (15.9%) patients with TST+/QFT-G+ results; 38 (16.3%) with TST+/QFT-G− results; eight (3.4%) with TST−/QFT-G+ results; and 150 (64.4%) with TST−/QFT-G− results (table 1). A higher proportion of patients with TST greater than 10 mm (31/37, 83.8%) was observed in the TST+/QFT-G+ subgroup than that (24/38, 63.2%, p=0.067) in the TST+/QFT-G− subgroup.

There were no significant differences in the percentage of women, the proportion of patients with BCG vaccination, positive rate of rheumatoid factor or anticyclic citrullinated peptide antibodies, DAS28, the daily dosage of corticosteroids, the proportion of DMARD used, or the frequency of comorbidities among the four subgroups of RA patients (table 1).

Characteristics of RA patients with the emergence of active tuberculosis during anti-TNFα therapy

Of the 233 RA patients who received anti-TNFα therapy, nine (3.9%) developed active tuberculous disease. As shown in table 2, five (56%) of them had extrapulmonary involvement, including miliary (2/5), pleura (2/5), and the 5th metatarsophalangeal joint (1/5). Our results showed the biphasic emergence of active tuberculous disease: four patients developed active tuberculosis within the first 3 months of anti-TNFα therapy, while the other five patients with baseline TST−/QFT-G− results developed active tuberculosis after 20–24 months of anti-TNFα therapy (table 2). None of the TST+/QFT− patients developed active tuberculous disease or had QFT-G conversion after 24 months' anti-TNFα therapy (figure 1). No significant differences were observed in demographic data, radiographic staging, or disease activity between RA patients with and without active tuberculosis (data not shown).

Change in IFNγ release levels in RA patients undergoing anti-TNFα therapy

After the exclusion of nine patients who developed active tuberculous disease, the mean duration of anti-TNFα therapy was 30.9±6.5 months. As shown in figure 1,a total of 233 RA patients received serial QFT-G assays. During the first 3-month anti-TNFα therapy, two patients with LTBI (TST+/QFT+) developed active tuberculosis despite INHP. One TST−/QFT+ patient with a high IFNγ release level in the absence of INHP subsequently developed active tuberculosis in the third month of anti-TNFα therapy. As shown in figure 2A, IFNγ release levels significantly decreased after the initiation of INHP, and declined continuously during the period of anti-TNFα therapy in QFT-G-positive patients without developing active tuberculosis. Moreover, seven TST−/QFT-G+ patients who did not develop active tuberculosis were found to have QFT-G reversion after 24 months' anti-TNFα therapy. In contrast, an early increased level of released IFNγ paralleled the development of active tuberculosis in three patients with QFT-G-positive results (figure 2B). Among the 150 patients without LTBI (baseline TST−/QFT-G−), the levels of released IFNγ showed no significant change in those who did not develop active tuberculosis during anti-TNFα therapy for more than 24 months (figure 3A). Three of those patients had indeterminate results at follow-up QFT-G assay (data not shown). However, the levels of released IFNγ markedly increased at the time of active tuberculosis infection in one patient after receiving 3 months of anti-TNFα therapy, and in another five patients after treatment for 20–24 months (figure 3B).

DISCUSSION

An increased prevalence of active tuberculosis has been reported among RA patients receiving anti-TNFα therapy.[4 5] In the present study, nine of 233 RA patients (3.9%) developed active tuberculous disease during the 2-year period of anti-TNFα therapy. Similar to the results of previous reports showing a high prevalence of extrapulmonary tuberculosis in patients receiving anti-TNFα therapy,[4] five (56%) of our nine patients who developed active tuberculosis had extrapulmonary involvement.

This study is the first attempt to investigate the kinetics of IFNγ release levels in RA patients with different TST/QFT-G status during their anti-TNFα therapy. Our results demonstrated the biphasic emergence of active tuberculous disease during long-term anti-TNFα therapy in RA patients: the early emergence occurred within the first 3 months and the late emergence after 20–24 months' therapy. Active tuberculous disease could be caused by LTBI reactivation in those with early emergence

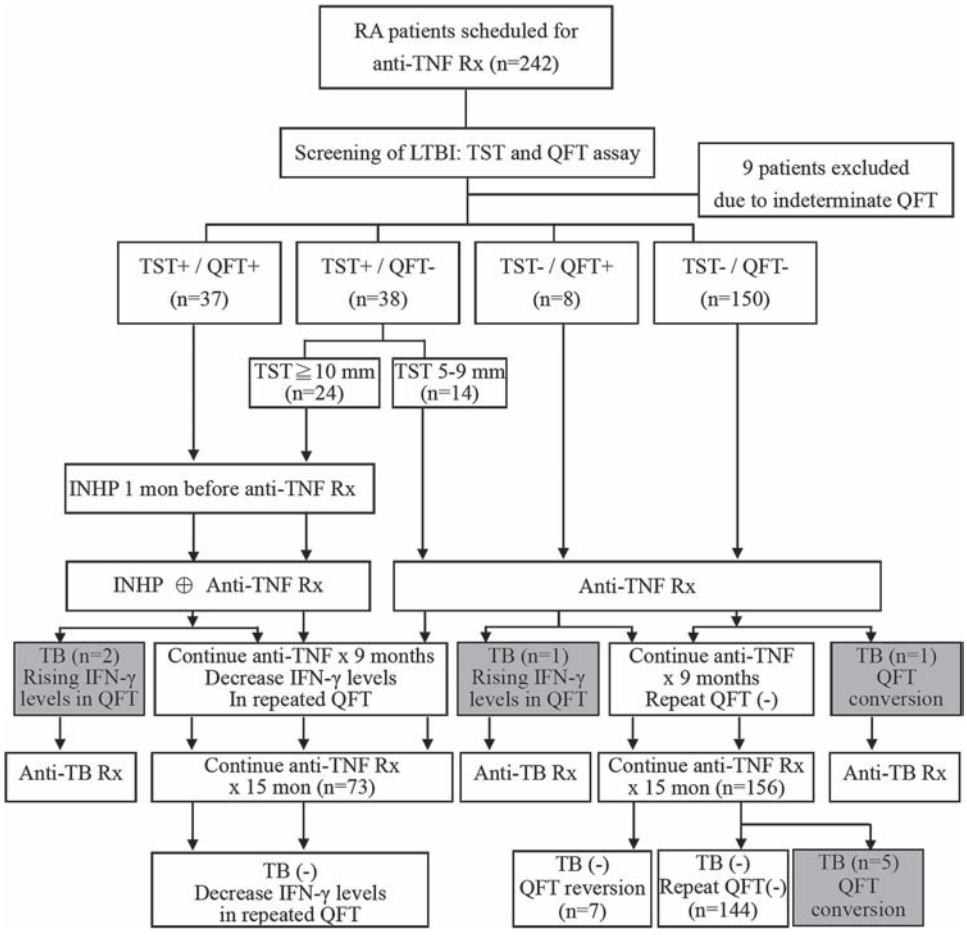

Figure 1 Flow chart shows the distribution of results of tuberculin skin test (TST) and QuantiFERON-TB Gold (QFT) in 242 patients with rheumatoid arthritis (RA) before and after anti-tumour necrosis factor alpha (TNFα) therapy. Nine patients developed active tuberculosis (TB) disease after anti-TNFα therapy. QFT-G conversions were defined as baseline interferon gamma (IFNγ) less than 0.35 IU/ml and follow-up IFNγ of 0.35 IU/ml or greater. QFT-G reversions were defined as baseline IFNγ of 0.35 or greater and follow-up IFNγ less than 0.35 IU/ml. INHP, isoniazid prophylaxis; LTBI, latent tuberculosis infection; QFT+, positive QFT result; QFT−, negative QFT result; TST+, positive TST; TST−, negative TST.

of tuberculosis, and by de-novo re infection in those with baseline TST−/QFT-G− results and late development of tuberculosis. We also showed that continuously rising levels of released IFNγ paralleled the early emergence of active tuberculous disease in certain QFT-G-positive patients undergoing anti-TNFα therapy, while QFT-G conversion was observed in QFT-G-negative patients who developed active tuberculosis late in the treatment course. In agreement with the results of previous studies showing that the QFT-G assay had better specificity than TST,[12 27] our data support the potential utility of serial QFT-G assays for detecting tuberculosis infection in BCG-vaccinated patients receiving long-term anti-TNFα therapy.

The lower positive rate of baseline TST (31.0%) in our RA patients when compared with the general population was in accordance with the results reported by Ponce de Leon *et al*[28] (29.4%). The use of immunosuppressive agents and immune dysfunction related to RA may be the reasons for the reported false-negative TST.[28 29] Our data support the findings of previous reports claiming the limited utility of TST for detecting LTBI in immunocompromised patients.[28 29] Among eight patients with TST−/QFT-G+ results, one patient who had a high baseline IFNγ level (2.07 IU/ml in ESAT-6-stimulated well) developed active tuberculosis. Although INHP was not given to our patients with TST−/QFT-G+ results according to Taiwan's Centers for Disease Control recommendation, we closely monitored them to detect active tuberculous disease early. In contrast, the other seven patients with low IFNγ levels at the baseline QFT-G test (mean±SD, 0.67±0.23 IU/ml) did not develop active tuberculosis and showed subsequent QFT-G reversion. Consistent with the results of recent studies,[30 31] our findings suggest that a QFT-G-positive assay with a low IFNγ level (0.35–1.0 IU/ml) in TST-negative patients may be considered to be a false-positive QFT-G result.[30] However, this hypothesis still needs to be validated in a future study. For better safety, INHP should be given to RA patients with TST−/QFT-G+ results at baseline before anti-TNFα therapy.

There were more patients with positive TST than those having QFT-G-positive results (31.0% vs 18.6%) before starting anti-TNFα therapy. As shown in the follow-up data for those with discordant TST+/QFT-G− results, no patients developed active tuberculosis even in the absence of INHP during anti-TNFα therapy (figure 1). The discordant TST+/QFT-G− results in our patients lacking an active tuberculosis exposure history seem to be due to universal BCG vaccination or a high prevalence of non-tuberculous mycobacteria in Taiwan.[32] Our results were consistent with those of recent studies showing that the QFT-G assay is a better diagnostic tool than TST for detecting LTBI in BCG-vaccinated populations.[12 33] The National Institute for Health and Clinical Excellence in the UK has recommended the IFNγ-release assay (IGRA) for confirming a positive TST or in

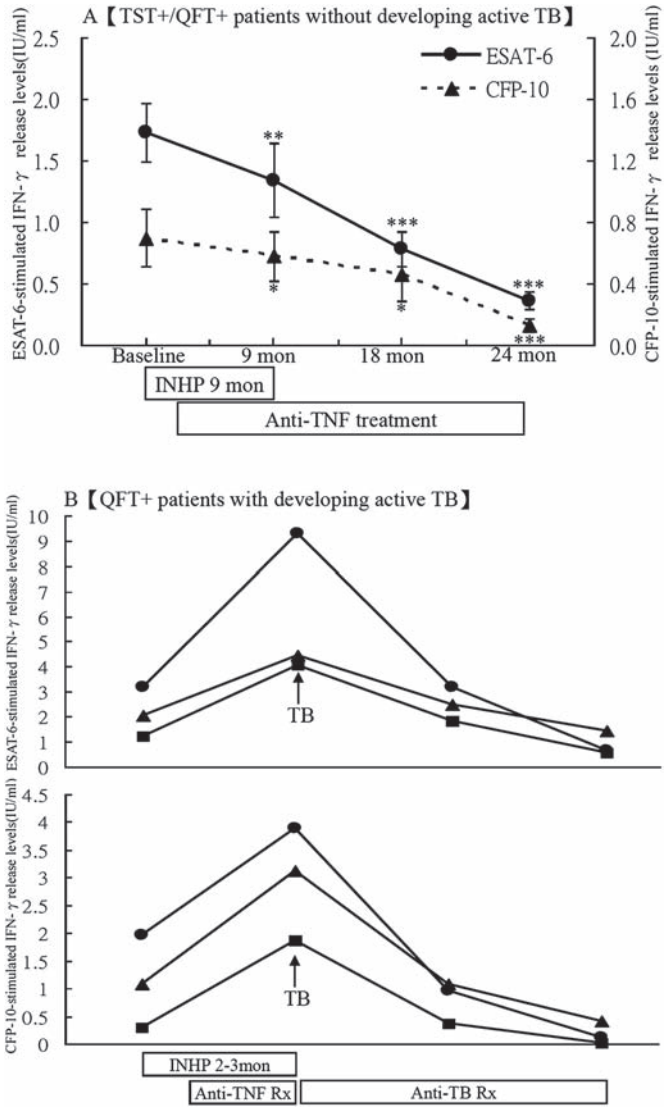

Figure 2 The kinetics of interferon gamma (IFNγ) release levels in response to tuberculosis (TB)-specific antigens (early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10)) in rheumatoid arthritis patients undergoing anti-tumour necrosis factor alpha (TNFα) therapy, including (A) 35 patients with baseline TST+/QFT+ results who did not develop active tuberculous disease, and (B) three patients with baseline QFT+ results who subsequently developed active tuberculous disease. Data are presented as mean±SEM. *p<0.05, **p<0.005, ***p<0.001, versus values at baseline. INHP, isoniazid prophylaxis; QFT, QuantiFERON-TB Gold assay; TST, tuberculin skin test.

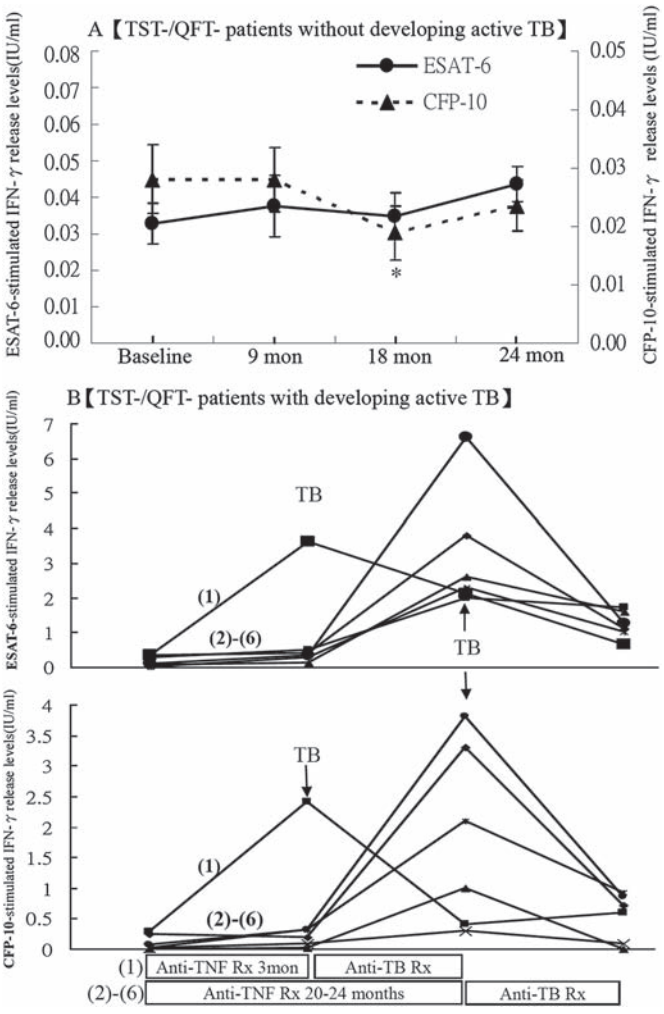

Figure 3 The kinetics in interferon gamma (IFNγ) release levels in response to tuberculosis (TB)-specific antigens (early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10)) in rheumatoid arthritis patients undergoing anti-tumour necrosis factor alpha (TNFα) therapy, including (A) 144 patients with baseline TST−/QFT− results who did not develop active tuberculous disease, and (B) six patients with baseline concordant TST−/QFT− results who subsequently developed active tuberculous disease ((1) patient developed tuberculosis pleurisy after receiving 3-month anti-TNFα therapy, and (2)–(6) patients developed active tuberculous disease after 20–24-month anti-TNFα therapy). Data are presented as mean ± SEM. *p<0.05, versus values at baseline. QFT, QuantiFERON-TB Gold assay; TST, tuberculin skin test.

individuals for whom the TST might be less reliable.[34] The false-positive TST results in our study also support the recently proposed TST/IGRA dual strategy for LTBI screening.[35] Physicians' growing awareness of the limited specificity of TST in vaccinated populations may reduce unnecessary INHP and the related risk of drug toxicity.[36 37] However, QFT-G-negative results should not be used to exclude the possibility of active tuberculosis in those with suggestive signs or symptoms because of the suboptimal sensitivity of the QFT-G assay.[18 19 38]

In the present study, 37 patients who had TST+/QFT-G+ results at baseline could be regarded as having LTBI and all received INHP. Consistent with the results of previous studies,[39] our findings indicate that the levels of released IFNγ significantly decreased after initial INHP therapy. Moreover, the levels of released IFNγ further declined after anti-TNFα therapy in our patients who did not develop active tuberculous disease. Our results support the findings of a recent study showing that TNF inhibitors could suppress IFNγ release in response to tuberculosis-specific antigens.[40] In contrast, continuously rising levels of released IFNγ were in parallel with the early emergence of active tuberculosis in the other two patients, suggesting reactivation of LTBI as the most probable cause. Our results were supported by the findings of epidemiological studies indicating a positive correlation between bacterial loads of *M tuberculosis* and IFNγ levels in response to ESAT-6.[41] The occurrence of active tuberculosis in our patients despite INHP may result from isoniazid resistance (as in one case) or inadequate chemoprophylaxis, which would be consistent with the findings of a recent report.[42]

Because 3 months' treatment with rifampicin and isoniazid has been shown to be as effective as 6–9 months of INHP,[43 44] rifampicin and isoniazid chemoprophylaxis seems to be a promising alternative to INHP before starting anti-TNFα therapy for RA patients with LTBI.

Previous studies reported a high negative predictive value (>95%) for active tuberculous disease in patients with baseline TST−/QFT-G− results.[45 46] In the present study, one patient with a baseline TST−/QFT-G− result developed active extrapulmonary tuberculosis after receiving 3 months' anti-TNFα therapy. Considering the extrapulmonary involvement and the short interval between the initiation of anti-TNFα therapy and the onset of active tuberculosis, the emergence of active tuberculosis in this patient could be due to LTBI reactivation. Our findings agree with the results of a meta-analysis showing relatively low sensitivity of the QFT-G assay and TST in detecting LTBI for immunocompromised hosts.[12 18 19]

Besides, our five patients with baseline TST−/QFT-G− results later developed active tuberculosis after 20–24 months' anti-TNFα therapy. Considering the long interval between the initiation of anti-TNFα therapy and the onset of active tuberculosis, a recent exposure to tuberculosis infection could be the probable cause of active tuberculous disease for immunocompromised patients living in an area with a high or intermediate tuberculosis burden such as Taiwan. In addition, all of them had QFT-G conversion with high levels of released IFNγ at the time of active tuberculous disease. Our findings were in keeping with the observation in reports that a cell-mediated immune response with high IFNγ levels could be detected as early as 2 weeks after infection with *M tuberculosis*.[47] Our data were also in agreement with the findings of a previous study showing that strong ESAT-6 responses shortly after exposure to *M tuberculosis* were associated with a 10-fold increased risk of the subsequent development of active tuberculosis.[48]

Just as sputum-smear microscopy cannot detect extrapulmonary tuberculosis that frequently occurs in RA patients receiving anti-TNFα therapy,[3 4] the usefulness of chest x-ray as a marker of LTBI is also inadequate.[17] The immune memory response identified by the QFT-G assay probably correlates better with the risk of the emergence of active tuberculosis compared with TST in patients with LTBI,[21] especially in the case of extrapulmonary tuberculous disease.[49] The real proof of the usefulness of the serial QFT-G assay to predict the emergence of active tuberculosis in such patients will require larger studies in various settings in the future.

There are some limitations in our study that need to be addressed. The sample size of active tuberculous disease was too small to determine the predictive value of serial QFT-G assays. Malnutrition is an important comorbid condition related to the emergence of active tuberculous disease; we disd not evaluate the nutritional status of the enrolled subjects. Although the QFT-G version used in this study is now less frequently in use, its diagnostic performance is similar to that of QFT-GIT,[12 50] and a high level of concordance has been shown between the QFT-G assay and T-SPOT.TB.[17] Considering that the 'delayed booster' response of repeated TST might be responsible for TST conversion in the BCG-vaccinated population,[49 50] serial TST was not performed in the present study.

In conclusion, our study shows the biphasic emergence of active tuberculosis in RA patients undergoing long-term anti-TNFα therapy. Our data indicate that monitoring IFNγ release levels by serial QFT-G assays may be a promising and useful tool for detecting LTBI reactivation or a recent tuberculosis infection in RA patients undergoing anti-TNFα therapy, and also seems to be an alternative to repeated TST, which has the possible drawback of a booster effect.[50] Based on our findings, we recommend that periodical IGRA screening may be performed every 3 months during the first 6 months for detecting LTBI reactivation, and after 18 months' anti-TNFα therapy for detecting acquired de-novo re-infection in high or intermediate tuberculosis burden areas.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was obtained from the Clinical Research Ethics Committee of Taichung Veterans General Hospital.

Provenance and peer review Not commissioned; externally peer reviewed.

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