T-CELL RESPONSES ASSESSED USING IGRA AND TST ARE NOT CORRELATED WITH AFB GRADE AND CHEST RADIOGRAPH IN PULMONARY TUBERCULOSIS PATIENTS

Kiatichai Faksri[1, 4], Wipa Reechaipichitkul[2, 4], Wilailuk Pimrin[3], Janpen Bourpoern[3] and Supapim Prompinij[3]

[1]Department of Microbiology, Faculty of Medicine, [2]Department of Medicine, Faculty of Medicine, [3]Tuberculosis Clinic, Srinagarind Hospital, [4]Research and Diagnostic Center for Emerging Infectious Diseases (RCEID), Khon Kaen University, Khon Kaen, Thailand

Abstract. A definitive marker determining the bacillary load of *Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis (TB), and hence disease severity, is required for patient monitoring and management. In this study, the association among T-cell responses based on the interferon-gamma release assay (IGRA) and the tuberculin skin test (TST), the sputum acid-fast bacilli (AFB) grade and types of radiological lesions were analyzed in new cases of pulmonary TB patients ($n = 54$) at Srinagarind Hospital, Khon Kaen, Thailand between September 1, 2012 and March 31, 2014. It was found that infiltrative and cavitary lesions from chest radiographs were associated with high sputum AFB grade ($p = 0.048$). T-cell responses from both IGRA and TST were not correlated with sputum AFB grade. Neither IGRA nor TST was correlated with the bacillary load as defined by AFB grade and chest radiographs. Patients with cavitary lesions on chest radiographs tended to have high IFN-γ concentrations and large TST indurations. In addition, TB patients with previous BCG vaccination showed significantly higher IFN-γ induction compared to the non-vaccinated group ($p = 0.001$). This study showed T-cell responses based on both IGRA and TST were not correlated with AFB grade and chest radiograph. In areas of high rates of BCG vaccination, as in Thailand, the BCG may affect IGRA and TST interpretations.

Keywords: tuberculosis, bacillary load, *Mycobacterium tuberculosis*, IGRA, TST

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease that is still a major public health problem. Finding a simple marker correlated with the bacillary load of *Mycobacterium tuberculosis* (MTB) and disease severity is important for patient management and monitoring. The most-used markers for monitoring pulmonary TB patients are chest radiographs and staining of sputum for acid-fast bacilli (AFB). These markers, however, are rather insensitive and not applicable for latent TB infections. An association between disease severity

Correspondence: Wipa Reechaipichitkul, Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand;
Tel: 66 (0) 43 363664; Fax: 66 (0) 43 203767
E-mail: wipree@kku.ac.th

and the extent of radiological lesions has previously been reported (Ralph *et al*, 2010). Sputum AFB grade in pulmonary TB patients is correlated with extensive lesions on chest radiographs (Gomes *et al*, 2003; Palaci *et al*, 2007). The association between radiological lesions and T-cell responses is mostly unknown.

Tuberculin skin tests (TST) and interferon-gamma release assays (IGRA) are standard screening tests for MTB infection. TST and IGRA tests are both based on the responses of T-cells after reacting with crude or specific MTB antigens (Trajman *et al*, 2013). The application of these tests for treatment monitoring and patient management could maximize their benefits in TB control. Attempts have been made to use IGRA for monitoring the outcomes of treatment (Ferrand *et al*, 2005; Wilkinson *et al*, 2006; Sauzullo *et al*, 2009; Bocchino *et al*, 2010; Lee *et al*, 2010) and to correlate IGRA results with sputum AFB grade (Palaci *et al*, 2007; Theron *et al*, 2012). It was hypothesized that MTB replication was associated with T-cell responses in the patients (Theron *et al*, 2012). Previous studies relied on AFB grade or CFU counts from sputum. However, the sputum AFB grade on any specific occasion might not accurately reflect the actual bacillary load due to temporal variation in AFB release from infected tissues (Palaci *et al*, 2007; Theron *et al*, 2012). Additionally, the associations among T-cell induction (based on IGRA and TST) and radiological lesions is still unclear, especially in a population with high coverage of Bacille Calmette-Guérin (BCG) vaccination. Determining the association between T-cell responses and actual bacillary load based on both AFB grade and types and extent of radiological lesions might point the way to the use of T-cell based assays for TB management.

Here, we investigate the association between bacillary load (defined by sputum AFB grade and by assessment of radiological lesions), and T-cell responses based on TST and IGRA in pulmonary TB patients in a tertiary care hospital, northeastern Thailand.

MATERIALS AND METHODS

Study population and setting

Fifty-four newly diagnosed pulmonary TB cases were enrolled in the study between September 1, 2012 and March 31, 2014. These volunteers were receiving treatment at Sirnagarind Hospital, Khon Kaen, Thailand. Diagnosis was based on positive AFB smears, or positive cultures, or PCR-TB positive results with nonreactive HIV serological status.

The volunteers completed written informed consent forms and a TB risk-assessment questionnaire, provided demographic and related data, and submitted to a chest radiograph and physical examination. Blood samples were collected at recruitment and then a TST was performed. This study was approved by Research Ethics Committee (HE551101), Khon Kaen University.

Classification of TB patients and bacillary-load groups

Pulmonary TB patients were defined as symptomatic if they had positive chest radiographs and sputum AFB and/or cultures for MTB and/or were PCR-TB positive. The identification of MTB was performed using a biochemical test and in-house PCR methods (based on RD9) from cultures, or using Xpert MTB/RIF (Cepheid, Sunnyvale, CA) testing from sputum samples. TB patients were classified into three bacillary-load groups based on sputum AFB grade (the higher grade from two tested sputum samples from each patient) and assessment of chest

radiographs (uni/bi-laterality, cavitary, infiltrative, nodular, miliary lesions and others) (Table 1). Radiological lesions seen in chest radiographs were measured. BCG status was defined as presence of a BCG scar in the patient. Potential TB patients were excluded from the study if they were HIV-positive or receiving immunosuppressants. Five AFB grades were recognized in increasing order: culture or PCR-positive, scanty, 1+, 2+ and 3+. Patients with scanty AFB, or who were culture- or PCR-positive only, were placed in a "low sputum AFB" group (n=13) for some analyses (as summarized in Table 2). Remaining patients were in the "high sputum AFB" group (n=41).

IGRA test

The QuantiFERON®-TB Gold In-Tube (QFT-G IT) (Qiagen, Valencia, CA) test was used according to the manufacturer's instructions. Briefly, venous blood samples were drawn from volunteers and placed into three 1 ml tubes. One was pre-coated with MTB-specific antigens (ESAT-6, CFP-10 and TB7.7), one was used for the phytohemagglutinin (PHA) test as a positive control, and the final tube was a negative control. Samples were mixed by hand after collection. The samples were then delivered to the laboratory and incubated at 37°C for 21 hours. After incubation, levels (IU/ml) of interferon-gamma (IFN-γ) in the samples were assayed. A result was considered positive when the IFN-γ level in the tube containing MTB-specific antigens minus the level in the negative control, was ≥ 0.35 IU/ml and ≥ 25% of the IFN-γ concentration in the negative-control plasma sample.

TSTs

The TST was performed according to Mantoux's technique. At the first visit of volunteers, after blood samples were taken, 5 TU/0.1 ml purified protein derivatives (RT23; Statens Serum Institute, Copenhagen, Denmark) were intradermally injected on the volar aspect of the forearm, and the sites were marked with a circle using a felt-tip pen. At 48 hours, the transverse diameters of TST induration, rather than erythema, were determined by trained nurses using the ball-point pen and ruler method. Results were considered positive when the induration size was ≥ 10 mm as the recommended cut-off for a high TB-burden country (American Thoracic Society, 1981).

Data analysis

Comparisons of numerical results (IGRA, TST, BCG vaccine, radiological lesions, AFB grade and bacillary-load groups) were performed using the chi-square test (or Fisher's exact test). Student's t-test was used to compare the mean IFN-γ concentrations based on IGRA and induration sizes based on TST between two groups, and one-way ANOVA was used to compare among three or more groups. Correlation analysis was performed using regression analysis. SPSS version 16.0, (SPSS, Chicago, IL) was used. A $p < 0.05$ was considered significant.

RESULTS

Demographic characteristics of the study population

In total, 34/54 cases (63.0%) of the TB patients were male. The mean age of the study population was 49.5 ± 15.6 years. Most cases (40/54, 74.1%) lived in Khon Kaen Province. Nine of 54 cases (16.7%) lived in Mahasarakham and five cases (9.3%) lived in other provinces in northeastern Thailand. The mean body mass index (BMI) of these TB cases was 20.32 ± 3.28 (19.86 ± 3.17 for males and 21.21 ± 3.39 for females). Most individuals (42/54,

Table 1
Classification of TB patients into three bacillary-load groups according to AFB grade and assessment of lesions seen using radiography.

Bacillary-load groups	AFB grade[a]	Lesions from chest radiographs		
		Uni/Bi-laterality	Cavitary lesion	Miliary lesion
High	3+	Any lesion	Any lesion	Neg
	1+ to 2+	Bilateral	Any lesion	Neg
	Any grading	Any lesion	Any lesion	Pos
Moderate	Scanty to 2+	Unilateral	Cavity	Neg
	Scanty or AFB-/Culture+ or PCR+	Bilateral	Any lesion	Neg
Low	Scanty or AFB-/Culture+ or PCR+	Minimal lesions[b]	No cavity	Neg

[a]The higher grade from two sputum samples.
[b]Minimal lesions: a unilateral lesion less than 3 mm in size, or lesions not seen.

77.8%) had received BCG vaccination.

There were 13 cases with an AFB grade lower than 1+ (six scanty AFB cases and seven cases who were AFB-negative but culture-positive or PCR-TB positive)("low sputum AFB" group) and 41 cases with sputum AFB grades equal to or higher than 1+ (19, 11 and 11 cases of AFB grades 1+, 2+ and 3+, respectively)("high sputum AFB" group). The most common lesion type seen in chest radiographs of these TB patients was the infiltrative type (31/54, 51.4%).

AFB grades and T-cell responses based on IGRA and TST

The correlation between AFB grade and T-cell induction was tested. The IFN-γ concentrations were slightly higher in the high sputum AFB group compared to the low sputum AFB group. The proportion of IGRA-positive results (≥ 3.5 IU/ml) was slightly higher in the former group, but not significantly so. Conversely, the induration size (≥ 10 mm) and proportion of positive results based on the TST was slightly larger in the low sputum AFB group, but not significantly so (Table 2).

Correlations among AFB grades, BCG vaccinations and chest radiographs

Approximately 83% of TB patients in the high sputum AFB group had received BCG vaccination, but only 62% of the low sputum AFB group had been vaccinated. This difference was not statistically significant ($p = 0.106$).

Overall, radiological lesions were not significantly associated with the sputum AFB group ($p = 0.058$). The TB cases with cavitary and infiltrative lesions however, were significantly higher in the high sputum AFB group ($p = 0.048$) (Table 2). This difference was not significant ($p = 0.517$) when comparing all cavitary cases between the high sputum AFB group (14/41 cases, 34.1%) and the low sputum AFB group (3/13 cases, 23.1%).

Correlation between chest radiographs, T-cell responses and BCG vaccination status

No correlations existed between T-cell responses and uni/bi-lateral lesions seen in chest radiographs (Table 3). The mean IFN-γ concentrations from IGRA in cavitary TB cases (2.66 ± 3.06) and induration

Table 2
Correlation analysis among sputum AFB groups, T-cell responses, BCG vaccination and radiological lesions of TB patients.

Characteristics	Sputum AFB groups		*p*-value
	Low sputum AFB (n = 13)	High sputum AFB (n = 41)	
T cell responses			
IGRA (IU/ml)			
Mean (±SD)[a]	1.73 (±2.89)	1.86 (±2.23)	0.872
≥3.5 IU/ml	6 (46.15)	12 (29.27)	0.260
>3.5 IU/ml	7 (53.85)	29 (70.73)	
TST (mm)			
Mean (±SD)	17.58 (±6.23)	16.52 (±7.32)	0.643
<10 mm	1 (7.69)	6 (14.63)	1.000
≥10 mm	12 (92.31)	35 (85.37)	
BCG vaccination status			
BCG vaccinated	8 (61.54)	34 (82.93)	0.106
Non BCG vaccinated	5 (38.46)	7 (17.07)	
Radiological lesions			
Uni/ Bi-laterality			
Unilateral	10 (76.92)	25 (60.98)	0.341
Bilateral	3 (23.08)	16 (39.02)	
Lesion types			
Infiltrative	8 (61.54)	23 (56.10)	0.730
Nodular	0	2 (4.88)	1.000
Cavitary	3 (23.08)	3 (7.32)	0.143
Infiltrative and cavitary	0	11 (26.83)	0.048
Others[b]	2 (15.38)	2 (4.88)	0.242

[a] Mean IGRA, mean IFN-γ concentration calculated as level in the value of MTB antigen tube minus the level in the negative control. SD, standard deviation. [b] Other lesion type, pulmonary effusion, military pattern and/or bronchial changes. AFB, acid-fast bacilli; Low sputum AFB, scanty AFB or PCR+ or culture+; High sputum AFB, AFB 1+, 2+ or 3+; BCG, Bacillus Calmette–Guéri; IGRA, interferon gamma release assays; TST, tuberculin skin test; IGRA, QuantiFERON®-TB Gold In-Tube (Qiagen, Valencia, CA). All values are shown as a number follow by percentages in brackets, as a mean values with the "±" indicating standard deviations.

size of TST in cavitary TB cases (18.42 ± 10.21) were the highest among types of lesions (Table 3).

Compared to non-vaccinated patients, the BCG-vaccinated TB patients showed significantly higher concentrations of IFN-γ based on the IGRA tests (p = 0.001). The mean induration size based on the TST of vaccinated TB patients was also higher than the non-vaccinated group (18.02 *vs* 12.42 mm), but not significantly (p = 0.074) (Table 3).

Correlation between bacillary load and T-cell responses based on TST and IGRA

The TB patients in low bacillary-load group showed lower IFN-γ induction

Table 3
Correlation analyses among T cell responses based on TST and IGRA, AFB grade, radiological lesion, and BCG vaccine of pulmonary TB patients.

Characteristics	T cell responses (mean ±SD)			
	IFN-γ concentration (IU/ml)	*p*-value	Induration size (mm)	*p*-value
Radiological lesion				
Uni/ Bilateral lesion				
Unilateral (*n* = 35)	2.08 (±2.85)	0.176	17.3 (±7.37)	0.465
Bilateral (*n* = 19)	1.36 (±0.96)		15.81 (±6.48)	
Lesion types				
Infiltrative (*n* = 31)	1.74 (±2.33)	0.754	16.55 (±6.89)	0.668
Nodular (*n* = 2)	0.76 (±1.07)		10.00 (±14.14)	
Cavitary (*n* = 6)	2.66 (±3.06)		18.42 (±10.21)	
Infiltrative and cavitary (*n* = 11)	1.47 (±1.13)		17.82 (±3.59)	
Others[a] (*n* = 4)	2.71 (±4.61)		16.63 (±8.82)	
BCG vaccination status				
BCG vaccinated (*n* = 42)	2.17 (±2.57)	0.001	18.02 (±5.71)	0.074
Non BCG vaccinated (*n* = 12)	0.64 (±0.72)		12.42 (±9.53)	
AFB grades				
Culture+ or PCR+ (*n* = 7)	1.26 (±2.20)	0.854	17.43 (±3.05)	0.438
Scanty AFB (*n* = 6)	2.29 (±3.67)		17.75 (±9.15)	
1+ (*n* = 19)	2.15 (±2.45)		16.68 (±7.94)	
2+ (*n* = 11)	1.25 (±1.03)		15.50 (±8.23)	
3+ (*n* = 11)	1.96 (±2.73)		17.27 (±5.56)	
Bacillary-load groups[b]				
Low (*n* = 9)	1.71 (±3.05)	0.149	19.22 (±4.09)	0.394
Moderate (*n* = 26)	1.83 (±2.36)		16.15 (±8.49)	
High (*n* = 19)	1.88 (±2.18)		16.47 (±5.89)	

[a]Other lesion types: pulmonary effusion, military pattern and bronchial changes.
[b]Bacillary-load groups were defined based on AFB grades and radiological lesions from chest radiographs (Table 1).

than the moderate and high bacillary-load groups. Conversely, the induration sizes based on the TST were smallest in the high bacillary-load group (Table 3). IFN-γ induction was not correlated with AFB grade ($p = 0.854$) or bacillary-load groups ($p = 0.149$). Similarity, TST was not correlated with the AFB grade ($p = 0.438$) or bacillary-load groups ($p = 0.394$) (Table 3).

DISCUSSION

T-cell based assays, especially IGRA, have been suggested as potential tools for determining bacillary load and treatment response (Lalvani, 2004). Information about the associations between T-cell response, sputum AFB grade and chest radiography, however, is limited, especially

in those high burden countries in which there has been high coverage of BCG vaccination. In this study, it was found that T-cell responses based on both IGRA and TST were not significantly associated with AFB grade and chest radiograph in pulmonary TB patients. However, the infiltrative and cavitary lesions seen in chest radiographs were associated with high sputum AFB grades. In addition, vaccinated TB patients showed higher IFN-γ induction compared to the non-vaccinated group.

It has been suggested that the T-cell response might be proportional to bacillary load and may serve as a surrogate marker for response following treatment (Lalvani, 2004; Oni *et al*, 2010). However, the T-cell responses induced by specific and non-specific MTB antigens (IGRA and TST respectively) did not correlate with sputum AFB (Theron *et al*, 2012). Similarly, many studies have also found that IFN-γ T-cell responses (Carrara *et al,* 2004; Hill *et al*, 2005; Sauzullo *et al,* 2009) were not associated (Ferrand *et al,* 2005; Bocchino *et al,* 2010; Lee *et al,* 2010) with sputum AFB and TB treatment. Results from the present study indicated that T-cell responses based on IGRA and TST showed no significant correlation with sputum AFB grades and bacillary-load groups (defined by AFB grade and chest radiograph). However, patients with high sputum AFB grades had high IFN-γ concentrations. Conversely, low sputum AFB grades patients had larger TST indurations. This might indicate an advantage of IGRA over TST for reflecting the bacillary load in pulmonary TB patients. The correlation analysis revealed that there was no correlation between AFB grades from PCR+ to sputum AFB 3+ and T-cell response based on either test. As BCG vaccination status affected both the IGRA and TST in this study, other factors such as non-tuberculous mycobacterial infections might affect the correlation between the sputum AFB grade and TST.

Previously, AFB grade was associated with cavitary lesions in pulmonary TB patients (Gomes *et al,* 2003; Matsuoka *et al,* 2004; Palaci *et al*, 2007). The lesion characteristics of chest radiographs were associated with the severity of pulmonary TB (Ralph *et al*, 2010). A higher sputum AFB grade of TB patients was also associated with drug-resistant TB (Sunita *et al*, 2010). No association was found between the IFN-γ response and the presence of cavitary lesions in TB patients (Theron *et al*, 2012). In a previous study, only the association between cavitary lesions and IFN-γ induction was analyzed (Theron *et al*, 2012). In the current study, the association between various radiological lesions (cavitary, bilateral, nodular, infiltrative and others) and sputum AFB grades were analyzed: no significant associations between cavitary lesions and AFB grades or bacillary-load groups were found. High AFB grades were not associated with cavitary lesions alone. However, cavitary and infiltrative lesions together were significantly associated with high AFB grade. Patients with cavitary lesions had slightly higher IFN-γ concentrations and larger induration sizes of TST than non-cavitary lesions. In addition, uni/bi-lateral lesions were not associated with AFB grade. This might indicate that certain characteristics of chest radiological lesions, associated with the pathology and severity (Ralph *et al*, 2010), are proportional to bacillary loads. An additional population or a larger patient group might provide clearer information.

False positive results of TST can be found due to cross-reactivity in the event of prior BCG vaccination (Pai *et al,* 2008).

Unlike TST, IGRA is based on T-cell responses to RD1, an MTB-specific antigen, which avoids the problem of cross reactivity (Cattamanchi *et al*, 2011). Although, the specificity of the IGRA was not affected by the response to the BCG vaccine, this study showed that TB patients who with prior BCG vaccination (identified by the presence of a BCG scar) had mean IFN-γ concentrations significantly higher than non-vaccinated patients. In the TST, the mean induration size in the BCG vaccinated group was also larger than in the non-vaccinated group. Thus, in countries with a high coverage of BCG vaccination with administration at birth, such as Thailand, prior vaccination with BCG may affect IGRA and TST interpretations. False positive results of IGRA in the BCG-vaccinated population was found from previous study (Pai *et al*, 2008) and in at least one case (Hermansen *et al*, 2014) was due to nontuberculous mycobacteria. Alternatively, other underlying conditions excluding HIV infection might interfere with the T cell response in this population.

There are limitations to this study. Firstly, the number of the patients in the low bacillary-load group is small. The number of non-vaccinated TB patients is also low due to a high coverage rate of BCG vaccine in Thailand. Much larger samples sizes might be needed to confirm the finding that BCG vaccine affected the IGRA interpretation.

In summary, this study found that co-occurrence of infiltrative and cavitary lesions on chest radiographs was associated with high sputum AFB grade. T-cell responses based on both IGRA and TST were not associated with sputum AFB grade, chest radiograph and bacillary-load groups defined based on both AFB grade and chest radiograph. Patients with cavitary lesions on chest radiographs tended to have high IFN-γ concentrations and large TST indurations. BCG vaccination may affect IGRA and TST interpretation.

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