Time Interval to Conversion of Interferon-γ Release Assay after Exposure to Tuberculosis

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ABSTRACT

The proper interval for repeating an interferon-γ release assay (IGRA) among tuberculosis contacts with initially negative results is unknown. We evaluated the interval for IGRA conversion after exposure to patients with active pulmonary tuberculosis in an outbreak setting.

In a platoon of 32 soldiers, four active pulmonary tuberculosis patients, in addition to one index patient, were diagnosed during a contact investigation. For the other 27 contacts, a tuberculin skin test (TST) and QuantiFERON® TB Gold In-Tube assay (QFT-GIT) were performed. For soldiers with a negative result on the initial QFT-GIT, the test was repeated at 2, 4, 8, 14, 18, and 30 weeks, until positive conversion occurred. When conversion was identified, the subject was treated for latent tuberculosis infection.

Initially, 17 (63.0%) soldiers had positive QFT-GIT results, whereas 21 (77.8%) showed positive TST results. Among 10 participants with initially negative QFT-GIT results, three had conversion at 2 weeks; three, at 4 weeks; and three, at 14 weeks. Conversion did not occur in one contact during 30-week observation period.

Based on the tuberculosis exposure time points among the contacts, IGRA conversion generally occurred 4–7 weeks after exposure, although it could occur as late as 14–22 weeks after exposure.

Keywords: contacts, infection control, IFN-γ assay, serial testing, tuberculosis
INTRODUCTION

Despite global efforts, the total number of new tuberculosis (TB) cases continues to rise, with 9.27 million new cases and 1.32 million deaths reported in 2007 [1]. For effective and efficient treatment of active TB and for disease control in developing countries, the rapid diagnosis and treatment of patients with sputum smear-positive TB are critical. In countries with low or intermediate rates of TB, the treatment of latent TB infection (LTBI) to prevent progression to active disease has been an essential component of public health efforts to eliminate the disease [2, 3].

Despite its well-known limitations, the tuberculin skin test (TST) has been the only method for diagnosing LTBI for more than 100 years. However, two interferon-gamma (INF-γ) release assays (IGRAs) that overcome several TST limitations were recently introduced in a routine clinical practice. The IGRAs appear to be unaffected by previous Bacille Calmette-Guérin (BCG) vaccination. They do not require intradermal injection or second visit. In addition, IGRAs provide objective results.

Compared with the TST, two commercially available tests, an ELISA-based QuantiFERON-TB Gold assay and an ELISPOT-based T.SPOT.TB test, produce more specific results [4, 5]. The U.K. National Institute for Health and Clinical Excellence (NICE) guidelines recommend the use of IGRA in individuals at risk for LTBI who have tested positive with TST [6]. The U.S. Centers for Disease Control and Prevention (CDC) guidelines recommend initial use of IGRA in all groups as a direct replacement of the TST [7].

Nevertheless, the interval for positive conversion of the IGRA after exposure to a patient with active TB is unclear, whereas conversion of the TST is known to occur
within 2–12 weeks [8-10]. NICE guidelines recommends to repeat an IGRA test after 6 weeks for contacts with a negative TST, although there is no concrete evidence for this recommendation [6]. Similarly, CDC guidelines recommend that negative IGRA results be confirmed with a repeat test performed 8–10 weeks after the end of exposure, while admitting that the best interval request to retest is unknown [7].

In this study, we evaluated the time interval for positive IGRA conversion after a TB outbreak in a Korean military platoon, which is a closed communal setting.
MATERIALS AND METHODS

Status of TB control in South Korea

In 2007, the incidence and prevalence of TB of South Korea were 90/100,000 and 126/100,000, respectively. In addition, the annual TB mortality was 4,887 [1], and the rate of BCG coverage was about 95% [11]. The prevalence of positive TST among young South Koreans without contact to TB patients was reported to be 28% in 2006 [12].

Index patient and identification of the TB outbreak

The first patient diagnosed with pulmonary TB was a 20-year-old male soldier in a platoon of two officers and 32 soldiers. He had no underlying disease and was negative for human immunodeficiency virus (HIV). He developed a productive cough at the beginning of January 2009 and had hemoptysis in March 2009. Subsequently, he was diagnosed with sputum smear-positive pulmonary TB in April 2009, and was evacuated from the platoon for isolation. We treated him for active pulmonary TB and 5 weeks later culture confirmed *M. tuberculosis* (MTB) infection. Within 7 days of this patient’s diagnosis, simple CXRs were performed for all officers and soldiers in the same platoon. Acid-fast staining and mycobacterial sputum cultures were also conducted for any contacts who could expectorate sputum.

Study protocol

On May 2009, this study investigation was initiated for all soldiers in the same platoon. Although the soldiers in the platoon lived in two adjacent rooms, they shared a
bathroom and dining room, and lived their daily lives together. Therefore, all were invited to participate in this study. However, two officers were excluded from the study because they did not have as close contact as the soldiers themselves. After giving written consent, each participant was asked to complete a questionnaire regarding demographics, previous history of TB, smoking status, and other factors. Additionally, low-dose chest computed tomography (LDCT), a TST, and a QuantiFERON® TB Gold In-Tube assay (QFT-GIT; Celletis Ltd., Victoria, Australia), as well as acid-fast bacilli (AFB) smears and mycobacterial cultures of sputum when possible, were performed for all close contacts (Fig. 1). LDCT was included to detect any active lesions that were not identified by CXR [13, 14]. This study was reviewed and approved by the institutional review board of the Armed Forces Medical Command.

**Tuberculin skin test**

After QFT-GIT blood sampling, the two-step TST was performed by trained personnel following standard procedures [9], with 0.1 ml (2 TU) of purified protein derivate (RT23; Statens Serum Institute, Copenhagen, Denmark). We defined a positive test as an induration of ≥10 mm [9].

**Interferon-γ release assay**

The QFT-GIT was performed according to the manufacturer’s instructions. The plasma concentration of IFN-γ was measured by ELISA, and the technician had no information about the subjects. Test results were interpreted as negative, indeterminate, or positive (cut-off, 0.35 IU/ml) using the manufacturer’s software. QFT-GIT conversion was
defined as a change from a negative (<0.35 IU/ml) to a positive (≥0.35 IU/ml) result [15].

**Low-dose chest CT**

LDCT was performed using a 64-channel multi-detector (Brilliance 64; Philips, Amsterdam, Netherlands) and a thickness of 5 mm. A board-certified radiologist, who was blinded to the participants’ clinical information, interpreted the results.

**Acid-fast bacilli smears and mycobacterium sputum cultures**

For participants who could expectorate sputum, we performed three AFB smears and mycobacterial cultures of the sputum. Sputum was decontaminated with 4% NaOH, homogenized, and concentrated by centrifugation (3,000 × g, 20 min). The processed sediment was stained using the Ziehl–Neelsen method [16]. Sputum was also cultured in 3% Ogawa medium and observed for growth every week for 8 weeks.

**Diagnosis and treatment of active and latent TB infection**

*Active TB*—Active TB was diagnosed when specimens showed positive AFB staining, when MTB was cultured, or when patient data met the definition of a clinical case of TB as defined by the WHO [17]. Because the isolated MTB showed susceptibility to all antituberculosis drugs, treatment with isoniazid, rifampicin, pyrazinamide, and ethambutol was initiated and continued for 6 months for participants diagnosed with active TB.
**LTBI**—Participants with positive QFT-GIT results, but without active TB lesions on CXR and LDCT, were diagnosed with LTBI, and isoniazid and rifampicin was recommended daily for 3 months [18].

**Follow-up evaluation**

Participants with negative QFT-GIT results at the initial screening were tested again at 2, 4, 8, 14, 18, and 30 weeks after the outbreak investigation had begun. When a positive QFT-GIT conversion occurred, the follow-up tests were stopped, and the participant was treated with isoniazid and rifampicin daily for 3 months (Fig. 1).

**DNA fingerprinting of isolated Mycobacterium tuberculosis**

To confirm an outbreak of MTB at the molecular level, DNA fingerprinting was performed using *Pvu* II restriction, as described previously [19].

**Statistical analysis**

Agreement between the TST results and the whole-blood IFN-γ levels was measured using the κ-statistic. Statistical significance was defined by a two-tailed *P* value ≤ 0.05. All statistical analyses were conducted using PASW 17.0 (SPSS Inc., Chicago, IL, USA).
RESULTS

Patients identified with active pulmonary TB

During the investigation, two more soldiers with productive cough were diagnosed with active pulmonary TB based on symptoms and CXRs, despite having a negative AFB smear of their sputa; after a few weeks, MTB was isolated from one of the two soldiers. In addition, two more soldiers with normal CXRs were diagnosed and treated for active TB based on abnormal LDCT lesions; although their AFB smears were negative, MTB was isolated from the sputum of one of the soldiers several weeks later. In total, five soldiers in this platoon, including the index case, were diagnosed with active pulmonary TB. Three of the soldiers had culture-confirmed pulmonary TB. All five patients were isolated from the platoon as soon as active TB was suspected. Their symptoms and radiographic findings improved with anti-TB treatment. MTB cultured from two patients (index patient and one subsequent patient who had lesions on a CXR) showed the same RFLP pattern; however, patient diagnosed having an active TB based on LDCT showed a different pattern of MTB. (Table 1).

Demographic characteristics of contacts without active TB

All 27 contacts in the platoon with a normal LDCT completed the study protocol. The median age was 21 years (range, 20–24 years), and all were male. Twenty-one (77.8%) contacts had BCG scars, and 19 (70.4%) were current smokers. All contacts were negative for HIV, and none had a previous history of TB. Sixteen contacts were residing with the platoon when the index case started to cough; seven contacts were deployed to the platoon before the diagnosis and evacuation of the index case; and four contacts
were deployed to the platoon after evacuation of the index case, but were exposed to the four other patients with active TB.

**Results of the initial investigation for contacts without active TB**

Among the 27 contacts, 17 (63.0%) had a positive QFT-GIT result and none had an indeterminate result, whereas 21 (77.8%) showed a positive response on the TST. Sixteen (59.3%) contacts had positive results on both the QFT-GIT and TST, and five contacts (18.5%) had negative results on both tests. The overall agreement between the TST and QFT-GIT results was moderate ($\kappa = 0.56$, $P = 0.001$) [20].

**Follow up interferon-\(\gamma\) release assays among contacts with initially negative results**

Ten contacts had negative QFT-GIT results at the initial study investigation. Among them, three participants (all with a positive TST in first step) showed conversion at 2 weeks, with IFN-\(\gamma\) levels of 0.51 (subject 18), >10.0 (subject 19), and 1.47 IU/ml (subject 20), respectively. Another three (all with a negative TST) had conversion at 4 weeks, with IFN-\(\gamma\) levels of 0.58 (subject 24), 0.90 (subject 25), and 0.96 IU/ml (subject 26), respectively. Three other participants (one with a positive TST in first step, two with a negative TST) had conversion at 14 weeks; the IFN-\(\gamma\) levels were 1.09 (subject 22), 1.92 (subject 23), and 2.74 IU/ml (subject 27), respectively. No conversion occurred in one contact (subject 21) during the 30-week observation period (Fig. 2).

**Subsequent development of active TB among participants**

None of the 26 contacts was diagnosed with active TB during the 10-month follow-up.
DISCUSSION

Current guidelines recommend repeating the IGRA at 6–10 weeks after TB exposure among contacts with an initially negative IGRA result [6, 7]. However, this recommendation did not emerge from observed data, but from speculation. Using the results of the present study, we could estimate the interval between exposure and IGRA conversion.

The results of the serial QFT-GIT for subjects 24, 25 and 26 showed the shortest interval to conversion. Given that these four subjects had been deployed to the platoon 3 weeks before the investigation began, and considering that conversion occurred at 4 weeks after the investigation began, the interval from exposure to conversion for these subjects must have been 4–7 weeks. We also estimated the longest interval to conversion using the data from subjects 22 and 23, who could have contacted persons with active TB as long as 2 months before the investigation and showed conversion 14 weeks after the investigation began. Based on our results, conversion is possible as early as 4–7 weeks and as late as 14–22 weeks after exposure to TB, although we checked contacts not more than 6 months.

This study was performed under ideal conditions to estimate the interval between exposure and IGRA conversion. First, the participants in this study had scant possibilities for further exposure to other patients with active TB. From a platoon of 32 soldiers, five with active TB were isolated as soon as active TB was suspected. Although two participants with initial IGRA negative had spent few days outside the barrack before IGRA conversion, they denied contacts with TB patients while they were
outside. All the other participants did not leave their platoon for vacation before IGRA conversion. Furthermore, the possibility of missing a patient with active TB was minimized by performing LDCT for all contacts, as LDCT has been shown to be superior to a simple CXR for diagnosing active TB [13, 14]. In fact, two patients in the present investigation were diagnosed with active pulmonary TB based on LDCT. Furthermore, contact between the participants and soldiers from other units were very unlikely, because the platoon had performed their duties independently in an isolated barracks. We believe that in this setting, the observed positive conversions resulted from exposure to the index patient or subsequent patients with TB, rather than from other sources. Second, none of contacts with a negative QFT-GIT were released from military service. This condition provided an ideal opportunity to observe the whole set of contacts and minimized selection bias. The high rate of positive TST (21 of 27) and QTF-GIT results among the contacts (17 of 27) at the first screening raised the possibility of widespread pre-existing LTBI among the soldiers; however, large proportion of LTBI among the participants was thought to be the result of the intense exposure to TB case, given that the prevalence of positive TST among young South Koreans without contact with TB patients was only 28% in South Korea [12].

Theoretically, the TST could affect the results of subsequent IGRA tests, because both ESAT-6 and CFP-10 used for the IGRA are included in tuberculin. However, the data about boosting-effect of TST on IGRA are inconsistent across the studies and some previous studies confute a definite boosting of IGRA response by TST [21-23].

Owing to within-subject variability in the IFN-γ responses [24], a higher threshold (≥0.70 IU/ml) has been suggested to define conversion for the QFT-GIT [25]. Without a
higher threshold, minor fluctuations around the current IFN-γ cut-off level of 0.35 IU/ml could be mistakenly regarded as conversions. In the present study, the participants with a conversion had IFN-γ levels >0.70 IU/ml, with the exception of two participants (IFN-γ level of 0.51 IU/ml in subject 6 and 0.58 IU/ml in subject 27). Considering previous studies, the high IFN-γ levels of participants in our study can be regarded as real conversions, rather than boosting from the TST or within-subject variability in the IFN-γ response.

Given the TB exposure time points for contacts, IGRA conversion generally occurred 4–7 weeks after exposure and occurred as late as 14–22 weeks after exposure in a smaller number of cases. We did not observe any conversion between 22 and 30 weeks after the outbreak investigation. Thus, for close contacts with an initially negative IGRA, the appropriate time over which to repeat the IGRA may be longer than the currently recommended 6–10 weeks.

Acknowledgements

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References


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<th>Patient No.</th>
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<th>Duration of respiratory symptoms before evacuation (weeks)</th>
<th>Time of diagnosis and evacuation before investigation (weeks)</th>
<th>Acid-fast staining of sputum</th>
<th>Mycobacterium culture of sputum</th>
<th>RFLP pattern</th>
<th>Radiographic findings</th>
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RUL, right upper lobe; RML, right middle lobe; LLL, left lower lobe; TB, tuberculosis; n-a, not-applicable.

Patients 3 and 5 were diagnosed based on abnormal LDCT lesions.
**Figure Legends**

Figure 1. Schematic of the study protocol.

TB, tuberculosis; CXR, chest X-ray; LDCT, low-dose chest computed tomography; QFT-GIT, QuantiFERON® TB Gold In-Tube assay; TST, tuberculin skin test; LTBI, latent tuberculosis infection.

Figure 2. The interval between TB exposure and QFT-GIT conversion among 27 contacts without active TB.

The lines with numbers in squares indicate each participant. Each line begins at the point when the participant started to reside in the platoon and ends when the participant first had a positive QFT-GIT result. The large circle indicates the results of the TST, and the small circle indicates the QFT-GIT results (black, positive; white, negative). Abbreviations as in legend for Fig. 1.