Discrepancy Between the Tuberculin Skin Test and the Whole-Blood Interferon γ Assay for the Diagnosis of Latent Tuberculosis Infection in an Intermediate Tuberculosis-Burden Country

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TBerculosis (TB) remains a significant global health problem. Tuberculosis in humans is the most frequent cause of death from a single infectious agent, and often causes severe morbidity. Despite the worldwide effort for eradicating TB, it is responsible for an estimated 8.3 million new infections and 1.8 million deaths each year.1,2 For effective and efficient treatment of active TB in developing countries, rapid diagnosis and treatment of patients with sputum smear-positive test results are the key points in disease control. However, in countries with low or intermediate rates of TB endemism, the treatment of latent TB infection to prevent progression to active disease has been an essential component of public health efforts to eliminate TB.3,4

The tuberculin skin test (TST), which has been used for years for the diagnosis of latent TB infection, has many limitations, including false-positive test results in individuals who were vaccinated with BCG and in individuals who have infections not related to Mycobacterium tuberculosis.5,6 Recently, a whole-blood interferon γ (IFN-γ) assay based on the M tuberculosis–specific antigens early secreted antigenic target 6 and culture filtrate protein 10 (ESAT-6) and culture filtrate protein 10 (CFP-10) has shown promise for the diagnosis of latent tuberculosis (TB) infection.

Context A recently developed whole-blood interferon γ (IFN-γ) assay based on stimulation with the Mycobacterium tuberculosis–specific antigens early secreted antigenic target 6 and culture filtrate protein 10 shows promise for the diagnosis of latent tuberculosis (TB) infection.

Objective To compare the tuberculin skin test (TST) and the whole-blood IFN-γ assay in the diagnosis of latent TB infection according to the intensity of exposure.

Design and Setting A prospective comparison between the whole-blood IFN-γ assay and the TST using a 2-TU dose of purified protein derivative RT23 in a population with intermediate TB burden was conducted sequentially between February 1, 2004, and February 28, 2005, in a Korean tertiary referral hospital.

Participants Of 273 participants, 220 (95.7%) had received BCG vaccine. Participants were grouped according to their risk of infection: group 1, no identifiable risk of M tuberculosis infection (n = 99); group 2, recent casual contacts (n = 72); group 3, recent close contacts (n = 48); group 4, bacteriologically or pathologically confirmed TB patients (n = 54).

Main Outcome Measures Levels of agreement between the TST and the IFN-γ assay and the likelihood of infection in the various groups.

Results For the TST with a 10-mm induration cutoff, the positive response rate in group 1 was 51%; group 2, 60%; group 3, 71%, and group 4, 78%. For the IFN-γ assay, the positive response rate in group 1 was 4%; group 2, 10%; group 3, 44%; and group 4, 81%. The overall agreement between the TST and the IFN-γ assay in healthy volunteers was κ = 0.16. The odds of a positive test result per unit increase in exposure across the 4 groups increased by a factor of 5.31 (95% confidence interval [CI], 3.62-7.79) for the IFN-γ assay and by a factor of 1.52 (95% CI, 1.20-1.91) for the TST (P<.001). Using a 15-mm induration cutoff for the TST did not make a substantial difference to the test results.

Conclusion The IFN-γ assay is a better indicator of the risk of M tuberculosis infection than TST in a BCG-vaccinated population.
been introduced for the diagnosis of latent TB infection. The whole-blood IFN-γ assay QuantiFERON-TB Gold (Cellestis Ltd, VIC, Australia) is based on the elaboration of inflammatory cytokines by T cells previously sensitized to mycobacterial antigens when they encounter ESAT-6 and CFP-10.12 In previous studies and when compared with TST, this assay showed high sensitivity and specificity in patients with active TB and correlated better with exposure to M tuberculosis.13-17 However, there has been no study on the usefulness of this assay in an intermediate TB-burden area. In addition, the utility of the commercialized IFN-γ assay has yet to be reported.

The aim of this study was to estimate the usefulness of the IFN-γ assay for the diagnosis of latent TB infection in Korea, where the incidence of active pulmonary TB is intermediate (92/105 per year) and BCG vaccination is mandatory.18 We hypothesized that this assay would correlate better with the risk of infection and the degree of exposure than the TST and could help to eliminate the limitations of the TST in this population.

METHODS

Participants
The participants were recruited between February 1, 2004, and February 28, 2005. The protocol was approved by the ethics review committee of the Seoul National University Hospital (Seoul, Republic of Korea). After providing written consent, each individual was asked to complete a questionnaire about his/her possible risk of exposure to M tuberculosis. All participants were prospectively recruited from the Seoul National University College of Medicine and from the Seoul National University Hospital.

The participants were classified into 1 of 4 groups that reflected the risk of infection. Group 1 consisted of healthy medical students without an identified risk for M tuberculosis exposure. Group 2 consisted of healthy hospital staff with a history of casual contact with active pulmonary TB patients. Group 3 consisted of individuals who had household contact with or who had worked in the same rooms as patients with smear-positive pulmonary TB for longer than 8 hours per day. Group 4 consisted of patients with active pulmonary TB. Diagnosis was confirmed by culture of M tuberculosis from sputum or by the presence of caseating granuloma in the lung tissue of patients in whom masslike consolidation was evident from chest radiographs. Participants in groups 1 through 3 were excluded if they showed abnormal simple chest radiographs, if they had taken immunosuppressive drugs during the previous 3 months, or if they had positive test results for the human immunodeficiency virus. The participants with indeterminate IFN-γ assay test results were excluded from further analysis.

Determination of the Sample Size
The sample size was determined by the following factors: the prevalence of TB infection in Korea, the κ coefficient, α and β errors, and the “supposed” missing rate. We used a value of 33% for the TB infection prevalence rate. This is a presumptive value based on the 44.4% infection rate in 1990 in the sixth nationwide TB prevalence survey of Korea. We consider the test results from the TST and the IFN-γ assay to be in agreement when κ coefficients are greater than 0.75. We assumed that the α error was .05, that the β error was .20, and that the “supposed missing” rate was 20%. Using these parameters and assumptions, and the method previously described,19 the minimum sample size for each group was estimated to be 51.

Tuberculin Skin Test
After collection of blood samples for the IFN-γ assay, the TST was performed on the volar side of the forearm according to the Mantoux method20 using a 2-TU dose of purified protein derivative RT23 (Statens Serum Institut, Copenhagen, Denmark), and any induration was measured in millimeters after between 48 and 72 hours using the ballpoint method.20 The investigator who performed the TST was blinded to the status of all groups except group 4 (patients with active pulmonary TB).

IFN-γ Assay
The IFN-γ assay was performed in 2 stages according to the manufacturer’s instructions. First, 1 mL of heparinized whole blood was incubated with aliquots of antigen-free control and antigens ESAT-6, CFP-10, or phytohemagglutinin for 16 to 24 hours at 37°C in a carbon dioxide incubator. Then, after overnight incubation, 200 µL of plasma was removed from each well and the concentration of IFN-γ was determined using the assay kit according to the manufacturer's instructions (Cellestis Ltd). A positive response value of 0.35 IU/mL of IFN-γ was used as the cutoff.13 The investigator who performed the IFN-γ assay was blinded to the group status of all participants.

Statistical Analysis
Concordance between test results from the TST and the IFN-γ assay was assessed using κ coefficients (κ>0.75, excellent agreement; κ<0.4, poor agreement; and κ between 0.4 and 0.75, fair to good agreement). Instead of calculating sensitivity and specificity of the TST and the IFN-γ assay, we measured the correlation of the 2 tests with the risk of latent TB infection by estimating the odds ratio (OR) and relating the test results to the likelihood of TB infection.

Given that there is no criterion standard for the diagnosis of latent TB infection, it is hard to estimate the sensitivities of the TST and the IFN-γ assay. Although sensitivity could be calculated from patients with active TB, active pulmonary TB would differ from latent TB infection in terms of the size of the bacterial inoculum, duration and period of exposure, and host immune status. In addition, it would be difficult to make an accurate estimate of the specificity of the 2 tests using Korean populations because the prevalence of latent TB infection can be as high as 33%. Furthermore, to adjust for the effects of age, sex, and BCG vaccination status on the TST and the IFN-γ assay, we performed a logistic regression analysis.
We estimated the increase in odds of a positive test result per unit increase in exposure by logistic regression. Matched-pairs logistic regression was used to assess any significant difference in the associations between the tests. We also calculated an OR adjusted for age, sex, and BCG vaccination status. Analyses were performed using SPSS version 12.0 (SPSS Inc, Chicago, Ill) and STATA version 7.0 (STATA Corp, College Station, Tex) software packages.

RESULTS
Characteristics of Participants
Group 1 contained 99 participants; group 2, 72; group 3, 48; and group 4, 58. All participants had negative test results for human immunodeficiency virus. Four patients with active TB (from group 4) were excluded from further analysis because of indeterminate IFN-γ assay test results. No one in groups 1 through 3 had an indeterminate result. The demographic characteristics of the enrolled participants are listed in Table 1.

Comparison Between TST and IFN-γ Assay
When a 10-mm induration cutoff was used for the TST, 50 participants (51%) had positive test results in group 1, whereas only 4 participants (4%) had positive test results for the IFN-γ assay (κ = 0.08). In group 2, 43 participants (60%) had positive test results with the TST, but only 7 participants (10%) had positive test results with the IFN-γ assay (κ = 0.14). In group 3, 34 participants (71%) had positive test results and an induration of 10 mm or more and 21 participants (44%) had a positive test result with the IFN-γ assay (κ = 0.17). The overall agreement between the TST and IFN-γ assay in healthy volunteers (groups 1 through 3) was κ = 0.16. In group 4, 42 participants (78%) had positive test results with the TST and 44 participants (81%) had positive test results with the IFN-γ assay (κ = 0.43; Figure). When using a 15-mm induration cutoff, the correlation between a positive test result on the TST and IFN-γ assay was κ = 0.13 in group 1, κ = 0.25 in group 2, κ = 0.25 in group 3, and κ = 0.40 in group 4.

Risk of M tuberculosis Infection
The odds of a positive test result for each increase in risk across the 4 groups increased by a factor of 5.31 for the IFN-γ assay (95% confidence interval [CI], 3.62-7.79; P < .001), by a factor of 1.52 for the TST with a 10-mm cutoff (95% CI, 1.20-1.91; P < .001), and by a factor of 1.74 for the TST with a 15-mm cutoff (95% CI, 1.39-2.18; P < .001). However, the IFN-γ assay correlated significantly better with the increased risk of infection across the groups compared with the TST using a 10-mm cutoff or a 15-mm cutoff (both P < .001). We estimated the relationship between the TST test results and BCG vaccination status for groups 1 through 3 using the χ² test. However, no significant relationship was found (P = .89 for TST with a 10-mm cutoff and P = .88 for a 15-mm cutoff). After adjustment for the effects of age, sex, and BCG vaccination status, the OR was 1.68 (95% CI, 1.24-2.62) for the TST with a 10-mm cutoff, 1.82 (95% CI, 1.38-2.41) for the TST with a 15-mm cutoff, and 4.23 (95% CI, 2.79-6.41) for the IFN-γ assay (Table 2 and Table 3).

COMMENT
Our study showed poor correlation between the TST and the IFN-γ assay among healthy volunteers in Korea.

Table 1. Characteristics of Healthy Participants and Patients With TB

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1: Low Risk of Infection (n = 99)</th>
<th>Group 2: Casual Contacts (n = 72)</th>
<th>Group 3: Close Contacts (n = 48)</th>
<th>Group 4: TB Patients (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
<td>25 (24-36)</td>
<td>28 (25-36)</td>
<td>41 (16-70)</td>
<td>43 (17-84)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>58 (59)</td>
<td>48 (67)</td>
<td>9 (19)</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>41 (41)</td>
<td>24 (33)</td>
<td>39 (81)</td>
</tr>
<tr>
<td>BCG scar</td>
<td>93 (94)</td>
<td>65 (95)</td>
<td>32 (67)</td>
<td>30 (56)</td>
</tr>
</tbody>
</table>

Abbreviation: TB, tuberculosis.

*Values are expressed as number (percentage) unless otherwise indicated.
addition, the correlations in each sub-
group of the participants according to the risk of infection were poor. This poor correlation between the TST and the IFN-\(\gamma\) assay is different from prior test results. In industrialized countries with low TB endemicity, the reported agreement was good between the TST and the IFN-\(\gamma\) assay based on the M tuberculosis-specific antigen in TB contacts (94% concordance in Denmark \(k = 0.87\) and 89% in the United Kingdom \(k = 0.72\)).15,16

Given that the proportions of positive test results with the TST (58% for a 10-mm cutoff and 37% for a 15-mm cutoff) and the IFN-\(\gamma\) assay (15%) in participants were much different, this discrepancy might be explained by the false-positive TST results and false-negative IFN-\(\gamma\) assay results.

In Korea, BCG vaccination is given at birth and again at age 12 or 13 years if the child proves to be a TST nonresponder. Most of the participants in group 1 and 2 had BCG vaccination scars. Therefore, the positive test result for the TST in 51% of participants in group 1 (least possibility of latent TB infection) might be explained by the confounding effects of previous BCG vaccinations,6,22 considering the predicted prevalence of TB infection (33%) in the Korean population.24 Non-TB mycobacteria infection could be another explanation for the observed discrepancy in this study because non-TB mycobacteria infections also give positive test results for the TST.24,25 Given that non-TB mycobacteria infection usually causes the TST false-positive result with indurations of 5 to 14 mm,24,25 the analysis using a positive result with the 15-mm cutoff for the TST could validate this possibility. The fact that the discrepancy between the TST and the IFN-\(\gamma\) assay was not corrected with a 15-mm induration cutoff for TST weakness the probability of cross-reactivity between non-TB mycobacteria and M tuberculosis infections.

However, the possibility of underestimation of latent TB infection by IFN-\(\gamma\) assay could not be excluded. The TB infection rate in 20- to 29-year-old Koreans was 59% in 1995 and the expected prevalence of latent TB infec-

### Table 2. Risk of Infection in Participants With Positive Response on Tuberculin Skin Test With a 10-mm Induration Cutoff Compared With Positive Response on Interferon \(\gamma\) Assay*

<table>
<thead>
<tr>
<th>Group 1: Low Risk of Infection (n = 99)</th>
<th>Group 2: Casual Contacts (n = 72)</th>
<th>Group 3: Close Contacts (n = 48)</th>
<th>Group 4: TB Patients (n = 54)</th>
<th>P for Trend</th>
<th>Continuous†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculin skin test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive response, No. (%)</td>
<td>50 (51)</td>
<td>43 (60)</td>
<td>34 (71)</td>
<td>42 (76)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>1.45 (0.79-2.68)</td>
<td>2.38 (1.14-4.97)</td>
<td>3.43 (1.62-7.28)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Adjusted‡</td>
<td>1.00</td>
<td>1.48 (0.79-2.74)</td>
<td>3.13 (1.33-7.36)</td>
<td>4.63 (1.77-12.13)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Interferon (\gamma) assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive response, No. (%)</td>
<td>4 (4)</td>
<td>7 (10)</td>
<td>21 (44)</td>
<td>44 (81)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>2.56 (0.72-9.10)</td>
<td>18.47 (5.84-58.43)</td>
<td>104.50 (31.06-351.62)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Adjusted‡</td>
<td>1.00</td>
<td>2.48 (0.69-8.90)</td>
<td>8.98 (2.54-31.68)</td>
<td>63.09 (17.09-232.95)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio; TB, tuberculosis.
*P<.001 for comparison of adjusted OR between the tuberculin skin test and the interferon \(\gamma\) assay.
†Based on the assumption that the risk of each group is a continuous variable.
‡Adjusted for age, sex, and BCG vaccination status.

### Table 3. Risk of Infection in Participants With Positive Response on Tuberculin Skin Test With a 15-mm Induration Cutoff Compared With Positive Response on Interferon \(\gamma\) Assay*

<table>
<thead>
<tr>
<th>Group 1: Low Risk of Infection (n = 99)</th>
<th>Group 2: Casual Contacts (n = 72)</th>
<th>Group 3: Close Contacts (n = 48)</th>
<th>Group 4: TB Patients (n = 54)</th>
<th>P for Trend</th>
<th>Continuous†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculin skin test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive response, No. (%)</td>
<td>27 (27)</td>
<td>31 (43)</td>
<td>23 (48)</td>
<td>38 (70)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>1.92 (1.01-3.63)</td>
<td>2.33 (1.14-4.77)</td>
<td>6.02 (2.90-12.49)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Adjusted‡</td>
<td>1.00</td>
<td>1.95 (1.02-3.72)</td>
<td>2.46 (1.10-5.50)</td>
<td>6.83 (2.82-16.55)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Interferon (\gamma) assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive response, No. (%)</td>
<td>4 (4)</td>
<td>7 (10)</td>
<td>21 (44)</td>
<td>44 (81)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>2.56 (0.72-9.10)</td>
<td>18.47 (5.84-58.43)</td>
<td>104.50 (31.06-351.62)</td>
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<td>Adjusted‡</td>
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<td>2.48 (0.69-8.90)</td>
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tion in all Koreans was about 33% in 2004,23,28 so the TB infection rates of 4% and 10% in groups 1 and 2 measured by the IFN-γ assay might be too low. A previous large evaluation comparing enzyme-linked immunospot assays and skin tests for the diagnosis of M tuberculosis infections in Gambia, a region with a high TB prevalence, also raised concern about the low sensitivity of assays based on M tuberculosis–specific antigens.27

The possible underestimation of latent TB infection by IFN-γ assay in our study could be understood in terms of the antigenicity of ESAT-6 and CFP-10. Although these antigens are specific for M tuberculosis, they do not represent the whole spectrum of antigenicity of M tuberculosis.28,29 In addition, the different response rates to the antigen is cleared.33,34 Thus, the memory cells producing IFN-γ and 10% in groups 1 and 2 measured a short period of exposure to antigens in an ex vivo assay.31,32 Moreover, activated lymphocytes and effector memory cells producing IFN-γ persist for a limited time in circulation once the antigen is cleared.14,15 Thus, the IFN-γ assay might reflect recent rather than remote TB infections.

The measurement of correlation with the risk of infection could be a more sensitive method to compare the utility of the TST and the IFN-γ assay than a detailed determination of the specificity and sensitivity of tests considering that there is no criterion standard for the diagnosis of latent TB infection, especially in endemic regions, because of high levels of environmental exposure to mycobacteria and M tuberculosis. In our study, the IFN-γ assay test results were more closely associated with the risk of infection than the TST (P<.001), and this was not significantly affected by BCG vaccination status, age, and sex. Therefore, the IFN-γ assay could be more helpful than the TST for the detection of latent TB infection in Koreans despite possible underestimation.

However, we should consider the relatively higher costs, practical inconvenience, and the presence of indeterminate test results of the IFN-γ assay. The tentatice test of the IFN-γ assay to process a sample from 1 patient would be between $20 and $30 US while the cost of the TST is as low as $1 US in Korea. In addition, samples of at least 22 patients should be analyzed per run not to waste wells. Because the IFN-γ assay is based on an enzyme-linked immunosorbent assay method that involves the use of a standard curve, minimal sample costs will only be achieved when all wells are used. Moreover, the presence of indeterminate test results of the IFN-γ assay, although rare, should be acknowledged.

In conclusion, the IFN-γ assay based on the ESAT-6 and CFP-10 M tuberculosis–specific antigens is a useful method for detecting latent TB infections and might help to eliminate the limitations of the TST in BCG-vaccinated populations in intermediate TB-burden countries. However, the negative IFN-γ assay test results should be cautiously appreciated because of its possible low sensitivity in the diagnosis of latent TB infections.

Author Contributions: Dr Yim had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Yim. Acquisition of data: Kang, Lee, Yoon, Cho. Analysis and interpretation of data: Kang, Han, Shim, Yim. Drafting of the manuscript: Kang, Lee. Critical revision of the manuscript for important intellectual content: Yoon, Cho, Han, Shim, Yim. Statistical analysis: Kang. Administrative, technical, or material support: Lee, Yoon, Cho, Han, Yim. Study supervision: Han, Shim, Yim. Financial Disclosures: None reported.

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Every good poem, in fact, is a bridge built from the known, familiar side of life over into the unknown. Science too is always making expeditions into the unknown. But this does not mean that science can supersede poetry. For poetry enlightens us in a different way from science; it speaks directly to our feelings or imagination. The findings of poetry are no more and no less true than science.

—C. Day-Lewis (1904-1972)