

Mycobacterium tuberculosis Infection in Health Care Workers in Rural India

Comparison of a Whole-Blood Interferon γ Assay With Tuberculin Skin Testing

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AN ESTIMATED ONE THIRD OF the world's population is infected with *Mycobacterium tuberculosis*,¹ presenting a major impediment to tuberculosis control. Despite the importance of latent tuberculosis infection (LTBI), the tuberculin skin test (TST) was, until recently, the only test available for its diagnosis.²⁻⁵ The TST measures hypersensitivity response to purified protein derivative (PPD), a crude mixture of antigens, many of which are shared among *M tuberculosis*, *Mycobacterium bovis* bacille Calmette-Guérin (BCG), and several nontuberculous mycobacteria (NTM). The TST has limitations with respect to accuracy and reliability.²⁻⁶

Advances in genomics and immunology have led to a promising alternative,

See also pp 2756 and 2785.

Context *Mycobacterium tuberculosis* infection in health care workers has not been adequately studied in developing countries using newer diagnostic tests.

Objectives To estimate latent tuberculosis infection prevalence in health care workers using the tuberculin skin test (TST) and a whole-blood interferon γ (IFN- γ) assay; to determine agreement between the tests; and to compare their correlation with risk factors.

Design, Setting, and Participants A cross-sectional comparison study of 726 health care workers aged 18 to 61 years (median age, 22 years) with no history of active tuberculosis conducted from January to May 2004, at a rural medical school in India. A total of 493 (68%) of the health care workers had direct contact with patients with tuberculosis and 514 (71%) had BCG vaccine scars.

Interventions Tuberculin skin testing was performed using 1-TU dose of purified protein derivative RT23, and the IFN- γ assay was performed by measuring IFN- γ response to early secreted antigenic target 6, culture filtrate protein 10, and a portion of tuberculosis antigen TB7.7.

Main Outcome Measures Agreement between TST and the IFN- γ assay, and comparison of the tests with respect to their association with risk factors.

Results A large proportion of the health care workers were latently infected; 360 (50%) were positive by either TST or IFN- γ assay, and 226 (31%) were positive by both tests. The prevalence estimates of TST and IFN- γ assay positivity were comparable (41%; 95% confidence interval [CI], 38%-45% and 40%; 95% CI, 37%-43%, respectively). Agreement between the tests was high (81.4%; $\kappa=0.61$; 95% CI, 0.56-0.67). Increasing age and years in the health profession were significant risk factors for both IFN- γ assay and TST positivity. BCG vaccination had little impact on TST and IFN- γ assay results.

Conclusions Our study showed high latent tuberculosis infection prevalence in Indian health care workers, high agreement between TST and IFN- γ assay, and similar association between positive test results and risk factors. Although TST and IFN- γ assay appear comparable in this population, they have different performance and operational characteristics; therefore, the decision to select one test over the other will depend on the population, purpose of testing, and resource availability.

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the in vitro interferon γ (IFN- γ) assay,⁶⁻⁸ based on the concept that T cells of infected individuals release IFN- γ . Although early IFN- γ assays used PPD, newer assays use antigens, such as the early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These proteins, encoded within the region of difference 1 (RD1) of the *M tuberculosis* genome, are significantly more specific to *M tuberculosis* than PPD, as they are not shared with BCG substrains or several NTM species that might cause nonspecific sensitization.⁶ The QuantiFERON-TB assay (Cellestis Limited, Carnegie, Australia) was the first commercially available IFN- γ assay. The PPD-based QuantiFERON-TB test was approved by the US Food and Drug Administration in 2001.⁹ The QuantiFERON-TB Gold, an enhanced assay based on RD1 antigens, was approved by the Food and Drug Administration in December 2004. A simplified variant of the QuantiFERON-TB Gold assay, the QuantiFERON-TB Gold In-Tube test (hereafter referred to as IFN- γ assay) has been recently developed. This assay uses overlapping peptides representing ESAT-6, CFP-10, and a portion of tuberculosis antigen TB7.7 (Rv2654), and stimulation by this antigenic mixture occurs within the tube used to collect blood. Other tests, including those using enzyme-linked immunospot, have also been evaluated.⁶⁻⁸

Several studies have evaluated IFN- γ assays (in a review article⁸); however, very few have been conducted in tuberculosis-endemic countries. In such countries, high BCG vaccine coverage, widespread NTM exposure, and high tuberculosis incidence pose challenges for the evaluation of any LTBI test. Because of the paucity of data from endemic countries and the need for improved tuberculosis diagnostic tests, we evaluated an RD1-based IFN- γ assay in a high-risk population in India, the country that accounts for one third of the global burden of tuberculosis.^{10,11} An estimated 40% of the Indian population is infected and the annual risk of infection is 1.5%.¹⁰⁻¹²

Health care workers are at high risk for tuberculosis.¹³ Despite India's tuberculosis burden, little is known about tuberculosis in health care workers.^{14,15} The Revised National Tuberculosis Control Programme of India is focused on expanding Directly Observed Treatment, Short-Course services. There are currently no national guidelines specific for reducing nosocomial tuberculosis risk. Because health care workers treat large numbers of patients¹⁶ and isolation facilities are scarce and expensive, Indian hospitals do not routinely isolate patients with tuberculosis.^{14,15} Health care workers often work in crowded hospitals with minimal infection control measures.¹⁴ The goals of our study were to estimate the burden of LTBI in Indian health care workers using the TST and an enhanced IFN- γ assay, determine the agreement between the tests, and compare their correlation with LTBI risk factors.

METHODS

Study Design and Setting

We conducted a cross-sectional comparison study from January to May 2004, at the Mahatma Gandhi Institute of Medical Sciences, a rural medical school in Sevagram, India. At this hospital, about 300 patients with tuberculosis are treated each year and the estimated community prevalence of smear-positive tuberculosis is 200 per 100 000,¹⁷ with the annual risk of infection at 1.2% to 1.6%.¹⁸ At the Mahatma Gandhi Institute of Medical Sciences hospital, there are limited tuberculosis infection control measures in place and health care workers are constantly exposed to patients with tuberculosis. Although no data were available on the incidence of tuberculosis among health care workers at the time of our study, 5 (33%) of 15 internal medicine residents had developed active tuberculosis during their training.

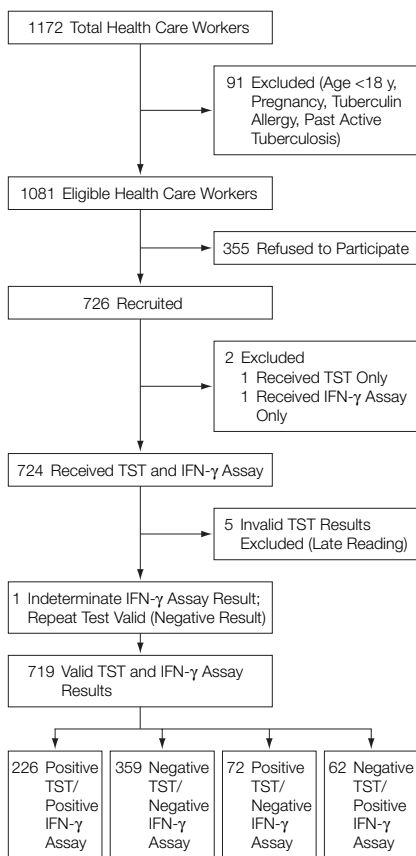
Participants

Our study was designed to recruit health care workers, including trainees, nurses, laboratory workers, orderlies, and attending physicians. Health care workers who met any of the following crite-

ria were excluded: age younger than 18 years, pregnancy, allergy to tuberculin, and active tuberculosis in the past. Because our goal was to recruit all health care workers at our institution, no formal sample size determination was made. All participants gave written informed consent, and the research was approved by the ethics committees at the Mahatma Gandhi Institute of Medical Sciences hospital and the University of California, Berkeley.

Each participant provided data on age, sex, education, employment, BCG vaccination, prior TST, and symptoms. We also assessed surrogate markers of *M tuberculosis* exposure, including year of training, years in the health care profession, job category, direct contact with sputum smear-positive tuberculosis (defined as contact between 2 people that is of sufficient distance to allow conversation between them⁵), training in internal medicine, and household tuberculosis contact. Education and job category were used as socioeconomic status indicators. After the interview, BCG vaccine scar was ascertained by inspection and blood was drawn for the IFN- γ assay, followed by TST administration. The tests were interpreted independently of each other and of exposure information. Human immunodeficiency virus testing was not performed because of the low prevalence of positive human immunodeficiency virus expected in this young, educated population.

Participants who were symptomatic or positive by either test were investigated (using chest radiographs and sputum microscopy) to rule out active disease. Although it is not standard practice to treat LTBI in India, health care workers diagnosed to have LTBI (n=360) in our study were offered the option of counseling on risks and benefits of LTBI treatment. Of these, 61 (17%) health care workers (mostly student and staff nurses) elected to undergo standard isoniazid treatment for 6 months.¹⁹ As of January 2005, 37 (61%) of 61 health care workers had completed therapy with no major adverse events.

Figure 1. Study Flow Diagram

TST indicates tuberculin skin test; IFN- γ , interferon γ . TST cutpoint is at least 10 mm; IFN- γ assay cutpoint is at least 0.35 IU/mL.

TST and IFN- γ Testing

As recommended by India's Revised National Tuberculosis Control Programme²⁰ and based on previous studies,^{3,21} we performed the TST using 1-TU dose of PPD RT23 (Statens Serum Institut, Copenhagen, Denmark). Tuberculin skin test was administered by a certified technician using the Mantoux method and read after 48 to 72 hours using a blinded caliper. Interreader variability was assessed using a second independent reader for a subset of the participants. The second trained reader used the same palpation technique as the first. As recommended by the Revised National Tuberculosis Control Programme²⁰ and consistent with the standard risk-stratified interpretation,¹⁹ an

induration of at least 10 mm was considered positive. For comparison, 5 mm and 15 mm cutpoints were also used.

An enhanced "in-tube" version of the IFN- γ assay was used for this study. The assay involves 2 stages: incubation of whole blood with antigens, and measurement of IFN- γ production in harvested plasma by enzyme-linked immunosorbent assay. Venous blood was directly collected into three 1-mL heparin-containing tubes. One tube contained only heparin as negative control, another also contained the T-cell mitogen phytohemagglutinin as positive control, and the third tube had overlapping peptides representing the entire sequences of ESAT-6 and CFP-10, and another peptide representing a portion of TB7.7. Within 2 to 6 hours of blood draw, the tubes were incubated at 37°C. After 24 hours of incubation, the tubes were centrifuged and plasma was harvested and frozen at -70°C until the enzyme-linked immunosorbent assay was performed. The amount of IFN- γ was quantified using the enzyme-linked immunosorbent assay. The IFN- γ values (IU/mL) for tuberculosis-specific antigens and mitogen were corrected for background by subtracting the value obtained for the respective negative control. As recommended by the manufacturer and based on previous studies,^{22,23} the cutoff value for a positive test was IFN- γ of at least 0.35 IU/mL. The enzyme-linked immunosorbent assay cannot accurately determine the absolute IFN- γ values when they exceed 10 IU/mL. Therefore, IFN- γ values of more than 10 IU/mL are shown as 10 IU/mL in our graphs. Testing was performed at the study site, and all assays met quality control standards.

Statistical Analysis

Statistical analyses were performed using Stata version 8 (Stata Corp, College Station, Tex). The outcomes included agreement between the tests, factors associated with discordance, and comparison of the tests with respect to their association with risk factors. Agreement between the tests was quantified using the κ statistic. Correlation was also

assessed between continuous IFN- γ values (IU/mL) and TST induration (mm). Factors associated with discordance were explored using a logistic regression model with any discordance (either positive TST/negative IFN- γ assay or negative TST/positive IFN- γ assay) as the outcome, and factors such as age, sex, exposure to tuberculosis, and BCG vaccine as covariates.

Risk factors for test positivity were evaluated using odds ratios (ORs). To adjust for multiple covariates, we used a logistic regression model with TST or IFN- γ assay results as the outcome and covariates such as markers of tuberculosis exposure. Logistic regression was performed using methods described elsewhere²⁴: covariates that were significant in bivariate analyses were included in the preliminary model. In addition, other covariates (such as age) that were considered biologically important were forced into the model irrespective of statistical significance. Interaction between covariates was evaluated by adding interaction terms to the model and evaluating its statistical significance using the Wald *P* value. From the initial model, covariates that did not contribute significantly were dropped 1 at a time until the best fitting, parsimonious model was identified. Model fit was evaluated using the Hosmer-Lemeshow test.²⁴ The results of the final model are presented as adjusted ORs with 95% confidence intervals (CIs). Because young trainees in the hospital were expected to have shorter and uncomplicated exposure histories, and recall of exposure might be better in students, we planned a prespecified subgroup analysis to evaluate risk factors among 353 medical and nursing students.

RESULTS

Participant Description

Of 1081 eligible health care workers, 726 (67%) participated in the study (FIGURE 1). Participation rates were high among students (91%) but low among attending physicians (10%). As shown in TABLE 1, medical and nursing students accounted for 48% of the

cohort. The median age was 22 years (range, 18-61 years). Only 36 of the health care workers (5%) had ever been tuberculin tested and 514 (71%) of 726 had BCG vaccine scars. A total of 493 (68%) of the participants reported direct contact with patients with sputum smear-positive tuberculosis. Exposure was particularly high among interns, residents, physicians, and nurses. On average, these health care workers reported at least 3 contacts with patients with tuberculosis in the week before their interviews.

TST Results

Valid TST results were available in 720 (99%) of 726 individuals. FIGURE 2 shows a bimodal TST distribution with the first peak centered on zero and a second peak centered on 15. With a cutpoint of at least 10 mm, 298 (41%) of 720 participants were positive (95% CI, 38%-45%). With a cutpoint of at least 15 mm, 167 (23%) of 720 participants were positive (95% CI, 20%-27%). A second TST reader independently read 254 (35%) of 720 participants and the interrater agreement was excellent ($\kappa=0.91$). TABLE 2 shows the risk factors for TST positivity. On multivariate analysis, age (21-30 vs 18-20 years) and number of years in the health care profession (>5 vs ≤ 1 year) were statistically significant. Previous BCG vaccination was not associated with TST positivity in bivariate and multivariate analyses.

IFN- γ Assay Results

Valid IFN- γ assay results were available for 725 (99%) of 726 participants (Figure 2). The median IFN- γ assay level was 0.11 IU/mL. With a cutpoint of at least 0.35 IU/mL, 291 (40%) of 725 participants were positive (95% CI, 37%-43%). Table 2 shows the risk factors for IFN- γ assay positivity. On multivariate analysis, age (≥ 41 vs 18-20 years), number of years in the health profession (>5 vs ≤ 1 year), and job category (orderlies vs medical students) were significant. Previous BCG vaccination was not associated with IFN- γ assay positivity in bivariate and multivariate analyses.

Subgroup Analysis

Among students, we evaluated covariates, including age, sex, student category, socioeconomic status, year of training, direct contact with active tuberculosis, household contact, training in internal medicine, and BCG vaccine scar. With TST as the outcome, the final model had only 2 covariates: age and sex. Students aged 21 to 23 years and older than 23 years had increased ORs compared with students aged 18 to 20 years (1.45; 95% CI, 0.8-2.6; and 4.2; 95% CI, 1.9-9.3; respectively). Although not statistically significant, female students had higher odds of TST positivity (OR, 1.7; 95% CI, 0.9-2.9). With IFN- γ assay as the outcome, only age and sex were retained in the final model. The ORs for those participants aged 21 to 23 years and older than 23 years were 1.36 (95% CI, 0.8-2.4) and 4.01 (95% CI, 1.8-8.8), respectively. Female students had higher odds of IFN- γ assay positivity (OR, 1.6; 95% CI, 0.9-2.8) but was not statistically significant.

Agreement Between TST and IFN- γ Assay Results

Data on agreement between the TST and IFN- γ assay results were available for 719 participants (TABLE 3). Of these, 360 (50%) were positive by either test and 226 (31%) were positive by both tests. With a 10-mm cutpoint, the agreement was 81.4% ($\kappa=0.61$; 95% CI, 0.56-0.67). Agreement was lower when 5-mm and 15-mm cutpoints were used. FIGURE 3 shows the distribution of IFN- γ assay values by TST category. A sharp increase in the IFN- γ assay values was observed when the induration exceeded 10 mm. FIGURE 4 shows the TST distribution by IFN- γ assay status. The TST distributions are well-separated, with a median of 3 mm and 15 mm in the negative IFN- γ assay and positive IFN- γ assay groups, respectively ($P<.001$ for difference between medians, using the Wilcoxon rank sum test).

TABLE 4 shows the agreement data stratified by covariates. Increasing age,

Table 1. Participant Characteristics (N=726)*

Characteristics	No. (%) of Participants
Age, y	
18-20	226 (31)
21-30	324 (45)
31-40	75 (10)
≥ 41	101 (14)
Women	453 (62)
Educational level	
Medical degree, master's, bachelor's degree, or diploma†	619 (85)
High school or lower	107 (15)
Job category	
Medical students	227 (31)
Interns	43 (6)
Residents	30 (4)
Nursing students	126 (17)
Nurses	161 (22)
Laboratory staff	39 (5)
Orderlies	87 (12)
Attending physicians/faculty	13 (2)
Years served in the health care profession	
≤ 1	96 (13)
2-5	343 (47)
6-10	118 (16)
>10	169 (23)
Tuberculin skin tested in the past	36 (5)
Ever tested for human immunodeficiency virus	22 (3)
BCG vaccine scar present	514 (71)
Ever had direct contact with a patient with tuberculosis‡	493 (68)
Ever lived in a household with a patient with tuberculosis	40 (6)

*Percentages may not add up to 100 due to rounding.
 †In India, diploma is completed after high school but is 1 level less than a bachelors degree; therefore, this category does not include high school.
 ‡Direct contact was defined as contact between 2 people that is of sufficient distance to allow conversation between them.

years in health profession, lower educational level, and specific job categories (laboratory staff, orderlies, and attending physicians/faculty) were associated with greater discordance (either positive TST/negative IFN- γ assay or negative TST/postive IFN- γ assay) on bivariate analyses. However, on multivariate analysis, only 2 covariates were important but not statistically significant: job category (attending physicians/faculty vs medical students: OR, 3.9; 95% CI, 0.9-15.6) and increasing years in health care (OR, 1.3; 95% CI, 0.6-2.8; 2.01; 95% CI, 0.8-5.4; and 2.1; 95% CI, 0.6-7.5; respectively) for those participants with 2 to 5 years, 6 to 10 years, and more than

10 years in the health profession compared with those with 1 year or less. A positive TST/negative IFN- γ assay pattern of discordance was more frequently observed with increasing age, increasing years in the health care profession, and in specific subgroups (nurses, laboratory staff, and attending physicians/faculty). A negative TST/positive IFN- γ assay pattern of discordance was more frequently observed in those participants with household tuberculosis contact. However, none of these differences were statistically significant on multivariate analyses (data not shown).

COMMENT

Our study demonstrated the expected high prevalence of LTBI in Indian health care workers. Although TST and IFN- γ assay use different antigen combinations, it was surprising that these tests had comparable prevalence estimates (41% and 40%, respectively) and a high level of agreement (81.4%, $\kappa=0.61$). The marginal bimodal TST distribution appears to be composed of 2 fairly well-separated component distributions of negative and positive IFN- γ assay individuals. Greater discordance between the PPD-based TST and the

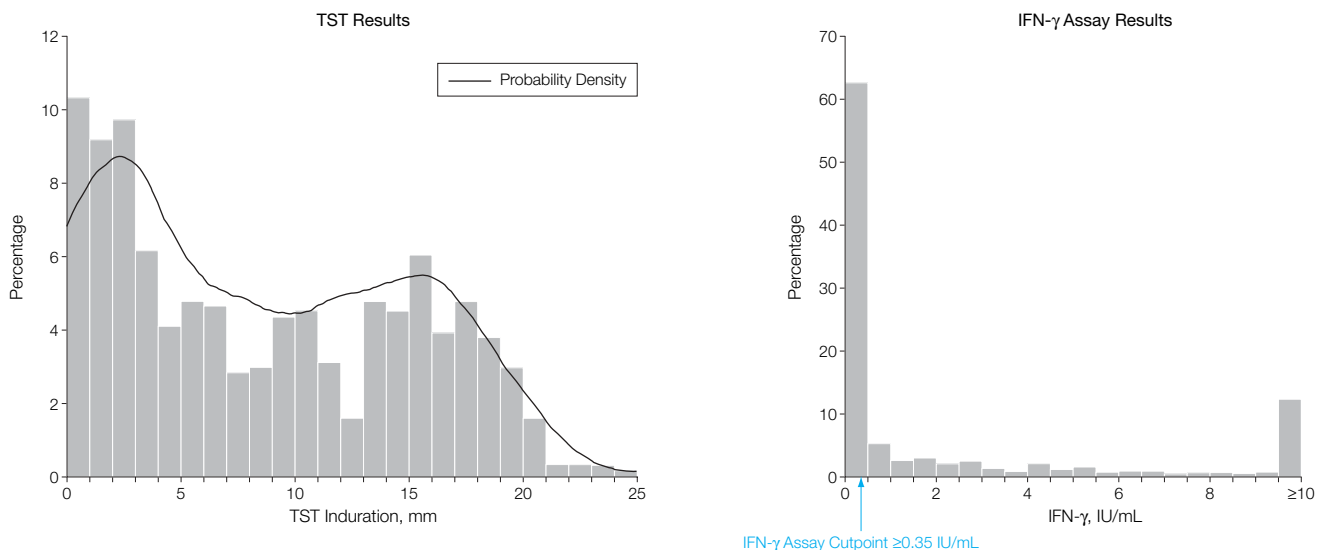
tuberculosis-specific antigen-based IFN- γ assay was expected. The use of 1-TU dosage of PPD in our study may have improved TST specificity, as many countries use a 2-TU dosage.²¹ Increasing age and years in the health profession were risk factors for both IFN- γ assay and TST positivity, and the risk factor associations were fairly similar for both tests. To our knowledge, our study is the largest evaluation of an IFN- γ assay among health care workers in a developing country and the first report of the In-Tube version of the QuantiFERON-TB Gold assay. This version of the assay is easier to perform and facilitates testing of people in remote locations.

The high prevalence of LTBI in our study is consistent with estimates from other developing countries.^{13,25-27} However, our prevalence of approximately 40% might be an underestimate because we recruited few senior physicians who were older and, presumably, more frequently exposed to *M tuberculosis*. Also, our study was not designed to determine if tuberculosis transmission occurred in the nosocomial setting. Although studies from India have suggested a high rate of tuberculosis among health care

workers,^{14,15} these studies did not test for LTBI.

The risk factors we identified have been shown to be important in several studies.^{13,25-29} Age and years in health care reflect cumulative exposure to *M tuberculosis*. Variability of risk across job categories may reflect variations in exposure frequency and intensity.^{28,29} Although direct contact with patients with tuberculosis has been shown to be a risk factor in previous studies,^{13,25-27} we did not find a strong effect. However, this is not surprising as nearly 70% of our health care workers reported direct contact, reducing the predictive ability of this exposure factor. Overall, our findings highlight the need to study tuberculosis among Indian health care workers. India's Revised National Tuberculosis Control Programme is the largest Directly Observed Treatment, Short-Course program in the world,¹⁶ and the safety and well-being of health care workers is important for the continued expansion of this successful program. Further research is needed to document the incidence of tuberculosis among Indian health care workers and determine the rate of nosocomial transmission. If such studies demonstrate a

Figure 2. Distribution of TST (n=720) and IFN- γ Assay (n=725) Responses



TST indicates tuberculin skin test; IFN- γ , interferon γ . The overlaid curve is a plot of the probability density function, a smoothed version of the histogram.

Table 2. Covariates Associated With Positive TST and IFN- γ Assay Results

Covariate	TST Cutpoint ≥ 10 mm			IFN- γ Assay Cutpoint ≥ 0.35 IU/mL		
	No. Positive/ Total No. Tested (%)	OR (95% CI)		No. Positive/ Total No. Tested (%)	OR (95% CI)	
		Unadjusted	Adjusted*		Unadjusted	Adjusted*
Age, y						
18-20	47/225 (21)	1.00	1.00	50/226 (22)	1.00	1.00
21-30	130/322 (40)	2.56 (1.73-3.79)	1.91 (1.12-3.25)	127/323 (39)	2.28 (1.55-3.35)	1.48 (0.88-2.48)
31-40	49/75 (65)	7.13 (4.02-12.67)	1.75 (0.63-4.80)	42/75 (56)	4.48 (2.57-7.79)	1.43 (0.54-3.80)
≥ 41	72/98 (73)	10.48 (6.04-18.20)	2.92 (0.94-9.01)	72/101 (71)	8.73 (5.12-14.89)	3.09 (1.04-9.21)
Sex						
Male	98/270 (36)	1.00	1.00	102/273 (37)	1.00	1.00
Female	200/450 (44)	1.40 (1.02-1.91)	1.49 (0.96-2.31)	189/452 (42)	1.20 (0.88-1.63)	1.22 (0.78-1.87)
Education level						
Medical degree, master's, bachelor's degree, or diploma	226/617 (37)	1.00		216/618 (35)	1.00	
High school or lower	72/103 (70)	4.02 (2.55-6.31)		75/107 (70)	4.36 (2.79-6.81)	
Job category						
Medical students	52/227 (23)	1.00	1.00	49/227 (22)	1.00	1.00
Nursing students	36/125 (29)	1.36 (0.82-2.23)	1.05 (0.60-1.83)	41/126 (33)	1.75 (1.07-2.85)	1.63 (0.94-2.83)
Interns	8/43 (19)	0.76 (0.33-1.76)	0.43 (0.18-1.06)	12/43 (28)	1.40 (0.67-2.94)	0.87 (0.39-1.91)
Residents	13/30 (43)	2.57 (1.17-5.64)	0.51 (0.19-1.32)	13/30 (43)	2.77 (1.26-6.11)	0.83 (0.32-2.14)
Nurses	104/161 (65)	6.14 (3.92-9.60)	1.55 (0.81-2.95)	88/160 (55)	4.43 (2.84-6.92)	1.56 (0.82-2.96)
Laboratory staff	23/39 (59)	4.83 (2.38-9.83)	0.93 (0.37-2.38)	21/39 (54)	4.23 (2.09-8.57)	1.07 (0.43-2.66)
Orderlies	57/83 (69)	7.37 (4.22-12.88)	1.49 (0.68-3.29)	63/87 (72)	9.53 (5.41-16.8)	2.71 (1.25-5.86)
Attending physicians/faculty	5/12 (42)	2.40 (0.73-7.89)	0.41 (0.10-1.59)	4/13 (31)	1.61 (0.47-5.46)	0.41 (0.10-1.61)
Years served in the health care profession						
≤ 1	23/95 (24)	1.00	1.00	17/96 (18)	1.00	1.00
2-5	86/343 (25)	1.04 (0.61-1.77)	0.76 (0.42-1.37)	98/343 (29)	1.85 (1.04-3.30)	1.61 (0.86-3.02)
6-10	72/117 (62)	5.00 (2.75-9.11)	2.78 (1.23-6.25)	67/117 (57)	6.22 (3.28-11.80)	4.15 (1.81-9.50)
> 10	117/165 (71)	7.63 (4.28-13.59)	3.20 (1.08-9.45)	109/169 (65)	8.44 (4.58-15.55)	3.34 (1.13-9.81)
BCG vaccine scar						
Absent	87/209 (42)	1.00		90/212 (42)	1.00	
Present	211/511 (41)	0.98 (0.71-1.36)		201/513 (39)	0.87 (0.63-1.20)	
Direct contact with tuberculosis						
No	84/229 (37)	1.00		84/233 (36)	1.00	
Yes	214/491 (44)	1.33 (0.96-1.84)		207/492 (42)	1.28 (0.93-1.77)	
Household contact with tuberculosis						
No	282/680 (42)	1.00		271/685 (40)	1.00	
Yes	16/40 (40)	0.94 (0.49-1.80)		20/40 (50)	1.52 (0.80-2.89)	

Abbreviations: CI, confidence interval; IFN- γ , interferon γ ; OR, odds ratio; TST, tuberculin skin test.

*From a multivariate logistic regression model with age, sex, job category, and years in health care as covariates.

problem, control strategies will be needed to address the issue.

Several studies have compared RD1-based IFN- γ assays with TST, mostly in low-endemic populations.⁸ The agreement in our high-endemic population (81.4%), interestingly, is consistent with those data reported by a majority of the studies.^{22,30-33} A recent study on an RD1-based IFN- γ assay that reported agreement data showed an agreement of 94%, but this analysis in-

Table 3. Agreement Between TST and IFN- γ Assay Results (n = 719)

Results*	TST Cutpoint, mm		
	≥ 5	≥ 10	≥ 15
Positive TST/positive IFN- γ assay	259	226	148
Negative TST/negative IFN- γ assay	254	359	412
Positive TST/negative IFN- γ assay	177	72	19
Negative TST/positive IFN- γ assay	29	62	140
Agreement, %	71.4	81.4	77.9
κ (95% CI)	0.45 (0.39-0.51)	0.61 (0.56-0.67)	0.51 (0.44-0.57)

Abbreviations: CI, confidence interval; IFN- γ , interferon γ ; TST, tuberculin skin test.*IFN- γ assay cutpoint was at least 0.35 IU/mL.

cluded participants who had not received BCG vaccine.²² Only 1 published study has estimated the prevalence of LTBI in India using an IFN- γ assay. Lalvani et al³⁴ showed that 80% of healthy adults (affluent corporate executives) in Bombay (Mumbai) tested enzyme-linked immunospot–positive to either ESAT-6 or CFP-10. In contrast, only 40% of our high-risk cohort was positive by IFN- γ assay, in line with the

epidemiological estimate for India of 40%¹⁰ and the 41% TST positivity in the present study. Differences in IFN- γ assay methodology might explain this difference.

Although previous studies have shown BCG vaccine to cause a positive TST/negative IFN- γ assay discordance,^{31,35} previous BCG vaccination had little impact on TST in our study. Our study largely comprised adults who received BCG vaccine (Danish 1331 strain) at birth. In individuals vaccinated in infancy, TST response due to BCG vaccine wanes rapidly.^{3,5,36,37} Another explanation could be misclassification of BCG vaccine status owing to the use of scar as a proxy.^{3,36} Recent tuberculin surveys from India, involving more than 100 000 children, have also shown the BCG vaccination does not influence the estimation of annual risk of infection.^{36,38,39} However, BCG vaccine might have an effect on TST in other populations, depending on vaccine strain, timing, frequency, and time since vaccination.^{4,5,37} Infection with NTM, which tends to cause nonspecific tuberculin sensitivity, is highly prevalent in India.^{3,40} Therefore, NTM infection might have caused false-positive TST results. Because the IFN- γ

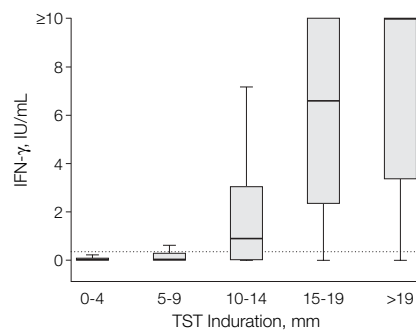
assay uses RD1 antigens, the effect of NTM on the test should be limited. However, ESAT-6 and CFP-10 have the potential to cross-react with certain NTM species,^{6,30} and it has been suggested that cross-reactivity may exist with *Mycobacterium leprae*.⁴¹ Because NTM and *M leprae* are endemic in India, research is needed to study the effect of NTM on IFN- γ assays.

Our cross-sectional data suggest that both tests are comparable in the population we studied. However, this does not necessarily indicate that these tests are completely equivalent because they do not measure exactly the same immunological phenomenon.³⁵ To put our findings in context, it is important to review the evidence on the performance characteristics of these tests.

The TST has moderate to high sensitivity and specificity, depending on the population screened, with specificity being more unpredictable.²⁻⁵ Several studies have shown a positive association between TST response and subsequent risk of active tuberculosis,^{2,4,42,43} and randomized trials have shown that treatment of LTBI, diagnosed using TST, reduces the risk of active tuberculosis by 60% to 90%.¹⁹ Furthermore, the TST is a simple test with low material costs that does not require a laboratory, but does require skilled testers.

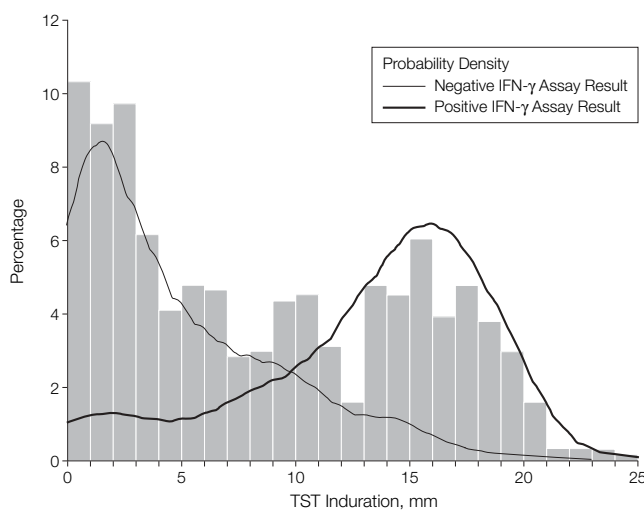
The RD1-based IFN- γ assays have higher specificity than TST, better correlation with exposure to *M tuberculosis* than TST, no cross-reactivity due to BCG vaccination, and limited cross-reactivity due to NTM infection.^{6-9,22,23,31,35} Other advantages include rapidity, the need for only a single visit, avoidance of subjective measurements, and boosting.^{6,7,35} There is limited evidence of an association between IFN- γ assay response to ESAT-6 and subsequent progression to active tuberculosis among contacts of patients with tuberculosis.⁴⁴ To date, no trial has demonstrated the efficacy of treatment based on IFN- γ assay results. A limitation of IFN- γ assays is its higher material costs and the need for laboratory infrastructure.⁸ Also, IFN- γ assays have been inadequately evaluated in children, individuals with im-

Figure 3. Correlation Between TST and IFN- γ Assay Responses (n=719)



TST indicates tuberculin skin test; IFN- γ , interferon γ . The boxes, which indicate the distribution of IFN- γ values for each category of TST induration, are bordered at the 25th and 75th percentiles of the IFN- γ values, with a median line at the 50th percentile. Outliers are not displayed. IFN- γ values of more than 10 IU/mL are displayed as 10 IU/mL. The horizontal dotted line indicates the IFN- γ assay cutpoint of 0.35 IU/mL.

Figure 4. Distribution of TST Responses by IFN- γ Assay Status (n=719)



TST indicates tuberculin skin test; IFN- γ , interferon γ . The overlaid curves are plots of the probability density functions, smoothed versions of the histograms.

munosuppressive conditions, and in high-burden countries.⁸

Our study has several limitations. Unlike the previous IFN- γ assay studies in outbreak and contact investigations,^{22,31-33} we were unable to quantify duration and proximity of contact with patients with tuberculosis. Such exposure measures might have enabled us to evaluate whether the IFN- γ assay was better associated with increasing exposure than TST, although in a high prevalence health care worker

population this may be difficult. Because of the cross-sectional design and the lack of follow-up data, we were unable to adequately resolve the discordance between the 2 tests.

We were also unable to measure important covariates, such as NTM and human immunodeficiency virus infection. Information on human immunodeficiency virus status would have helped to better evaluate the performance of the IFN- γ assay. Although nonresponse among senior physicians

might have led to an underestimation of LTBI prevalence, it is unlikely to affect the comparisons between TST and the IFN- γ assay. Unfortunately, due to lack of data from nonparticipants, we could not compare their characteristics with those who did participate.

Although consistent with the Indian policy, the use of 1-TU dose of PPD RT23 limits our ability to compare our data with studies that have used 2-TU dosages. Our study consisted of health care workers in a high prevalence coun-

Table 4. Discordance Between TST and IFN- γ Assay Results*

Covariates	Agreement Between TST and IFN- γ Assay		No. of Discordant Results/Total No. Tested	%			
	%	κ (95% CI)		Positive TST/Positive IFN- γ Assay	Negative TST/Negative IFN- γ Assay	Positive TST/Negative IFN- γ Assay	Negative TST/Positive IFN- γ Assay
Age, y							
18-20	85.3	0.57 (0.43-0.70)	33/225	14	71	7	8
21-30	81.9	0.62 (0.54-0.71)	58/321	31	51	10	8
31-40	77.3	0.53 (0.34-0.72)	17/75	49	28	16	7
≥ 41	73.5	0.34 (0.13-0.54)	26/98	59	14	14	12
Sex							
Male	81.1	0.59 (0.49-0.69)	51/270	27	54	9	10
Female	81.5	0.62 (0.55-0.70)	83/449	34	48	11	8
Education level							
Medical degree, master's, bachelor's degree, or diploma	82.6	0.62 (0.56-0.69)	107/616	27	56	10	8
High school or lower	73.8	0.37 (0.18-0.57)	27/103	57	17	13	14
Job category							
Medical students	85.5	0.58 (0.45-0.71)	33/227	15	70	8	7
Nursing students	81.6	0.57 (0.41-0.72)	23/125	22	60	7	11
Interns	90.7	0.74 (0.51-0.97)	4/43	19	72	0	9
Residents	86.7	0.73 (0.48-0.98)	4/30	37	50	7	7
Nurses	80.0	0.59 (0.46-0.71)	32/160	50	30	15	5
Laboratory staff	69.2	0.38 (0.08-0.67)	12/39	41	28	18	13
Orderlies	75.9	0.42 (0.20-0.63)	20/83	59	17	10	14
Attending physicians/faculty	50.0	-0.09 (-0.60 to 0.42)	6/12	8	42	33	17
Years served in the health care profession							
≤ 1	87.4	0.62 (0.43-0.81)	12/95	15	73	9	3
2-5	84.3	0.60 (0.50-0.70)	54/343	19	65	6	10
6-10	77.6	0.54 (0.38-0.69)	26/116	48	29	14	9
> 10	74.6	0.42 (0.27-0.56)	42/165	55	19	16	10
BCG vaccine scar							
Absent	84.2	0.68 (0.57-0.78)	33/209	34	50	8	8
Present	80.2	0.59 (0.52-0.66)	101/510	30	50	11	9
Direct contact with tuberculosis							
No	81.7	0.60 (0.50-0.71)	42/229	27	55	10	9
Yes	81.2	0.62 (0.55-0.69)	92/490	33	48	10	9
Household contact with tuberculosis							
No	81.4	0.61 (0.55-0.67)	126/677	31	50	10	8
Yes	80.0	0.60 (0.36-0.84)	8/40	35	45	5	15

Abbreviations: CI, confidence interval; IFN- γ , interferon γ ; TST, tuberculin skin test.
*TST cutpoint was at least 10 mm and IFN- γ assay cutpoint was at least 0.35 IU/mL.

try. Although this may limit our ability to generalize the results to settings with different baseline prevalences, we believe our data will be helpful in understanding the performance of IFN- γ assays in high-burden settings for which data are scarce.⁸

In conclusion, our study showed a high prevalence of LTBI in Indian health care workers and high agreement between TST and an RD1-based IFN- γ assay. Our results and the available evidence suggest that both tests have advantages and limitations and, as of this time, both may have a useful role, depending on factors unique to each setting. The emergence of IFN- γ assays is a welcome development that has expanded the armamentarium of LTBI diagnostics. The decision to select one or the other test will depend on the population, the goal of testing, and the resources available. For example, for serial testing of health care workers, the IFN- γ assay will be appropriate. It will eliminate the need for repeat visits, avoid boosting, and minimize interpretational difficulties. Because of its high specificity, RD1-based assays will be helpful in populations where cross-reactivity due to BCG vaccine and NTM pose problems in TST interpretation and/or where true tuberculosis infection rate is relatively low. Furthermore, the IFN- γ assay may be helpful in screening populations in which low return rates for TST reading are a concern.

On the other hand, in high-burden, resource-limited settings, the TST might serve a useful purpose. In India, the TST is widely used in diagnosing childhood tuberculosis,^{3,20} and in epidemiological studies on annual risk of infection in which blood testing will be difficult.^{3,10,12,18,21,36} In India, a 15-year follow-up of 280 000 individuals showed that TST response is significantly associated with development of active tuberculosis.⁴² Our findings and evidence from Indian studies^{3,10,18,21,36} suggest that the TST remains a useful test, particularly because of the low cost and since BCG vaccine has a limited effect on TST.^{36,38,39} The relationship between BCG vaccine and subsequent TST response may vary in

other populations. To fully evaluate the use of IFN- γ assays, long-term cohort studies to determine the association between positive IFN- γ assay results and the subsequent risk of active tuberculosis are required in diverse settings.⁸ If such studies demonstrate a strong consistent association, IFN- γ assays might have the potential to replace TST.

Author Contributions: Dr Pai 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:* Pai, Kalantri, Narang, Daley, Granich, Mazurek, Riley, Colford. *Acquisition of data:* Pai, Gokhale, Joshi, Dogra, Kalantri, Mendiratta, Granich. *Analysis and interpretation of data:* Pai, Joshi, Kalantri, Narang, Granich, Mazurek, Reingold, Riley, Colford. *Drafting of the manuscript:* Pai, Kalantri, Colford. *Critical revision of the manuscript for important intellectual content:* Pai, Gokhale, Joshi, Dogra, Kalantri, Mendiratta, Narang, Daley, Granich, Mazurek, Reingold, Riley, Colford. *Statistical analysis:* Pai, Colford. *Obtained funding:* Pai, Reingold. *Administrative, technical, or material support:* Gokhale, Joshi, Dogra, Mendiratta, Narang, Granich, Mazurek, Reingold, Riley, Colford. *Study supervision:* Pai, Joshi, Kalantri, Mendiratta, Daley, Granich, Reingold, Riley, Colford.

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There is an art of reading, as well as an art of thinking, and an art of writing.
—Isaac D'Israeli (1766-1848)