Serial Testing of Health Care Workers for Tuberculosis Using Interferon- γ Assay

Madhukar Pai, Rajnish Joshi, Sandeep Dogra, Deepak K. Mendiratta, Pratibha Narang, Shriprakash Kalantri, Arthur L. Reingold, John M. Colford, Jr., Lee W. Riley, and Dick Menzies

Divisions of Epidemiology and Infectious Diseases, School of Public Health, University of California, Berkeley; Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, University of California, San Francisco, California; Departments of Medicine and Microbiology, Mahatma Gandhi Institute of Medical Sciences, Sevagram, India; and the Montreal Chest Institute, McGill University, Montreal, Quebec, Canada

Rationale: Although interferon- γ (IFN- γ) assays are promising alternatives to the tuberculin skin test (TST), their serial testing performance is unknown.

Objective: To compare TST and IFN- γ conversions and reversions in healthcare workers.

Methods: We prospectively followed-up 216 medical and nursing students in India who underwent baseline and repeat testing (after 18 mo) with TST and QuantiFERON-TB Gold In-Tube (QFT). TST conversions were defined as reactions greater than or equal to 10 mm, with increments of 6 or 10 mm over baseline. QFT conversions were defined as baseline IFN- γ less than 0.35 and follow-up IFN- γ greater than or equal to 0.35 or 0.70 IU/ml. QFT reversions were defined as baseline IFN- γ greater than or equal to 0.35 and follow-up IFN- γ less than 0.35 IU/ml.

Results: Of the 216 participants, 48 (22%) were TST-positive, and 38 (18%) were QFT-positive at baseline. Among 147 participants with concordant baseline negative results, TST conversions occurred in 14 (9.5%; 95% confidence interval [CI] = 5.3–15.5) using the 6 mm increment definition, and 6 (4.1%; 95% CI = 1.5–8.7) using the 10 mm increment definition. QFT conversions occurred in 17/147 participants (11.6%; 95% CI = 6.9–17.9) using the definition of IFN- γ greater than or equal to 0.35 IU/ml, and 11/147 participants (7.5%; 95% CI = 3.8–13.0) using IFN- γ greater than or equal to 0.70 IU/ml. Agreement between TST (10 mm increment) and QFT conversions ($\geq$ 0.70 IU/ml) was 96% (κ = 0.70). QFT reversions occurred in 2/28 participants (7%) with baseline concordant positive results, as compared with 7/10 participants (70%) with baseline discordant results (p < 0.001).

Conclusions: IFN- γ assay shows promise for serial testing, but repeat results need to be interpreted carefully. To meaningfully interpret serial results, the optimal thresholds to distinguish new infections from nonspecific variations must be determined.

Keywords: health care workers; interferon- γ assay; serial testing; tuberculin skin test; tuberculosis

Tuberculosis (TB) infects an estimated one third of the world's population, and about 9 million cases occur every year (1, 2). Because these individuals eventually present to health care pro-

viders, health care workers (HCWs) are especially vulnerable to TB exposure and infection (3, 4). Therefore, in many developed countries (e.g., the United States and Canada), HCWs are screened with tuberculin skin testing (TST) to identify and treat latent TB infection (LTBI) (3–6). However, effective screening requires a test that can accurately and reliably diagnose LTBI and predict those most likely to progress to disease. Unfortunately, the TST does not meet all these expectations; interpretation of serial TST is particularly complicated because of boosting, conversions, and reversions (7, 8).

With the emergence of IFN- γ release assays (IGRAs) for LTBI, there is interest in using them for screening contacts, immigrants, and other high-risk groups (9–11). Available evidence, extensively reviewed elsewhere (9–14), suggests that IGRAs have higher specificity than TST and are unaffected by previous bacillus Calmette-Guérin (BCG) vaccination. IGRAs avoid subjective measurements, can be repeated without boosting, and eliminate the need for repeat visits and two-step testing; these features are ideal for serial testing (9–12). In addition, IGRAs may actually detect recent, as compared with remote, infection, and T-cell responses may be related to bacterial burden (15–18). In that case, IGRAs may be particularly well suited to detecting new infections (i.e., conversions).

Two commercial IGRAs are now available: QuantiFERON-TB Gold In-Tube (QFT; Cellestis Ltd, Carnegie, Australia) and T-SPOT.TB (Oxford Immunotec, Oxon, UK), and institutions in North America and Europe are beginning to replace TST with IGRAs (11, 12). In December 2005, the US Centers for Disease Control and Prevention (CDC) published its interim guidelines on the US Food and Drug Administration (FDA)-approved version of QFT assay (11), as well as TB infection control in health care facilities (5). The CDC guidelines state that QFT can replace the TST in all circumstances in which the TST is currently used, including serial testing (5, 11). The infection control guidelines suggest that health facilities can directly switch to QFT for serial state (without overlapping with TST), with a single QFT at baseline; a QFT conversion was defined as change from negative to positive result (5).

To date, there are no published data on the performance of IGRAs in serial testing. There is a need to generate evidence on issues such as variability of IFN- γ responses during serial testing, frequency of conversions and reversions, and thresholds to distinguish new infection from nonspecific variation. We conducted a preliminary study of the performance of a commercial IGRA during serial testing by following-up a cohort of Indian HCWs. Some of the results have previously been reported in the form of an abstract (19).

METHODS

Study Population and Test Procedures

In 2004, we established a cohort of HCWs at the Mahatma Gandhi Institute of Medical Sciences, a rural hospital in Sevagram, India (20).

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Correspondence and requests for reprints should be addressed to Madhukar Pai, M.D., Ph.D., McGill University, Department of Epidemiology, Biostatistics & Occupational Health, 1020 Pine Avenue West, Montreal, Canada H3A 1A2. E-mail: madhukar.pai@mcgill.ca

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Between January and May 2004, 726 HCWs underwent TST and IGRA testing. Additional information on this cohort and baseline results are reported elsewhere (20) and described in the online supplement. At baseline, HCWs underwent a one-step TST using 1 TU PPD-RT23, the standard dose in India (21). They also underwent the QFT assay. As recommended by the manufacturer and based on previous studies (22–25), a positive QFT was defined as IFN- γ greater than or equal to 0.35 IU/ml. Because QFT ELISA cannot accurately measure absolute IFN- γ values greater than 10 IU/ml, such values were treated as 10 IU/ml. Details of TST and QFT methods, and distributions of IFN- γ responses (Figure E1 in the online supplement) are described further in the online supplement.

In July 2005, we invited the 353 medical and nursing students who had undergone baseline testing in January 2004 to undergo follow-up TST and QFT testing. Follow-up TST was offered only to those who had a TST less than 10 mm at baseline. Follow-up QFT was offered to everybody. To minimize test-related variability, identical test protocols were used for baseline and follow-up tests. Follow-up TST and QFT were performed by the same tuberculin reader and technician, respectively, blinded to the previous results.

Definitions of Conversions and Reversions

We used two definitions for TST conversions: (1) baseline TST less than 10 mm and follow-up TST of greater than or equal to 10 mm, with an increment of 6 mm; and (2) baseline TST less than 10 mm, and follow-up TST of greater than or equal to 10 mm, with an increment of 10 mm. Although the more sensitive 6 mm increment has been suggested because random variations will result in differences of less than 6 mm (7), the more stringent 10 mm increment threshold is more specific (4–6).

In contrast to the data available on TST thresholds (3, 4, 6–8), no data exist on serial IGRA testing in HCWs. Our choice of IFN- γ thresholds, therefore, was exploratory: (1) baseline IFN- γ less than 0.35 IU/ml and follow-up IFN- γ greater than or equal to 0.35 IU/ml (i.e., CDC definition of QFT conversion [(5)]; and (2) baseline IFN- γ less than 0.35 IU/ml and follow-up IFN- γ greater than or equal to 0.70 IU/ml (twice the manufacturer's diagnostic cut-off point); and (3) baseline IFN- γ less than 0.35 IU/ml and an absolute increase of at least 0.35 IU/ml over the baseline value. However, because this third definition produced results similar to those of the first, the data from the third definition are not reported. QFT reversions were defined as baseline IFN- γ greater than or equal to 0.35 IU/ml and follow-up IFN- γ less than 0.35 IU/ml (i.e., change from positive to negative). Because participants who were TST-positive (≥ 10 mm) at baseline did not undergo repeat testing, TST reversions were not determined.

All participants gave informed consent, and the research was approved by ethics committees at Mahatma Gandhi Institute of Medical Sciences, Sevagram, India, and the University of California, Berkeley. Participants with TST conversions were evaluated for TB disease and offered isoniazid therapy.

Statistical Analyses

Statistical analyses involved estimation of incidence of TST and QFT conversions using varying definitions, and incidence of QFT reversions. Concordance between dichotomized TST and QFT conversions were evaluated using agreement and κ statistics. We also evaluated the association between absolute TST and IFN- γ changes, with TST (mm induration) and QFT (IFN- γ levels in IU/ml) treated as continuous measures.

RESULTS

Participant Description

Of the 246 medical and nursing students eligible for follow-up TST and QFT testing, 216 (88%) participated (Figure 1). The median age of the cohort was 21 yr (range, 19–27 yr), and 138 (64%) were women. A total of 153 of 216 (71%) were medical students (49% women), and the remaining 63 (29%) were nursing students (100% women). BCG scars were observed on 153 of 216 (71%) participants, and 16 of 216 (7%) had received isoniazid therapy after the baseline survey. A total of 164 of 216 (76%) participants reported direct contact (i.e., within conversa-

tional distance) with patients with smear-positive TB since the baseline survey.

As shown in Figure 1, at baseline, 48 of 216 (22%) HCWs were TST-positive, and 38 of 216 (18%) were QFT-positive (86% agreement; $\kappa = 0.57$); 28 of 216 (13%) were positive by both TST and QFT (concordant positive), and 158 of 216 (73%) were negative by both tests (concordant negative). At baseline, 30 of 216 (14%) were discordant; 20 were TST⁺/QFT⁻, and 10 were TST⁻/QFT⁺. The distribution of absolute IFN- γ responses at baseline and after repeat testing is shown in Figure E1.

TST and QFT Conversions

TST and QFT conversions were compared in the baseline concordant negative group with valid follow-up TST and QFT results ($n = 147$). As seen in Table 1, there were more QFT than TST conversions, using either set of definitions. The estimated conversion rates ranged from 4.1 to 14.9%, depending on the definitions used.

As seen in Table 2, when less stringent thresholds were used for both tests, the agreement between TST and QFT conversions was high ($\kappa = 0.53$; 95% CI = 0.31–0.76). Agreement was higher when stringent thresholds were used for both tests ($\kappa = 0.70$; 95% CI = 0.44–0.94). When stringent thresholds were used for both tests (Table 2, *lower section*), every HCW who had a TST conversion (10 mm increment) had QFT conversion (IFN- $\gamma \geq 0.70$ IU/ml). As shown in Table 3 (*lowest section*), large increases (≥ 10 mm increases over the baseline) in TST induration were always accompanied by massive increases in IFN- γ that were much higher than the diagnostic threshold (Figure E2). However, there were five individuals who had QFT conversions but did not reach the 10-mm increment threshold on TST. As seen in Table 3 (*middle section*), of these five individuals, three had TST increments of 7–9 mm, associated with substantial IFN- γ increases. These may have been new TB infections, but missed by the use of the more stringent threshold for the TST.

When less stringent thresholds were used for both tests, there was greater discordance between TST and QFT (Table 2, *top section*). There were 5 HCWs who had TST but not QFT conversion. Table 3 (*top section*) shows that most individuals in this subgroup had modest increases in TST (3 of 5 just reached 10 mm on follow-up testing), with small or no increases in IFN- γ responses. Another eight HCWs had QFT conversion but no TST conversion. Table 3 (*top section*) shows that in most individuals in this subgroup, the increases in IFN- γ were modest (6 of 8 had IFN- $\gamma < 0.70$ IU/ml in the follow-up testing), with small or no increases in TST.

Concordance between TST (6-mm increment) and QFT (≥ 0.70 IU/ml) conversions was 95% ($\kappa = 0.69$), and concordance between TST (10-mm increment) and QFT (≥ 0.35 IU/ml) conversions was 92% ($\kappa = 0.49$). These data are shown in Table E1 in the online supplement. At baseline and repeat testing, previous BCG vaccination had no significant effect on either TST or QFT results (data not shown).

QFT Reversions

QFT reversions were determined in the groups with baseline-concordant positive (QFT⁺/TST⁺), and discordant (QFT⁺/TST⁻) results. Reversions occurred in 2 of 28 (7%) individuals with concordant positive results. In contrast, QFT reversions occurred in a significantly higher proportion (7/10 [70%]) of participants with baseline discordant results ($p < 0.001$). The absolute changes in TST and IFN- γ results are shown in Table E2. Overall, QFT reversion rates were significantly higher in those who had baseline IFN- γ levels close to the cut-off point (Table 4). The discordant group had significantly lower baseline

Figure 1. Study flow diagram and baseline test results. TST cut-off point greater than or equal to 10 mm; QFT cut-off point IFN- γ greater than or equal to 0.35 IU/ml. QFT = QuantiFERON-TB Gold In-Tube assay; TST = tuberculin skin test.

IFN- γ levels (median = 0.63 IU/ml) than the concordant group (median = 5.6 IU/ml; $p < 0.001$).

Annual Risk of TB infection

Recognizing the limitations of our current understanding of the optimal IFN- γ threshold to define new infection, we generated a range of plausible estimates of risk of new infection (Table 1). Based on the data presented in Tables 2 and 3, the use of less stringent thresholds for TST or QFT could potentially result in misclassification of nonspecific variations as new infections. Therefore, a QFT value of greater than or equal to 0.70 IU/ml on repeat testing might be more specific for new infections. Using this stringent definition, which identified all HCWs

with a TST increment of greater than or equal to 10 mm, 11 of 147 (7.5%) HCWs were newly infected over an 18-mo period, equivalent to an annual risk of TB infection (ARTI) of 5% (95% CI = 2–9%). Because the average community ARTI in India is about 1.5% (26), the 3.5% excess risk among HCWs may be attributable to nosocomial exposure.

DISCUSSION

Screening of HCWs for TB is an important component of infection control programs (3–5, 27). In North America alone, an estimated 13–14 million individuals are employed in the health sector (28, 29); most undergo testing at least when recruited. The

TABLE 1. INCIDENCE OF TUBERCULIN SKIN TEST AND QUANTI-FERON-TB GOLD IN-TUBE ASSAY CONVERSIONS OVER A 18-MONTH PERIOD AMONG PARTICIPANTS WHO WERE CONCORDANTLY NEGATIVE BY BOTH TESTS AT BASELINE*

Definition of Conversion	No. Serially Tested	No. of Conversions	% Incidence of Conversions (95% CI)
TST			
1. Baseline induration of < 10 mm and follow-up TST of $\geq$ 10 mm, with increment of $\geq$ 6 mm	147	14	9.5 (5.3–15.5)
2. Baseline induration of < 10 mm and follow-up TST of $\geq$ 10 mm, with increment of $\geq$ 10 mm	147	6	4.1 (1.5–8.7)
QFT			
3. Baseline IFN- γ < 0.35 IU/ml and follow-up IFN- γ $\geq$ 0.35 IU/ml	147	17	11.6 (6.9–17.9)
4. Baseline IFN- γ < 0.35 IU/ml and follow-up IFN- γ $\geq$ 0.70 IU/ml	147	11	7.5 (3.8–13.0)
Combinations of TST and QFT			
1 or 3	147	22	14.9 (9.6–21.8)
2 or 4	147	11	7.5 (3.8–13.0)
1 and 3	147	9	6.1 (2.8–11.3)
2 and 4	147	6	4.1 (1.5–8.7)

Definition of abbreviations: CI = confidence interval; QFT = QuantiFERON-TB Gold In-Tube assay; TST = tuberculin skin test.

* $n = 147$.

TABLE 2. CONCORDANCE BETWEEN TUBERCULIN SKIN TEST AND QUANTIFERON-TB GOLD IN-TUBE ASSAY CONVERSIONS AMONG PARTICIPANTS WHO WERE CONCORDANTLY NEGATIVE BY BOTH TESTS AT BASELINE*

Definition of TST Conversion [†]	Yes/No	Definition of QFT Conversion [†]		Total (n)	% Agreement between TST and QFT Conversion	κ (95% CI)
		QFT Conversion				
		Yes (n)	No (n)			
TST conversion, ≥ 6-mm increment		IFN-γ ≥ 0.35 IU/ml threshold			91	0.53 (0.31–0.76)
	Yes	9	5	14		
	No	8	125	133		
	Total	17	130	147		
TST conversion, ≥ 10-mm increment		IFN-γ ≥ 0.70 IU/ml threshold			96	0.70 (0.44–0.94)
	Yes	6	0	6		
	No	5	136	141		
	Total	11	136	147		

For definition of abbreviations see table 1.

* n = 147.

†see METHODS for a detailed description of the definitions used for conversion.

current screening approach relies on the imperfect TST (7, 8). IGRAs are more specific than TST, and have characteristics suited for serial testing (9, 13, 20).

Although QFT has been recommended for serial testing in the United States (5, 11), there are currently no data on how much IFN- γ responses will increase with new TB infection as opposed to increases due to test-related error and biological variations. To our knowledge, this is the first report on conversions, reversions, and ARTI among HCWs screened using the

“In-Tube” version of QFT; this assay, a simplified, improved version of the FDA-approved QFT assay, uses antigen TB7.7 (Rv2654) in addition to the early-secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) peptides, and this may result in higher positivity (30).

Our preliminary data suggest that QFT, when used alone with a less stringent threshold, produced a higher conversion rate than the TST with the 10-mm increment threshold. Although this might suggest that QFT has a higher sensitivity for detecting

TABLE 3. CHANGES IN TUBERCULIN SKIN TEST REACTIONS AND IFN- γ RESPONSES AMONG PARTICIPANTS WHO HAD EITHER TST OR QUANTIFERON-TB GOLD IN-TUBE CONVERSION*

ID No.	Age/Sex	BCG Scar	Student Group	TST Induration (mm)		IFN- γ Response (IU/ml)	
		(Y/N)	(Nursing/Medical)	Baseline	Follow-Up	Baseline	Follow-Up
TST conversion (≥ 6 mm increment) or QFT conversion (≥ 0.35 IU/ml) (n = 22)							
1	21/M	Y	Medical	3	10	0	0.24
2	21/M	Y	Medical	6	6	0	0.62
3	22/F	N	Medical	3	10	0	0
4	24/M	N	Medical	5	7	0.11	0.42
5	22/M	Y	Medical	3	10	0.08	0.07
6	20/F	Y	Nursing	2	3	0	0.42
7	19/F	Y	Nursing	2	1	0	0.60
8	19/F	N	Nursing	5	11	0	0
9	20/F	Y	Nursing	2	5	0.14	0.35
10	21/M	Y	Medical	5	12	0.20	0
11	21/F	Y	Nursing	1	4	0	0.57
TST conversion (≥ 6 mm increment) or QFT conversion (≥ 0.70 IU/ml) (n = 11) [†]							
12	21/M	N	Medical	6	7	0	0.97
13	21/M	Y	Medical	5	12	0	2.34
14	21/F	Y	Nursing	4	13	0	10.0
15	21/M	Y	Medical	1	10	0.27	0.93
16	19/F	Y	Medical	1	3	0.01	0.72
TST conversion (≥ 10 mm increment) and QFT conversion (≥ 0.70 IU/ml) (n = 6) [†]							
17	20/F	Y	Nursing	2	17	0	10.0
18	21/F	Y	Nursing	0	15	0.22	3.88
19	21/F	Y	Nursing	2	17	0.08	1.34
20	20/F	Y	Nursing	4	14	0.06	10.0
21	19/F	N	Nursing	2	19	0.05	10.0
22	22/F	Y	Medical	0	12	0.03	10.0

Definition of abbreviations: BCG = bacillus Calmette-Guérin; CI = confidence interval; QFT = QuantiFERON-TB Gold In-Tube assay; TST = tuberculin skin test.

* n = 22.

†Subset of the preceding group.

TABLE 4. INCIDENCE OF QFT REVERSIONS AMONG HEALTH CARE WORKERS WITH EITHER QUANTIFERON-TB GOLD IN-TUBE ASSAY-POSITIVE/TUBERCULIN SKIN TEST-POSITIVE RESULTS, OR QUANTIFERON-TB GOLD IN-TUBE ASSAY-POSITIVE/TUBERCULIN SKIN TEST-NEGATIVE RESULTS AT BASELINE*

Baseline IFN- γ Response, IU/ml	No. Retested after 18 Mo	No. That Received INH after Baseline Survey (%)	Total Reversions	Incidence of Reversions (%)	p Value for Trend in Reversion Rates across IFN- γ Categories
0.35–0.69	11	1 (9)	6	6/11 (55)	< 0.01
0.7–1.0	2	0 (0)	1	1/2 (50)	
1.1–5.0	9	3 (33)	1	1/9 (11)	
> 5.0	16	9 (56)	1	1/16 (6)	
Total	38	13 (34)	9	9/38 (24)	

Definition of abbreviations: TST = tuberculin skin test; QFT = QuantiFERON-TB Gold In-Tube assay; INH = isoniazid.

* n = 38.

new infections, it is also plausible that this represents lower specificity for conversions. Of the HCWs who had a QFT conversion with the threshold of greater than or equal to 0.35 IU/ml, nearly half did not meet the 6-mm TST conversion criterion, suggesting that some of these were probably false-positive QFT conversions resulting from nonspecific variations around the threshold.

It is important to note that there is strong evidence that QFT has high specificity for diagnosis (9, 11–13); our concern is about specificity for *conversion* when the less stringent, CDC-recommended negative-to-positive definition is used (5). As an illustration, one HCW with a baseline IFN- γ level of 0.14 IU/ml had a repeat IFN- γ of 0.35 IU/ml (concurrent TST indurations were 2 and 5 mm, respectively). Does this relatively small increase in IFN- γ responses (with only minor TST increase) constitute a true conversion? What if, instead, the IFN- γ level had increased from 0.34 to 0.35 IU/ml? A TST increase from 9 mm to 10 mm will usually not be considered a true conversion. Applying the same logic, a minor increase from 0.34 to 0.35 IU/ml may not be a conversion; it may merely reflect nonspecific IFN- γ variability. We approached this problem by setting a higher threshold for QFT conversions. Even with a stringent threshold, QFT produced a higher conversion rate than the TST with a 10-mm increment threshold, raising the possibility that IGRAs may be more sensitive for recent conversions (17, 31). This hypothesis deserves further study. However, it is important to consider the possibility that IGRA sensitivity for existing LTBI may be different from IGRA sensitivity for recent infection.

The TST, when used alone with the 10-mm increment threshold, produced the lowest conversion rate. The TST may be less sensitive for conversions, but possibly more specific. The TST, when used with a stringent threshold, may have missed some individuals who had substantial increases in IFN- γ responses, as well as increases in TST reactions, but just failed to make the 10-mm threshold. It is noteworthy that TST and QFT conversions were strongly concordant when stringent thresholds were used for both tests. Large increases (≥ 10 mm) in TST indurations were always accompanied by substantial increases in IFN- γ . This is an interesting finding, as it suggests that individuals with recent exposure have vigorous increases in T-cell responses, probably due to active bacterial replication. As with a TST conversion, it is plausible that a QFT conversion with strong increases in IFN- γ responses might be predictive of progression to active disease. This is a critical area for future research (9–11, 13).

Our data on reversions, although limited, suggest that QFT reversions were significantly more likely when the baseline test results were discordant than concordant. Individuals who were concordant-positive (TST⁺/QFT⁺) at baseline had very high

IFN- γ levels to begin with, in contrast to those who were discordant at baseline (TST[−]/QFT⁺). Thus, reversions were less likely in those who had high baseline IFN- γ responses; this is probably because IFN- γ responses have to drastically drop for the result to become negative. In contrast, reversions were frequent in those with baseline IFN- γ levels close to the diagnostic cut-point. Thus, most of the reversions in the discordant group were probably due to nonspecific variations around the diagnostic threshold. If a QFT test reverted, this may well have been a false-positive result at baseline. Alternatively, some of these reversions may reflect spontaneous clearing of TB infection. Because we did not repeat TST on participants who were previously TST positive, we were unable to compare QFT reversions with TST reversions.

Overall, our results show that conversions, reversions, and nonspecific variations occur with serial IGRA testing, as they do with TST. Although IGRAs are often thought of as tests that produce simple yes/no results, our data suggest that these tests are threshold dependent, and that the optimal thresholds to distinguish new infections from nonspecific variation are yet to be defined. Analogous to the TST, different thresholds may be appropriate for different populations or settings (e.g., threshold for *diagnosis* versus *conversion*). Further research is needed to validate IGRA thresholds (13, 32).

Our study had limitations. First, because of the small sample size, we were unable to adequately evaluate risk factors for conversions and reversions. Second, although standard in India (21), the use of 1-TU dose of PPD limits our ability to compare our results with studies that have used the 2-TU dose. However, use of the 1 TU dose permits a comparison of our ARTI with the community estimate for India (26). Third, because we did not perform a two-step baseline TST, the first TST may have boosted the follow-up TST results, and, potentially affected the second QFT results, since ESAT-6 and CFP-10 are present in PPD (12). Because repeat TST and QFT were performed 18 mo after the baseline TST, the effect may be limited. In addition, there is some evidence that a previous TST is unlikely to increase T-cell responses in a subsequent IGRA (33). Fourth, due to the lack of previous data on within-subject IFN- γ variability and test reproducibility, our choice of thresholds for conversions was arbitrary; these thresholds need validation in larger, prospective studies. Finally, despite using a trained TST reader and blinded calipers, some amount of digit preference was noted, and this might have influenced the assessment of TST conversions; digit preference is a known limitation of the TST (8), and unlikely to affect IGRAs.

Acknowledging these limitations, our data provide a useful starting point for understanding the complexity of serial IGRA

testing. Also, our study provides new information on TB among Indian HCWs. Even with the most stringent definition, ARTI among HCWs was higher than the community ARTI in India (26). In fact, because we included only young trainees with limited work experience, our study probably underestimates the true ARTI among Indian HCWs. Our data underscores a need to study nosocomial TB in India and devise control strategies appropriate for the high-burden, resource-limited setting (20, 27, 34, 35).

In conclusion, IGRAs show promise for serial testing, and may facilitate novel approaches to nosocomial TB control. However, our results suggest that health care facilities that switch to IGRAs for serial testing might observe higher conversion rates than those testing with TST, especially if the less stringent definition is used for conversion. There is a risk that such clusters of conversions might be interpreted as nosocomial outbreaks. There is also a potential risk of overtreatment if nonspecific increases in IFN- γ are misinterpreted as conversions. Therefore, research is needed to understand the biological basis of IGRA conversions and reversions, to optimize test reproducibility and thresholds, and to determine risk factors for conversions and reversions.

Such studies are being initiated—the Tuberculosis Epidemiologic Studies Consortium of the CDC is planning a multicentric cohort study on serial testing of HCWs using the TST and both commercially available IGRAs (Rachel Albalak, Tuberculosis Epidemiologic Studies Consortium, personal communication). Also, the CDC is planning a mechanism for postmarketing surveillance of QFT (11). These important initiatives should enable a more evidence-based approach to serial IGRA testing. Until then, health professionals should be cautious about using a simplistic negative-to-positive definition of conversion, and instead consider the amount of change in absolute IFN- γ responses, as well as relevant clinical information (e.g., likelihood of exposure and concurrent TST results, if available) to detect and treat conversions. Health facilities that plan to switch to IGRA for serial testing should seek expert help from local and regional TB control programs, at least during the initial phase of implementation.

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