JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2006, p. 2844–2850 Vol. 44, No. 8
0095-1137/06/$08.00+0 doi:10.1128/JCM.02411-05

Sensitivity Analysis and Potential Uses of a Novel Gamma Interferon Release Assay for Diagnosis of Tuberculosis

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Received 21 November 2005/Returned for modification 5 January 2006/Accepted 14 June 2006

Sputum smears for acid-fast bacilli (AFB) are the primary methods for diagnosis of tuberculosis (TB) in many countries. The tuberculin skin test (TST) is the primary method for diagnosis of latent TB infection (LTBI) worldwide. The poor sensitivity of the former and the poor specificity of the latter warrant the development of new tests and strategies to enhance diagnostic capabilities. We evaluated the sensitivity of an "in-tube" gamma interferon release assay (IGRA) using TB-specific antigens in comparison to the TST and the sputum smear for AFB in TB cases in South Africa. The sensitivity of the IGRA for TB was considered a surrogate of sensitivity in LTBI. Among 154 patients with a positive culture for *Mycobacterium tuberculosis*, the sensitivity of the IGRA for the diagnosis of TB varied by clinical subgroup from 64% to 82%, that of the TST varied from 85% to 94%, and that of two sputum smears for AFB varied from 35% to 53%. The sensitivity of the IGRA in human immunodeficiency virus (HIV)-infected TB cases was 81%. HIV-infected TB patients were significantly more likely to have indeterminate IGRA results and produced quantitatively less gamma interferon in response to TB-specific antigens than HIV-negative TB patients. The overall sensitivity of the TST in all TB cases was higher than that of the IGRA (90% versus 76%, respectively). The combined sensitivities of the TST plus IGRA and TST plus a single sputum smear were 96% and 93%, respectively. The TST combined with IGRA or with a single sputum smear may have a role in excluding the diagnosis of TB in some settings.

Worldwide, there were 8.8 million new cases of active tuberculosis (TB) and an estimated 1.7 million deaths from TB in 2003 (32). With increased numbers of TB cases, health systems in resource-limited settings face difficulties in coping with the large patient and specimen loads (16). Sputum smear for acid-fast bacilli (AFB) (two or three smears) is the primary microbiologic method used for diagnosis of TB in resource-limited settings. Cultures for AFB require more extensive laboratory facilities, and their cost can be prohibitive. However, there is concern regarding this dependence on the sputum smear for AFB due to its limited sensitivity. Human immunodeficiency virus (HIV) coinfection can further complicate TB diagnosis with an atypical clinical picture of pulmonary TB and a higher likelihood of negative sputum smears for AFB (7, 8, 12, 13, 25). The tuberculin skin test (TST) is the standard for diagnosis of latent tuberculosis infection (LTBI), is the only test available for that purpose in resource-limited settings, and is sometimes used to aid in the diagnosis of active TB; however, its specificity is limited by cross-reactivity with nontuberculous mycobacteria and BCG vaccine strains of *Mycobacterium bovis*, and its sensitivity can be affected by malnutrition and immunosuppression (17, 27).

Because of the efficacy of current therapeutic options, the need is great for affordable and rapid diagnostic methods with high sensitivity and specificity for TB and latent TB infections, especially in settings of high HIV prevalence. The gamma interferon (IFN-γ) release assay (IGRA) is an in vitro test based on release of IFN-γ by foreign epitope-stimulated T cells. Newly discovered antigens that are specific to the *Mycobacterium tuberculosis* complex and are not produced by *Mycobacterium bovis* BCG vaccine strains offer the opportunity to develop IGRAs with high sensitivity for TB and LTBI and high specificity for LTBI and, potentially, for TB as well (6, 10, 18, 29). Three promising antigens for use in such assays are the 6-kDa early secreted antigenic target (ESAT-6), 10-kDa culture filtrate protein (CFP-10), and the Rv2654 antigen (TB7.7), which are absent from BCG strains and from most nontuberculosis mycobacteria (2). ESAT-6 and CFP-10 have been shown to elicit strong IFN-γ responses from the T cells of persons infected with *M. tuberculosis* but not from the T cells of those vaccinated with BCG or at low risk of infection (3, 4, 10, 18, 23). In one recent study, conducted among hospitalized HIV-negative patients with TB and asymptomatic subjects considered at low risk for LTBI, the sensitivity and specificity of a new IGRA based on two of these antigens were found to be 89% in the former group and 98% in the latter (22), while in another study in patients with pulmonary TB, the sensitivity of a similar assay was 81% (19).

We evaluated the sensitivity of a new-generation IGRA in adult patients with culture-confirmed TB in South Africa and compared it with the sensitivity of the TST and sputum smear for AFB. Sensitivity of the IGRA in active TB was considered a surrogate of sensitivity of the IGRA in LTBI. The perfor-

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mance of this IGRA was also examined in a subgroup of HIV-infected patients. Furthermore, we examined the combined sensitivity of this IGRA with the TST or sputum smear for AFB.

MATERIALS AND METHODS

Setting. The setting was a primary care facility in Gugulethu, a township of about 70,000 people within the City of Cape Town in the Western Cape Province of South Africa, where the 2003 tuberculosis case notification rate was 678 per 100,000. A 2003 survey for the district estimated the HIV seroprevalence to be 29%. Between 55 and 61% of TB patients in South Africa are estimated to be HIV infected (32).

Patient recruitment and eligibility. Newly registered adult TB patients were approached for participation in the study. Patients were eligible if they were at least 18 years of age. Patients found subsequently not to have positive sputum cultures were excluded from these analyses. This study complied with all federal guidelines regarding human subject research as well as with the policies of the individual institutions involved. All participants signed an informed consent form approved by the institutional review boards (IRBs) of Columbia University, the University of Cape Town, and the South African Medical Research Council. These IRBs all approved the study protocol.

Study assessments and measurements. (i) Gamma interferon release assay. Manufacturer's instructions were followed precisely for the IGRA. The IGRA (QuantiFERON-TB Gold In-Tube; Cellestis Ltd., Carnegie, Australia) was supplied with three heparinized blood collection tubes precoated with the three TB-specific antigens (ESAT-6, CFP-10, and an 18-mer peptide [AWRTAAVE LARALVRAVA] of TB7.7), a mitogen (phytohemagglutinin), and a negative control, respectively. Three milliliters of blood (1 ml for each tube) was collected prior to TST administration. The tubes were mixed by shaking vigorously for 5 to 10 s to ensure that all surfaces of the tube were coated with blood and that complete mixing of the contents had occurred. The tubes were incubated upright overnight (16 to 24 h) at 37°C and then centrifuged at 2,200 × *g* (relative centrifugal force) for 10 min. Plasma (300 to 500 μl) was harvested from each tube and then refrigerated at 4°C for up to, but no more than, 4 weeks. Plasma samples from up to 28 subjects at a time were tested by an enzyme-linked immunosorbent assay (ELISA), whose IFN-γ limit of detection is 0.05 IU/ml, using manufacturer-provided microtiter plates, IFN-γ standard, conjugate, wash buffer, enzyme substrate solution, and enzyme stopping solution. Stored plasma was vortexed to ensure that IFN-γ was evenly distributed throughout the sample, and then 50-μl portions of subject plasma samples were mixed with 50 μl of conjugate in the wells of the microtiter plates, which were coated with anti-human IFN-γ murine monoclonal antibody. Each ELISA included a set of recombinant IFN-γ standards measured in triplicate. Microtiter plates were mixed thoroughly on a plate shaker and then incubated at room temperature for 120 min. Microtiter plate wells were then washed with 300 to 400 μl of wash buffer for nine cycles at room temperature with an ELx50 Autostrip washer (Bio-Tek Instruments Inc., Winooski, VT). One hundred microliters of enzyme substrate solution was added to each well, and the plates were incubated at room temperature for a further 30 min. Following the final 30-min incubation, 50 μl of enzyme stopping solution was added to each well and mixed by gentle agitation to terminate the ELISA. A VersaMax Tunable microplate reader (Molecular Devices Corp., Sunnyvale, CA) was used for the reading of ELISA plates. The optical density of each plasma sample was read using a 450-nm filter with a 620-nm reference filter within 5 min of terminating the ELISA. QuantiFERON TB Gold Analysis software was used to calculate results from the raw optical densities. The manufacturer's cutoff for a positive test, indicating likely *M. tuberculosis* infection, is 0.35 IU/ml of IFN-γ for the TB-specific antigen-stimulated plasma sample above the amount of IFN-γ in the negative control sample. The mitogen sample is the positive control. A positive response to the TB-specific antigens without a response to the mitogen is a valid and positive result. If there is no detectable IFN-γ response to the mitogen and the TB-specific antigens, the test is deemed "indeterminate." The negative control adjusts for background, heterophile antibody effects, and/or nonspecific IFN-γ in blood samples.

(ii) Tuberculin skin test. Two tuberculin units of PPD RT23/Tween 80 (Statens Serum Institut, Copenhagen, Denmark) in 0.1 ml were planted on the volar surface of the left forearm via intradermal injection using a 25-gauge needle and 1-ml syringes graduated in hundredths of milliliters. Only the amount of tuberculin necessary for the day's testing was taken out of cold storage at any given time. While in the field, tuberculin was stored in insulated coolers with ice packs. Two independent and experienced readers assessed the reaction 72 h later; each was blinded to the other's results and to the patient's BCG scar status. Indurations were read transversely in millimeters using calipers. Results of ≥10 mm and ≥5 mm of induration were considered positive in HIV-negative or status unknown patients and HIV-infected patients, respectively.

(iii) Smears and cultures. AFB sputum smear and culture analyses were done at the National Health Laboratory Services in Cape Town, South Africa. Sputa for smears were processed with NaCl-NaOH prior to auramine staining. Cultures were done using the BACTEC 460TB system (BD Diagnostic Systems, Sparks, MD) with 12B liquid medium as well as conventional Lowenstein-Jensen medium. Midway through the study, BACTEC 460TB was replaced with BACTEC MGIT 960. Cultures were incubated for 6 weeks. Positive cultures were confirmed as *M. tuberculosis* using the Accuprobe *Mycobacterium tuberculosis* complex culture identification test (Gen-Probe Inc., San Diego, CA).

Information on the site of TB (pulmonary versus extrapulmonary) and categorization of cases as new or retreatment was abstracted from patient charts. New TB cases were patients who had not been previously registered as having TB; retreatment cases were ones previously registered. Retreatment cases included three subcategories: (i) retreatment after cure, i.e., when previous TB treatment was completed and a fifth-month sputum smear for AFB was negative, (ii) retreatment after completion when previous TB treatment was completed but no final sputum smear was performed, and (iii) retreatment after interruption of previous TB treatment for over 2 months. Number of days on antituberculosis therapy prior to phlebotomy for IGRA was recorded. HIV status was obtained via patient self report. Presence of BCG scars was noted by physical examination. Age and duration of symptoms prior to presentation, including fever, night sweats, cough, hemoptysis, and loss of weight or appetite, were recorded, as were patients' Karnofsky performance scores (21).

(iv) Sample size and power calculations. The sample size goal was 300 patients registered as TB cases. We assumed 50% of the patients would have a positive sputum culture and that the IGRA would be ~90% sensitive for the diagnosis of TB disease in adults. This would provide 150 culture-positive TB patients with whom to determine the sensitivity of the IGRA, allowing for narrow confidence intervals of approximately ±4%. We assumed the IGRA would have 95% sensitivity for the diagnosis of TB in HIV-negative adults. Given knowledge of the TB-HIV coinfection rate and the likelihood of HIV disclosure by patients, we estimated that, of the 300 subjects, 140 would know and disclose their HIV status and be culture positive, and of these, half would be HIV infected. We calculated that the study would have 77% power to detect a 20% difference in sensitivity between HIV-infected and HIV-negative groups with an n of 70 in each group.

(v) Statistical analysis. Data were entered into Access (Microsoft Corp., Redmond, WA) and analyzed using SPSS v12.0 (SPSS Inc., Chicago, IL). Means were compared using the Student's t test for two independent samples. Proportions were compared using the chi-square test and the Fisher's exact test, as appropriate. Univariate analysis of the effect of age on outcome was analyzed using univariate logistic regression. Multivariate analyses were done via multivariate logistic regression. All analyses were two sided, with $P < 0.05$ considered significant.

(vi) Sensitivity analyses. For all sensitivity analyses, a positive culture for *M. tuberculosis* indicated true positive TB. Sensitivity was calculated by dividing the number of individuals with a positive test (IGRA, TST, or sputum smear for AFB) by the number of true positives. The combined sensitivity of two tests (IGRA and TST, IGRA and a single sputum smear for AFB, or TST and a single sputum smear for AFB) was calculated by dividing the total number of individuals with a positive result to either of the two tests by the number of true positives. Confidence intervals (CI) for sensitivity were calculated using Wilson's procedure without a continuity correction. The calculated sensitivity of the IGRA for culture-confirmed active TB was used as a surrogate for its sensitivity for LTBI, as has been done for the TST in the past (11) given the lack of a gold standard for the diagnosis of LTBI.

RESULTS

In this study, we intended to determine with high precision the sensitivity of a new, whole-blood, in-tube gamma interferon release assay using tuberculosis-specific antigens for active TB and, as a corollary, LTBI by analyzing the results of this assay in patients with culture-confirmed tuberculosis. We also wished to examine what effect, if any, HIV status may have on the sensitivity of the IGRA. For these reasons, we enrolled TB suspects from a primary care clinic in a high-TB-burden area

TABLE 1. Characteristics of patients

Characteristic	Value for group		
	Initially enrolled	Culture not sent	Retained in sensitivity analysis
Total no. of patients	249	62	154
Female (*n* [%])	96 (38.6)	23 (37)	58 (37.7)
Mean age (yr)			
Male	38[a]	36.5	38
Female	34.9	33	36.5
TB type[b] (*n* [%])			
Pulmonary	215 (87.4)	45 (72.6)	143 (92.9)
Pleural	19 (7.7)	8 (12.9)	7 (4.5)
Other	8 (3.3)	5 (8.1)	4 (2.6)
Lymph	4 (1.6)	4 (6.4)	0 (0)
TB registration[c] (*n* [%])			
New	154 (62.6)	46 (74.2)	97 (63.4)
Retreatment			
After completion	61 (24.8)	13 (21)	38 (24.8)
After cure	20 (8.1)	0	13 (8.5)
After interruption	11 (4.5)	3 (4.8)	5 (3.3)
HIV status (*n* [%])			
Status unknown	179 (71.9)	45 (72.6)	112 (72.7)
Infected	42 (16.9)	11 (17.7)	26 (16.9)
Negative	28 (11.2)	6 (9.7)	16 (10.4)
BCG scars[d] (*n* [%])			
None	58 (24.5)	14 (23.3)	39 (25.7)
Doubtful	22 (9.3)	5 (8.3)	13 (8.6)
1	107 (45.1)	31 (51.7)	63 (41.4)
2	36 (15.2)	7 (11.7)	25 (16.4)
≥3	14 (5.9)	3 (5)	12 (7.9)
Symptoms (median wk [IQR[e]])			
Cough	4 (2–4)	3 (1–4)	4 (2–6)
Hemoptysis	0	0	0
Fever	0 (0–2)	0 (0–1)	0 (0–3)
Night sweats	3 (2–4)	3 (1–4)	3 (2–4)
Loss of appetite	3 (1–4)	1 (0–3)	3 (0–4)
Loss of weight	2 (0–4)	2 (1–3)	3 (2–4)
Median Karnofsky score (range)	90 (70–90)	90 (90–90)	90 (80–90)

[a] Difference in mean ages between male and female significant; Student's *t* test, $P = 0.019$.
[b] TB type was unavailable for 3 of the initial 249 patients.
[c] TB registration was unavailable for 3 of the initial 249 patients.
[d] BCG scar status was unavailable for 12 of the initial 249 patients.
[e] IQR, interquartile range.

with a prevalence of HIV coinfection estimated at around 60%.

Two hundred sixty-four adult patients registered as having TB, out of a potential 725 registered in the clinic between 15 July 2003 and 30 May 2004, were approached due to availability of research staff only 3 days per week. Eleven patients refused to participate. Four of the 253 patients who agreed to participate were excluded because, on review, they were found to be less than 18 years of age. For the remaining 249, median age was 37 years, 38.6% were female, 87.4% had pulmonary TB, and 62.6% were new cases (Table 1). HIV status was known (through self report) for 70 participants, of whom 42 (60%) were HIV infected.

Of the 249 patients initially enrolled, 170 had sputum samples sent for culture. Of these, 143 had at least one positive culture for *M. tuberculosis*. In addition, culture results were available for 15 other patients with extrapulmonary TB, of which 11 were positive for *M. tuberculosis*. No patients had positive sputum smear results with negative culture results. Thus, there were 154 patients with culture-positive TB. We restricted all further analyses to this group of patients.

Of the 154 patients with culture-positive TB, 26 reported being HIV infected, 15 reported being HIV negative, and 113 were status unknown. Among those who knew their HIV status, women were more likely than men to be HIV infected (odds ratio [OR], 3.43; 95% CI, 1.25 to 9.40; $P = 0.027$, Fisher's exact test). The median Karnofsky scores were 80 in the HIV-infected group and 90 in the other two groups.

One hundred thirty-eight patients with culture-positive pulmonary TB had results from at least one sputum smear for AFB, and 100 patients had results from two sputum smears available. Forty-nine of 138 cases of pulmonary TB had a positive first sputum smear for AFB, resulting in a single sputum smear sensitivity of 36%, while two sputum smears increased the sensitivity to 52% (Table 2). The sensitivity of two smears was 35% in HIV-infected patients.

Of the 154 patients with positive cultures for *M. tuberculosis*, 23 had indeterminate IGRA results. The remaining 131 had interpretable IGRA results, and of these, 100 had a positive IGRA result for an overall sensitivity of 76% (95% CI, 68% to 83%), which increased to 82% (95% CI, 71% to 89%) when analysis was restricted to new pulmonary TB cases. There was a median of 3 days (interquartile range, 0 days and 6 days) between start of TB treatment and phlebotomy for the IGRA. In a subgroup analysis of patients with known HIV status, the sensitivity of the IGRA was higher in HIV-infected patients than in HIV-negative patients (81% versus 73%) (Table 2). However, of the 41 patients with known HIV status (26 HIV infected and 15 HIV negative), five had indeterminate results, all of whom were HIV infected (19% versus 0%, respectively; $P = 0.139$, Fisher's exact test). If these five indeterminate results were considered negative, then the sensitivity of the IGRA in HIV-infected individuals with culture-proven TB would be 65% (95% CI, 46% to 81%). IFN-γ levels were significantly lower in HIV-infected patients than in HIV-negative patients with culture-positive TB ($P = 0.033$; Table 3). There were 18 patients with indeterminate IGRA results for whom HIV status was unknown.

On univariate analysis, sex, age, days from start of TB therapy to phlebotomy, Karnofsky score, HIV status, and site of TB were not associated with IGRA result. However, patients with at least one BCG scar on physical examination were more likely to have a positive IGRA result (OR, 2.1; 95% CI, 1.1 to 4.2; $P = 0.032$, Fisher's exact test). In addition, patients with new cases of TB were more likely to have a positive IGRA than those retreated for TB (OR, 2.3; 95% CI, 1.3 to 4.2; $P = 0.008$, Fisher's exact test). In multivariate analysis adjusting for sex, age, HIV status, and type of TB, the BCG scar association was no longer statistically significant (OR, 2.0; 95% CI, 0.99 to 4.0; $P = 0.052$), while patients with new cases of TB remained

TABLE 2. Sensitivity analysis

Type of TB and patient group	IGRA		TST		One sputum smear for AFB		Two sputum smears for AFB	
	No. of patients with positive result/no. with true-positive result[a]	Sensitivity[e] (95% CI[b])	No. of patients with positive result/no. with true-positive result	Sensitivity (95% CI)	No. of patients with positive result/no. with true-positive result	Sensitivity (95% CI)	No. of patients with positive result/no. with true-positive result	Sensitivity (95% CI)
All types[c]	100/131	76 (68–83)	131/146	90 (84–94)	NA[d]	NA	NA	NA
New	67/82	82 (72–89)	84/92	91 (84–96)	NA	NA	NA	NA
Retreatment	33/49	67 (53–79)	47/54	87 (76–94)	NA	NA	NA	NA
HIV negative	11/15	73 (48–89)	15/16	94 (72–99)	NA	NA	NA	NA
HIV infected	17/21	81 (60–92)	22/26	85 (66–94)	NA	NA	NA	NA
All pulmonary TB	90/120	75 (67–82)	122/135	90 (84–94)	49/138	36 (28–44)	52/100	52 (42–62)
New	62/76	82 (71–89)	79/86	92 (84–96)	28/88	32 (22–43)	47/88	53 (42–64)
Retreatment	28/44	64 (49–76)	43/49	88 (76–945)	21/50	42 (28–57)	25/50	50 (36–64)
HIV negative	10/13	77 (50–92)	13/14	93 (69–99)	2/13	15 (2–45)	6/13	46 (19–75)
HIV infected	15/19	79 (57–91)	21/24	88 (69–96)	3/24	13 (3–32)	7/20	35 (15–59)

[a] True positive was defined as culture positive for *Mycobacterium tuberculosis.*
[b] The Wilson procedure was used to determine confidence intervals (without continuity correction).
[c] Includes pulmonary and extrapulmonary TB cases.
[d] NA, not applicable.
[e] Values are percentages.

significantly more likely to have a positive IGRA than retreatment patients (OR, 2.2; 95% CI, 1.1 to 4.5; $P = 0.028$).

One hundred forty-six (95%) of the 154 patients with culture-positive TB had TST results. Of these, only two patients, one who was HIV negative and another with unknown HIV status, had indurations of >0 mm but <10 mm. For this reason, all further analyses were done with induration of ≥10 mm considered positive irrespective of HIV status. Thirty-three patients had only one TST reading. Of the 121 with two TST readings, overall agreement between the two readers was 99%, with a kappa statistic of 0.961. Because of this high level of agreement, all further analysis was done using the result from the first TST only. One hundred thirty-one of the 146 patients had a positive TST for an overall sensitivity of 90% (95% CI, 84% to 94%) (Table 2). On univariate analysis, sex, age, days from start of TB therapy, Karnofsky score, HIV status, site of TB, type (new versus retreatment) of TB, and BCG scar status were not associated with TST result. In multivariate analysis, no other associations were found.

There were 126 patients with culture-positive TB who had both a valid IGRA result and a TST result. The absolute agreement between the tests in these 126 patients was 74.6%, with a kappa statistic of 0.118. The combined sensitivity of the IGRA and TST in culture-confirmed TB was 96% (95% CI, 91% to 98%) (Table 4). In the subset of patients with pulmonary TB, the combined sensitivity remained at 96% (110/115; 95% CI, 90% to 98%). The sensitivities of the IGRA and the TST for diagnosis of pulmonary TB exceeded that of one or two sputum smears for AFB (Table 5). A single sputum smear combined with the IGRA resulted in a sensitivity of 86% (95% CI, 79% to 91%) for culture-proven pulmonary TB. A single sputum smear combined with the TST resulted in a sensitivity of 93% (95% CI, 87% to 96%) for culture-positive pulmonary TB (Table 6).

TABLE 3. Comparison of mean IFN-γ responses to antigens in 26 HIV-infected versus 15 HIV-negative patients with culture-positive TB

Antigens	HIV status	Mean IFN-γ (IU/ml)	SD (IU/ml)
Negative control	Positive	0.25	0.27
	Negative	0.72	1.38
ESAT-6, CFP-10, and TB7.7	Positive	1.29	1.37
	Negative	8.19[a]	11.24
Mitogen	Positive	4.23	7.49
	Negative	5.91	6.08
(ESAT-6, CFP-10, and TB7.7b) − negative control	Positive	1.05	1.38
	Negative	7.47[a]	10.51

[a] $P = 0.033$; Student's t test, equal variances not assumed.

DISCUSSION

The diagnosis of TB in many resource-limited settings is restricted by the low sensitivity of the sputum smear for AFB, the most widely available test for the diagnosis of TB. The diagnosis of LTBI worldwide is limited by the poor specificity of the TST. The development of new TB diagnostic methods which are feasible and affordable to use in areas with limited resources and high HIV seroprevelance has become a worldwide priority. Studies of in vitro tests which measure the release by immune cells of gamma interferon when stimulated with TB-specific antigens have focused on their usefulness for

TABLE 4. Agreement between and combined sensitivity of the IGRA and the TST in culture-proven cases of active TB[a]

TST result	No. of patients tested by IGRA		Total
	Negative	Positive	
Negative	5	7	12
Positive	25	89	114
Total	30	96	126

[a] Absolute agreement, 74.6%; kappa, 0.118; combined sensitivity, 96%.

TABLE 5. Head-to-head comparison of sensitivities of IGRA, TST, and sputum smear for AFB in culture-positive pulmonary TB

Test	No. of patients with positive result/no. with true-positive result[a]	Sensitivity[b] (95% CI[c])
One sputum smear for AFB	37/112	33 (25–42)
IGRA	84/112	75 (66–82)
TST	102/112	91 (84–95)
Two sputum smears for AFB	42/83	51 (40–61)
IGRA	66/83	80 (70–87)
TST	75/83	90 (82–95)

[a] A sputum culture that was positive for *Mycobacterium tuberculosis* was considered a true positive result.
[b] Values are percentages.
[c] The Wilson procedure for confidence intervals without continuity correction was used.

TABLE 6. Combined sensitivity of IGRA and TST with a single sputum smear for AFB in culture-positive pulmonary TB

Test and result	No. of patients with smear result that was:		Total
	Negative	Positive	
IGRA[a]			
Negative	16	13	29
Positive	60	28	88
Total	76	41	117
TST[b]			
Negative	9	4	13
Positive	78	39	117
Total	87	43	130

[a] Combined sensitivity of the IGRA and smear, 101/117 (86%).
[b] Combined sensitivity of the TST and smear, 121/130 (93%).

the diagnosis of LTBI. We assessed the sensitivity of a new whole-blood "in-tube" IFN-γ release assay to active TB, a measure which has been used as a surrogate for sensitivity to LTBI, and also examined possible strategies to maximize the sensitivity of this assay in combination with the TST and sputum smear for AFB in the diagnosis of TB.

The overall sensitivity of the IGRA in our study was 75% in all the patients with pulmonary TB, which increased to 82% in new cases of pulmonary TB. This finding is in contrast to the sensitivity of the sputum smear for AFB, the standard methodology for diagnosis of TB in this setting, which was 52% and 53% for two smears in these same two groups of TB patients, respectively. The sensitivity of the TST, at 90% in all pulmonary TB cases and 92% in new pulmonary TB cases, was the highest of all three modalities. The IGRA and TST both had higher sensitivities than the sputum smear in a population in whom HIV status was largely unknown but in a setting where the rate of TB cases with HIV coinfection has been estimated at around 60% (32).

Prior studies have reported the sensitivity of sputum smear for AFB to vary widely between 50 and 80% (5, 15, 20, 33). The low sensitivity of the sputum smear for the diagnosis of pulmonary TB noted in our study is consistent with these reports but may also reflect the effect of high TB-HIV coinfection rates on clinical features of pulmonary TB and associated sputum smear positivity.

The sensitivity of the IGRA was slightly, although not significantly, higher in self-reported HIV-infected individuals than in those self reported as HIV negative. The subgroup of participants with known HIV status was small, leading to very wide confidence intervals for these two estimates. Further studies are needed to examine this finding. It is important to note that the mean levels of IFN-γ produced in response to exposure to the TB-specific antigens were significantly lower in HIV-infected patients than in HIV-negative patients. In addition, all indeterminate results occurred among HIV-infected patients. This was due to lack of response to both the TB-specific antigens as well as to the mitogen, most likely reflecting the effect of HIV infection on the number and function of T cells. Thus, the sensitivity (and potentially the usefulness) of this test for the diagnosis of LTBI and as an aid in the diagnosis of TB in HIV-infected patients may be reduced. More studies in HIV-infected individuals, stratified by level of immunosuppression, are needed.

The IGRA was less sensitive (67%) in the small subgroup of patients who were being retreated for TB for unclear reasons. As the percentage of patients with concomitant HIV infection is likely to be higher among patients with recurrent TB disease than new cases, the observed reduction in the sensitivity among retreatment cases may be due to HIV infection and low CD4 counts. It is important to note that retreatment cases constituted nearly one third of the patients in this study and at least 10% of TB patients in South Africa (31). Thus, new diagnostic tests for TB would ideally have high sensitivity in both new TB cases and in retreatment of TB. The TST displayed remarkably stable sensitivity, ranging from 85% to 94%, regardless of clinical subgroup.

Evidence of BCG scar among study participants was surprisingly associated with a nonstatistical trend for the IGRA to be positive. Further studies are needed to confirm this finding. This is unexpected, given that the TB-specific antigens used in the IGRA are on genes not conserved in the genome of *M. bovis* strains used in BCG vaccines. While 66% of patients had evidence of BCG scars, it is recognized that not all vaccinated individuals develop a scar (14, 24), potentially leading to misclassification. Therefore, BCG scar status may not accurately reflect prior BCG vaccination.

We found overall agreement between the IGRA and TST to be moderate, at 74.6%, with a weak kappa statistic of 0.118. However, these two tests may not be measuring the same phenomena. It is thought that new-generation IFN-γ assays, with their short incubation periods, measure mostly effector T-cell responses to antigens, as opposed to the TST, which measures both effector and memory T-cell responses (9). Thus, inconsistent or low levels of agreement between the two tests may not be surprising. This possible difference in what immune responses the two tests measure may also warrant caution when interpreting the meaning of a positive IGRA in patients being tested for LTBI, as the exact predictive value of a positive IGRA (i.e., its correlation with future risk of development of TB) awaits more extensive study in prospective cohorts (28).

Strategies for screening individuals suspected of having pulmonary TB using both the IGRA and TST or IGRA and single sputum smear for AFB could have attractive negative predictive values, given the high sensitivity obtained by using combinations of these tests. Further studies of such strategies in regions of varying TB prevalence are warranted. In this study, of the 30 TB suspects with results available for both the IGRA and TST whose cultures eventually came back negative, 17 had a positive IGRA and 25 had a positive TST. Although these 30 TB suspects may not be representative of all TB suspects, this suggests that in high-TB-burden areas, strategies for screening TB suspects using combinations of these tests may not be very useful, given the inability of both the TST and IGRA to distinguish between active TB and LTBI. However, this does not preclude their utility in low- or medium-TB-burden areas, where the positive predictive value of a screening strategy for active TB using combined testing may be higher. Whether any of these strategies for screening patients with suspected TB, irrespective of level of TB burden, could be useful in reducing overall programmatic costs through the exclusion of TB and the reduction in the need for multiple sputum smears, cultures, and other diagnostic modalities will require cost effectiveness analyses of these new diagnostic assays (30), as has been done for other new rapid TB-diagnostic technologies (1).

The main advantage of IGRAs which use TB-specific antigens are their increased specificity for the diagnosis of LTBI compared to the TST. A limitation of our study was the inability, by design, to assess the specificity of the IGRA we studied. The IGRA's requirement of a single visit to obtain a test result and ability to analyze samples from multiple individuals at once are attractive characteristics for use in LTBI prevalence surveys and for diagnosis of LTBI in clinical settings where treatment of LTBI is offered. On the other hand, the need for venipuncture could limit its usefulness in children (26). Its current cost and the need for trained laboratory staff and a laboratory equipped to perform ELISA may limit these advantages in resource-limited settings.

Conclusions. This study examined the sensitivity of a new generation of IGRA using "in-tube" TB-specific antigens. The sensitivity of the IGRA in individuals with active TB was used as a surrogate for its sensitivity in LTBI. The IGRA demonstrated high overall sensitivity, although not as high as that of the TST, with highest sensitivity in new pulmonary TB patients. HIV-infected TB patients were more likely to have indeterminate IGRA results than HIV-negative patients. The sensitivity of the TST combined with the IGRA or sputum smear for AFB was high in pulmonary TB suspects, which may make it suitable, in some settings, to use such a combined testing strategy to exclude a diagnosis of pulmonary TB, given its potentially high negative predictive value. Newer diagnostics, such as this IGRA, have created exciting possibilities for improvements in the diagnosis of LTBI and for use as adjuncts in the diagnosis of TB disease.

ACKNOWLEDGMENTS

This study was supported by a grant from the Aeras Global TB Vaccine Foundation (formerly the Sequella Global Tuberculosis Foundation). Kits for the IFN-γ release assay used in the study were provided free of charge by Cellestis Ltd. of Australia. S.J.T. was supported by a National Institutes of Health Infectious Disease Epidemiology Research training grant, number T-32 A149821-01, from the National Institute of Allergy and Infectious Disease, during the planning and execution of the research study.

The funding sources did not have any involvement in the design and conduct of this study or in the analysis and interpretation of data from this study.

We thank Linda-Gail Bekker and Monica Vogt at the University of Cape Town for laboratory and technical support, Khunjuzwa Khume for field support, Marcel Behr and Anne-Marie Demers at McGill University for their help with the sputum smear and culture database, Karin Weyer at the South African Medical Research Council, and Ivan Toms at the City of Cape Town Department of Health and the staff of the NY1 Clinic, without all of whom this study would not have been possible. Sincerest appreciation is also due to all patients who participated in this study.

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