Low Sensitivity of a Whole-Blood Interferon-γ Release Assay for Detection of Active Tuberculosis

Puneet K. Dewan,[1] Jennifer Grinsdale,[2] and L. Masae Kawamura[2]

[1]International Research and Programs Branch, Division of Tuberculosis Elimination, National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia; and [2]San Francisco Department of Public Health, San Francisco, California

(See the editorial commentary by Pai and Menzies on pages 74–7)

The sensitivity of an interferon-γ assay (Quantiferon-TB Gold; Cellestis) was evaluated for the detection of tuberculosis among 242 persons with suspected tuberculosis in San Francisco, California. Thirty-seven subjects had culture-confirmed tuberculosis. Excluding 1 indeterminate result, 23 (64%; 95% confidence interval, 48%–78%) of 36 subjects had positive results using the QuantiFERON-TB Gold assay. The 64% sensitivity suggests that the QuantiFERON-TB Gold assay should not be used alone to exclude active tuberculosis.

In December 2004, the US Food and Drug Administration approved the Quantiferon-TB Gold (QFT-G; Cellestis), a new assay for the detection of *Mycobacterium tuberculosis* infection. The QFT-G assay measures the amount of IFN-γ released after blood is incubated with synthetic antigens (early secretory antigen 6 and culture filtrate protein 10) that simulate proteins present in *M. tuberculosis*. Although the QFT-G assay is reported to detect *M. tuberculosis* infection with high specificity, it does not distinguish between active tuberculosis and latent tuberculosis infection [1–7]. Furthermore, the sensitivity of QFT-G for detection of active tuberculosis has not been well defined among individuals with suspected tuberculosis in the United States.

The San Francisco Department of Public Health (San Francisco, CA) has used the QFT-G assay as a component of routine evaluation for patients with suspected tuberculosis since February 2005. We sought to evaluate the sensitivity and predictive value of QFT-G for the detection of active tuberculosis among persons with suspected tuberculosis at a public health clinic.

Methods. San Francisco Department of Public Health Tuberculosis Clinic guidelines require QFT-G testing of patients who are suspected of having active tuberculosis (defined as American Thoracic Society tuberculosis class 5), in conjunction with routine clinical, microbiologic, and radiographic examinations [8]. An individual with suspected tuberculosis is defined as a patient with clinical or radiographic evidence consistent with active tuberculosis for whom laboratory confirmation of the final disease classification has not been received. Because specimens require same- and subsequent-day processing, the QFT-G assay is not used if patients are evaluated at the San Francisco Department of Public Health Tuberculosis Clinic after 3 P.M., on Fridays or weekends, or if phlebotomy is unsuccessful or declined. The assay is performed in accordance with the manufacturer's instructions: specimens are processed ⩽8 h after phlebotomy, and incubation time was standardized at 16–18 h [9]. A tuberculin skin test (TST) is not required for patients with suspected tuberculosis, but results are often available.

In this retrospective evaluation, we reviewed clinical records for all consecutive patients from San Francisco who were reported to have suspected tuberculosis during the period from 1 March through 31 December 2005. We excluded patients who transferred out of the jurisdiction or were treated for >14 days with antituberculosis drugs before QFT-G testing. We collected information about TST results obtained within 2 weeks of the QFT-G test date that were reported to San Francisco Department of Public Health by the referring provider or that were obtained at the tuberculosis clinic using standard methods [8]. TST indurations ⩾5 mm in diameter were classified as positive results. We defined "high clinical suspicion" as suspected tuberculosis in a patient who was receiving empirical antituberculosis medication before the availability of diagnostic microbiologic test results. Cases were classified by final diagnosis as either a verified case of tuberculosis or as negative for tuberculosis [10].

Ninety-five percent CIs for sensitivity were calculated using the Wilson score method [11]. Proportions were compared using the χ^2 test or, if there were ⩽5 observations, Fisher's test.

Results. In the evaluation period, the San Francisco Department of Public Health noted 522 consecutive patients with suspected tuberculosis; 41 (8%) were excluded as transfers out,

Received 19 July 2006; accepted 23 August 2006; electronically published 28 November 2006.

Reprints or correspondence: Dr. Puneet K. Dewan, International Research and Programs Branch, Div. of Tuberculosis Elimination, National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention, 1600 Clifton Rd., MS E10, Atlanta, GA 30333 (pdewan@cdc.gov).

Clinical Infectious Diseases 2007; 44:69–73

1058-4838/2007/4401-0012

and 39 (7%) had not yet received a final diagnosis as of 15 March 2006. Of the remaining 442 subjects with suspected tuberculosis, 242 (55%) were tested using the QFT-G assay (table 1). Persons who underwent QFT-G testing did not significantly differ from those who did not with regard to distribution of age, HIV infection status, high clinical suspicion of tuberculosis, or final diagnosis of tuberculosis. Patients who underwent QFT-G testing were more likely to be foreign born or Asian and were less likely to declare non-Hispanic black or Hispanic white ethnicity/race.

Among the 242 persons with suspected tuberculosis, 45 (19%) received a diagnosis of tuberculosis, including 37 (82%) who had culture-confirmed disease (table 2). Among these 45 patients with tuberculosis, QFT-G results were positive for 25 (55%), negative for 17 (38%), and indeterminate for 3 (7%). Excluding indeterminate results, we calculated an overall QFT-G sensitivity for this population of 60% (95% CI, 44%–73%) and a negative predictive value of 86% (95% CI, 79%–91%). False-negative QFT-G results were distributed equally throughout the 46-week evaluation period (data not shown). We observed similar results among the 37 patients with culture-confirmed tuberculosis (sensitivity, 64% [95% CI, 48%–78%]; negative predictive value, 89% [95% CI, 83%–94%] and among persons with no history of prior tuberculosis infection (including those with latent tuberculosis infection and active disease) or tuberculosis treatment (table 2). Only 3 HIV-infected patients received a diagnosis of tuberculosis, precluding meaningful evaluation of this subgroup.

We compared the clinical characteristics of patients with verified tuberculosis and true-positive QFT-G results (25 patients) with those who had false-negative QFT-G results (17 patients) (table 3). We observed no significant differences with regard to age distribution, proportion of patients without chronic medical conditions, HIV infection status, or duration of treatment of active tuberculosis at the time of testing. Patients with false-negative QFT-G results were more likely to have extrapulmonary tuberculosis (35% vs. 4%; $P < .05$). We did not observe cases of multiple-site or miliary tuberculosis in this cohort of patients who underwent QFT-G testing.

TST results were available for 24 (53%) of the 45 patients with verified tuberculosis who were tested using the QFT-G. Of these 24 patients, the QFT-G and TST results were both positive for 12 (50%) and both negative for 1 (4%). Nine patients (38%) had negative QFT-G and positive TST results, and 2 (8%) had indeterminate QFT-G and negative TST results. No patient with verified tuberculosis had negative TST and positive QFT-G results. Eighteen (75%) of the 24 patients' TST results were verified by the San Francisco Department of Public Health, and 6 (25%) results were reported in induration (in mm) by community providers; between these groups, the proportion of discordant TST and QFT-G results did not significantly differ (data not shown).

Table 1. Characteristics of patients evaluated for tuberculosis, San Francisco, California, March–December 2005.

Characteristic	No. (%) of patients: Tested by QFT-G (n = 242)	Not tested by QFT-G (n = 200)	P
Age, years			
0–5	3 (1)	3 (2)	
6–15	1 (0)	0 (0)	
16–35	29 (12)	40 (20)	
36–55	111 (46)	79 (40)	
56–75	85 (35)	62 (31)	
⩾76	13 (5)	16 (8)	.15[a]
Race/ethnicity			
Asian	172 (71)	99 (50)	<.01
Black, Hispanic	1 (0)	1 (0)	1.0
Black, non-Hispanic	17 (7)	31 (16)	<.01
Native American	4 (2)	4 (2)	1.0
White, Hispanic	18 (7)	35 (18)	<.01
White, non-Hispanic	30 (12)	30 (15)	.43
Foreign born			
All patients	189 (78)	131 (66)	<.01
In United States <5 years	147 (61)	55 (28)	<.01
Immigration evaluation[b]	116 (48)	20 (10)	<.01
Homeless	15 (6)	23 (12)	.05
Injection drug use	3 (1)	2 (1)	1.0
HIV infection status			
Positive	21 (9)	26 (13)	.18[c]
Negative	164 (68)	84 (42)	
Unknown	57 (24)	90 (45)	<.01
History of prior TB treatment			
Treatment for LTBI	10 (4)	25 (13)	<.01
Treatment for active TB	71 (29)	37 (19)	<.01
Level of clinical suspicion for TB			
High	85 (35)	83 (42)	.17
Low	157 (65)	117 (59)	
Final diagnosis			
TB	45 (19)	51 (26)	.09
Not TB	197 (81)	149 (75)	

NOTE. Patients with QuantiFERON-TB Gold (QFT-G; Cellestis) results obtained after receipt of >14 days of antituberculosis treatment were classified as not tested. P values were calculated using the Mantel-Haenszel χ^2 test or, if there were ⩽5 observations, with Fisher's exact test. LTBI, latent tuberculosis infection; TB, tuberculosis.

[a] P value by χ^2 test for difference in trend.
[b] Persons classified as class B during immigration health evaluation.
[c] Calculated in comparison with the HIV infection status "not positive."

Discussion. The QFT-G IFN-γ assay was demonstrated to have 64% sensitivity and 89% negative predictive value for culture-confirmed cases of active tuberculosis among patients with suspected tuberculosis in San Francisco. Excluding indeterminate results, we observed negative QFT-G results in 17

Table 2. QuantiFERON-TB Gold (QFT-G; Cellestis) results among 242 persons with suspected active tuberculosis, by patient population subgroup.

Patient population, QFT-G result	No. (%) of subjects		Sensitivity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
	With TB (%)	Without TB (%)			
All patients					
All	45	197	60 (44–73)	23 (16–32)	86 (79–91)
Positive	25 (56)	84 (43)	…	…	…
Negative	17 (38)	106 (54)	…	…	…
Indeterminate	3 (7)	7 (4)	…	…	…
Patients with high clinical suspicion of TB[a]					
All patients	40	45	57 (41–71)	65 (48–80)	67 (53–79)
Positive	21 (53)	11 (25)	…	…	…
Negative	16 (40)	33 (73)	…	…	…
Indeterminate	3 (8)	1 (2)	…	…	…
Patients with low clinical suspicion of TB					
All patients	5	152	80 (38–96)	05 (02–13)	99 (93–100)
Positive	4 (80)	73 (48)	…	…	…
Negative	1 (20)	73 (48)	…	…	…
Indeterminate	0	7 (5)	…	…	…
Foreign-born newcomers					
All patients	19	128	55 (33–75)	14 (8–24)	89 (79–94)
Positive	10 (53)	61 (48)	…	…	…
Negative	8 (42)	63 (49)	…	…	…
Indeterminate	1 (5)	4 (3)	…	…	…
HIV-infected patients					
All patients	3	18	50 (09–91)	50 (9–90)	94 (73–99)
Positive	1 (33)	1 (6)	…	…	…
Negative	1 (33)	16 (89)	…	…	…
Indeterminate	1 (33)	1 (6)	…	…	…
Patients with culture-confirmed TB					
All patients	37	205	64 (48–78)	21 (14–30)	89 (83–94)
Positive	23 (62)	86 (42)	…	…	…
Negative	13 (35)	110 (54)	…	…	…
Indeterminate	1 (3)	9 (4)	…	…	…
Patients with no known prior TB or treatment[b]					
All patients	25	116	60 (41–76)	28 (18–41)	88 (79–93)
Positive	15 (60)	39 (34)	…	…	…
Negative	10 (40)	74 (64)	…	…	…
Indeterminate	0	3 (3)	…	…	…

NOTE. Patient population subgroups are not mutually exclusive. Persons with indeterminate QFT-G results are excluded from calculations of test accuracy. NPV, negative predictive value; PPV, positive predictive value; TB, tuberculosis.

[a] Patients with abnormal chest radiograph findings and/or clinical symptoms of active tuberculosis who started receiving antituberculosis treatment immediately, regardless of the availability of microbiological examination results.

[b] Patients had no known prior positive tuberculin skin test result or IFN-γ release assay result, no prior treatment history for active tuberculosis or latent tuberculosis infection, and no current use of antituberculosis medications for >5 days. Prior tuberculosis infection was defined as either (1) documented history of a tuberculin skin test induration >10 mm at least 1 year prior to notification as having suspected tuberculosis, or (2) prior self-reported history of active tuberculosis disease at any time in the past.

(40%) of 42 patients with tuberculosis, including 13 (36%) of 36 patients with culture-confirmed tuberculosis and 10 (40%) of 25 patients with no prior history of tuberculosis infection, disease, or treatment. Our results reinforce the recent recommendation that negative results should not be used alone to exclude active tuberculosis and that results should be interpreted in conjunction with other clinical and diagnostic findings [12]. The combination of low sensitivity for active tuberculosis and the intrinsic inability of the test to distinguish between latent infection and active disease suggests that the QFT-G assay has a limited role in the evaluation of patients with suspected tuberculosis.

The sensitivity of the QFT-G assay for the detection of active tuberculosis cases observed in San Francisco was slightly lower

Table 3. Characteristics of patients who had verified cases of tuberculosis diagnosed.

Characteristic	Patients with positive QFT-G results (n = 25)	Patients with negative QFT-G results (n = 17)
Age, median years (range)	51 (22–81)	39 (23–88)
Prior history active TB treatment	4 (16)	0 (0)
Chronic medical conditions		
Absent	14 (56)	10 (59)
Present		
All	11 (44)	7 (41)[a]
Alcoholism	1	3
Chronic obstructive lung disease	0	1[b]
Diabetes mellitus	7	3
HIV infection	1	1
Lung cancer (untreated)	1	0
Rheumatoid arthritis	1	0
HIV infection status		
Positive	1 (4)	1 (6)
Negative	17 (68)	14 (82)
Unknown	7 (28)	2 (12)
Pulmonary TB[c]		
All cases	24 (96)	11 (65)
Smear positive for acid-fast bacilli, *n/N* (%)	11/24 (46)	4/11 (36)
Culture positive for *Mycobacterium tuberculosis, n/N* (%)	22/24 (92)	10/11 (91)
Extrapulmonary TB[c]		
All cases	1 (4)	6 (35)
Culture positive for *M. tuberculosis, n/N* (%)	1/1 (100)[d]	3/6 (50)[e]
Current treatment for TB		
None	17 (68)	9 (53)
1–7 days	4 (16)	5 (29)
8–14 days	4 (16)	3 (18)

NOTE. Data are no. (%) of patients, unless otherwise indicated. Patients with indeterminate QFT-G results are excluded. QFT-G, QuantiFERON-TB Gold (Cellestis); TB, tuberculosis.

[a] One patient had both diabetes mellitus and alcoholism.
[b] Patient was not taking oral corticosteroids at time of testing.
[c] $P < .05$.
[d] There was 1 case of culture-confirmed tuberculous lymphadenitis.
[e] Culture-confirmed diagnosis in case of tuberculous lymphadenitis (1 patient), colitis (1 patient), and peritonitis (1 patient), as well as pathological (culture-negative) diagnosis of tuberculous lymphadenitis (2 patients) and osteomyelitis (1 patient).

but similar to that observed in referral hospitals [13]. Lee et al. [5] reported a sensitivity of 70% among 87 patients who received a diagnosis of tuberculosis; the subjects in that study differed from our population in that 55 patients (63%) in that study had culture-confirmed tuberculosis, and 29 (33%) were classified as immunocompromised. Among patients with culture-confirmed tuberculosis, Kang et al. [3] reported 81% sensitivity among 54 patients, and Mori et al. [4] reported 89% sensitivity among 118 patients. Lee et al. [5] suggested that using lower IFN-γ response values for classification of a positive QFT-G result may increase the assay's sensitivity with minimal trade-off in specificity and that this should be considered when testing patients with a high pretest probability of active tuberculosis, such as close contacts or patients with suspected tuberculosis. This approach, however, has not been validated.

Among patients with extrapulmonary tuberculosis, we observed that 6 (86%) of 7 had negative QFT-G results, including 3 (75%) of 4 with culture-confirmed cases. Poor IFN-γ response to culture filtrate protein 10 has been previously reported among patients with extrapulmonary tuberculosis [14].

Our retrospective evaluation had several limitations. Our results may have been affected by the fact that 8 (47%) of 17 patients with tuberculosis diagnoses who had false-negative QFT-G results had received 1–14 days of antituberculosis treatment. Our evaluation lacked the power to assess the possible effects of treatment [15, 16]. However, similar QFT-G sensitivity was observed among the subset of patients with no prior history of antituberculosis treatment or *M. tuberculosis* infection; thus, this limitation has likely not affected our conclusions. Because the TST was only performed for a subset of

patients and by multiple health care providers, our evaluation should not be interpreted as a comparative study with the TST. The limitations of the TST in the diagnosis of active tuberculosis are well established, and clinical recommendations suggest that the TST should not be used alone to exclude active tuberculosis [17].

Our results are specific for the QFT-G assay and should not be assumed to apply to other IFN-γ assays. Newer commercial assays, such as the T-SPOT.TB (Oxford-Immunotec) and the QuantiFERON-TB Gold In Tube (Cellestis) assays, have many differences and may perform with higher sensitivity [5, 18]. Nevertheless, the QFT-G assay is currently the only IFN-γ assay commercially available in the United States, and providers should be aware of the limitations we have observed. Similarly, these findings should not be extrapolated to assume that the QFT-G or other IFN-γ assays lack adequate sensitivity for the detection of latent tuberculosis infection. Prospective studies are critically needed to characterize the future risk of tuberculosis among persons with different responses to IFN-γ in these commercial assays.

In conclusion, we observed a low sensitivity for the QFT-G assay for the detection of active tuberculosis among persons with suspected tuberculosis at a public health clinic in San Francisco. As with the TST, providers should be aware of the limitations of this assay and should not use negative QFT-G results alone to rule out active disease. Prospective, community-based clinical trials should be conducted for newer IFN-γ assays before any adjustments are made in current diagnostic practices [19].

Acknowledgments

We acknowledge Sally Liska and Ernest H. Wong (Public Health Laboratory of the San Francisco Department of Public Health), for their invaluable contributions to IFN-γ assay implementation in San Francisco; Robert Fallstead, for data entry assistance; and the frontline staff of the Tuberculosis Control Section, San Francisco Department of Public Health. We also thank Dr. Andrew Vernon (Centers for Disease Control and Prevention [CDC]; Atlanta, GA), for his review of this manuscript, and Ann Lanner (CDC), for editorial assistance.

Potential conflicts of interest. All authors: no conflicts.

References

1. Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis **2004**; 4:761–76.
2. Ferrara G, Losi M, Meacci M, et al. Routine hospital use of a new commercial whole blood interferon-γ assay for the diagnosis of tuberculosis infection. Am J Respir Crit Care Med **2005**; 172:631–5.
3. Kang YA, Lee HW, Yoon HI, et al. Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA **2005**; 293:2756–61.
4. Mori T, Sakatani M, Yamagishi F, et al. Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. Am J Respir Crit Care Med **2004**; 170:59–64.
5. Lee JY, Choi HJ, Park IN, et al. Comparison of two commercial interferon gamma assays for diagnosing *Mycobacterium tuberculosis* infection. Eur Respir J **2006**; 28:24–30.
6. Tsiouris SJ, Coetzee D, Toro PL, Austin J, Stein Z, El-Sadr W. Sensitivity analysis and potential uses of a novel gamma interferon release assay for diagnosis of tuberculosis. J Clin Microbiol **2006**; 44:2844–50.
7. Ravn P, Munk ME, Andersen AB, et al. Prospective evaluation of a whole-blood test using *Mycobacterium tuberculosis*-specific antigens ESAT-6 and CFP-10 for diagnosis of active tuberculosis. Clin Diagn Lab Immunol **2005**; 12:491–6.
8. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med **2000**; 161:1376–95.
9. Cellestis International. Quantiferon-TB Gold [package insert]. **2005**. Available at: http://www.cellestis.com. Accessed 10 February 2006.
10. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. MMWR Recomm Rep **1997**; 46(RR-10):1–55. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/00047449.htm. Accessed 20 August 2006.
11. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med **1998**; 17:857–72.
12. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. MMWR Recomm Rep **2005**; 54(RR-15):49–55.
13. Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: Part I. Latent tuberculosis. Expert Rev Mol Diagn **2006**; 6:413–22.
14. Hussain R, Kaleem A, Shahid F, et al. Cytokine profiles using whole-blood assays can discriminate between tuberculosis patients and healthy endemic controls in a BCG-vaccinated population. J Immunol Methods **2002**; 264:95–108.
15. Al-Attiyah R, Mustafa AS, Abal AT, Madi NM, Andersen P. Restoration of mycobacterial antigen-induced proliferation and interferon-gamma responses in peripheral blood mononuclear cells of tuberculosis patients upon effective chemotherapy. FEMS Immunol Med Microbiol **2003**; 38:249–56.
16. Carrara S, Vincenti D, Petrosillo N, Amicosante M, Girardi E, Goletti D. Use of a T cell–based assay for monitoring efficacy of antituberculosis therapy. Clin Infect Dis **2004**; 38:754–6.
17. Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med **2000**; 161:S221–47.
18. Mahomed H, Hughes EJ, Hawkridge T, et al. Comparison of mantoux skin test with three generations of a whole blood IFN-gamma assay for tuberculosis infection. Int J Tuberc Lung Dis **2006**; 10:310–6.
19. Pai M, O'Brien R. Tuberculosis diagnostics trials: do they lack methodological rigor? Expert Rev Mol Diagn **2006**; 6:509–14.