Negative and Positive Predictive Value of a Whole-Blood Interferon-γ Release Assay for Developing Active Tuberculosis

An Update

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***Rationale*: Only limited data are available on the predictive value of interferon-γ release assays for progression from latent tuberculosis infection to active tuberculosis (TB).**

***Objectives*: To build on our initial study comparing the QuantiFERON-TB Gold in-tube assay (QFT) with the tuberculin skin test (TST) in close contacts of patients with TB and evaluating progression to active TB for up to 4 years.**

***Methods*: A cohort of close contacts of smear-positive index cases established between May 2005 and April 2008 was tested with QFT and TST. Through April 2010, progressors to active TB were consecutively recorded.**

***Measurements and Main Results*: Of the 1,414 contacts (141 children), 1,033 were still resident in Hamburg at the end of the study period, and results of both tests were available for 954. QFT, but not TST, results were associated with exposure time ($P < 0.0001$). For QFT, 198 of 954 (20.8%) were positive; 63.3% (604) were TST positive at greater than 5 mm and 25.4% at greater than 10 mm. Nine hundred and three contacts refused chemoprevention and 19 developed active TB. All 19 (100%) had been QFT positive with a progression rate of 12.9% (19 of 147) over the observation period. Corresponding values for the TST were significantly lower: 89.5% (17 of 19) and 3.1% (17 of 555) at greater than 5 mm, and 52.6% (10 of 19) and 4.8% (10 of 207) at greater than 10 mm, respectively. The progression rate of 28.6% (6 of 21) for QFT-positive children was significantly higher than 10.3% (13 of 126) for adults ($P = 0.03$).**

***Conclusions*: Results suggest that QFT is more reliable than the TST for identifying those who will soon progress to active TB, especially in children.**

Keywords: tuberculosis; latent infection; interferon-γ release assay; tuberculin skin test; predictive value

Evidence is growing to support the hypothesis of superior sensitivity and specificity of interferon-γ release assays (IGRAs) over the tuberculin skin test (Mantoux; TST) for the detection of infection with *Mycobacterium tuberculosis* (MTB) (1, 2). Despite the promise that the use of a more specific IGRA in screening groups for MTB infection will greatly reduce the number of subjects testing positive and thus reduce unnecessary preventive treatment, the important clinical question remains as to whether IGRAs will more precisely identify those infected who are destined to later progress to active tuberculosis (TB) if not prescribed preventive treatment.

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Only limited data are available for progression of contact persons tested positive by an interferon-γ release assay (IGRA) to active tuberculosis (TB) disease.

What This Study Adds to the Field

This study provides evidence on the ability of an IGRA to identify contacts who will soon progress to active TB given *Mycobacterium tuberculosis* infection. In addition, it provides information about the respective negative predictive value for progression among close contacts with negative test results.

A further question raised since the advent of the more specific IGRAs is that of the reliability of their negative results. Where, in low-burden countries, so many fewer positive results are generally produced with IGRAs than with the TST, is it not probable that the new tests are "missing positives"? That is, do the IGRAs fail to detect people who will progress to active TB and who would have been identified by the TST? Only a few studies have evaluated the outcome of high-risk subjects with negative commercial IGRA results over a significant observation period (3–6). Results from these studies are promising and suggest that commercial IGRAs may have very high negative predictive values (NPVs) for the development of active TB, but this requires further validation.

In 2008, we first reported that a commercially available IGRA (QuantiFERON-TB Gold in-tube; QFT; Cellestis Ltd, Chadstone, Australia) had a high predictive value for progression to active TB in 601 close contacts in a low-burden country, who had been monitored for 2 years (3). This study found a 2-year rate of progression to active TB of nearly 15% for those who were QFT positive, compared with 2.3% for those who were TST positive. The major limitation was that of the 601 contacts studied, only a small number (41) were IGRA positive and untreated and only 6 individuals progressed to active TB.

This study builds on that earlier publication, monitoring close contacts in Hamburg, Germany, and reports on a substantially larger cohort tested with QFT and the TST, now monitored for up to 4 years to determine whether active TB developed.

METHODS

Study Design and Inclusion Criteria

The present report is the latest from an ongoing study, the initial phase of which was conducted from May 2005 through April 2006 to evaluate

the predictive value of QFT for progression to active TB disease in contact investigations in a low-incidence setting. The results of that initial phase were published previously in the *Journal* (3).

In accordance with the original strategy, we continued to recruit close contacts of acid-fast bacilli (AFB) smear–positive, subsequently culture-confirmed source MTB cases in Hamburg through April 1, 2008. As previously described (3), to increase pretest probability of MTB infection in subjects, a second study inclusion criterion was an aggregate exposure time of the contact in the 3 months before the diagnosis of his or her respective index case, that is, the presumed period of infectiousness, of not less than 40 hours indoors with shared air. All subjects were offered QFT and TST during the ninth week after their last possible exposure.

The resulting cohort was monitored until April 1, 2010, with a focus on TB development, through passive follow-up based on reports to the Public Health Department. All tested subjects were monitored, regardless of whether they received chemoprevention, which was offered to QFT-positive subjects only in the form of isoniazid (INH) and/or rifampicin (RIF) treatment.

To estimate contact time for enrollment and analysis purposes, any contact during each of six 4-hour blocks in a day was counted as 4 hours of contact time. This allowed us to make more precise estimates of the duration of exposure, both in household settings and in nondomestic contact situations. Contact exposure time was determined by interviewing both the contact person and, if possible, his or her respective index case. Where more than one possible source case was considered for a contact, the longest cumulative exposure time to a single case was used. Sociodemographic and clinical data of the individuals were recorded, using a standardized questionnaire.

Contacts with an exposure time of less than 40 hours to the source case were not included in the study. All individuals were informed of the nature of the study, which was approved by the Hamburg local ethics committee, and all agreed to participate.

Progression to TB disease in members of the cohort was obligatorily reported by physicians or hospitals to the Public Health Department of the City of Hamburg according to Section 7 of the German Infectious Diseases Law. Reports were filed on average within 1 week (range, 2–16 d during the study period) of diagnosis and a search of the registry of contact persons was promptly undertaken by the receiving Public Health Department. At the end of this study phase, April 2010, we further verified, through positive checks of the Hamburg Residents Registration Office, the residency status of each cohort member. Participants who had moved away from Hamburg in the course of the follow-up period were eliminated from the final analysis (*see* the online supplement).

Diagnosis of Tuberculosis

TB disease was diagnosed on the basis of chest radiographic (and computerized tomographic) findings, identification of AFB in sputum samples, by bronchoscopy or lavage of gastric secretions, conventional culture of *M. tuberculosis*, and/or nucleic acid amplification assays and/or histopathology, together with the assessment of preceding clinical suspicion of TB. In culture-negative cases, and given a chest radiograph consistent with TB, subsequent clinical and radiographic response to multidrug therapy over an appropriate time course (1–3 mo) was considered sufficient to confirm the diagnosis of tuberculosis.

Confirmation by Restriction Fragment Length Polymorphism Fingerprinting

To confirm that subjects progressing to TB disease represented fresh transmission, all available isolates from the presumed source patient and contact persons developing culture-confirmed TB disease were analyzed by IS*6110* restriction fragment length polymorphism (RFLP) fingerprinting or by mycobacterial interspersed repetitive-unit (MIRU) typing by the National Reference Center for Mycobacteria (Borstel, Germany).

IGRA and Mantoux TST

QFT was performed according to the manufacturer's instructions (Cellestis Ltd, Chadstone, Australia) and the Mantoux TST was administered as previously described (3; and *see* the online supplement).

Statistical Analysis

Statistical analyses were performed as described in the online supplement.

Preventive Therapy

The decision to start preventive treatment was taken by private practice pneumologists to whom the QFT-positive patients had been referred, and without the influence of the study investigators, who only received notification of treatment decisions.

RESULTS

In addition to the 601 close contacts from the initial phase of this study (3), a further 816 close contacts from April 2006 to the end of March 2008 fulfilled the inclusion criteria. The contacts came from 101 different source cases (all with culture-proven MTB infection), 4 of whom had INH-resistant strains and 2 of whom had RIF resistance. The study recruitment profile is shown in Figure 1. Of the 1,417 enrolled study participants, 79 refused testing with the TST in favor of the IGRA only and 3 contacts, all without known immunosuppression, had indeterminate QFT results; thus data from 1,335 subjects were available for direct comparison of QFT and TST results. A total of 381 subjects moved away from Hamburg during the observation period, 276 QFT negative and 105 QFT positive (16 of these after finishing INH therapy), and no information was available on possible progression to active TB. Of the 954 contact persons remaining in Hamburg and with results for both tests, 51 completed preventive therapy. Thus, 903 subjects for whom both QFT and TST results were available could be observed for at least 2 years through April 1, 2010 for subanalysis examining positive and negative predictive value for progression to active TB.

The composite of the 954 contacts for whom results of both tests were available and who did not move is shown in Figure 1: 342 (35.8%) household or intimate contacts, 296 (31.0%) employees exposed in their workplace to a colleague with TB disease, 190 (19.9%) pupils or students of traditional and vocational school classes, 67 (7.0%) health care workers working in pulmonary or other infection wards and exposed to an infectious patient before that patient's TB diagnosis, 46 (4.8%) members of sports clubs where a comember had active TB, 9 (0.9%) patients sharing a ward with an initially undetected patient with infectious TB, and 4 (0.4%) were classified simply as exposed intravenous drug users sharing time in bars (to buy or consume drugs) with a subsequently diagnosed patient with infectious TB.

As shown in Table 1, there were equivalent numbers of male and female participants and only slightly more bacillus Calmette-Guérin (BCG)–vaccinated persons than BCG-unvaccinated persons (495 and 459, respectively). The proportion of foreign-born contacts, most of them coming from intermediate- or high-incidence countries, was 40.6% (387 of 954). Although half of the contacts (48.8%) exceeded the minimal cumulative time for inclusion into the study only marginally (<60 h), the mean exposure time (±SD) was 113.6 (±132.6) hours (data not shown). The mean age of the contacts was 29.02 ± 11.8 years (range, 1–62 yr; data not shown), with the majority of contacts aged between 16 and 39 years (632 of 954, 66.2%). However, there were 106 close contacts (11.1%) who were children under 16 years of age, most of them (76 of 106, 71.7%) household contacts.

For the total number of contacts who were tested by QFT (1,414), 314 (22.2%) were positive and were referred to pneumologists for evaluation and possible preventive therapy, which was finished by 67 contacts (21.3%). Rates for acceptance

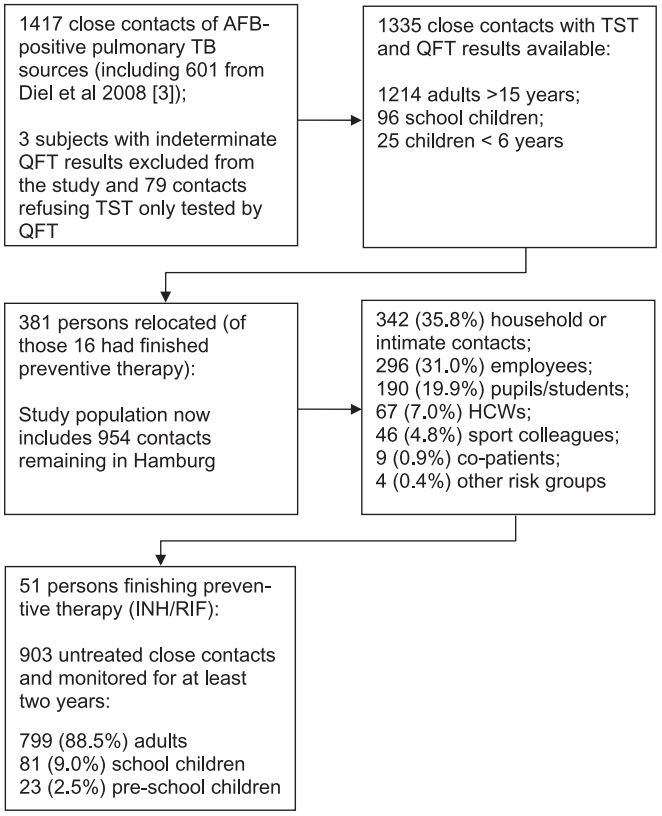

Figure 1. Study recruitment profile. AFB = acid-fast bacilli; HCWs = health care workers; INH = isoniazid; QFT = QuantiFERON-TB Gold in-tube assay; RIF = rifampicin; TB = tuberculosis; TST = tuberculin skin test. Percentages are rounded and may not sum up to 100%.

of preventive treatment did not differ between foreign-born and German-born contacts; (37 of 193 [19.2%] foreign-born and 30 of 121 [24.8%] German-born contacts; not significant). The 247 QFT-positive contacts who refused chemoprevention did not differ from those 67 contacts taking INH and/or RIF with respect to age (31.82 ± 12.52 vs. 30.37 ± 10.30 yr), sex (40.4 vs. 49.3% female, respectively), origin (36.8 vs. 44.8% German born, respectively), or previous BCG vaccination (42.5 vs. 44.8% vaccinated, respectively).

QFT and TST Results

At the time of contact investigation, 314 of the 1,414 contacts (22.2%) had a positive QFT result and 65.2% (870 of the 1,335 tested), almost three times more, had a positive TST. The cumulative exposure time among QFT-positive contacts was on average 85 hours longer than among those who were QFT negative (mean, 174.1 ± 162.1 vs. 89.6 ± 110.6 h; $P < 0.0001$), whereas there was no difference in exposure time with TST results (107.5 ± 128.7 vs. 116.2 ± 134.7 h; not significant).

For those subjects remaining in Hamburg during the follow-up period, 209 of 1,033 (20.2%) were QFT positive. Of the 954 persons who had both a QFT and a TST and could be monitored for the study period, 198 (20.8%) were QFT positive, 604 (63.3%) had a TST induration greater than 5 mm, and 242 (25.4%) were positive at a 10-mm cutoff (Table 1). Using a greater than 15-mm cutoff, 78 of 954 (8.2%) were TST positive (data not shown). More than two-thirds (409 of 604; 67.7%) of those who were TST positive (>5 mm) had received BCG vaccination. The BCG vaccination rate was nearly identical for TST-positive contacts who were foreign born and those of German origin (207 of 308 [67.2%] vs. 202 of 296 [68.2%], respectively).

On the basis of the assumption that the longer a contact is exposed to his/her index case, the greater the chance is that he/she will become infected, we stratified test results against exposure time gradients (40–59, 60–99, 100–199, and 200+ h) (Table 1). In multiple logistic regression analysis, no significant increase in odds ratios (ORs) for test positivity was found for the TST at a 5-mm cutoff, taking the first exposure category (40–59 h) as reference and adjusting for age, sex, origin, and

TABLE 1. RESULTS OF MULTIPLE LOGISTIC REGRESSION OF DEMOGRAPHIC AND BEHAVIORAL PARAMETERS OF THE 954 STUDY PARTICIPANTS WITH BOTH TUBERCULIN SKIN TEST AND QUANTIFERON-TB GOLD IN-TUBE ASSAY RESULTS FOR TEST POSITIVITY

		QFT Positive			TST Positive (>*5 mm*)			TST Positive (>*10 mm*)		
Variable	N (%)	No. (%)	OR*	*P* Value*	No. (%)	OR*	*P* Value*	No. (%)	OR*	*P* Value*
Number of subjects	954	198 (20.8)			604 (63.3)			242 (25.4)		
Sex										
Male	489 (51.3)	109 (22.3)	—		321 (65.6)	—		135 (27.6)	—	
Female	465 (48.7)	89 (19.1)	0.99	n.s.	283 (60.9)	0.999	n.s.	107 (23.0)	0.93	n.s.
Age, yr										
0 to <6	24 (2.5)	7 (29.2)	—		8 (33.3)	—		5 (20.8)	—	
6 to <16	82 (8.6)	16 (19.5)	0.87	n.s.	34 (41.5)	1.58	n.s.	16 (19.5)	1.26	n.s.
16 to <30	404 (42.3)	70 (17.3)	0.60	n.s.	251 (62.1)	2.48	n.s.	94 (23.3)	1.08	n.s.
30 to <40	228 (23.9)	57 (25.0)	1.42	n.s.	150 (65.8)	3.16	0.03	68 (29.8)	1.90	n.s.
40 to <50	194 (20.3)	44 (22.7)	1.23	n.s.	143 (73.7)	6.09	0.001	49 (25.3)	1.52	n.s.
50+	22 (2.3)	4 (18.2)	0.84	n.s.	18 (81.8)	5.63	0.03	10 (45.5)	3.39	n.s.
BCG vaccinated										
No	459 (48.1)	113 (24.6)	—		195 (42.5)	—		87 (19.0)	—	
Yes	495 (51.9)	85 (17.2)	0.64	<0.01	409 (82.6)	7.20	<0.0001	155 (31.3)	2.33	<0.0001
Exposure time, h										
40 to <60	466 (48.8)	25 (5.4)	—		301 (64.6)	—	.	76 (16.3)	—	
60 to <100	194 (20.3)	50 (25.8)	6.12	<0.0001	114 (58.8)	0.88	n.s	50 (25.8)	2.01	<0.0001
100 to <200	143 (15.0)	52 (36.4)	9.76	<0.0001	94 (65.7)	1.18	n.s	51 (35.7)	3.20	<0.0001
200+	151 (15.8)	71 (47.0)	14.50	<0.0001	95 (62.9)	1.08	n.s	65 (43.0)	4.41	<0.0001
German origin										
Yes	567 (59.4)	79 (13.9)	—		296 (52.2)	—		89 (15.7)	—	
No	387 (40.6)	119 (30.7)	2.40	<0.0001	308 (79.5)	4.19	<0.0001	153 (39.5)	3.13	<0.0001

Definition of abbreviations: BCG = bacillus Calmette-Guérin; n.s. = not significant; OR = odds ratio.

* Adjusted for sex, age, BCG, exposure time, and origin.

BCG vaccination, whereas stratifying the QFT-positive subjects with respect to the four gradients of exposure resulted in an increase in adjusted ORs of 6.12, 9.76, and 14.50 ($P < 0.0001$). Positive QFT and TST results at both 5- and 10-mm cutoffs were significantly associated with being born outside of Germany (OR, 2.4, 4.19, and 3.13, respectively; $P < 0.0001$ for each). Notably, positive TST results were strongly associated with a history of BCG vaccination (OR, 7.20 and 2.33; $P < 0.0001$ for both), but positive QFT results were inversely associated (OR, 0.64; $P < 0.01$). Only TST results at a 5-mm cutoff were associated with increasing age of contacts for those who were at least 30 years old, taking the first age group (0 to <6 yr) as reference.

Agreement between QFT and TST results, stratified by BCG vaccination status, is shown in Table 2. Overall agreement was poor (56%; $\kappa = 0.238$) using the greater than 5-mm cutoff for the TST. When the population was stratified according to BCG status, agreement proved bipolar. It was low in those vaccinated (33.7%; $\kappa = 0.072$), but good in nonvaccinated contacts (80.0%; $\kappa = 0.566$). Using the 5-mm cutoff, there were only seven contacts who were QFT positive, but TST negative. Agreement between the two tests improved markedly if a greater than 10-mm cutoff was used for the TST (Table 3), but resulted in a large number of QFT-positive contacts (60 of 198; 30.3%) who were TST negative.

Follow-up and Progression to Active TB

Of the 1,033 contacts who remained in Hamburg for the duration of the study, 209 were QFT positive, with 51 completing preventive therapy and 158 not treated. The mean time period of observation per untreated contact was more than 3.5 person-years (186.5 ± 49.6 wk), comprising observation of subjects from the initial phase of the study for a mean of 233.3 ± 13.8 weeks (4.48 person-years) and contacts included since then (April 2006) with a mean time of 148.3 ± 32.6 weeks (2.85 person-years).

By April 1, 2010, 19 of the untreated contacts had been diagnosed with active TB, 6 during the initial phase of the study (3). With the exception of one 30-year-old contact from the initial phase, all of the new TB cases reported here came from the subcohort of contacts recruited between April 2006 and March 2008. All 19 active TB cases came from the 982 untreated contacts still listed as Hamburg residents in the Residents' Registration Office, and all 19 had been QFT positive at initial screening, yielding progression rates for the whole group of 1.9 and 12.0% (19 of 158) for those who were QFT positive and untreated, respectively.

Whereas QFT screening picked up all 19 of the patients (100%) who would later progress to active TB, only 17 of those (89%) were TST positive if using a greater than 5-mm cutoff, 10 of 19 (53%) if using a greater than 10-mm cutoff, and 2 of 19 (11%) at greater than 15 mm. The magnitudes of each contact's QFT and TST responses are shown in Figure 2, as are the responses of the 19 individuals who progressed to active TB. Sixteen of the 19 people who developed active TB were strong responders in the QFT assay, with IFN-γ levels more than 10-fold higher than the cutoff of 0.35 IU/ml. Ten of the 19 had levels above the 10-IU/ml upper limit for the test's ELISA. There were, however, three patients whose QFT levels—determined 8 weeks after the last possible exposure toward their respective index cases—were comparatively low (0.41, 0.52, and 0.78 IU/ml).

Details of the 19 individuals who developed TB disease, of whom 7 were born outside of Germany, are given in Table 3. Eighteen (94.8%) of the patients had pulmonary TB and 1 suffered from pleurisy. The diagnosis of TB was confirmed by culture of *M. tuberculosis* or histopathologically for 12 of the 19 patients (63.2%). The seven patients, five of them children, with epidemiological evidence of transmission of TB by their presumed source cases, but for whom *M. tuberculosis* could not be positively detected, were all treated for active TB; all resolved their symptoms and radiological lesions. IS*6110* genotyping of *M. tuberculosis* isolates revealed that for all 11 culture-positive patients the infective strain matched that of the respective index case.

A reduction of our analysis to the 954 contacts for whom both QFT and TST results were available revealed that 903 did not receive treatment, with 147 (16.3%) positive by QFT and 555 (60.1%) by the TST (207 [22.9%], using a greater than 10-mm cutoff; and 63 [7.0%], at greater than 15 mm; *see* Table 4). The corresponding rates for progression to active TB during the follow-up period were 12.9% (19 of 147) for QFT-positive contacts, significantly higher than the 3.1% rate (17 of 552) of those positive by the TST, using a greater than 5-mm cutoff ($P < 0.0001$). The TST progression rate was higher when a greater than 10-mm cutoff (4.8%; 10 of 207) was used, but still significantly lower than that for QFT ($P < 0.01$). More importantly, use of a greater than 10-mm cutoff would have identified only 10 of the 19 (53%) persons who progressed to active TB. The use of a greater than 15-mm cutoff would have limited the success of the screening to only 2 individuals of the 19 (11%) who progressed. Progression rates for the 11 contacts who developed active TB, confirmed by culture of MTB, were 7.5% (11 of 147), 1.6% (9 of 552), and 1.9% (4 of 207) for QFT and the TST at cutoffs greater than 5 and 10 mm, respectively. The rate for those who were QFT positive was significantly higher than that for the TST at either cutoff ($P < 0.001$ and $P = 0.015$, respectively).

Two of the subjects who progressed to disease were QFT positive, but TST negative, each with a 0-mm induration. One 14-year-old Turkish girl who was infected by her 35-year-old father in 2007 (confirmed by RFLP fingerprinting) was also TST negative and progressed to active TB 6 months later, but QFT

TABLE 2. AGREEMENT BETWEEN QUANTIFERON-TB GOLD IN-TUBE ASSAY AND TUBERCULIN SKIN TEST, STRATIFIED BY BACILLUS CALMETTE-GUERIN VACCINATION STATUS

		QFT				QFT		
	TST (>5 mm)	Positive	Negative	Agreement	TST (*>10 mm*)	Positive	Negative	Agreement
All subjects, n = 954	Positive	191 (20.0%)	413 (43.3%)	Agreement = 56.0%	Positive	138 (14.5%)	104 (10.9%)	Agreement = 82.8%
	Negative	7 (0.7%)	343 (36.0%)	κ = 0.238	Negative	60 (6.3%)	652 (6.84%)	κ = 0.517
BCG vaccinated, n = 495	Positive	83 (16.8%)	326 (65.9%)	Agreement = 33.7%	Positive	63 (12.7%)	92 (18.5%)	Agreement = 77.0%
	Negative	2 (0.4%)	84 (17.0%)	κ = 0.072	Negative	22 (4.4%)	318 (64.2%)	κ = 0.390
No BCG, n = 459	Positive	108 (23.5%)	87 (19.0%)	Agreement = 80.0%	Positive	75 (16.3%)	12 (2.6%)	Agreement = 89.1%
	Negative	5 (1.1%)	259 (56.4%)	κ = 0.566	Negative	38 (8.3%)	334 (72.8%)	κ = 0.682

Definition of abbreviations: BCG = bacillus Calmette-Guérin; QFT = QuantiFERON-TB Gold in-tube assay; TST = tuberculin skin test.

TABLE 3. DEMOGRAPHICS AND TEST RESULTS FOR THE 19 CONTACTS WHO PROGRESSED TO ACTIVE TUBERCULOSIS

Patient No.	Age (*yr*)	Sex	BCG	Place of Origin	Place of Contact	Contact Time (*h*)	Time from Testing to TB (*mo*)	TST (*mm*)			QFT		MTB Mapped to Index Case	Site of MTB	Culture/NAA/ Histology
								>5	>10	Size	Result	IU/ml			
1	1	Female	No	Germany	Household	196	4	Pos	Neg	8	Pos	10	—	Pulmonary	No
2	2	Female	No	Germany	Household	120	3	Pos	Pos	12	Pos	10	—	Pulmonary	No
3	3	Female	No	Germany	Household	52	4	Pos	Neg	9	Pos	3.59	Yes	Pulmonary	Yes
4	6	Female	No	Macedonia	Household	48	6	Pos	Pos	13	Pos	7.44	—	Pulmonary	No
5	10	Male	No	Germany	Household	520	7	Pos	Pos	14	Pos	10	—	Pulmonary	No
6	15	Male	No	Germany	Household	120	3	Pos	Pos	20	Pos	10	—	Pulmonary	No
7	22	Male	No	Germany	Household	120	3	Pos	Pos	12	Pos	10	—	Pulmonary	No
8	24	Female	No	Germany	Household	96	16	Neg	Neg	0	Pos	10	Yes	Pulmonary	Yes
9	24	Male	No	Poland	Household	440	9	Pos	Pos	12	Pos	8.66	Yes	Pulmonary	Yes
10	25	Male	Yes	Myanmar	Household	248	10	Pos	Pos	12	Pos	6.09	Yes	Pulmonary	Yes
11	27	Male	Yes	Turkey	Workplace	40	5	Pos	Neg	7	Pos	0.52	—	Pleuritis	Histology
12	29	Female	No	Germany	Household	480	18	Pos	Neg	9	Pos	0.41	Yes	Pulmonary	Yes
13	30	Female	Yes	Turkey	Household	238	22	Neg	Neg	0	Pos	0.78	Yes	Pulmonary	Yes
14	30	Male	No	Germany	Household	52	17	Pos	Pos	11	Pos	4.72	Yes	Pulmonary	Yes
15	30	Female	Yes	Germany	Household	496	9	Pos	Pos	17	Pos	10	Yes	Pulmonary	Yes
16	39	Male	No	India	Workplace	48	18	Pos	Neg	8	Pos	4.54	Yes	Pulmonary	Yes
17	44	Male	No	Germany	Household	328	23	Pos	Neg	9	Pos	10	Yes	Pulmonary	Yes
18	48	Female	No	Germany	Household	244	6	Pos	Neg	8	Pos	10	Yes	Pulmonary	Yes
19	62	Male	Yes	Turkey	Household	204	5	Pos	Pos	11	Pos	10	—	Pulmonary	No

Definition of abbreviations: BCG = bacillus Calmette-Guérin; MTB = *Mycobacterium tuberculosis*; NAA = nucleic acid amplification; Neg = negative; Pos = positive; QFT = QuantiFERON-TB Gold in-tube assay; TB = tuberculosis; TST = tuberculin skin test.

had not been initially performed and her data were thus not included in the study outcome.

Independent Predictors of Progression to Disease

When progression to disease was taken as a dependent variable in multiple logistical regression analysis, two independent factors were found to be significantly associated: IFN-γ level in QFT and age (Table 5). The chance of developing TB nearly doubled with every unit of IFN-γ per milliliter (OR, 1.93; $P < 0.0001$) and decreased by 6% (OR, 0.94; $P < 0.02$) with each year of age. In contrast, after adjustment for confounding, TST induration diameter, exposure time of the contacts to their index case(s), origin outside of Germany, sex, and BCG vaccination were not found to be independent predictors of progression to active TB.

Children

Our subjects included 141 children less than 16 years of age, of whom 15 moved away during the observation period. Of the remaining 126 children (89.4%), the mean age was 10.4 ± 4.3 years; 84 (66.7%) were born in Germany and 45 (35.7%) were BCG vaccinated.

Both TST and QFT results were available for 106 of the 126 contacts less than 16 years of age. Of those, 23 of 106 (21.7%) were positive by QFT, 40 (37.8%) by TST using a greater than 5-mm cutoff, and 20 (18.9%) by TST using a greater than 10-mm cutoff. All but 1 of the 23 QFT-positive children were also positive by TST and there were 20 of the 106 children (18.9%) who were TST positive, but QFT negative. Only 2 of the 23 QFT-positive children accepted chemoprevention (8.7%).

Both QFT and TST results were available for 104 children who did not take preventive therapy (Figure 3). During the follow-up period, six children were diagnosed with active TB (ages 1, 2, 3, 6, 10, and 15 yr). All six of these children were QFT positive, resulting in a progression rate of 28.6% (6 of 21), significantly higher than that found for contacts 16 years of age and older at risk (13 of 126; 10.3%; $P = 0.03$). The level of IFN-γ response for these six children was high: four of them greater than 10 IU/ml, one 7.44 IU/ml, and one 3.59 IU/ml. In comparison, none of the QFT-positive children who did not develop active TB had responses greater than 4.5 IU/ml and their mean level of response was 2.5 IU/ml (±1.24 SD). For the TST, all six children progressing to active TB were positive at greater than 5 mm, equating to a progression rate of 15% (6 of 40) for those who were untreated, not significantly different from the rate for QFT ($P = 0.33$). However, if a greater than 10-mm TST cutoff was applied, only four of the six children who developed active TB would have been identified.

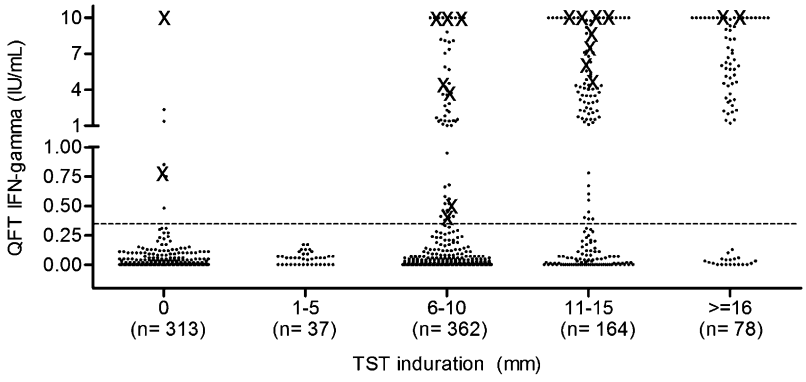

Figure 2. Comparison of the level of responses for QuantiFERON-TB Gold in-tube assay (QFT) and tuberculin skin test (TST) for the 954 subjects with both results available. The QFT response is the level of IFN-γ (IU/ml) in the tuberculosis (TB) antigen–stimulated plasma sample with that for the negative control subtracted. The 19 individuals who developed TB disease are marked (×). Responses equal to or greater than 10 IU/ml for the QFT assay are shown as 10 IU/ml. The *dotted line* represents the 0.35-IU/ml cutoff for the QFT test.

TABLE 4. RATE OF PROGRESSION TO ACTIVE TUBERCULOSIS FOR THOSE QUANTIFERON-TB GOLD IN-TUBE ASSAY POSITIVE OR POSITIVE BY THE TUBERCULIN SKIN TEST AT VARIOUS INDURATION CUTOFFS AMONG THE 903 UNTREATED CONTACT PERSONS

	No. of Untreated Contacts	Progressed to Active TB	Progression Rate (%)
QFT			
Positive	147	19	12.9
Negative	756	0	0
TST, mm			
0–5	348*	2	0.6
>5	555	17	3.1
>10	207	10	4.8
>15	63	2	3.2

Definition of abbreviations: QFT = QuantiFERON-TB Gold in-tube assay; TB = tuberculosis; TST = tuberculin skin test.

* Two TST-negative but QFT-positive contact persons received preventive chemotherapy.

TABLE 5. RESULTS OF MULTIPLE LOGISTIC REGRESSION ANALYSIS FOR PROGRESSION TO ACTIVE DISEASE FOR THE UNTREATED 903 SUBJECTS WHO HAD QUANTIFERON-TB GOLD IN-TUBE ASSAY AND TUBERCULIN SKIN TEST RESULTS AND REMAINED IN HAMBURG

Progression to TB	Odds Ratio	95% Confidence interval	*P* Value
IFN-γ level, IU/ml*	1.93	1.551–2.40	<0.0001
TST induration diameter, mm	0.89	0.78–1.01	0.08 (n.s.)
Age, yr	0.94	0.89–0.99	<0.02
Sex	0.75	0.23–2.44	0.63 (n.s.)
Origin outside Germany	1.88	0.52–6.75	0.33 (n.s.)
BCG vaccination	0.76	0.22–2.61	0.66 (n.s.)
Cumulative exposure time, h	1.002	0.998–1.005	0.29 (n.s.)

Definition of abbreviations: BCG = bacillus Calmette-Guérin; n.s. = not significant; QFT = QuantiFERON-TB Gold in-tube assay; TB = tuberculosis; TST = tuberculin skin test.

* The level of IFN-γ (IU/ml) in the QFT TB antigen–stimulated plasma sample with that for the negative control subtracted.

There was some evidence of BCG having a protective effect in children under 16 years of age. The QFT-positive rate in BCG-vaccinated children was 13.9% (5 of 36) and higher in those who were unvaccinated (26.5%; 18 of 68), although this did not reach statistical significance. In addition, all 6 children who developed active TB were among the 68 who were unvaccinated, whereas none of the 36 BCG-vaccinated children progressed. This finding, too, failed to reach statistical significance ($P = 0.09$).

Negative Predictive Value

None of the 824 untreated contacts who were QFT negative (410 of them were TST positive) developed active TB over the 3.7 ± 0.92 person-years of follow-up, yielding an NPV of 100%. Using a greater than 5-mm cutoff for the TST, 2 of the 351 negative and untreated contacts developed active TB, resulting in an NPV of 99.4%. If a greater than 10-mm cutoff were to be applied for the TST, 9 of the 720 TST-negative contacts developed active TB, for an NPV of 98.8%. In 393 of the 824 contacts (47.7%) with negative QFT results, no IFN-γ response to the TB antigens was detected above background, and 711 (86.3%) of the contacts with negative QFT results had an IFN-γ level below 0.1 IU/ml. For the TST, 316 of the total of 351 contacts (89.3%) who tested negative had 0 mm of induration measured.

DISCUSSION

The results of this update confirm, with greater statistical power provided by the larger count of 1,033 untreated contacts monitored for progression to active TB, the key results of our study's initial phase. First, the progression rate of close contacts who were QFT positive was outstanding in comparison with that of the TST, irrespective of the TST cutoff chosen (12.9 vs. 3.1% at a >5-mm cutoff and 4.8% at a >10-mm cutoff). Second, all of the 19 contacts who progressed to active TB were positive by QFT at the time of contact screening, compared with 17 of 19 (89.5%) for the TST using a greater than 5-mm cutoff and only 10 of 19 (52.6%) using a greater than 10-mm cutoff. Third, the QFT had maximal negative predictive value, with none of the 413 TST-positive, QFT-negative subjects progressing to active TB disease.

Apart from our publication of the initial phase of this study, only four published works using the newest commercial IGRAs (QFT or the enzyme-linked immunospot–based test T-SPOT.*TB*; Oxford Immunotec, Abingdon, UK) have evaluated the progression of untreated persons to active TB. Clark and colleagues (4) and Aichelburg and colleagues (5) reported progression rates in approximately 2 years of 10% (95% confidence interval [CI], 12.3–31.7) and 8.3% (95% CI, 1.8–22.5), respectively, among QFT-positive, outpatient HIV-1–positive adults for whom the date and extent of presumed prior MTB exposure was unknown. The statistical power of these two studies was limited as they reported only two and three subjects, respectively, progressing to active TB. Kik and colleagues (6) presented lower rates of progression, 2.8% (95% CI, 0.9–6.4) and 3.3% (1.2–7.1), among QFT- and T-SPOT.*TB*-positive immigrant and BCG-vaccinated Dutch-born contacts. However, these estimates were probably biased by the fact that IGRA testing was conditional to prior TST positivity and that TST was repeated if not positive initially, whereas the IGRA was not. Leung and colleagues (7) found progression in 7.4% of male Hong Kong patients with silicosis with positive T-SPOT.*TB* results, but TB risk in this group differs with respect to severity of silicosis and cannot be directly compared with that of close contacts. Overall, and despite the relatively small number of reported subjects progressing to active TB, the findings of these publications are commensurate with the high rate of progression we observed for QFT-positive close contacts.

There are challenges to conducting population-based epidemiological studies in metropolises with highly mobile populations. These are underlined by the fact that approximately one-third of the QFT-positive contacts our study identified had moved out of our sphere of influence by the time we closed our files for this update. In the years 2005 to 2007, a total of 187,156 persons left Hamburg (57,338 foreign born). A national TB registry in Germany that would allow searching for the names of those lost to follow-up (i.e., those who had moved from Hamburg), to ascertain whether any had in fact developed TB, does not exist, but even then this would be of only limited help as 38,350 (20.4%) of these moved directly beyond the German borders, many of them foreign guest workers or asylum seekers moving back to their home countries or to neighboring European Union countries (8). In such settings, simply counting test-positive contacts cumulatively and dividing the reported TB patients by this denominator leads to an underestimation of the true progression rate; and even a nationwide study would be subject to this selection bias. We used available public records to determine, as much as realistically possible, which members of our study populations had remained in Hamburg for the duration of the study period. As such, our observed progression rate of 12.0% for those who were QFT positive is probably

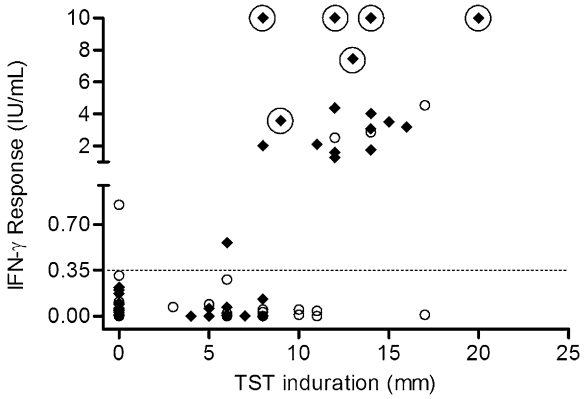

Figure 3. QuantiFERON-TB Gold in-tube assay (QFT) and tuberculin skin test (TST) data from the 104 untreated children (<16 yr of age) with both results available. The QFT response is the level of IFN-γ (IU/ml) in the tuberculosis (TB) antigen–stimulated plasma sample with that for the negative control subtracted. Results for those who were vaccinated with bacillus Calmette-Guérin (BCG) are represented by *open circles* and those who were unvaccinated are represented by *solid diamonds*. Results for the six children who progressed to active TB are highlighted with a *circled diamond*. The *dotted line* represents the 0.35-IU/ml cutoff for the QFT test.

a good estimate of the true rate. Other factors may also hamper epidemiological studies such as ours; for example, failure to perform the required testing at initial contact screening. In addition to the one juvenile subject progressing to active TB and confirmed by fingerprinting, but not included in the study because only a false-negative TST was available and no QFT was performed, we had 79 of the total 1,414 contacts (5.6%) declining to have the TST performed, in favor of QFT.

Although a number of publications have demonstrated that IGRAs can be used reliably in children (9–13), there are relatively limited data available from this age group. This has led to appropriate conservatism in published IGRA guidelines and to a general call for more studies regarding the use of IGRAs before the TST is abandoned in its favor. Concern is especially strong with respect to children less than 5 years of age (14). In our study cohort, there were 141 children aged 15 years or less, of whom 26 were less than 5 years of age. Notably, the rate of progression to active TB in untreated QFT-positive children was significantly higher than that of adults (28.6%, 6 of 21 vs. 10.3%, 13 of 126; $P = 0.03$). The rate of progression for the 24 untreated children aged 5 years or less was even more remarkable, with 3 of the 7 (43%) who were QFT positive developing active TB. The TST also had a high predictive value, with all six children developing active TB being positive at a greater than 5-mm cutoff. However, if using a greater than 10-mm cutoff, two children would have been missed, both of them younger than 5 years. These findings suggest that the test is at least commensurate with the TST in this age group. Our data highlight the high risk of progression to active TB for children, as compared with adults, and provides further evidence of the need for preventive therapy in children contacts.

Although we found that contacts progressing to active TB had significantly higher initial IFN-γ levels, there was a wide overlap in IFN-γ values between those QFT-positive contacts who progressed and those QFT-positive contacts who remained healthy. Although our results clearly indicate that the risk of progression—especially among children—rises with increasing level of QFT response, some QFT-positive immune-competent persons with initial IFN-γ levels less than 1.0 IU/ml progress to TB disease. Although, as Andersen and colleagues postulate (15), QFT responses may reflect the dynamics of mycobacterial load, their exact significance appears to be subjective and remains poorly understood. Thus, a positive QFT response, whatever the magnitude, indicates an MTB infection that is the prerequisite for TB disease. This should be taken into account when considering the prescription of preventive therapy.

Many countries worldwide have adopted a higher TST cutoff for screening close contacts than the 5-mm cutoff recommended by bodies such as the U.S. Centers for Disease Control and Prevention (16) and the German Central Committee against Tuberculosis (17). Our results demonstrate that although the use of a greater than 10-mm cutoff may mitigate the specificity problems inherent with the greater than 5-mm TST cutoff, it introduces a dangerous compromise in terms of sensitivity. Nine of our 19 (47%) close contacts who progressed to active TB would have been overlooked if we had limited our concern to persons with indurations of 10 mm or more; resulting in an NPV for progression of only 98.7%. Clearly such information is highly relevant for policy makers who are considering replacing the TST with an IGRA.

It has been suggested that the advent of IGRAs, which are unaffected by BCG vaccination, may allow us to better determine the protective efficacy of BCG vaccination (13). Our cohort, in which approximately one-half of the subjects had received BCG vaccination at some time in their life, offers a good opportunity to address that possibility. Where results for both tests were available, our data demonstrate that, as expected, the rate of TST positivity, even when adjusted for confounding in multiple logistic regression analysis, is significantly higher for those who are BCG vaccinated when using either a greater than 5- or 10-mm cutoff ($P < 0.0001$ for both). In total contrast, the rate of QFT positivity was significantly lower in those who were BCG vaccinated ($P < 0.01$), which indicates that the vaccine has some protective efficacy. For the juvenile cohort, in which the efficacy of BCG might be expected to be at its highest, the rate of QFT positivity was also lower in those vaccinated, and all six children who progressed to active TB were unvaccinated. Neither of these important trends, however, reached statistical significance. Although these findings support protective efficacy of BCG vaccination, evaluating this was not an aim of the study, and a number of uncontrolled factors may be confounding the outcome. Our findings do raise the possibility, however, that IGRAs could be useful tools for monitoring vaccine efficacy in clinical studies of new vaccines.

Our study did have limitations: Contact persons were passively monitored via Public Health Department records to determine those had been reported with active TB. Three contact persons, who later developed culture-confirmed TB and whose epidemiological relationship to a source case in Hamburg could be documented by fingerprinting of the isolates, had not been reported to the Public Health authorities by their index cases and therefore could not participate in the contact investigations done around the respective source case. They had thus received neither test and could not be included in this study. This imperfection in our TB control methods probably had the effect of underestimating the true positive predictive value of both TST and QFT.

In a further limitation, genomic DNA of the causative strain of MTB was not available from all test-positive contacts who developed active TB, and so 100% identification of source cases could not be achieved. However, five of the seven cases not confirmed by isolation of MTB were in children, a population in which isolation of MTB is notoriously difficult (18, 19). The MTB strains of the 11 culture-positive patients, on the other hand, were all confirmed by RFLP mapping as being identical to those of the respective index cases.

In conclusion, our results demonstrate the benefits of using the highly specific QFT assay in place of the TST in populations at risk and with a high pretest probability of MTB infection. QFT yielded a higher positive predictive value, not only for determination of latent tuberculosis infection status, but, more importantly, for identifying those most likely to develop active TB disease in the near future. Moreover, whereas in clinical settings the usefulness of IGRAs falls short of that of a "rule-out" test for active TB, given a diagnostic sensitivity of only 81 to 88% (2), in healthy subjects with intact immune function the sensitivity of the tests for detecting latent tuberculosis infection is likely much higher. This is supported by our finding that the QFT had a 100% negative predictive value for progression to active TB in our study of a large body of close contacts with a high pretest likelihood of infection.

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