Predictive Value of a Whole Blood IFN-γ Assay for the Development of Active Tuberculosis Disease after Recent Infection with *Mycobacterium tuberculosis*

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***Rationale*: Numerous studies have been published on the new *Mycobacterium tuberculosis* (MTB)–specific IFN-γ release assays. However, their prognostic value for progression from latent tuberculosis infection (LTBI) to active TB has yet to be established.**

***Objectives*: To compare the QuantiFERON-TB Gold In-Tube assay (QFT) with the tuberculin skin test (TST) in recently exposed close contacts of active TB cases with respect to their development of TB disease within 2 years.**

***Methods*: Close contacts (n = 601) of MTB-positive source cases underwent both TST and QFT testing and were subsequently observed for 103 (±13.5) weeks. Risk factors for MTB infection were evaluated by multivariate analysis.**

***Measurements and Main Results*: For the TST, 40.4% (243/601) of contacts were positive at a 5-mm cutoff, whereas only 66 (11%) were QFT positive. QFT positivity, but not TST, was associated with exposure time ($P < 0.0001$). Six contacts progressed to TB disease within the 2-year follow-up. All were QFT positive and had declined preventive treatment, equating to a progression rate of 14.6% (6/41) among those who were QFT positive. The progression rate for untreated TST-positive subjects was significantly lower ($P < 0.003$), at 2.3% (5 of 219), and one subject who progressed was TST negative.**

***Conclusions*: Results suggest that QFT is a more accurate indicator of the presence of LTBI than the TST and provides at least the same sensitivity for detecting those who will progress to active TB. The high rate of progression to active TB of those who are QFT positive (14.6%), which is far greater than the 2.3% found for those who are TST positive, has health and economic implications for enhanced TB control, particularly if this higher progression rate is seen in studies of other at-risk populations.**

Keywords: tuberculosis; latent infection; IFN-γ release assay; predictive value; disease development

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

In contrast to IFN-γ release assay (IGRA) tests, the tuberculin skin test (TST) suffers from high rates of false-positive responses in contacts of active tuberculosis (TB) cases. However, the key question that remains unanswered is whether IGRAs are better than the TST in predicting the development of TB.

What This Study Adds to the Field

IGRA testing appears to be a more accurate indicator of the presence of latent TB infection than the TST and provides at least the same sensitivity for detecting individuals who will progress to active TB.

In low-incidence countries, identification and treatment of individuals recently infected with *Mycobacterium tuberculosis* (MTB) is central to tuberculosis (TB) control (1), but until recently, the detection of latent TB infection (LTBI) could only be based on tuberculin skin testing (TST). Because the TST is intrinsically confounded by cross-reactive immune responses after vaccination with bacillus Calmette-Guérin (BCG) strains or infection with nontuberculous mycobacteria, *in vitro* IFN-γ release assays (IGRAs) that are highly specific for MTB have been developed as alternative screening tools (2, 3).

Numerous recent studies have shown that the degree of exposure of contacts to their source cases is usually more closely correlated with a positive result in an early secreted antigenic target 6 and culture filtrate protein 10 (ESAT-6/CFP-10)–based IGRA than with reactivity to the TST (4–8). This is especially true in those vaccinated with BCG, where the TST suffers from high rates of false-positive responses. In contrast, IGRAs are unaffected by BCG vaccination and are at least as sensitive as the TST for detecting active TB (2, 3, 9, 10).

To date, the key unanswered question is whether IGRAs are better than the TST in predicting the development of TB disease. If an IGRA has the same (or better) sensitivity of predicting future TB disease, its use would lead to fewer people being "identified" as positive and, thereby, fewer people to evaluate for active TB and fewer being offered chemoprevention for LTBI. Such an outcome would focus scarce TB-control resources on those individuals most likely to progress to active TB, likely improving compliance with LTBI treatment and thereby diminishing transmission routes.

Because the only gold standard for LTBI is the eventual development of active TB, the predictive value for progression can be ascertained only through longitudinal cohort studies that follow clinical outcomes of tested individuals. This requires extensive follow-up of a large, well-characterized population of persons whose prognosis suggests the possible development of manifest disease within the study period. Contacts of sputum smear–positive TB cases can constitute such a population, when observed for 2 years after infection.

The site of the present study, Hamburg, is one of the German federal states, and with 1.8 million residents, the second largest

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city in Germany. Hamburg has the highest TB incidence rate (10.8 per 100.000 inhabitants in 2005) of all 16 federal states (11). It also has the highest proportion of foreign residents (14.2%) compared with a German national average of 8.8% reported for December 31, 2005 (12). In the present prospective study, we performed a head-to-head comparison of a whole blood IGRA (QuantiFERON-TB Gold In-Tube [QFT]) and TST as measures of infection in immunocompetent close contacts of patients with pulmonary TB. Association of test results with measures of extent of exposure was evaluated as was prognostic accuracy of the two tests for those who subsequently developed TB disease.

METHODS

Study Design and Inclusion Criteria

The present study is an extension of an ongoing pilot study initiated in 2005 to evaluate the QFT assay for routine use in contact investigations in a low-incidence setting; preliminary results of this study have been published elsewhere (10). Close contacts of acid-fast bacilli (AFB) smear-positive, subsequently culture-confirmed source MTB cases were prospectively enrolled in the study from May 2005 until April 2006. All contacts were tested by QFT and the TST at least 8 weeks after identification of their respective index case. The resulting cohort was observed until September 2007, with a focus on TB development, with or without isoniazid (INH) chemoprevention.

To maximize probability of contacts enrolled into our study being infected, we used the restrictive definition of Behr and colleagues (13) to define "close" contacts. The principal study inclusion criterion was an aggregate exposure time of the contact, before diagnosis of his or her respective index case, of not less than 40 hours in closed rooms. The estimated minimum time of contact was recorded for each contact person in 4-hour windows (40–43 h, 44–47 h, etc.). Contacts with unclear or only occasional exposure and an exposure time of less than 40 hours to the source case during the presumed period of infectiousness 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 agreed to participate.

Questionnaire

Sociodemographic and clinical data were derived from interviews of the individuals by trained public health staff using a standardized questionnaire (*see* the online supplement).

Confirmation by Restriction Fragment Length Polymorphism Fingerprinting

Because a portion of patients with LTBI in contact investigations may have been infected outside of the current exposure episode (14, 15), contacts with a history of previous TB disease or any contact tracing in the past were excluded from the study population. To confirm that a positive screening result in a subject progressing to TB disease represented fresh transmission, all 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 and/or spoligotyping by the National Reference Center for Mycobacteria, Borstel, Germany.

IGRA and Mantoux TST

The QFT test was performed according to the manufacturer's instructions (Cellestis Ltd, Carnegie, Australia) with a cutoff value for a positive test of IFN-γ of 0.35 IU/ml or greater. From May until October 2005, venous blood was collected from each subject (before administration of Mantoux TST) into two evacuated and heparinized blood tubes calibrated to draw 1 ml of blood. From November 2005 onward, an additional 1 ml of whole blood was drawn in a third tube containing phytohemagglutinin (mitogen), as a positive control (*see* the online supplement). The maximal level of IFN-γ accurately detected by the QFT ELISA is 10 IU/ml, and thus values greater than this are reported as 10 IU/ml.

TST was administered by the Mantoux method and, from May to October 2005, 0.1 ml of Tuberculin-10-GT (Chiron Behring, Marburg, Germany; bioequivalent to 5 units of the international purified protein derivative-Seifert [PPD-S] standard) was used, and subsequently (after Chiron Behring tuberculin was no longer available) 0.1 ml (2 tuberculin units) of purified protein derivative RT23 (Statens Serum Institute, Copenhagen, Denmark) was used, which is equivalent to Tuberculin-10-GT (Chiron Behring). TST reaction was scored as positive if induration diameter was greater than 5 mm, according to the German guidelines (16), regardless of BCG vaccination status. Individuals performing the TST were blinded to QFT results and vice versa. Induration was read by trained and well-experienced public health nurses. If there was a borderline result (e.g., 5 mm exactly), a second reading was performed by a different nurse to verify this result. If there was disagreement, a third nurse read the TST and the consensus result used.

Statistical Analysis

Categorical data were compared by the Pearson's χ^2 test (or Fisher's exact test, when expected sample sizes comprised fewer than five subjects). The Wilcoxon rank sum test was performed to determine whether the distribution of continuous variables differed between two groups; the Kruskal-Wallis test was used for multiple group comparison. Concordance between the results of the TST and QFT was assessed by using κ coefficients, for contacts both with and without BCG vaccination. Kappa values below 0.4 indicate weak correlation, values of 0.41–0.60 indicate good agreement, and values above 0.6 strong agreement (17). Independent predictors of the primary outcomes were identified through logistic regression models. All potential predictors or confounders of interest were entered simultaneously and model building was performed backward using the chance criteria for variable selection, but variables deemed to be clinically significant were retained regardless of statistical significance (18). Variables included were age, sex, country of origin (German or foreign), history of BCG vaccination (determined by vaccination record or presence of a scar), and exposure time of the contacts to a source case. Relations are expressed as odds ratios (ORs) and 95% confidence intervals (CIs) for each risk factor, with significance assessed by *P* values computed from Wald statistics. All *P* values reported are based on two-tailed comparisons with statistical significance set at $P < 0.05$.

Statistical analyses were performed using SPSS version 14.0 for Windows (SPSS, Inc., Chicago, IL) and BIAS version 8.3.6 for Windows (Epsilon, Inc., Frankfurt, Germany).

RESULTS

Study Population

Between May 2005 and April 2006, a total of 1,794 contact persons related to 107 source patients with at least microbiologically confirmed pulmonary TB were reported to the public health department. Of those, 629 were confirmed contacts of AFB smear- and culture-positive source cases meeting the inclusion criteria of at least 40 hours of exposure in a closed room. Two hundred eighty-seven contacts had *a priori* been excluded due to unclear or only casual contact to their related source cases. Nine contacts who had been subjects in previous contact tracing exercises and three who had been treated due to manifest TB disease did not fulfill the study criteria as described above and were excluded as study participants. None of the other subjects reported prior exposure to people with TB. Nineteen contacts did not come back for their TST to be read and were not used in the final analysis; they did not develop active TB disease during the study period.

In summary, 601 contacts of a total of 47 different AFB- and culture-positive source cases (24 foreign born and 23 German born), with complete results available for TST and QFT, formed the study population (Figure 1). Three MTB strains of the source cases were INH resistant. There was no evidence of immunosuppression for any of the contacts, and there were no indeterminate QFT results for the 292 subjects (48.6%) who were tested using the mitogen control tube. None of the contacts reported that they were seropositive for HIV, undergoing hemodialysis, currently

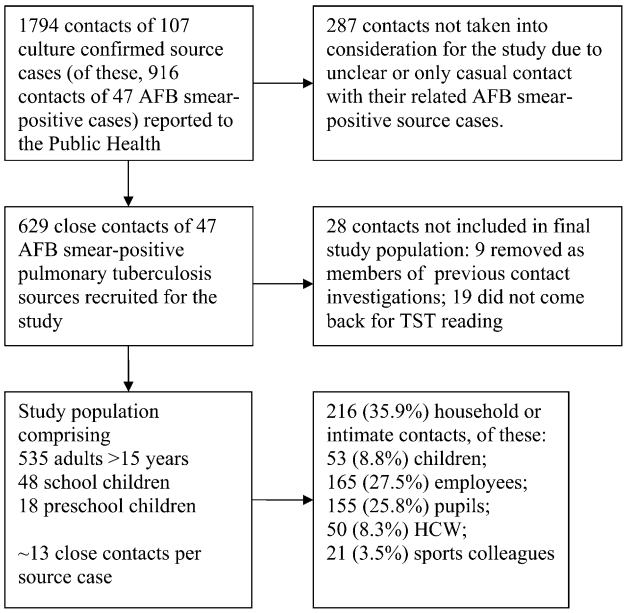

Figure 1. Study recruitment profile. AFB = acid-fast bacilli; HCW = health care workers; TST = tuberculin skin test.

being treated with immunosuppressive medication, known to have a malignant disease or diabetes mellitus, or had been recently immunized with live vaccines. The clinical outcome of all contacts was observed until September 1, 2007, the mean time period per contact being nearly 2 person-years (103.3 ± 13.5 wk).

The characteristics of the contacts are shown in Table 1. The number of close contacts per source case varied between 1 and 39 individuals; the mean cumulative exposure time (±SD) was 140.8 (±161.7) hours, with a range of 40 to 1,080 hours (the latter corresponding to 45 d). The mean age of the contacts was 27.7 ± 12.0 years (range, 1–56 yr), and there were only slightly more female contacts (n = 305; 50.7%) than male. Of the subjects, 216 were household or intimate (in a sexual relationship with their index case) contacts, 165 were colleagues of a source case within a company, 155 were pupils or teachers of traditional and vocational school classes, 50 were health care workers, and 21 were members of sports clubs.

The household contacts included 53 children under the age of 15; 18 of these were preschool children under the age of 6. Household contacts were thus younger than other contacts (26.3 ± 13.8 vs. 28.4 ± 10.9 yr, $P = 0.04$), but did not differ in sex, origin, and cumulative exposure time (150.6.0 ± 182.9 vs. 135.3 ± 148.5 h, not significant [NS]) compared with nonhousehold contacts.

Of the 601 contacts, 433 (72.0%) were born in Germany, whereas 168 (28%) had migrated to Germany from 29 different countries. The mean period between the date of entry to Germany and the date of contact tracing was 500.2 ± 346 weeks, with a range from 37 to 1,601 weeks.

Mantoux Tuberculin Skin Test

Overall, 243 of the 601 contacts (40.4%) developed induration greater than 5 mm at the TST site (Table 1). Using the recommended 5-mm cutoff for contacts in Germany, TST-positive individuals' mean age (±SD) of 28.1 (± 10.4) years was only slightly higher than that for those with negative TST (27.4 ± 13.1, NS). TST results were not significantly associated with exposure time because there was no difference between the length of exposure of the TST-positive contacts to their index case and the length of exposure for those who were TST negative (149.7 ± 182.0 vs. 134.7 ± 146.4 h, NS). If a 10-mm cutoff was used, there was a significantly longer exposure for those who were TST positive (177.4 ± 205.8 vs. 132.7 ± 149.2 h, $P < 0.01$; Table 1), which became even more significant when applying a 15-mm cutoff (224.3 ± 255.9 vs. 133.9 ± 1,249.7 h, $P < 0.0001$; data not shown).

Nearly one-half of the contacts (278 of 601; 46.3%) had received a BCG vaccination (*see* Table 1) and, of these, 187 (67.3%) were TST positive at greater than 5 mm. Only 56 of 323 (17.3%) of the unvaccinated contacts were TST positive ($P < 0.0001$). The rate of BCG vaccination was similar for foreign-born subjects and those of German origin (52.3 vs. 43.9%, respectively). For those 80 contacts who were foreign born and positive by the TST alone, 68 (85%) were reported as being

TABLE 1. DEMOGRAPHIC AND BEHAVIORAL CHARACTERISTICS OF THE STUDY PARTICIPANTS

Variables	n	QFT Positive, n (%)	*P*	TST Positive, n (% > *5 mm*)	*P*	TST Positive, n (% > *10 mm*)	*P*
No. of subjects	601	66 (11.0)		243 (40.4)		110 (18.3)	
Sex							
Male	296	37 (12.5)	NS	130 (43.9)	NS	45 (15.2)	NS
Female	305	29 (9.5)		113 (37.0)		65 (21.3)	
Age, yr							
0 to <6	18	1 (5.6)	0.03	1 (5.6)	<0.0001	1 (5.6)	<0.04
6 to <16	48	2 (4.2)		5 (10.4)		2 (4.2)	
16 to <30	287	24 (8.4)		152 (53.0)		55 (19.2)	
30 to <40	118	16 (13.6)		36 (30.5)		21 (17.8)	
40 to <50	121	22 (18.2)		44 (36.4)		28 (23.1)	
50 to <60	9	1 (11.1)		5 (55.6)		3 (33.3)	
BCG vaccinated							
Yes	278	32 (11.5)	NS	187 (67.3)	<0.0001	81 (29.1)	< 0.0001
No	323	34 (10.5)		56 (17.3)		29 (9.0)	
Exposure time, h (hours)							
40 to <60	232	2 (0.9)	<0.0001	85 (36.6)	NS	22 (9.5.0)	<0.0001
60 to <100	124	28 (22.6)		51 (41.1)		31 (25.0)	
100 to <200	121	15 (12.4)		59 (48.7)		29 (24.0)	
200+	124	21 (16.9)		48 (38.7)		28 (22.6)	
Born outside Germany							
Yes	168	29 (17.3)	0.03	109 (64.9)	<0.0001	65 (38.7)	<0.0001
No	433	37 (8.5)		134 (30.9)		45 (10.4)	

Definition of abbreviations: BCG = bacillus Calmette-Guérin; NS = not significant; QFT = QuantiFERON-TB Gold In-Tube assay; TST = tuberculin skin test.

BCG vaccinated, compared with only 4 of the 59 (6.8%) who were negative by both tests. The equivalent BCG vaccination rates for those of German origin were 86.1% for those who were TST positive only and 29.5% for those who were negative by both tests.

QFT In-Tube Test

Sixty-six of the 601 contacts (11%) showed a positive QFT result. The cumulative exposure time among QFT-positive contacts was on average 92 hours longer than among those who were QFT negative (222.7 ± 237.4 vs. 130.7 ± 147.0 h, $P < 0.0001$). QFT-positive subjects were older than those who were QFT negative (32.1 ± 11.2 vs. 27.12 ± 12.0, $P = 0.02$). BCG vaccination was not associated with the probability of having a positive QFT result (32/278 vs. 34/323, $P = 0.7$; *see* Table 1).

Agreement between TST and QFT

Table 2 shows that overall agreement between TST and QFT was low ($\kappa = 0.28$; 95% CI, 0.22–0.33), with concordant results in only 416 (354 negative and 62 positive) of 601 contacts (69.2%). Broken down by TST result, this corresponded to a high concordance in 354 of 358 (98.9%) contacts with TST-negative results, but only 62 of 243 contacts (25.5%) with TST-positive results. Concordance between the two tests was poor in the 278 BCG-vaccinated subjects (123 [91 negatives and 32 positives]/278; 44.2%; $\kappa = 0.12$; 95% CI, 0.06–0.17), but was high (293 [263 negatives and 30 positives]/323; 90.7%; $\kappa = 0.62$; 95% CI, 0.51–0.72) in those who had not been vaccinated (Table 2).

Factors potentially associated with discordant results between QFT and the TST were examined. The average exposure time for contacts with concordant positive results for both tests was 231 hours, whereas it was 135 hours for those with concordant negative results. The mean exposure time for the 181 contacts with discordant, TST-positive responses was 122 hours, significantly less than for those with concordant positive test results. Similarly, those with a TST-positive/QFT-negative response profile were significantly more likely to be BCG vaccinated (156/183; 85.2%) than those with concordant positive (31/60; 51.7%) or negative (91/354; 25.7%) response profiles ($P < 0.0001$ and $P < 0.0001$, respectively). The rate of reported origin outside of Germany was significantly higher for those contacts positive to both tests (29/60; 48.3%) and also for those positive by the TST only (84/183; 45.9%), as compared with those with concordant negative responses (59/354; 16.7%; $P \leq 0.0001$ and $P < 0.0001$, respectively). There were only four contacts who were positive by QFT only, precluding any meaningful statistical analysis for this response profile. These four had an average exposure time of 91 hours, all were born in Germany, and none had been BCG vaccinated.

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

		QFT, n (%)		
	TST	Positive	Negative	
All subjects	Positive	62 (10.3)	181 (30.1)	Raw agreement = 69.2%
	Negative	4 (0.7)	354 (58.9)	$\kappa = 0.276$
BCG vaccinated	Positive	32 (11.5)	155 (55.8)	Raw agreement = 44.2%
	Negative	0 (0)	91 (32.7)	$\kappa = 0.119$
No BCG	Positive	30 (9.3)	26 (8.1)	Raw agreement = 90.7%
	Negative	4 (1.2)	263 (81.4)	$\kappa = 0.616$

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

Independent Predictors of Test Positivity

Multiple logistical regression analysis revealed significant association between positive TST results (5-mm cutoff) and both BCG vaccination and foreign origin (Table 3). Those who were BCG vaccinated were more than 12 times more likely (OR, 12.5; 95% CI, 8.1–19.3; $P < 0.0001$) to be TST positive, whereas those of foreign origin had a nearly sixfold increase in the likelihood of a positive TST (OR, 5.8; 95% CI, 3.6–9.5; $P < 0.0001$). After adjustment for confounding, neither exposure time of the contacts to their index case(s) nor age was associated with a positive TST. If a 10-mm TST cutoff had been used, BCG vaccination, increasing age, origin outside of Germany, and exposure time were all significantly associated with a positive result (Table 3).

Positive QFT test results were associated with foreign origin, increasing age, and exposure time as independent predictors (Table 3). The chance of having a positive QFT increased by 4.2% (OR, 1.042; 95% CI, 1.02–1.07) with each year of age. For each additional hour of exposure of contacts to their index case(s), the likelihood of having a positive QFT increased by 0.2% (OR, 1.002; 95% CI, 1.001–1.003).

Follow-up and Progression to Active TB

In anticipation of the new national recommendations coming into effect in early 2007 in Germany (19), INH chemopreventative treatment was offered to those who were QFT positive only. INH therapy was not offered to those who were only positive by the TST. Twenty-five of the contacts with a positive QFT (37.8%) started chemoprevention and were followed up every 6 months by their chosen pulmonologists. Rates of preventive treatment did not differ between foreign-born and German-born contacts (8 of 29 foreign-born and 17 of 37 German-born contacts, NS). At the time of writing, none of the treated persons has developed TB disease. The 41 QFT-positive contacts who refused INH chemoprevention did not differ from contacts taking INH with respect to age, sex, origin, or previous BCG vaccination.

Six of the 41 QFT-positive individuals (14.6%) who refused INH treatment had developed active TB by September 1, 2007. All six were strong responders in the QFT assay, with IFN-γ levels above the 10-IU/ml upper limit for the test's ELISA (Figure 2). The mean cumulative exposure time for the QFT-positive subjects who fell ill did not differ from that of the QFT-positive subjects who did not (245.3 ± 203.8 vs. 243.5 ± 238.6 h, NS).

For the TST, there were 219 contacts who had an induration greater than 5 mm, and who were not treated with INH, and 5 of these (2.3%) developed active TB (indurations of 12, 12, 14, 17, and 20 mm, respectively), a significantly lower rate than that found for QFT (5/219 vs. 6/41, $P < 0.003$). One of the subjects who progressed to disease was QFT positive, but TST negative, with a 0-mm induration. If a 10-mm cutoff had been used for the TST, 90 contacts would have been TST positive and not offered prophylaxis. The same five contacts would have been TST positive, equating to a progression rate of 5.6% (5/90), however, which was not significantly lower than that found for QFT (5/90 vs. 6/41, $P = 0.1$; Table 4).

Details of the six individuals who developed TB disease, all of whom were born in Germany, are given below.

Case 1. In July 2005, a 66-year-old Turkish patient was admitted to the hospital with interstitial pneumonia. In early August, the patient died of general respiratory failure, and, as confirmation of his AFB-positive sputum, a culture-confirmed diagnosis of TB was obtained after autopsy. Contact tracing was performed on 28 family members and health care workers who had had regular contact with the patient. His 24-year-old

TABLE 3. RESULTS OF MULTIPLE LOGISTICAL REGRESSION ANALYSIS

	Unadjusted OR (*95% CI*)	*P*	Adjusted OR (*95% CI*)	*P*
TST (5-mm cutoff)				
BCG vaccination	9.8 (6.7–14.4)	<0.0001	12.5 (8.1–19.3)	<0.0001
Age	1.005 (0.99–1.02)	0.45 (NS)	1.017 (1.00–1.03)	0.55 (NS)
Origin (foreign born/German born)	4.4 (3.0–6.4)	<0.0001	5.81 (3.6–9.1)	<0.0001
Cumulative exposure time	1.001 (0.99–1.002)	0.27 (NS)	1.001 (0.99–1.002)	0.33 (NS)
TST (10-mm cutoff)				
BCG vaccination	4.2 (2.6–6.6)	<0.0001	4.6 (2.8–7.6)	<0.0001
Age	1.029 (1.01–1.05)	0.001	1.044 (1.02–1.07)	<0.0001
Origin (foreign born/German born)	5.2 (3.4–8.0)	<0.0001	5.2 (3.2–8.4)	<0.0001
Cumulative exposure time	1.001 (1.00–1.003)	0.01	1.001 (1.00–1.003)	0.03
QFT				
BCG vaccination	1.11 (0.7–1.8)	0.7 (NS)	1.14 (0.7–1.9)	0.66 (NS)
Age	1.035 (1.013–1.058)	0.002	1.04 (1.016–1.064)	0.001
Origin (foreign born/German born)	2.3 (1.4–3.9)	0.002	2.28 (1.3–3.9)	0.004
Cumulative exposure time	1.002 (1.001–1.004)	<0.0001	1.002 (1.001–1.003)	<0.0001

Definition of abbreviations: BCG = bacillus Calmette-Guérin; CI = confidence interval; NS = not significant; OR = odds ratio; QFT = QuantiFERON-TB Gold In-Tube assay; TST = tuberculin skin test.

daughter, who had frequently visited her father, had a negative TST (0 mm), but a highly positive QFT result (>10 IU/ml), but did not accept INH treatment. In January 2007, the daughter developed AFB-positive, culture-confirmed pulmonary TB. RFLP fingerprinting revealed the same MTB strain as that carried by her father.

Case 2. In early November 2005, a 31-year-old Turkish housewife was admitted to the hospital with AFB- and culture-positive pulmonary TB. In December 2005, her 10-year-old, BCG-unvaccinated son had a 14-mm TST and a strongly positive QFT (>10 IU/ml), but chest X-ray examination at that time was unsuspicious. In July 2006, the follow-up chest X-ray revealed streaky infiltrates in the right upper pulmonary lobe and an enlargement of the left hilus that disappeared promptly during chemotherapy. Confirmation by culture was not obtained; no verification by fingerprinting was possible.

Cases 3, 4, and 5. In February 2006, a 31-year-old Turkish cleaner who had suffered from persistent coughing and night sweats since early January of that year fell ill with AFB-positive and culture-confirmed pulmonary TB. Contact investigation of his BCG-unvaccinated nephews (both of Turkish ethnicity), aged 15 and 22, revealed a TST of 20 and 12 mm, respectively, and QFT responses of greater than 10 IU/ml for both. Three months later, at their follow-up chest X-rays, both nephews presented infiltrations in the middle lobe that resolved with anti-TB chemotherapy. At the same time, a 2-year-old niece of the patient, who had also not been BCG vaccinated and who was of Turkish ethnicity, presented a TST induration of 12 mm and a QFT response of more than 10 IU/ml. A hilus enlargement on the left side of her lung developed within 3 months of the first examination and resolved with TB therapy. Culture confirmation of TB was not obtained.

Case 6. In early May 2006, an Angolan man was hospitalized with fever reaching 40°C and a productive cough that had been present for at least 20 days. He was diagnosed with culture-positive, INH-resistant TB. His 30-year-old, BCG-vaccinated girlfriend of German ethnicity had a strongly positive QFT (>10 IU/ml) and a TST result of 17 mm, but because the MTB strain was INH resistant and the woman of good health, no chemoprevention was started. In March 2007, the woman had a short episode of swelling of the glandula submandibularis and supplementary chest X-ray and computed tomography scan revealed pulmonary lesions consistent with TB. Sputum smear–negative, culture-positive pulmonary TB was confirmed and found to have the same INH resistance profile as that of the index case. The MTB strains were found to be identical by spoligotyping.

BCG Vaccination and Development of Disease

Only one of the six individuals who developed TB disease (17%) had a BCG scar, compared with 264 of 576 (45.8%) in the cohort at risk (after exclusion of the 25 persons who were started on INH chemoprevention). For QFT-positive subjects, 1 of the 17 (5.6%) who were BCG vaccinated developed TB disease, compared with 5 of the 23 (21.7%) nonvaccinated subjects. Among the TST-positive contacts (at >5 mm), 1 of the 172 (0.6%) BCG-vaccinated subjects developed TB disease, compared with 4 of 42 (8.7%) who were not vaccinated. Overall, only 1 (0.4%) of the 264 BCG-vaccinated contacts, compared with 5 (1.6%) of the 312 unvaccinated individuals, developed active

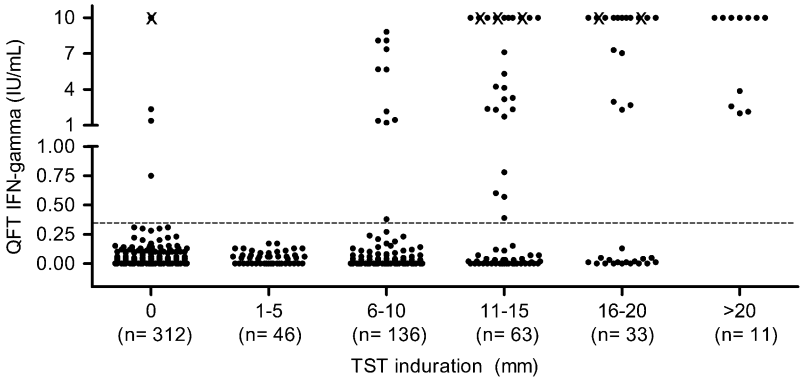

Figure 2. Comparison of the level of responses for QuantiFERON-TB Gold In-Tube assay (QFT) and the tuberculin skin test (TST). The six individuals who developed tuberculosis disease are marked by an X. Responses ⩾ 10 IU/ml for the QFT assay are shown as 10 IU/ml. The *dotted line* represents the cutoff for the QFT test.

TABLE 4. NUMBER OF CASES OF ACTIVE TUBERCULOSIS THAT DEVELOPED IN UNTREATED CONTACTS WITH RESPECT TO DIFFERENT TUBERCULIN SKIN TEST CUTOFFS

Induration Diameter (*mm*)	No. of Contacts		Progressed to Active TB	
	n	Untreated	n	%
0–5	358	357*	1	0.3
>5	243	219	5	2.3
>10	110	90	5	5.6
>15	46	38	2	5.3
Total	601	576		

Definition of abbreviation: TB = tuberculosis.

* One tuberculin skin test–negative but QuantiFERON-TB–positive contact person received preventive chemotherapy.

TB. The calculated relative risk reduction of 76.4%, however, was not statistically significant ($P = 0.15$).

DISCUSSION

The aim of LTBI screening in developed countries is to select high-risk subjects for preventive treatment or intensified surveillance programs. The epidemiologic rationale of treating LTBI—aiming to decrease the incidence of TB and subsequently diminish further MTB transmission—is clear, and the highly specific IGRA tests offer new possibilities for following that rationale. Confidence in treating persons at TB risk on the basis of IGRA testing would be greater, however, if the ability of the tests to identify those most likely to develop TB disease could be confirmed.

Limited studies (4, 20) have shown that QFT-positive individuals do develop active TB. So far, however, no study comparing traditional TST and the new IGRA tool, longitudinally and prospectively following a larger cohort through to clinical outcomes, has been completed. This contact study, which followed 601 immunocompetent contacts of infectious TB cases in routine contact tracing in a German metropolis, revealed a statistically significant higher predictive value for progression for QFT than the TST. The progression rate for those who were QFT positive was 14.6% (6/41), compared with 2.3% (5/219) for the TST. The TST failed to identify one of the six contacts who developed active TB. These data suggest comparable sensitivity for the two tests in their ability to detect those who will progress to active TB, and allow the possibility of higher sensitivity for QFT.

The stronger association of QFT with exposure time to index cases and the lower number of positive results as compared with the TST is consistent with previous studies (4–10). This suggests that the QFT test may more accurately identify those truly infected with MTB. A probable explanation for the larger number of TST positives and poorer association with exposure is cross-reactivity of the test with BCG vaccine, as evidenced by the finding that 187 of the 243 (77%) TST-positive subjects were BCG vaccinated (*see* Table 1). An association of positive TST results with BCG is supported by the finding that increasing the TST cutoff to 10 mm resulted in a significant association of a positive results with exposure time to the source, whereas the association with BCG vaccination decreased significantly by more than half (Table 3). Cross-reactivity to nontuberculous mycobacteria, as previously reported for the TST (21–24), could account for some or all of the positive TST responses of the 26 contacts who were neither BCG vaccinated nor QFT positive, especially because 18 of the 26 had a TST induration of less than 10 mm and all had a response of 15 mm or less. However, there remains the possibility that QFT is missing infection that is detected by the TST, a question that is in part addressed by the present study, and other studies involving long-term follow-up of TST-positive, QFT-negative contacts (9).

Only four contacts were QFT positive but TST negative, and one of the three of these who declined INH therapy progressed to active TB. The other five individuals who developed active TB were both QFT and TST positive. Although direct progression of those who were QFT positive is a definitive marker of predictive sensitivity, lack of progression of those who were QFT negative (but TST positive) provides further evidence of the sensitivity of QFT for detecting those likely to progress. None of the 181 contacts with QFT-negative/TST-positive responses progressed to active TB over the follow-up period. This is in agreement with the results of Higuchi and colleagues (9) who found that none of 91 TST-positive, QFT-negative contacts, not given prophylaxis, developed TB within a 3.5-year time frame.

The rate of progression of QFT-positive contacts to active TB was remarkably high (14.6%; 95% CI, 6.9 to 28.4%) for the first 2 years after infection with MTB, and compared with the rate found for those who were TST positive (2.3%; 95% CI, 1.0 to 5.2%) using a 5-mm cutoff. Although this difference in progression rates is highly significant ($P < 0.003$), a limitation of the present study is the comparatively small number of 41 QFT-positive persons not receiving INH and thus having a reasonable possibility of developing TB. Other potential limitations were that only six contacts progressed to active TB and that four of the six had their diagnosis made on clinical grounds, including knowledge of TST and QFT results, and without confirmation by mycobacterial culture. However, all four of these patients met standard criteria for TB disease, both pre- and post-therapy, with all showing clinical and radiologic resolution after anti-TB treatment, and thus incorrect diagnoses of TB are unlikely.

There are several implications for TB control of a test that has a 2-year predictive rate for active TB of 14.6%. By using QFT in place of the TST in our study, 66 contacts would have been identified for INH chemoprevention, instead of the 243 identified by the TST. This reduction would allow focus of scarce TB-control resources on those individuals most likely to progress to active TB, possibly improving compliance with LTBI treatment and thus diminishing transmission routes. By using QFT as the standard by which INH is prescribed, use of that drug, with its risks of hepatotoxicity and other side effects could be reduced by as much as 75%. By solely using QFT to screen for LTBI, testing costs would be reduced. Chest X-rays, medical consultations, INH-associated costs, such as liver function tests, and numerous other medical and managerial costs would be limited to a smaller group (25). However, the above potential benefits of QFT, and the findings of the present study, need to be validated in further prospective studies of large cohorts of QFT-positive close contacts.

Conclusions

In conclusion, the data presented here suggest that the MTB-specific whole blood IFN-γ test (QFT) was at least as sensitive as the TST in identifying those recent TB contacts who are truly infected and at risk of progressing to active TB. The QFT test was highly specific and unaffected by BCG vaccination status, a major cause of false-positive TST responses in this study. The high specificity of QFT and the high sensitivity of the test for those likely to progress, as suggested by our data, offer the possibility of limiting LTBI treatment to those who are truly infected. The high rate of progression to active TB of those who were QFT positive (14.6%) in our study, far greater than the 2.3% found for those who were TST positive (at >5 mm), sug-

gests significant health and economic implications for enhanced TB control, particularly if this higher progression rate is seen in studies of other at-risk populations.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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