Predicting the Development of Tuberculosis with the Tuberculin Skin Test and QuantiFERON Testing

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Abstract

Rationale: The identification of patients with latent tuberculosis infection, who are at higher risk to develop active disease, is an important component of disease control.

Objectives: We aim to compare the usefulness of the QuantiFERON-TB Gold in-tube assay and the tuberculin skin test to predict the development of active tuberculosis during follow-up, using positive and negative predictive values, positive likelihood ratios, and stratified level of risk.

Methods: The study included contacts of tuberculosis cases diagnosed between 2007 and 2009. All contacts included were from the first circle of exposure. Tuberculin skin test and QuantiFERON test were performed and a chest radiograph was obtained during the contact's study.

Measurements and Main Results: A total of 1,335 contacts were followed up for 4 years: a smear-positive index case was identified for 937 contacts, of whom 15 developed active tuberculosis and had initially presented with positive tuberculin skin test/QuantiFERON results, a normal chest radiograph, and no symptoms. The positive predictive value was 4% for QuantiFERON and 2% for the tuberculin skin test (when $\geqslant$5 mm). The probability of developing active disease was 2.36 times higher with a positive QuantiFERON, and 1.3 times higher with a positive tuberculin skin test. The positive predictive value was 17%, and the positive likelihood ratio was 7.53 for untreated contacts with a positive QuantiFERON. Stratifying according to initial QuantiFERON results showed a 6.36 times higher risk of developing active tuberculosis for patients with a QuantiFERON result greater than or equal to 10 IU/ml. Among bacillus Calmette-Guérin–vaccinated patients, a tuberculin skin test induration greater than or equal to 15 mm correlated better with a positive QuantiFERON.

Conclusions: QuantiFERON results were more accurate than tuberculin skin test results in predicting tuberculosis. Although all contacts with QuantiFERON-positive results are at risk of developing tuberculosis, those with a tuberculin skin test induration greater than or equal to 15 mm and QuantiFERON greater than or equal to 10 IU/ml are at highest risk. This has important implications in the clinical management of tuberculosis contacts.

Keywords: tuberculosis; IFN-γ release assays; tuberculosis development

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An individual with latent tuberculosis infection has an estimated 10% risk of developing active tuberculosis throughout his or her lifetime, and the risk is highest during the first 2 years after infection (1, 2). However, existing data are heterogeneous and influenced by individual variation, index case characteristics, disease incidence in the community, and the use of latent tuberculosis treatment (1, 2).

There is currently no gold standard test to diagnose latent tuberculosis. Until

recently, the tuberculin skin test was the only test available to detect latent tuberculosis. The tuberculin skin test is known to have low sensitivity, especially among immunosuppressed individuals, and low specificity, producing false positives to environmental mycobacteria and *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccine strains (1).

In vitro IFN-γ release assays, such as QuantiFERON TB-Gold *In tube* (Qiagen, Düsseldorf, Germany) and T-SPOT.TB (Oxford Immunotec Limited, Abingdon, UK), use antigens that are expressed mainly by *Mycobacterium tuberculosis* (3). Although it appears that they can identify latent tuberculosis with more accuracy than the tuberculin skin test, they do not distinguish between latent tuberculosis and active disease (4–6).

Individuals recently infected with tuberculosis are at the highest risk of developing active disease. Therefore, its detection and treatment will prevent new cases of active tuberculosis in the future (2, 7). The usefulness of IFN-γ release assays in predicting the development of active disease has been analyzed using positive and negative predictive values, with some of them suggesting superiority of the IFN-γ release assays over the tuberculin skin test (8–11). However, additional studies are needed to confirm these promising results, and to assess their influence on the future management of contact tracing studies (12).

The objective of the present study is to determine the usefulness of QuantiFERON testing compared with the tuberculin skin test in the diagnosis of latent tuberculosis during contact tracing studies, by calculating sensitivity, specificity, positive and negative predictive values, likelihood ratios, and risk stratification for both testing modalities, to predict the development of active tuberculosis during a 4-year follow-up.

Methods

Study Subjects

The study included contacts of tuberculosis cases diagnosed between 2007 and 2009 who were registered in the Vall d'Hebrón-Drassanes Tuberculosis Control Program and the Public Health Agency of Barcelona. All contacts included were from the first circle of exposure: individuals with daily exposure, or with a minimum exposure of 6 hours/week. Demographic and clinical variables of index cases and contacts were collected. Contacts were excluded if a previous positive tuberculin skin test was documented, preventive tuberculosis chemotherapy was prescribed, or active tuberculosis had been diagnosed. Informed consent was obtained from each study subject. The study was approved by the Ethics Committee of the Instituto de Investigación en Atención Primaria Jordi Gol (Barcelona, Spain).

Contact tracing studies were performed according to Spanish guidelines (13). Initial tuberculin and QuantiFERON tests were performed and a chest radiograph was obtained during the contact's first visit. If the chest radiograph was abnormal, further studies were obtained to rule out active disease.

In children under 5 years of age, in whom tuberculosis infection was ruled out, a primary prophylactic treatment was prescribed until the second phase of the contact tracing study. The second phase of the contact tracing studies was performed 8–12 weeks after the initial visit for contacts with an initial negative QuantiFERON result, independently of the tuberculin skin test result. A second QuantiFERON and/or tuberculin skin test (when the initial tuberculin skin test was negative), a chest radiograph, and other further studies were performed, if necessary. A "coprevalent" or secondary tuberculosis case was defined as an active tuberculosis case that was diagnosed during the contact tracing study within 90 days of the index case diagnosis. Latent tuberculosis treatment (or preventive chemotherapy) was initiated for contacts with a positive tuberculin skin test and/or positive QuantiFERON result (13).

All contacts were followed up for 4 years, and visited immediately if there was clinical suspicion of active disease. Contacts undergoing preventive chemotherapy made monthly visits during treatment and annual visits after treatment. Contacts who did not require preventive chemotherapy were visited at 6, 12, 24, and 48 months. Study follow-up was performed using clinical records, telephone calls, contact with primary care physicians, and reviewing the Tuberculosis Control Program database.

Tuberculin Skin Testing

Tests were performed using 2 tuberculin units of PPD RT23 (Statens Serum Institut, Copenhagen, Denmark). The result was read within 48–72 hours by trained staff. All tuberculin skin test indurations greater than or equal to 5 mm were classified as positive regardless of BCG vaccination status (13). The results were analyzed using the following tuberculin skin test induration cutoffs: greater than or equal to 5, 10, and 15 mm, indicating that all the contacts over this cutoff had been analyzed.

IFN-γ Release Assay

QuantiFERON was performed and interpreted according to the manufacturer's instructions.

Statistical Analysis

Qualitative variables were analyzed by absolute number and percentage, and quantitative variables were analyzed by mean and standard deviation. Qualitative variables were compared using the chi-square test and Fisher's exact test. The odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to determine associated risk; the variables with a *P* value less than 0.05 were analyzed at a multivariate level by logistic regression. Qualitative variables were compared by category, using nonparametric tests (Mann–Whitney, Kolmogorov–Smirnov, Kruskal–Wallis). Cohen's kappa coefficient (κ) was used to analyze concordance, *P* value, and standard error, according to Landis and Koch estimation. The risk of developing tuberculosis was analyzed and calculated as a rate per 1,000 person-years according to tuberculin skin test and QuantiFERON results. Positive and negative predictive value, sensitivity, specificity, likelihood ratio, and posttest positive probability of developing active tuberculosis were also calculated. Data were analyzed with Epi Info 7.1.2 (www.cdc.gov/epiinfo/7).

Results

Diagnosing Latent Tuberculosis Infection by Tuberculin Skin Test and QuantiFERON

A total of 1,335 contacts from 103 tuberculosis index cases were included in the study. Of those, 1,047 (78.4%) were contacts of smear- and culture-positive index cases, 229 (17.2%) of smear-negative and culture-positive index cases, and 59 (4.3%) of both smear- and culture-negative index cases. Diagnosis of cases without bacteriological confirmation was based on clinical evaluation,

radiological study (chest radiograph and/or thoracic computed tomography scan), and response to antituberculous chemotherapy. Characteristics of the study population can be found in Table 1. BCG vaccination was present in 97.3% of the immigrants and 25.8% of the Spanish autochthonous contacts. In the study, 91 (6.8%) cases were contacts of patients with a drug-resistant tuberculosis strain (but not multidrug-resistant tuberculosis), and 46 (3.5%) were contacts for multidrug-resistant patients with tuberculosis. Therefore, 137 contacts were contacts of index cases with resistance (10.26%). Six subjects were coinfected with HIV, and managed in their reference hospital.

Figure 1 shows the results obtained in the first and second rounds of tuberculin skin testing and QuantiFERON testing. Almost 60% (797 individuals) of the study subjects had an initial positive tuberculin skin test, and only 34% (453 individuals) had an initial positive QuantiFERON result. Among the 538 subjects with an initial negative tuberculin skin test result, a second tuberculin skin test was performed on 515 subjects, with 214 of them being positive. Among the 882 patients with an initial negative QuantiFERON result, a second QuantiFERON test was performed on 827 subjects, with 115 of them being positive. Interestingly, of the 346 patients with an initial positive tuberculin skin test and negative QuantiFERON result, a second QuantiFERON test was performed on 289, with only 30 of them being positive.

Of the 1,011 subjects with a positive tuberculin skin test result, 64.5% were immigrants and 35.5% were autochthonous ($P < 0.001$). On the other hand, of the 558 subjects with a positive QuantiFERON result, 43% were immigrants and 41.9% were autochthonous ($P = 0.37$).

Table 1. Characteristics of contacts included in study

Variable	n (%)
Total no. of contacts studied	1,335 (100)
Age group, yr	
0–4	82 (6.1)
5–14	374 (28.2)
15–35	535 (40.1)
⩾35	344 (25.8)
Sex	
Female	660 (49.4)
Male	675 (50.6)
Immigrant	
Yes	772 (57.8)
No	563 (42.2)
Presence of BCG vaccination	
Yes	896 (67.1)
No	439 (32.9)
Smoking exposure	
Yes	404 (45.9)
No	476 (54.1)
Smoker	
Yes	374 (40.8)
No	542 (59.2)
Alcohol consumption (>40 g/d)	
Yes	311 (34.1)
No	602 (65.9)
Cohabitation with index case	
Yes	448 (33.6)
No	887 (66.4)
Type of contact	
Family	676 (50.7)
Recreational/friendship	78 (5.8)
School	204 (15.3)
Work	377 (28.3)
Degree of contact	
Daily for >6 h	391 (29.3)
Daily for <6 h	392 (29.4)
Nondaily (>6 h/wk)	552 (41.3)
Duration of exposure	
<30 d	598 (44.8)
⩾30 d	737 (55.2)
Index case status	
Negative smear and negative culture	58 (4.3)
Negative smear and positive culture	230 (17.2)
Positive smear and positive culture	1,047 (78.4)
Diagnostic delay for index case	
<50 d	179 (13.4)
⩾50 d	1,156 (86.6)

Definition of abbreviation: BCG = bacillus Calmette-Guérin.

Factors Associated with Positive Tuberculin Skin Test and QuantiFERON

Results can be found in Table 2. BCG vaccination is predictive of a tuberculin skin test induration greater than or equal to 5 and 10 mm. Contacts of a smear-positive index case and exposure for more than 30 days were associated with positive tuberculin skin test and QuantiFERON results. In the univariate analysis, BCG vaccination was a risk factor at all three tuberculin skin test cutoffs considered, but not for QuantiFERON (relative risk, 0.98; 95% confidence interval, 0.86–1.12; nonsignificant P value), and thus BCG vaccination was not included in the multivariate analysis. A lower proportion of positive tuberculin skin test and positive QuantiFERON results was found among contacts under 15 years of age.

Table 3 shows the impact of tuberculosis exposure (estimated as the median of hours exposed), and the microbiologically active tuberculosis diagnosis characteristics of the index case, in the risk of latent tuberculosis infection. A progressive linear trend of latent tuberculosis diagnosis and the two analyzed variables can be observed: the highest latent tuberculosis rates correspond to contacts with a daily contact of more than 6 hours with a patient with active tuberculosis diagnosed by positive smear and positive culture.

A correlation was also found between the amount of IFN-γ released in the initial QuantiFERON test and the tuberculin skin test induration result. When the tuberculin skin test induration was between 0 and 4 mm the median IFN-γ released was 0.01 IU/ml (range, 0–0.03); and when the tuberculin skin test induration was greater than or equal to

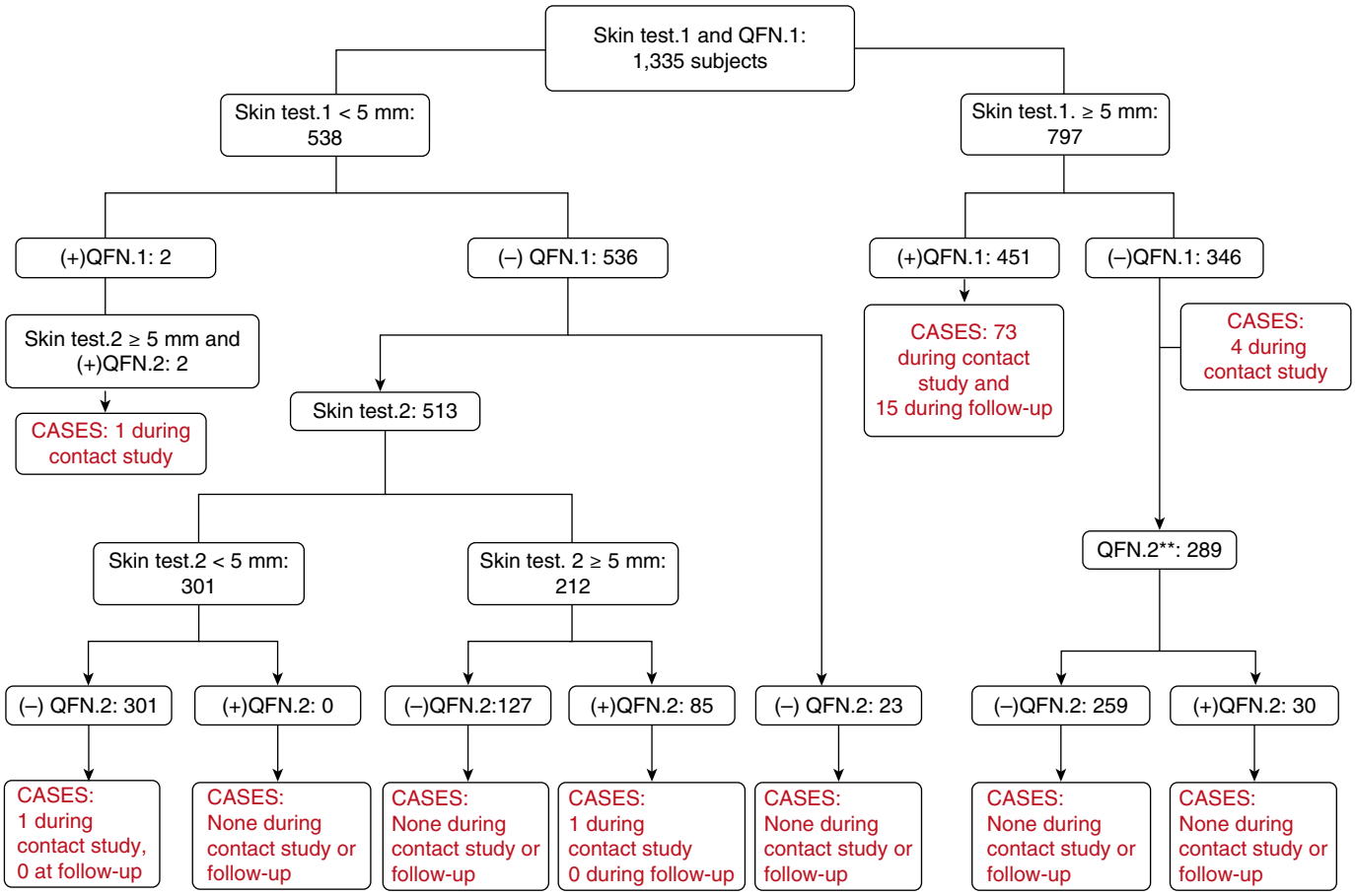

Figure 1. Results obtained in the initial and second rounds of testing in the contact tracing studies. Initial tuberculin skin test and QuantiFERON (QFN) tests were performed at the beginning of contact tracing. The second tuberculin skin test and QFN tests were performed 8–12 weeks after the first visit. + = positive; – = negative; Skin test.1 and QFN.1 = tests performed at the beginning of contact tracing; Skin test.2 and QFN.2 = tests performed 8–12 weeks after the first visit.

5 mm, the median IFN-γ released was 0.33 IU/ml (range, 0.01–4.38). On the other hand, in patients diagnosed with active tuberculosis during contact tracing studies, the median was 4.06 IU/ml (range, 1.24–13.41), and the median obtained during the contact tracing studies for the individuals that progress to active tuberculosis during follow-up was 3.09 IU/ml (range, 1.19–13.25). The difference in IFN-γ released in patients diagnosed with active tuberculosis during contact tracing studies and during the follow-up was not significant.

Secondary (Coprevalent) Cases Diagnosed during Contact Tracing Studies

Eighty secondary cases of active tuberculosis (6%) were diagnosed during contact tracing studies (Figure 1 and Table 3). Seventy-four secondary cases (92.5%) were contacts of a smear-positive index case. In our population, 6.9% of daily contacts were detected as secondary tuberculosis cases, and 4.7% of nondaily contacts. Among contacts of patients with positive smear and culture results, those with a daily exposure of more than 6 hours had a statistically significant higher risk of developing active tuberculosis than those with a daily exposure of less than 6 hours (OR, 3.17; 95% CI, 1.79–5.65; $P < 0.001$) or those with a nondaily exposure of more than 6 hours per week (OR, 3.88; 95% CI, 1.92–8.01; $P < 0.001$).

Primary Preventive Chemotherapy Treatment and Latent Tuberculosis Treatment

Primary preventive chemotherapy consisting of isoniazid for 3 months was prescribed to 166 children, none of whom had a converted tuberculin skin test or QuantiFERON, or developed active tuberculosis during contact tracing studies, except one. This patient refused the preventive chemotherapy, converted according to both tuberculin skin test and QuantiFERON, and then progressed to active tuberculosis in the follow-up.

The recommended latent tuberculosis preventive chemotherapy was 3 months of isoniazid and rifampicin (93.5%). Eighty cases were contacts of drug-resistant tuberculosis index cases: 34 of isoniazid-resistant tuberculosis, and 46 of multidrug-resistant tuberculosis. Twenty-eight cases had positive QuantiFERON results, and preventive chemotherapy was prescribed. The preventive therapy was decided, where possible, according to their pattern of drug resistance; however, in some cases this was prescribed before knowing the drug resistance pattern of the tuberculosis index case. No cases progressed to active tuberculosis during follow-up.

Table 2. Predictive factors by adjusted odds ratio for latent tuberculosis infection according to QuantiFERON results and tuberculin skin test results*

Variable	TST ⩾ 5 mm aOR (95% CI); *P* Value	TST ⩾ 10 mm aOR (95% CI); *P* Value	TST ⩾ 15 mm aOR (95% CI); *P* Value	QFN Positive aOR (95% CI); *P* Value
BCG vaccination, yes/no	5.10 (3.72–7.00); <0.0001	2.38 (1.83–3.09); <0.0001	1.32 (0.99–1.77); NS	—
Male, yes/no	1.27 (0.95–1.69); NS	1.43 (1.12–1.81); <0.005	1.42 (1.10–1.82); < 0.01	1.74 (1.36–2.22); <0.0001
Age < 15 yr, yes/no	0.41 (0.30–0.55); <0.0001	0.72 (0.55–0.93); <0.05	0.68 (0.51–0.91); <0.05	0.70 (0.53–0.92); <0.01
Social class 4–6, yes/no	1.01 (0.73–1.39); NS	1.29 (1.00–1.67); <0.05	1.34 (1.03–1.74); <0.05	1.47 (1.13–1.90); <0.005
Cohabitation, yes/no	0.48 (0.33–0.70); <0.005	0.89 (0.66–1.19); NS	1.66 (1.24–2.22); <0.001	1.49 (1.12–1.99); <0.001
Smear-positive index case, yes/no	3.02 (2.16–4.22); <0.0001	2.37 (1.78–3.16); <0.0001	2.21 (1.58–3.08); <0.0001	3.83 (2.76–5.31); <0.0001
Exposure > 30 d, yes/no	3.94 (2.79–5.57); <0.0001	2.70 (2.07–3.51); <0.0001	2.65 (1.99–3.52); <0.0001	3.95 (3.02–5.18); <0.0001

Definition of abbreviations: aOR = adjusted odds ratio; BCG = bacillus Calmette-Guérin; CI = confidence interval; NS = nonsignificant; QFN = QuantiFERON; TST = tuberculin skin test.
*Variables that were significant in the univariate analysis were included in the multivariate analysis. The National Classification of Occupations Groups 7–9 of professional qualifications from Group III was used to define social class (www.ine.es/daco/daco42/clasificaciones/cno11_estructura.xls).

Tuberculosis Incidence during Follow-Up among Contacts of Smear-Positive Index Cases

There were no relapsed coprevalent cases during the 4 years of follow-up. Excluding coprevalent cases and patients lost to follow-up (39 cases), a total of 937 contacts with a smear-positive index case were followed up for 4 years, with 15 cases of developed active tuberculosis. These subjects initially presented with a tuberculin skin test induration greater than or equal to 5 mm and positive QuantiFERON, were asymptomatic, and had normal chest radiographs. Ten subjects developed tuberculosis during the first year of follow-up, and 5 subjects developed TB between 12 and 24 months after the contact tracing study. Seven of these subjects were under 15 years of age, six were smear negative but culture positive, and two had positive pleural adenosine deaminase levels.

Subjects with initial negative QuantiFERON and negative tuberculin skin test results did not develop active tuberculosis in the follow-up. The positive predictive value was 4% among subjects with a positive QuantiFERON, and 2% among subjects with a positive tuberculin skin test. The positive likelihood ratio indicates that contacts with a positive QuantiFERON, and tuberculin skin test induration greater than or equal to 5, 10, and 15 mm were 2.36, 1.3, 1.55, and 2.64 times more likely to develop active tuberculosis, respectively. Table 4 shows the comparison between QuantiFERON and tuberculin skin test results in predicting the development of active tuberculosis in patients who did not undergo treatment.

Fourteen of the 15 cases that developed active tuberculosis did not undergo latent tuberculosis treatment (refused or not recommended). The remaining case was a child who underwent latent tuberculosis treatment with isoniazid for 6 months, but developed tuberculosis in the fourth month. In this last case, the index case was susceptible to all antituberculous drugs. The child received only isoniazid, instead of 3 months of isoniazid plus rifampicin, as decided by the pediatrician. The initial clinical and radiological study ruled out active tuberculosis from the outset.

The difference between the proportion of cases that developed active tuberculosis without (14 of 81) and with (1 of 412) latent tuberculosis treatment was significant ($P < 0.001$). The positive predictive value was 17% for a positive QuantiFERON and 4, 6, and 10% for a tuberculin skin test induration greater than or equal to 5, 10, and 15 mm, respectively. The positive likelihood ratio was much higher for a positive QuantiFERON than for a positive tuberculin skin test: the probability of developing active tuberculosis was 7.53 times higher with a positive QuantiFERON, compared with 1.35, 1.86, and 3.59 times for a tuberculin skin test induration greater than or equal to 5, 10, and 15 mm, respectively.

Table 5 presents tuberculin skin test and QuantiFERON results in the BCG-vaccinated and unvaccinated contacts. Twelve subjects developed active tuberculosis among the 607 subjects with BCG vaccination, and 3 subjects developed tuberculosis among the 330 contacts without BCG vaccination. The difference was not statistically significant ($P = 0.33$). The positive likelihood ratio was similar by QuantiFERON result among both groups: subjects with a positive QuantiFERON and with and without a history of BCG vaccination had a 2.37 and 2.35 increased probability of developing tuberculosis, respectively. The positive predictive value and likelihood ratio calculations indicate that a tuberculin skin test induration greater than or equal to 15 mm represented a similar risk to that of a positive QuantiFERON test.

The risk of developing tuberculosis according to the initial IFN-γ concentration and tuberculin skin test induration is shown in Table 6. All subjects with a positive QuantiFERON (independent of the amount of IFN-γ released) and tuberculin skin test induration greater than or equal to 5 mm were at risk of developing active disease. However, the positive predictive value doubled for contacts who presented with an amount of IFN-γ released greater than or equal to 10 IU/ml, and the posttest probability showed a 6.66 times higher risk of developing tuberculosis compared with the pretest probability. Positive predictive value and positive likelihood ratio calculations by stratified IFN-γ concentration were higher than those stratified by tuberculin skin test diameter. The positive predictive value was more similar between a tuberculin skin test induration greater than or equal to 15 mm and a positive QuantiFERON result.

Discussion

Previous studies have demonstrated a positive association between tuberculin skin test reactivity and the development of

Table 3. Impact of contact degree of exposure and microbiological diagnosis index case characteristics on prevalence of latent infection diagnosed by QuantiFERON test and in tuberculosis cases developed during contact study and at follow-up

Contact Degree of Exposure	No. of Cases (%)	Median (25–75%) Number of Exposure Hours	Positive QFN according to Degree of Exposure		Microbiological Diagnosis Index Case Characteristics (n)	Positive QFN according to Degree of Exposure and Microbiological Index Case Diagnosis		TB Cases during Follow-Up: n (%)
			n (%)	Odds Ratio (95% CI); *P* Value		n (%)	Linear Trend *P* Value	
Daily, ⩾6 h	391 (29.3)	448 (385–692)	216 (55.2)	2.4 (1.8–3.2); <0.0001	S pos, C pos (289) S neg, C pos (75) S neg, C neg (27)	19 (66.1) 24 (32.0) 1 (3.7)	<0.001	14 (4.8) 0 (0) 0 (0)
Daily, <6 h	392 (29.4)	106 (87–125)	165 (42.1)	1.4 (1.1–1.9); <0.05	S pos, C pos (302) S neg, C pos (73) S neg, C neg (17)	142 (47.0) 20 (27.4) 3 (17.6)	<0.001	0 (0) 1 (0.25) 0 (0)
Nondaily, ⩾6 h/wk	552 (41.3)	60 (52–82)	187 (33.9)	1	S pos, C pos (456) S neg, C pos (81) S neg, C neg (15)	167 (36.6) 20 (24.7) 0 (0)	<0.001	0 (0) 0 (0) 0 (0)

Definition of abbreviations: C = culture; CI = confidence interval; neg = negative; pos = positive; QFN = QuantiFERON; S = smear; TB = tuberculosis.

active tuberculosis, such that the risk of developing tuberculosis is higher with increasing diameter of the induration (14). The present study supports these findings for the tuberculin skin test, and also correlates them with QuantiFERON results. It was observed that a positive QuantiFERON is also associated with increased risk of developing active disease, particularly during the first 2 years after infection, and is higher among contacts with an elevated initial amount of IFN-γ concentration released. Conversely, it was also found that none of the subjects with a negative QuantiFERON and negative tuberculin skin test result developed active tuberculosis during follow-up. Factors were identified that are significantly associated with a positive tuberculin skin test or positive QuantiFERON result, and it was also found in the present study that BCG vaccination does not protect against tuberculosis development. In fact, according to the QuantiFERON results, the incidence of tuberculosis development among BCG-vaccinated subjects is higher, with no difference in positive likelihood ratio from nonvaccinated subjects.

This study also shows that QuantiFERON testing has a greater capacity than tuberculin skin test in predicting the development of tuberculosis, as has also been reported in systematic reviews and meta-analyses (9, 15–17). One meta-analysis published an estimated positive predictive value of 6.8% among the subgroups at highest risk, and 8.5% among subjects who were not treated (9). In the present study, it was shown that the positive predictive value for those contacts not treated was 17%. Similar results have been published by other authors (8, 11, 15, 16, 18–20), although the number of untreated subjects in the study by Diel and colleagues (18, 21) was significantly higher than in the present study. Rangaka and colleagues (17) also evaluated the positive predictive value of QuantiFERON testing with an end point of incidence in person-years, but only analyzed the diagnosis of active tuberculosis without taking treatment into account. Their results showed an incidence rate of 4–48 cases per 1,000 person-years of follow-up.

Table 4. Follow-up of contacts of smear-positive index cases over 4 years: accumulated incidence and incidence rate of subjects who developed tuberculosis during follow-up of patients who did not undergo treatment*

Test Results	No. of Contacts	No. of Cases	AI	IR	PPV (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Total	453	14	3.09	7.72	—	—	—	—	—	—
Pos QFN	81	14	17.28	43.21	17 (9–26)	100 (100–100)	100 (100–100)	85 (81–88)	7.53 (5.91–9.58)	0 (0–0)
Neg QFN	372	0	0	0						
TST ⩾ 5 mm	340	14	4.12	10.29	4 (2–6)	100 (100–100)	100 (100–100)	26 (22–30)	1.35 (1.27–1.42)	0 (0–0)
TST < 5 mm	113	0	0	0						
TST ⩾ 10 mm	233	13	5.58	13.95	6 (3–9)	100 (99–100)	93 (79–100)	50 (45–55)	1.86 (1.56–2.21)	0.14 (0.02–0.95)
TST < 10 mm	220	1	0.45	1.14						
TST ⩾ 15 mm	107	11	10.3	25.70	10 (5–16)	99 (98–100)	79 (57–100)	78 (74–82)	3.59 (2.59–4.98)	0.27 (0.1–0.75)
TST < 15 mm	346	3	0.86	2.17						

Definition of abbreviations: AI = accumulated incidence (%); CI = confidence interval; IR = tuberculosis incidence rate during follow-up (cases per 1,000 person-years); LR = likelihood ratio; neg = negative; NPV = negative predictive value; pos = positive; PPV = positive predictive value; QFN = QuantiFERON; TB = tuberculosis; TST = tuberculin skin test.
Note: The 80 subjects diagnosed with active tuberculosis during the contact tracing studies have been excluded.
Note: One child developed active tuberculosis during the fourth month of 6 months of isoniazid treatment for latent TB infection and therefore was considered a treatment failure.
*Quality assessment of TST and QFN results in the diagnosis of tuberculosis.

Table 5. Evaluation of tuberculin skin test and QuantiFERON results in predicting the development of tuberculosis during follow-up of contacts of smear-positive index cases stratified by history of bacillus Calmette-Guérin vaccination

		n	No. of TB Cases	AI	IR	PPV (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
History of BCG vaccination	Total	607	12	1.98	8.72						
	Pos QFN	263	12	4.56	11.40	5 (2–7)	100 (100–100)	100 (100–100)	58 (54–62)	2.37 (2.16–2.6)	0 (0–0)
	Neg QFN	344	0	0	0						
	TST ⩾ 5 mm	538	12	2.23	5.6	2 (1–3)	100 (100–100)	100 (100–100)	12 (9–14)	1.13 (1.1–1.16)	0 (0–0)
	TST < 5 mm	69	0	0	0						
	TST ⩾ 10 mm	423	11	2.6	6.5	3 (1–4)	99 (98–101)	92 (76–100)	26 (22–29)	1.24 (1.03–1.47)	0.32 (0.05–2.12)
	TST < 10 mm	144	1	0.7	1.74						
	TST ⩾ 15 mm	199	9	4.52	11.31	5 (2–7)	99 (98–100)	75 (51–100)	68 (64–72)	2.35 (1.66–3.32)	0.37 (0.14–0.98)
	TST < 15 mm	408	3	0.73	1.83						
No history of BCG vaccination	Total	330	3	0.9	2.27						
	Pos QFN	143	3	0.02	5.24	2 (0–4)	100 (100–100)	100 (100–100)	57 (52–63)	2.34 (2.06–2.65)	0 (0–0)
	Neg QFN	187	0	0	0						
	TST ⩾ 5 mm	187	3	1.6	4.01	2 (0–3)	100 (100–100)	100 (100–100)	44 (38–49)	1.78 (1.62–1.96)	0 (0–0)
	TST < 5 mm	143	0	0	0						
	TST ⩾ 10 mm	147	3	2.04	5.10	2 (0–4)	100 (100–100)	100 (100–100)	56 (51–61)	2.27 (2.02–2.57)	0 (0–0)
	TST < 10 mm	183	0	0	0						
	TST ⩾ 15 mm	68	2	2.94	7.35	3 (0–7)	100 (99–100)	67 (13–100)	80 (75–84)	3.3 (1.44–7.56)	0.42 (0.08–2.07)
	TST < 15 mm	262	1	0.4	0.95						

Definition of abbreviations: AI = accumulated incidence (%); BCG = bacillus Calmette-Guérin; CI = confidence interval; IR = tuberculosis incidence rate during follow-up (cases per 1,000 person-years); LR = likelihood ratio; neg = negative; NPV = negative predictive value; pos = positive; PPV = positive predictive value; QFN = QuantiFERON; TB = tuberculosis; TST = tuberculin skin test.
Note: The 80 cases diagnosed with active tuberculosis during the contact tracing studies have been excluded.

Predictive value depends on disease prevalence in the analyzed population, and thus the positive predictive value is higher in low-resource countries compared with high-resource countries (16, 22, 23). For this reason, in addition to the predictive value results, the likelihood ratio was analyzed, because the sensitivity and specificity of a test are independent of disease prevalence (24). It was found that a positive QuantiFERON result is associated with a 4- to 6.5-fold increase in the probability of developing tuberculosis compared with a negative test result. In addition, it was also found that the highest latent tuberculosis infection rates correspond to contacts with high exposure (greater than 6 h/d) to a smear-positive patient with active tuberculosis.

A study performed in Ethiopia (25) showed a strong association between an *in vitro* ESAT-6 (6-kD early secretory antigenic target) response and the later development of tuberculosis among previously healthy contacts. Andersen and colleagues (3) proposed that IFN-γ release assays could be used to identify contacts at risk of developing active disease. A high IFN-γ concentration should be a biomarker for future development of active tuberculosis, because it represents bacterial replication and antigenic load. In the present study, it was observed that a high positive QuantiFERON result is predictive of developing tuberculosis during follow-up. It was also observed that the probability of developing tuberculosis is increased 5- to 6.7-fold depending on the amount of IFN-γ released in the initial QuantiFERON result. These results correspond with those from del Corral and colleagues (26) and from Diel and colleagues (18), who found that 6

Table 6. Tuberculosis incidence among contacts of smear-positive index cases monitored for 4 years, stratified by baseline tuberculin skin test induration and IFN-γ concentration

Test and Range of Results	n	No. of TB Cases	AI	IR	Sensitivity	Specificity	PPV (95% CI)	Positive LR (95% CI)	Pretest Probability (%)	Positive Posttest Probability (%)
IFN-γ concentration, IU/ml										
0–0.34	531	0	0	0	—	100	Reference	Reference	—	—
0.35–5	135	4	3.0	7.41	100	80	3 (0–6)	4.95 (4.33–5.89)	0.6	3
5.0–10	166	5	3.01	7.53	100	77	3 (0–6)	4.3 (3.75–4.92)	0.7	3
⩾10	105	6	5.7	14.3	100	84	6 (1–10)	6.36 (5.3–7.63)	0.9	6
TST induration, mm										
0–4	212	0	0	0	—	100	Reference	Reference	—	—
5–9	155	1	0.64	1.61	100	58	1 (0–2)	2.38 (2.11–2.68)	0.27	1
10–14	303	3	0.99	2.5	100	41	1 (0–2)	1.71 (1.59–1.84)	0.6	1
⩾15	267	11	4.3	10.74	100	45	4 (2–7)	1.83 (1.68–1.99)	2.3	7

Definition of abbreviations: AI = accumulated incidence (%); CI = confidence interval; IR = tuberculosis incidence rate during follow-up (cases per 1,000 person-years); LR = likelihood ratio; PPV = positive predictive value; TB = tuberculosis; TST = tuberculin skin test.

of 41 patients (14.6%) with an initial IFN-γ concentration greater than or equal to 10 IU/ml and who refused latent tuberculosis preventive chemotherapy developed tuberculosis disease during follow-up.

Among the 406 subjects with a positive QuantiFERON result, 15 developed tuberculosis during follow-up. Fourteen subjects were not provided with latent tuberculosis preventive chemotherapy (refused or not recommended) and 1 subject developed tuberculosis despite adequate compliance with treatment. If preventive chemotherapy had not been administered to the 406 subjects with a positive QuantiFERON result, 70 secondary cases of tuberculosis would have been expected. Thus, the administration of treatment reduced the number of tuberculosis cases by 98.6%.

The high tuberculosis incidence in the present study can be attributed, partly, to the average diagnostic delay of 45 days for smear-positive cases in the region studied (27). The diagnostic delay during contact tracing studies in the present study was more than 60 days for 40% of the contacts, which represented 51 of the subjects (63.5%) who developed tuberculosis during contact tracing studies, and 12 subjects (80%) who developed tuberculosis during follow-up.

Among the BCG-vaccinated subjects in the present study, the positive and negative predictive values and positive likelihood ratio for a tuberculin skin test induration greater than or equal to 15 mm were similar to those of subjects with a positive QuantiFERON result. Three tuberculosis cases were diagnosed during follow-up among 330 contacts with no history of BCG vaccination, and 12 TB cases were diagnosed among the 607 BCG-vaccinated contacts. Contrary to the results of the present study, the pTB-NET study (28), which included 1,093 European children, found that BCG vaccination had a protective effect against tuberculosis infection. On the other hand, the data presented here highlight the high risk of progression to active tuberculosis for children (7 cases from 299 children; and 6 cases from 72 children without preventive therapy), as compared with adults (8 cases from 638 adults; and 8 cases from 381 adults without preventive therapy), and strengthen the need for preventive therapy in children contacts.

One strength of the present study is that it was performed on first-ring contacts with 4 years of follow-up independent of treatment administration and, thus, the methodology is free of a convenience sampling bias. According to American Thoracic Society recommendations (1) and Spanish guidelines (13), contacts with a tuberculin skin test induration greater than or equal to 5 mm were considered to be infected, regardless of a history of BCG vaccination (13). This differs from the European Consensus recommendations (29), which state that a cutoff greater than or equal to 15 mm should be used among BCG-vaccinated individuals over 12 months of age when IFN-γ release assays are not available, and greater than or equal to 10 mm for infants under 12 months of age. In the present study, nine coprevalent tuberculosis cases with a tuberculin skin test induration diameter less than 15 mm among the BCG-vaccinated contacts could have been missed if QuantiFERON testing had not been available.

In conclusion, QuantiFERON was a more specific test than tuberculin skin testing because the results were not affected by BCG vaccination. The number of conversions 8–12 weeks after the first visit was significantly lower with QuantiFERON testing. Thus, its use reduces the diagnosis of latent tuberculosis and unnecessary treatments. Both tests, tuberculin and QuantiFERON, have an excellent negative predictive value. No patient with negative test results developed active tuberculosis during the follow-up. The positive predictive value and the likelihood ratio were higher for the QuantiFERON test in comparison with the tuberculin skin test. The results of the present study demonstrate that contacts with an initial positive tuberculin skin test and/or positive QuantiFERON result have a risk of developing active disease, and that this risk increases when the tuberculin skin test induration and the amount of IFN-γ are higher, especially when the tuberculin skin test induration is greater than or equal to 15 mm and the QuantiFERON result is greater than or equal to 10 IU/ml. These data may have an important impact on clinical practice in developed countries where latent tuberculosis preventive therapy is used in control programs. ■

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References

1 American Thoracic Society (ATS); Centers for Disease Control and Prevention (CDC). Targeted tuberculin testing and treatment of latent tuberculosis infection. *Am J Respir Crit Care Med* 2000;161: S221–S247.

2 Leung CC, Rieder HL, Lange C, Yew WW. Treatment of latent infection with *Mycobacterium tuberculosis*: update 2010. *Eur Respir J* 2011; 37:690–711.

3 Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* 2000;356:1099–1104.

4 Diel R, Goletti D, Ferrara G, Bothamley G, Cirillo D, Kampmann B, Lange C, Losi M, Markova R, Migliori GB, *et al*. Interferon-γ release assays for the diagnosis of latent *Mycobacterium tuberculosis* infection: a systematic review and meta-analysis. *Eur Respir J* 2011; 37:88–99.

5 Latorre I, De Souza-Galvão M, Ruiz-Manzano J, Lacoma A, Prat C, Fuenzalida L, Altet N, Ausina V, Domínguez J. Quantitative evaluation of T-cell response after specific antigen stimulation in active and latent tuberculosis infection in adults and children. *Diagn Microbiol Infect Dis* 2009;65:236–246.

6 Sester M, Sotgiu G, Lange C, Giehl C, Girardi E, Migliori GB, Bossink A, Dheda K, Diel R, Dominguez J, *et al*. Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2011;37:100–111.

7 Broekmans JF, Migliori GB, Rieder HL, Lees J, Ruutu P, Loddenkemper R, Raviglione MC; World Health Organization, International Union against Tuberculosis and Lung Disease, and Royal Netherlands Tuberculosis Association Working Group. European framework for tuberculosis control and elimination in countries with a low incidence: recommendations of the World Health Organization (WHO), International Union Against Tuberculosis and Lung Disease (IUATLD) and Royal Netherlands Tuberculosis Association (KNCV) Working Group. *Eur Respir J* 2002;19:765–775.

8 Bakir M, Millington KA, Soysal A, Deeks JJ, Efee S, Aslan Y, Dosanjh DP, Lalvani A. Prognostic value of a T-cell-based, interferon-γ biomarker in children with tuberculosis contact. *Ann Intern Med* 2008;149:777–787.

9 Diel R, Loddenkemper R, Nienhaus A. Predictive value of interferon-γ release assays and tuberculin skin testing for progression from latent TB infection to disease state: a meta-analysis. *Chest* 2012;142: 63–75.
10 Hill PC, Jackson-Sillah D, Fox A, Brookes RH, de Jong BC, Lugos MD, Adetifa MO, Donkor SA, Aiken AM, Howie SRC, *et al*. Incidence of tuberculosis and the predictive value of ELISPOT and Mantoux tests in Gambian case contacts. *PLoS One* 2008;3:e.1379.
11 Kik SV, Franken WP, Mensen M, Cobelens FG, Kamphorst M, Arend SM, Erkens C, Gebhard A, Borgdorff MW, Verver S. Predictive value for progression to tuberculosis by IGRA and TST in immigrant contacts. *Eur Respir J* 2010;35:1346–1353.
12 Lange C, Rieder HL. Intention to test is intention to treat. *Am J Respir Crit Care Med* 2011;183:3–4.
13 Ruiz-Manzano J, Blanquer R, Calpe JL, Caminero JA, Caylà J, Domínguez J, García JM, Vidal R. SEPAR guidelines: diagnostic and treatment of tuberculosis. *Arch Bronconeumol* 2008;44:551–566.
14 Watkins RE, Brennan R, Plant AJ. Tuberculin reactivity and the risk of tuberculosis: a review. *Int J Tuberc Lung Dis* 2000;4:895–903.
15 Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, Hatherill M, Moyo S, Hanekom W, Mahomed H. The utility of an interferon γ release assay for diagnosis of latent tuberculosis infection and disease in children: a systematic review and meta-analysis. *Pediatr Infect Dis J* 2011;30:694–700.
16 Mandalakas AM, Detjen AK, Hesseling AC, Benedetti A, Menzies D. Interferon-γ release assays and childhood tuberculosis: systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2011;15: 1018–1032.
17 Rangaka MX, Wilkinson KA, Glynn JR, Ling D, Menzies D, Mwansa-Kambafwile J, Fielding K, Wilkinson RJ, Pai M. Predictive value of interferon-γ release assays for incident active tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;12: 45–55.
18 Diel R, Loddenkemper R, Meywald-Walter K, Niemann S, Nienhaus A. Predictive value of a whole-blood IFN-γ assay for the development of active TB disease. *Am J Respir Crit Care Med* 2008;177:1164–1170.
19 Haldar P, Thuraisingam H, Patel H, Pereira N, Free RC, Entwisle J, Wiselka M, Hoskyns EW, Monk P, Barer MR, *et al*. Single-step QuantiFERON screening of adult contacts: a prospective cohort study of tuberculosis risk. *Thorax* 2013;68:240–246.
20 Higuchi K, Harada N, Mori T, Sekiya Y. Use of QuantiFERON-TB Gold to investigate tuberculosis contacts in a high school. *Respirology* 2007;12:88–92.
21 Diel R, Loddenkemper R, Niemann S, Meywald-Walter K, Nienhaus A. Negative and positive predictive value of a whole-blood IGRA for developing active TB: an update. *Am J Respir Crit Care Med* 2011; 183:88–95.
22 Machingaidze S, Verver S, Mulenga H, Abrahams DA, Hatherill M, Hanekom W, Hussey GD, Mahomed H. Predictive value of recent QuantiFERON conversion for tuberculosis disease in adolescents. *Am J Respir Crit Care Med* 2012;186:1051–1056.
23 Sollai S, Galli L, de Martino M, Chiappini E. Systematic review and meta-analysis on the utility of interferon-γ release assays for the diagnosis of *Mycobacterium tuberculosis* infection in children: a 2013 update. *BMC Infect Dis* 2014;14:S6.
24 Chang KC, Leung CC. Systematic review of interferon-γ release assays in tuberculosis: focus on likelihood ratios. *Thorax* 2010;65:271–276.
25 Doherty TM, Demissie A, Olobo J, Wolday D, Britton S, Eguale T, Ravn P, Andersen P. Immune responses to the *Mycobacterium tuberculosis*–specific antigen ESAT-6 signal subclinical infection among contacts of tuberculosis patients. *J Clin Microbiol* 2002;40:704–706.
26 del Corral H, París SC, Marín ND, Marín DM, López L, Henao HM, Martínez T, Villa L, Barrera LF, Ortiz BL, *et al*. IFNγ response to *Mycobacterium tuberculosis*, risk of infection and disease in household contacts of tuberculosis patients in Colombia. *PLoS One* 2009;4:e8257.
27 Subdirecció General de Vigilància i Resposta a Emergències de la Salut Publica, Agència de Salut Publica de Catalunya. Informe anual 2013. Situació epidemiològica i tendència de l'endèmia tuberculosa a Catalunya. Barcelona, Spain: Departament de Salut, Generalitat de Catalunya; 2015.
28 Basu Roy R, Sotgiu G, Altet-Gómez N, Tsolia M, Ruga E, Velizarova S, Kampmann B. Identifying predictors of interferon-γ release assay results in pediatric latent tuberculosis: a protective role of bacillus Calmette-Guerin? A pTB-NET collaborative study. *Am J Respir Crit Care Med* 2012;186:378–384.
29 Erkens CG, Kamphorst M, Abubakar I, Bothamley GH, Chemtob D, Haas W, Migliori GB, Rieder HL, Zellweger JP, Lange C. Tuberculosis contact investigation in low prevalence countries: a European consensus. *Eur Respir J* 2010;36:925–949.