Observational Study of QuantiFERON®-TB Gold In-Tube Assay in Tuberculosis Contacts in a Low Incidence Area

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Abstract

Background: QuantiFERON®-TB Gold in-Tube (QFT) assay is a recently developed test to assess latent tuberculosis infection in contagious tuberculosis (TB) contact subjects. To assess the QFT assay in recently exposed contacts of active tuberculosis patients in a French area with low TB incidence but high Bacille Calmette-Guerin coverage, and evaluate progression rates to TB disease.

Methodology/Principal Findings: Between January 2007 and December 2009, 687 contacts of culture-confirmed tuberculosis cases underwent the QFT assay, with tuberculin skin test (TST) in 473, and a 34 months mean follow-up. Of 687 contacts, 148 were QFT positive, while 526 were negative and 13 indeterminate. QFT was positive in 35% of individuals with TST ≥10 mm, 47.5% with TST ≥15 mm or phlyctenular, but in 21% of cases in which two-step TST (M0 and M3) remained negative. Conversely, QFT was negative in 69% of cases with two-step TST showing conversion from negative to positive. All indeterminate QFT were associated with TST induration <10 mm in diameter. For 29 QFT-positive subjects, no chemoprophylaxis was given due to medical contraindications. Of the remaining 119 QFT-positive contacts, 97 accepted chemoprophylaxis (81.5%), and 79 (81.4%) completed the treatment. Two contacts progressed to TB disease: one subject was QFT positive and had declined chemoprophylaxis, while the other one was QFT negative. QFT positive predictive value for progression to TB was 1.96% (1/51) with a 99.8% (525/526) negative predictive value.

Conclusions/Significance: Our results confirm the safety of the QFT-based strategy for assessing the TB chemoprophylaxis indication, as only one contact developed TB disease out of 526 QFT-negative subjects.

Introduction

To prevent progression to active tuberculosis (TB) disease, the early diagnosis and treatment of recent TB infections were shown to be efficacious measures for identified contact individuals, leading to a good control of the TB burden, in countries with low TB incidence [1,2]. A tuberculin skin test (TST) has long been the only reference test for the diagnosis of recent TB infection [3]. However, TST interpretation may be difficult in patients vaccinated with the Bacille Calmette-Guerin (BCG) vaccine [4], as the tuberculin protein shares common antigenic epitopes with the native Mycobacterium tuberculosis bacillus and BCG vaccine strain. Since the early 2000s, new assays have developed to detect in vivo gamma interferon production in response to specific Mycobacterium tuberculosis antigens (ESAT-6, CFP-10, TB7.7), which are not produced by the BCG strain. These interferon-gamma release assays (IGRAs) seemed to be a good alternative to the unspecific TST for the diagnosis of latent tuberculosis infection (LTBI) [5]. Indeed, the lack of a gold standard for the diagnosis of LTBI led to perform studies which compared TST and IGRA, particularly in tuberculosis contacts. In these studies, the concordance between IGRAs and TST was low, especially for the subgroup of contact individuals who previously received the BCG vaccine [6–8]. However, a strategy based on IGRA results still needs to be evaluated in terms of reducing the number of prophylaxis treatments administered compared to a TST-based strategy and in terms of the progression rates to active TB disease for both IGRA positive and negative groups. Only two published studies treat specifically the issue of contacts and use of IGRA in low incidence TB areas, but their diverging results led to conflicting interpretations as to the interest of IGRAs compared with TST for predicting the progression risk to active TB in the case of a positive assay. Both studies nonetheless confirmed the low risk of active TB in the case of negative IGRA [9,10]. However,
these studies included a limited number of contact subjects, 324 and 1033, respectively, with varying vaccination rates (91% and 52% BCG-vaccinated individuals, respectively), in 100% and 40% of migrants, respectively [9,10], with such discrepancies justifying the discordant findings. Additional studies were deemed necessary to reassess the advantages of an IGRA-based strategy, which could eventually replace TST, since IGRA was shown to exhibit a better cost/benefit ratio in two recent pharmaco-economic studies [11,12]. Our study aimed to evaluate QuantiFERON®-TB Gold In-Tube assay in individuals who were recently exposed to index cases with contagious pulmonary TB in Basse-Normandie, an area with low TB incidence (incidence of 7.3 cases per 100,000 inhabitants) but high BCG vaccine coverage (>80%) [13] and a low proportion of migrants (<5%) from high incidence countries. We report adhesion, compliance to chemoprophylaxis, and progression rates to active TB disease for both IGRA non-treated-positive and negative groups. Finally, we also compared QFT and TST results in the subgroup of contact subjects having both procedures.

Materials and Methods

Study population

Between 1st January 2007 and 31st December 2009, this observational, prospective study enrolled all contacts of consecutive index cases of culture-confirmed pulmonary TB patients. Contact subjects were tested using QFT in the regional Centre for Infectious Disease Prevention (Centre de Prévention des Maladies Infectieuses, CPMI) in Caen, France. As part of the systematic investigation initiated after the diagnosis of a pulmonary TB case, the CPMI research nurses collected social and clinical data from contact subjects, and retrospectively estimated their contact time with the index case individual. If the cumulated contact time with the index case over the 3 months preceding the TB diagnosis was <8 hours, the contact was defined as “occasional”, if between 8 and 40 hours, “regular”, and if >40 hours, “close” [14]. As part of the screening procedure for recent TB infection in contact subjects, an initial evaluation (M0) comprising functional symptom assessment and chest X-ray with or without TST was proposed to each subject with regular or close contact. Additionally, the same evaluation procedure was proposed to 82 (11.9%) subjects with only occasional contact on account of their underlying immunocompromised disease (n = 7) or because the index case was a health-care professional (n = 75) (Table 1). A second evaluation was performed 3 months later (M3), comprising a complete medical examination, chest X-ray, QFT and new TST, especially if the first TST revealed skin induration <10 mm in diameter, with QFT always performed prior to TST in such cases. Patients with a final diagnosis of active tuberculosis at M0 or M3 were excluded from the longitudinal study.

The management strategy of contacts without evidence for active tuberculosis at M3 was based on the QFT results according to manufacturer’s instructions (Cellestis Ltd, Chadstone, Australia) in the microbiology laboratory of Caen University Hospital. QFT results were expressed as positive or negative, using the cut-off value of ≥0.35 international units (IU)/mL.

QFT was considered as indeterminate if either an unprovoked IFN-γ level of ≥8.0 IU/mL in the negative-control plasma or a IFN-γ ≤0.5 IU/mL on phytohemagglutinin stimulation with a level of IFN-γ in the tuberculosis antigen-exposed sample minus the level in the negative control of either <0.35 IU/mL or <25% of the IFN-γ concentration in the negative control plasma supplied by the manufacturer.

Incident cases of tuberculosis disease

To define the positive or negative predictive values (PPV or NPV) of QFT, only cases of TB that occurred in contact subjects after the second clinical evaluation were considered to be “incident” and taken into account for evaluating the risk of progression towards TB.

Diagnosis presumption was based on clinical examination, chest x-ray or computed tomography scan, early-morning sputum, early-morning gastric aspiration (for subjects unable to spit), bronchoscopy with bronchoalveolar lavage (BAL) fluid specimens for acid-fast bacilli (AFB) smear examinations, Lowenstein-Jensen mycobacterial cultures, or histo-pathological data from tissue biopsies. Isolated contaminating strains from the index and incident cases were sent to the National Reference Centre for Mycobacteria and Resistance to Anti-tuberculosis Antibiotics (CHU Pitié-Salpêtrière, Paris) for molecular genotyping using the mycobacterial interspersed repetitive-unit (MIRU) typing to verify whether the strains were identical.

Ethical considerations

The DRASS validated the CPMI annual report. All participants received written information describing the prospective screening strategy, which followed the national HAS recommendations [15]. They were informed that they were free to attend the follow-up medical visits or not, respond to telephone calls from CPMI nurses or not, and decline the scheduled x-chest exams. Clinical assessment was blinded as to the contact subject identity.

Statistics

Concordance between TST and QFT results was assessed using κ coefficients for contacts. Kappa values <0.4 indicated weak correlation, values between 0.41–0.60 good agreement, and values >0.6 strong agreement.
QFT positive and negative predictive values (PPV and NPV) were calculated. QFT PPV for progression to tuberculosis is defined as the proportion of QFT positive untreated contacts who developed active tuberculosis during the follow-up. QFT NPV is defined as the proportion of QFT negative untreated contacts who do not progress to an active tuberculosis disease.

**Results**

**Study population**

176 compulsory declaration certificates were received by the DRASS from the whole Basse-Normandie region, between 1st January 2007 and 31st December 2009. 690 contact individuals, corresponding to 46 index cases, were referred to CPMI, since living in Caen area and were enrolled in the current study. All 46 index cases of active TB disease were confirmed by positive Mycobacterium tuberculosis culture, with 82% having a positive AFB smear. Only one isolated strain was found to be resistant to isoniazid. Three cases of active TB were diagnosed at the second evaluation (M3) following chest X-ray, and excluded from the study. Those three subjects were close contacts, and had positive M3 QFT. Thus, 687 individuals were included in the final analysis (Figure 1). Contact subject characteristics are summarised in Table 1.

Median age was 42 years (11.5–97 years), with five contact subjects being younger than 15 years and 94 older than 65 years. The population subset aged 30–49 years was the most represented (312/687, 45.4%). Overall, 479 women and 208 men were enrolled. BCG vaccination status was proven by national or international vaccination certificates in only 140 subjects (20.4%).

QFT in TB Contacts

**Table 1. Characteristics of close contacts undergoing QuantiFERON-TB® Gold in-tube assay.**

| Characteristics               | QuantiFERON-TB® Gold in-Tube M3 |
|-------------------------------|---------------------------------|
|                               | Total n (%) | QFT positive n (%) | QFT negative n (%) | QFT indeterminate n (%) |
| Close contacts population     | 687 (100)   | 148 (21.5)         | 526 (76.6)         | 13 (1.9)                |
| Age (years)                   |              |                    |                    |                         |
| 0–14                          | 44.5±18      | 47.5±17.3          | 42.6±17.9          | 0 (0)                   |
| 15–30                         | 5 (0.7)      | 0 (0)              | 5 (1)              | 0 (0)                   |
| 30–49                         | 149 (21.7)   | 23 (15.5)          | 126 (24)           | 0 (0)                   |
| 50–64                         | 312 (45.4)   | 61 (41.2)          | 251 (47.7)         | 0 (0)                   |
| ≥65                           | 126 (18.3)   | 40 (27)            | 86 (16.3)          | 13 (100)                |
| Sex                           |              |                    |                    |                         |
| Male                          | 208 (30.3)   | 62 (41.9)          | 144 (27.4)         | 2 (15.4)                |
| Female                        | 479 (69.7)   | 86 (58.1)          | 382 (72.6)         | 11 (84.6)               |
| French origin                 |              |                    |                    |                         |
| Yes                           | 660 (96.1)   | 138 (93.2)         | 509 (96.8)         | 13 (100)                |
| No                            | 26 (3.8)     | 10 (6.8)           | 16 (3)             | 0 (0)                   |
| Unknown                       | 1 (0.1)      | 0 (0)              | 1 (0.2)            | 0 (0)                   |
| Immune suppression            |              |                    |                    |                         |
| Yes                           | 6 (0.9)      | 1 (0.7)            | 5 (1)              | 0 (0)                   |
| HIV                           | 0 (0)        | 0 (0)              | 0 (0)              | 0 (0)                   |
| Corticosteroids               | 4 (0.6)      | 1 (0.7)            | 4 (0.8)            | 0 (0)                   |
| Other treatment               | 2 (0.3)      | 0 (0)              | 2 (0.4)            | 0 (0)                   |
| BCG vaccination               |              |                    |                    |                         |
| Confirmed                     | 140 (20.4)   | 28 (18.9)          | 112 (21.3)         | 0 (0)                   |
| Unknown                       | 547 (79.6)   | 120 (81.1)         | 414 (78.7)         | 13 (100)                |
| Contact                       |              |                    |                    |                         |
| Household or intimate         | 135 (19.7)   | 40 (27)            | 83 (15.8)          | 12 (92.3)               |
| Professional                  | 457 (66.5)   | 76 (51.4)          | 280 (53.2)         | 1 (7.7)                 |
| Health care worker            | 240 (34.9)   | 30 (20.3)          | 209 (39.7)         | 1 (7.7)                 |
| Other                         | 95 (13.8)    | 32 (21.6)          | 63 (12)            | 0 (0)                   |
| Exposure time                 |              |                    |                    |                         |
| Occasional                    | 82 (11.9)    | 16 (10.8)          | 65 (12.4)          | 1 (7.7)                 |
| Regular                       | 368 (53.6)   | 76 (51.4)          | 292 (55.5)         | 0 (0)                   |
| Intimate                      | 236 (34.4)   | 56 (37.8)          | 168 (31.9)         | 12 (92.3)               |
| Unknown                       | 1 (0.1)      | 0 (0)              | 1 (0.2)            | 0 (0)                   |

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BCG scars were not systematically looked for. This low rate contrasts with the well-known BCG vaccine coverage in France, estimated to exceed 80%, since from 1950 until 19th July 2007, BCG vaccination was mandatory for children starting school at the age of 6, meaning that it was given to almost 100% of French people aged between 4 and 61 years. Among the 687 contacts, 135 (19.7%) originated in the household or were related to the place of residency, while 457 (66.5%) derived from the occupational environment, with 240

Figure 1. Flow chart of the study population. QFT: QuantiFERON®-TB Gold in-tube; TST: tuberculin skin test; M: month. doi:10.1371/journal.pone.0043520.g001

Figure 2. Concordance of QuantiFERON-TB® Gold in-tube (QFT) per tuberculin skin test (TST). QFT: QuantiFERON®-TB Gold in-tube; TST: tuberculin skin test; P: phlyctenular; M: month. * Three patients with 10–14 mm and phlyctenular TST. doi:10.1371/journal.pone.0043520.g002
of a medical or social profession. Only 26 contact subjects (3.8%) were born outside of France. None were HIV positive, while only seven (1%) received an immunosuppressive treatment for chronic disease.

Taking into account the estimated time of contact, there were 236 close contacts (34.4%), 369 (53.7%) regular, and only 82 (11.9%) occasional.

**QuantiFERON®-TB Gold in-Tube and TST**

Among the 687 QFT performed at M3, 148 (21.5%) were considered as positive and 526 (76.6%) as negative, while 13 (1.9%) had indeterminate results. Population characteristics according to the QFT result are summarised in Table 1.

TST was conducted in 473 contact individuals: 313 at M0 alone, 45 at M3 alone, 114 at both M0 and M3. One contact with TST qualitative result was excluded from the comparative study (Figure 1). In contacts, the French Conseil Supérieur d’Hygiène Publique recommendations(3) defined tuberculosis infection as probable if skin induration was $\geq 10$ mm. The tuberculosis infection was considered as “probably recent” if skin induration was $\geq 15$ mm, or showed phlyctenular feature, or if the difference between an initial TST and a second TST at M3 was $\geq 10$ mm in a two-step strategy. The tuberculosis infection was considered as only “possible” if skin induration was comprised between10 and 15 mm. Thus, to compare the QFT results with those different TST cut-offs, individuals who only had a TST at M0 with induration $< 10$ mm in diameter(91 subjects), were excluded from the study. For individuals for whom TST are available at both M0 and M3, the skin induration diameter at M3 was used for this comparison. Consequently, 381 TST results were taken into account: 222 individuals with TST only at M0 with skin induration $\geq 10$ mm in diameter, 45 individuals with TST only at M3, and 114 with TST performed at both M0 and M3 (one contact subject was excluded due to only a qualitative result at M0 and M3) (Figure 1).

QFT results expressed as a function of TST are provided in Figure 2. QFT was positive in 35% (106/300) of TST $\geq 10$ mm and 47.5% (57/120) of TST $\geq 15$ mm or phlyctenular. Moreover, QFT was negative in 69% of cases with two-step TST being positive. Conversely QFT was positive in 21% of cases (21/101) in which two-step TST was negative.

All indeterminate QFT were associated with skin induration $< 10$ mm in diameter. The concordance between M3 TST and M3 QFT was weak whatever the cut-off for positive TST (10 mm, 15 mm, or positive reaction) and whatever the subset according to age (Table 2 and 3).

**Table 2. Agreement between QuantiFERON-TB® Gold in tube and tuberculin skin tests, analysed by BCG vaccination status, age, and health care worker status.**

| QFT | TST (≥10 mm) | Positive | Negative | Agreement | QFT | TST (≥15 mm or P.) | Positive | Negative | Agreement |
|-----|--------------|----------|----------|-----------|-----|-------------------|----------|----------|-----------|
| Total | Positive | 28 | 50 | Agreement = 61% | Positive | 19 | 19 | Agreement = 76% |
| N = 147 | Negative | 7 | 62 | $\kappa = 0.249$; 95% CI (0.115; 0.382) | Negative | 16 | 93 | $\kappa = 0.362$; 95% CI (0.201;0.524) |
| ≥65 years | Positive | 10 | 4 | Agreement = 85% | Positive | 5 | 1 | Agreement = 82% |
| N = 62 | Negative | 5 | 43 | $\kappa = 0.595$; 95% CI (0.340; 0.844) | Negative | 10 | 46 | $\kappa = 0.392$; 95% CI (0.176;0.608) |
| <65 years | Positive | 18 | 46 | Agreement = 44% | Positive | 14 | 18 | Agreement = 72% |
| N = 85 | Negative | 2 | 19 | $\kappa = 0.109$; 95% CI (-0.013; 0.232) | Negative | 6 | 47 | $\kappa = 0.350$; 95% CI (0.149;0.551) |

TST: tuberculin skin test; QFT: QuantiFERON®-TB Gold in-tube; BCG: Bacille Calmette-Guérin.

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**Table 3. Agreement between QuantiFERON-TB® Gold in tube and two-step tuberculin skin tests, analysed by BCG vaccination status, age, and health care worker status.**

| QFT | Two step TST | Positive | Negative | Agreement |
|-----|--------------|----------|----------|-----------|
| Total | Positive | 4 | 9 | Agreement = 71% |
| N = 102 | Negative | 21 | 68 | $\kappa = 0.051$; 95% CI (-0.128;0.231) |
| ≥65 years | Positive | 1 | 2 | Agreement = 73% |
| N = 62 | Negative | 14 | 45 | $\kappa = 0.033$; 95% CI (-0.138;0.204) |
| <65 years | Positive | 3 | 7 | Agreement = 65% |
| N = 40 | Negative | 7 | 23 | $\kappa = 0.067$; 95% CI (-0.243;0.377) |

TST: tuberculin skin test; QFT: QuantiFERON®-TB Gold in-tube; BCG: Bacille Calmette-Guérin.

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Contact management according to QuantiFERON®-TB Gold in-Tube results and active TB incidence

According to the 2006 HAS recommendations authorising the use of QFT instead of TST for screening contacts of an index TB case [15], the management strategy for such individuals was based on the qualitative QFT results. Antibiotic chemoprophylaxis was only proposed to contact subjects with positive QFT regardless their TST results.

Among the 148 contact subjects with positive QFT, 97 (65.5%) were treated with the isoniazid plus rifampicin doublet (95%) or isoniazid alone (5%), whereas 51 subjects did not receive treatment on account of refusal in 22 cases or medical decision in 29. The treatment adhesion rate was 81.5% (97/119). Treatment compliance and tolerance were good for 79 out of the 97 (81.4%) individuals who received a full course of chemoprophylaxis. Chemoprophylaxis was interrupted due to adverse events in 10 cases (56%) or spontaneously in eight (44%) owing to their personal decision. No individuals with negative QFT received chemoprophylactic therapy.

Mean follow-up for the 687 contact subjects was 34 ± 11 months, with two individuals presenting active TB during follow-up, as confirmed by mycobacterial culture. Molecular characterisation revealed both contacts to be infected by the same *M. tuberculosis* strain as their index case (Table 4). One of the patients had positive QFT and initial TST <10 mm in diameter at M0, but refused chemoprophylaxis. The other patient had negative QFT and TST induration of 13 mm at M0. Therefore, the rate of progression (PPV) towards active TB in contact individuals with positive QFT and who did not receive TB prophylaxis was estimated at 1.96% (1/51). Conversely, only one contact among 526 individuals with negative QFT subsequently developed active TB disease, providing a NPV of 99.8%. No active TB case was observed in contact subjects with undetermined QFT (Table 5).

**Table 4. Characteristics of the two contacts with incident tuberculosis disease.**

| Case | Sex | Age yrs | Type of contact | TST M0/M3, mm | QFT M3 (UI.ml⁻¹) | ID Type of contact | Time to TB, months | Type of TB | Culture Strain identical to index case |
|------|-----|---------|----------------|---------------|-----------------|------------------|-------------------|------------|-------------------------------------|
| 1    | M   | 34      | Intimate (Household) | 5-9/ND        | Positive (10)   | No Intimate (Household) | 17               | P          | Positive (10) | Refusal 17 P Positive (10) |
| 2    | F   | 23      | Intimate (Household) | 13/ND         | Negative (0.12) | No Intimate (Household) | 18               | P          | Negative (0.12) | No 18 P Positive (0.12) |

BCG: Bacille Calmette-Guérin; UK: Unknown; ID: immunodepression; TST: Tuberculin skin test; ND: not done; QFT: QuantiFERON®-TB Gold in-Tube; CP: Chemoprophylaxis; TB: Tuberculosis; P: Pulmonary.

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Discussion

In this cohort study including recently exposed contacts of culture-proven tuberculosis patients from a low-incidence tuberculosis area, our results confirm the interest of QFT in the diagnosis strategy of recent tuberculosis infection. Indeed, the high NPV for subsequent development of TB disease (99.8%) showed the accuracy of IGRA as recently shown in two meta-analyses [16,17]. As in those meta-analyses, our study also showed a low PPV for IGRA. However a direct comparison between the current study and those papers can hardly be drawn, since the latest included studies with individuals having very various risks of evolution toward active tuberculosis (contact subjects, immunocompromised patients, adolescents, patients with chronic renal insufficiency...), and from areas of low to high tuberculosis incidence. Only three published works using a commercial IGRA have evaluated the risk of progression of contacts to active TB in a low incidence country [9,10,18]. Nevertheless, in the study by Bradshaw and al. [15], the infectiousness of the index case and the time of contact was not precisely described.

Thus, in our study, 21.6% of individuals had positive QFT. Several prior studies focused on similar populations living in low TB incidence areas. Our results reflect the findings of Diel et al. [10] (20.8% of positive QFT), while differing from those published by Kik or Bradshaw who respectively reported 52.5% and 8.6% rates of contact subjects with positive QFT [9,18]. However, the discrepancy with the study by Kik et al. is probably due to the higher proportion of migrant individuals from high TB incidence countries (100% of subjects), compared with our study, in which only 3.8% of subjects were born in high-TB endemic areas, such as...
occurred prior to the 23rd month of follow-up [10]. In the Kik's study [9], the progression rate was 2.8% (5/178) and only involved adult migrants. Originating from a high TB incidence area is not a factor for positive QFT, being related to past infection and leading to an over- or underestimation of the progression risk to active TB. In our study, the low proportion of children (28.6% of children versus 10.3% of adults (p = 0.03) with 28.6% of children versus 10.3% of adults (p = 0.03) developing TB. In our study, the low proportion of children under 15 (0.7%) may account for the difference in progression rates, with the five children included in the study all having negative QFT. In Diel's study [10], interferon-γ levels were also associated with the risk of progression to active TB (OR 1.93 for each unit of interferon-γ.L.ml⁻¹, p<0.0001), with 16 of the 19 TB disease cases observed during follow-up having concentrations >3 U.L.ml⁻¹, with the remaining three <1 U.L.ml⁻¹ [10].

In our study, this difference could not be explained by a larger proportion of contact subjects having low interferon-γ concentrations, since for untreated contacts with positive QFT, interferon-γ levels <1 U.L.ml⁻¹ were only observed for 13, levels between 1 and 3 U.L.ml⁻¹ for 12, while levels >3 U.L.ml⁻¹ were found for 25. The duration of follow-up may have biased our study, since the mean follow-up period was 34 months in our study versus 46.6 months in that of Diel et al. However, all TB cases reported by Diel et al. occurred prior to the 23rd month of follow-up [10]. In the Kik's study [9], the progression rate was 2.8% (5/178) and only involved adult migrants. Originating from a high TB incidence area is not only an independent risk factor for active TB [25], but also a risk factor for positive QFT, being related to past infection and leading to an over- or underestimation of the progression risk to active TB. The low proportion of migrants (3.8%) in our study makes this bias unlikely and therefore, our evaluation of QFT PPV may be considered reliable. However, we were not able to reliably evaluate the protective role of the BCG vaccine despite the high vaccination coverage in our region, since our methodology requiring a vaccination certificate to prove BCG history was obviously biased, underestimating the true rate of BCG coverage in our study population. Nevertheless, given the differences in BCG vaccination coverage as observed by Diel et al. (51.9%) [10], Kik et al. (80.8%) [10], and the estimated rate of 80% in France [13], the impact of these differences should be evaluated, as 74% of TB cases in Diel's study occurred in non-vaccinated patients [10]. None of 37 contacts with QFT positive progressed to TB in the Bradshaw's study [18].

After the beginning of the current study, many publications reported a possible impact of a preceding TST on subsequent IGRA results. However those procedures were not performed in the context of a contact or outbreak study. A recent review, including thirteen studies, raised conflicting issues since five concluded to the absence of boosting of interferon-γ levels and seven to its occurrence [26]. In the studies reporting the occurrence of boosting, the risk was higher when IGRA was made shortly after TST (seven days). This risk persists up to 3 months after TST and wanes after this time. Boosting incidence was also higher in subjects who had a positive QFT before TST whereas conversions only occurred in 2 to 12% oﬁnitally QFT-negative subjects [26]. In the latter group, subjects would receive inappropriate chemoprophylaxis on the basis of this falsely positive IGRA. In two recent studies, conversion persisted at day 84 or week 6, but occurred only in subjects with a positive TST (≥10 mm) [27,28]. These false positives could induce a bias in our study, and might explain our low PPV. Indeed, among the 148 M3 QFT-positive contacts, 119 had a TST at M0. 96 had a TST with an induration ≥10 mm. Thus, according to this risk, we could derive from our data that the maximum number of false positive cases in this series could be 2 to 13 (2 to 12% of 96) among the QFT-positive population. However, we feel this risk is probably overestimated, since IGRA was performed at least three months after TST. Moreover the most positive TST, especially phlyctenular or ≥15 mm induration, corresponded to subjects with recent tuberculosis infection and would have shown positive QFT, if IGRA had been made at M0. Therefore we deeply think the impact of the TST at M0 on subsequent QFT positivity is low in our study. However, whereas TST had limited influence on QFT conversion from negative to positive, in QFT-positive subjects, M0 TST could actually have altered the absolute interferon-γ levels in our study, impairing the use of those quantitative values for predicting the risk of active tuberculosis.

Our results reassessed the safety of the QFT-based strategy, since for negative QFT our high NPV (99.8%) was similar to previously published studies on QFT (98 to 100%) [9,10,18].

Only one out of 526 contact subjects with negative QFT who did not receive prophylaxis subsequently developed active TB

| QFT | Contacts | Progression to tuberculosis disease |
|-----|----------|-------------------------------------|
|     | n       | Untreated, n | n | Sp, % (95% CI) | PPV, % (95% CI) | NPV, % (95% CI) |
| Positive | 148 | 51 | 1 | 87 (65.9; 100) | 1.96 (0; 4.2) | 99.8 (99.4; 100) |
| Negative | 526 | 526 | 1 | NA | NA | NA |
| Indeterminate | 13 | 13 | 0 | NA | NA | NA |

QFT: QuantiFERON®-TB Gold in-tube; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value.

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Table 5. Number of cases of incident tuberculosis disease in untreated contacts.
Conclusions

Our results confirm the advantage of QFT for the strategic management of TB contact subjects in a low TB incidence area with high BCG vaccination coverage and low rate of migrants or immuno-compromised individuals. However, larger studies are still required to confirm the advantage of QFT in identifying contact subjects with a higher risk of developing active TB according to population characteristics, such as vaccination coverage and geographic origin, and define the best management strategy for cases with undetermined QFT results.

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Author Contributions

Conceived and designed the experiments: EB. Performed the experiments: EB BM MAS GZ. Analyzed the data: EB BM GZ. Contributed reagents/materials/analysis tools: BM. Wrote the paper: EB GZ.

References

1. (2000) American Thoracic Society : Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med 161: s21–s24.
2. Erken CG, Kamphorst M, Abushaker I, Boathamley GH, Chemtob D, et al. (2010) Tuberculosis contact investigation in low prevalence countries: a European consensus. Eur Respir J 36: 925–949.
3. [2003] Intradermal reaction to tuberculin (IDR) or tuberculin test. Rev Mal Resp 20: S27–33.
4. Wang L, Turner MO, Elwood RK, Schulzer M, FitzGerald JM (2002) A meta-analysis of the effect of Bacille Calmette Guerin vaccination on tuberculin skin test measurements. Thorax 57: 804–809.
5. Diel R, Golebi D, Ferrara G, Boathamley G, Cirillo D, et al. (2011) Interferon-gamma release assays for the diagnosis of latent Mycobacterium tuberculosis infection: a systematic review and meta-analysis. Eur Respir J 37: 88–99.
6. Kik SV, Franken WP, Arend SM, Mensen M, Cobelens FG, et al. (2009) Interferon-gamma release assays in immigrant contacts and effect of remote exposure to Mycobacterium tuberculosis. Int J Tuberc Lung Dis 13: 820–828.
7. Arred SM, Thujen SF, Leyten EM, Bouwman JJ, Franken WP, et al. (2007) Comparison of two interferon-gamma assays and tuberculin skin test for tracking tuberculosis contacts. Am J Respir Crit Med 175: 616–627.
8. Diel R, Loddenkemper R, Meywald-Walter K, Niemann S, Niemann A (2000) Predictive value of a whole blood IFN-gamma assay for the development of active tuberculosis disease after recent infection with Mycobacterium tuberculosis. Am J Respir Crit Care Med 177: 1164–1170.
9. Kik SV, Franken WP, Mensen M, Cobelens FG, Kamphorst M, et al. (2010) Predictive value for progression to tuberculosis by IGRA and TST in immigrant contacts. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology 35: 1346–1355.
10. Diel R, Loddenkemper R, Niemann S, Meywald-Walter K, Niemann A (2011) Negative and Positive Predictive Value of a Whole-Blood Interferon-(gamma) Release Assay for Developing Active Tuberculosis: An Update. Am J Respir Crit Care Med 183: 88–95.
11. Diel R, Wrightson-Smith P, Zellweger JP (2007) Cost-effectiveness of interferon-gamma release assay testing for the treatment of latent tuberculosis. Eur Respir J 30: 321–332.
12. Deuffic-Burban S, Atou K, Vigeat N, Melleiz H, Rouvet E, et al. (2010) Cost-effectiveness of QuantiFERON-TB test vs. tuberculin skin test in the diagnosis of latent tuberculosis infection. Int J Tuberc Lung Dis 14: 471–481.
13. (2001) Mesure de la couverture vaccinale en France. Bilan des outils et des méthodes en l’an 2000. Institut de veille sanitaire. 1–57 p.
14. Enquête autour d’un cas cas. Recommandations pratiques.