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Borderline QuantiFERON results and the distinction between specific responses and test variability

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ABSTRACT

Background: QuantiFERON (QFT) results near the cut-off are subject to debate. We aimed to investigate which borderline QFT results were due to *Mycobacterium tuberculosis* (*Mtb*)-specific responses or to test variability.
Methods: In a contact investigation, tuberculin skin test (TST), QFT and T-SPOT.*TB* (T-SPOT) were performed in 785 BCG-unvaccinated contacts. Contacts with a low-negative (< 0.15), borderline (0.15–0.35), low-positive (0.35–0.70) or high-positive QFT (≥0.70 IU/mL) were compared with respect to exposure, TST and T-SPOT results. Development of active tuberculosis was assessed.
Results: Borderline QFT results occurred in threefold excess over test variability (p = 0.0027). In contacts with low-negative, borderline or positive QFT results, a positive TST occurred in 24.9%, 62.1% and 91.4% (p < 0.0001) and a positive T-SPOT result in 6.3%, 41.3% and 86.4%, respectively (p < 0.0001). Two-third (20/29) of contacts with a borderline and 14/16 (88%) with a low-positive QFT had a positive TST and/or T-SPOT, indicating probable *Mtb*-infection. During 12 years of follow-up, seven patients were diagnosed with active tuberculosis, two of whom after a low-positive QFT.
Conclusions: In this study, most borderline and low-positive QFT results were *Mtb*-specific, showing the biological significance of a borderline QFT. The clinical relevance, however, will be most distinct in patients who are or will be immunocompromised.

1. Introduction

Interferon-gamma release assays (IGRAs) measure *Mycobacterium tuberculosis* (*Mtb*) specific responses and are mainly used for the evaluation of latent tuberculosis infection (LTBI). Commercially available IGRAs are not affected by prior *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccination, which is an important advantage compared to the tuberculin skin test (TST) [1,2]. Commercial IGRAs are T-SPOT.*TB* (T-SPOT) (Oxford Immunotec, Abingdon, UK) which measures the number of interferon-γ (IFN-γ) producing peripheral blood mononuclear cells, and QuantiFERON-TB Gold (QFT) (Qiagen, Hilden, Germany) which measures produced IFN-γ in whole blood culture [3]. Both tests use similar *Mtb*-specific antigens.

Per manufacturer's instructions, QFT results are interpreted as positive or negative using 0.35 IU/mL as a cut-off but this dichotomy is subject to debate [4–6]. In studies of repeated QFT testing of healthcare workers (HCW), results near the cut-off had a high conversion or reversion rate [5,7–10], and such varying results were mostly attributed to random assay variation. This has resulted in a zone of uncertainty, variably named the “borderline zone” or “gray zone”, ranging between 0.20-0.25 and 0.70–1.0 IU/mL [5,7,9,11], and has led to repeated testing in some settings.

In two previous meta-analyses, the predictive value of IGRA for development of active tuberculosis was modest [12,13]. In a recent study, conversion of QFT from less than 0.20 IU/mL to greater than 0.70 IU/mL was associated with the highest incidence of TB disease [14] and this was proposed as a better definition of conversion. The authors acknowledged that a proportion of QFT results just below the

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regular cut-off might reflect weak yet *Mtb*-specific responses but stated that borderline results "cannot further be teased apart" [14].

In a previous study we provided proof of concept that borderline results actually include true antigen specific responses, showing that borderline QFT results (0.15–0.35 IU/mL) occurred in threefold excess over the number expected based on random variability and that the presence of various non-IGRA risk factors and parameters of *Mtb* infection showed a gradient along increasing quantitative QFT results [15]. The clinical relevance of a distinction between *Mtb*-specific responses and test variability on an individual basis will be highest if an individual is or will become immunocompromised [16–18]. The aim of the present study was to investigate whether it is possible to determine which QFT results near the regular cut-off were *Mtb* antigen-specific immune responses. We used a large cohort of BCG-unvaccinated contacts from two previous interconnected studies. All contacts were supermarket customers in a large-scale contact investigation in the Netherlands, in whom TST, QFT as well as T-SPOT results had been performed simultaneously.

By comparing quantitative QFT results with corresponding TST and T-SPOT results we were able to differentiate between infection and random variation. In addition, development of active TB was assessed in a 12-years follow-up in these unique cohorts.

2. Methods

2.1. Study population

Two cohorts of BCG-unvaccinated individuals from previous studies were used. The combined studies included 829 individuals, of which 78 patients took part in both studies (Fig. 1).

2.1.1. Cohort 1

In 2005, 785 contacts were included during a large contact investigation after exposure to a supermarket employee with smear-positive tuberculosis. The study protocol was approved by the ethical review board of Hospital Diakonessenhuis Utrecht/Zeist, The Netherlands (protocol 2004.23) and all participants had provided written informed consent. The results were published in 2007 [19], the key finding being that both IGRA were more strongly associated with exposure, expressed as the cumulative shopping time in the supermarket, than the TST.

2.1.2. Cohort 2

As a follow up study of the abovementioned contact investigation, 122 individuals with a positive TST result were followed prospectively during two years with repeated QFT and T-SPOT. The study protocol was approved by the ethical review board of the Leiden University Medical Center (protocol P05.53) and all participants provided written informed consent. The results were published in 2008, including a table with all individual follow-up IGRA responses [20]. The results showed the variable kinetics of IGRA responses which were unaffected by treatment of LTBI, indicating limited value of IGRA during follow-up.

2.2. Study design

All valid IGRA results in both abovementioned cohorts were evaluated to determine whether there was an excess of borderline results (in this study defined as ≥ 0.15 and < 0.35 IU/mL) compared to the corresponding negative range (-0.35 to -0.15 IU/mL). Subsequently, individuals with a borderline QFT result (in this study defined as ≥ 0.15 and < 0.35 IU/mL) were compared to those with a low-negative (< 0.15 IU/mL) or a positive result (≥ 0.35 IU/mL) with respect to demographic data, exposure risk, T-SPOT and TST results. All data were extracted from the existing databases from aforementioned studies [20,21]. From individuals with at least one borderline QFT result during the two-year IGRA follow-up study, the time course of simultaneous, previous or later IGRA responses was evaluated.

2.3. IGRA and interpretation

Both IGRAs (QFT-GIT and T-SPOT.*TB*) were performed according to the manufacturer's instructions [22]. QFT-GIT was considered positive if ≥ 0.35 IU/mL. In 2005, a T-SPOT result ≥ 6 spots was considered positive according to the package insert [19,20]. However, the package insert was later changed to a cut-off of ≥ 8 spots for a positive result, 5–7 spots being labelled as 'borderline ', and these new criteria were used for the present study [23]. Either a TST ≥ 10 mm or a positive T-SPOT result was interpreted as having a high likelihood of infection with *Mtb*.

2.4. Follow-up

From all 829 contacts (785 in cohort 1 and 44 additional contacts in cohort 2) it was checked in the medical records of the MHS whether they had developed active TB between 2005 and March 2017. In case of active TB, the localization of TB, immune status including HIV serology, culture results and *Mtb* variable number of tandem repeats (VNTR) genotype of those with a positive culture were recorded. Due to the study protocols it was not possible to check for possible active TB diagnosed elsewhere.

2.5. Statistical analyses

To compare categorical data, chi-square test or Fishers Exact Probability test were used. Continuous data were compared using one-way ANOVA, Mann-Whitney U or Kruskal-Wallis test as appropriate. Statistical analyses were performed using IBM SPSS Statistics version 23 and GraphPad Prism 7.

3. Results

3.1. Distribution of QFT results

From cohort 1, 704/785 (89.7%) QFT results were negative and 81/785 (10.3%) were positive. The distribution of quantitative QFT results

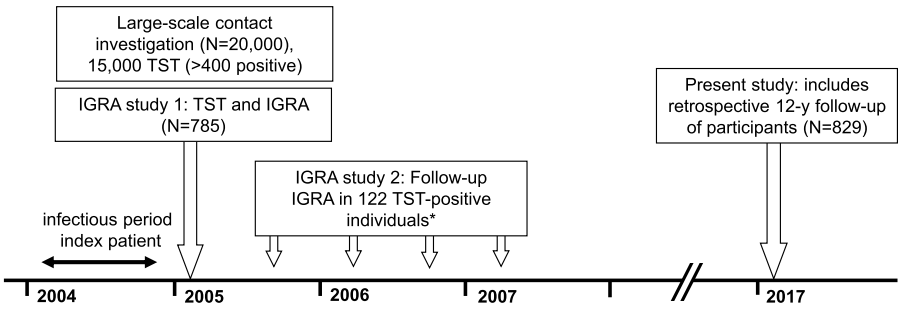

Fig. 1. Time line of the study; Abbreviations: TST, tuberculin skin test; IGRA, interferon-gamma release assay.

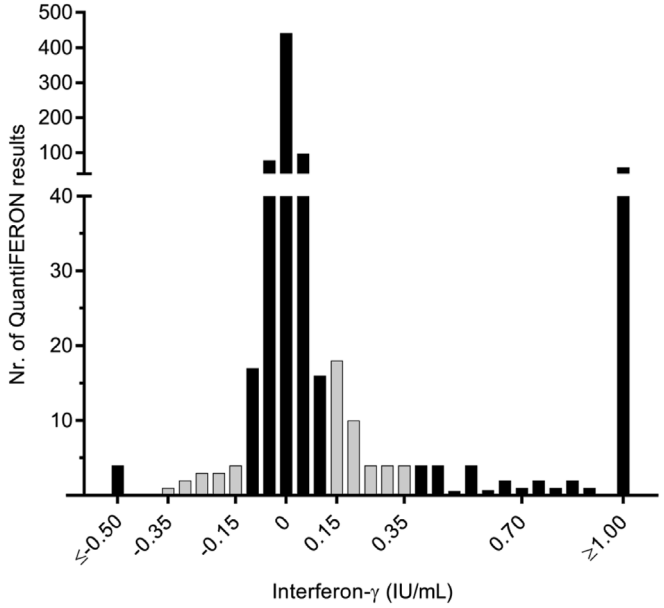

Fig. 2. Distribution of QuantiFERON results. QuantiFERON-TB Gold In-Tube® (QFT) result below −1.0 IU/mL is depicted as −1.0 (N = 1), results higher than 8.0 IU/mL are depicted as 8.0 (N = 10). The number of borderline results was significantly higher than the number of results within the corresponding range of minus values (p < 0.0001).

is shown in Fig. 2. Of the 704 negative results, ten (1.3%) were between −0.35 and −0.15 IU/mL; 271 (34.5%) were between −0.15 and 0 IU/mL; 123 (15.7%) were exactly zero; 266 (33.9%) were between zero and 0.15 IU/mL; 29 (3.7%) were ≥0.15 and <0.35 (the latter defined as borderline results). There was a threefold excess of borderline test results (n = 29 vs. 10 in the corresponding opposite range between −0.35 and −0.15 IU/mL, p = 0.0027).

3.2. Comparison between contacts with low-negative, borderline and positive QFT results

Contacts from cohort 1 with low-negative, borderline or positive QFT results were compared (Table 1). There were no significant differences between the QFT categories with regard to socio-demographic characteristics, travel to a TB-endemic country or professional risk. A minority of individuals (23/772, 3.0%) had an impaired immune status, which was independent of the QFT result. There was a trend towards an association between the cumulative shopping time and higher QFT results (p = 0.056). Among contacts with a low-negative, borderline, or positive QFT result, a positive TST (≥10 mm) was found in 24.9%, 62.1% respectively 91.4% (p < 0.0001) and a positive T-SPOT result in 6.3%, 41.3% respectively 86.4% (p < 0.0001). A similar gradient was found for TST size, median number of spots in T-SPOT, and the proportion of contacts with responses to both T-SPOT antigen panels (Fig. 3). An analogous analysis based on classification of T-SPOT results as low negative (< 5 spots), borderline (5–7 spots) or positive (≥8 spots) showed similar results, as is shown in Table S1.

3.3. Kinetics

Of the 122 individuals from cohort 2, 17 had at least one borderline QFT result, of whom four had also been included in cohort 1. Fig. S1 shows the variable kinetics of QFT and T-SPOT in these 17 individuals. In 6/17 individuals, all repeated QFT were negative, 5/17 converted to positive, 3/17 reverted from positive to negative and a combination of conversion and reversion was observed in 3/17 individuals. A positive T-SPOT result was observed at least once in 11/17 contacts. Of four contacts with a borderline QFT result during the initial contact investigation, all had a simultaneous or later positive TST and/or T-SPOT result. In the remaining 105 persons without any borderline result, conversion and reversion rates were 1.9% resp. 2.8%.

3.4. Borderline versus low-positive QFT results

Table 2 shows the characteristics of the 29 contacts with a borderline, and 16 contacts with a low-positive QFT result from cohort 1. Ten subjects also participated in the follow up study and repeated IGRA results were available after six, 12 and/or 24 months. A combination of a TST ≥10 mm and a positive T-SPOT result was seen in 10/29 (34.4%) subjects with a borderline result compared to 12/16 (75%) individuals with a low-positive result (p = 0.013). At least one earlier or later positive QFT result was observed in 3/4 (75%) individuals with a borderline result compared to 4/6 (67%) in patients with a low-positive result. A positive TST and/or a positive T-SPOT result was present in 20/29 (69.0% [95% CI: 52–86%]) contacts with a borderline result and 14/16 (87.5% [95% CI: 71–100%]) contacts with a low-positive QFT result.

3.5. 12-Year follow-up

During the 12 years since the contact investigation in 2005, active tuberculosis had been diagnosed in seven contacts (Table 3). Two patients with active tuberculosis (case 5 and case 6) had been included in both cohort 1 and 2, the remaining five only in cohort 1. The interval between QFT testing and time of diagnosis ranged between 0 and 132.5 months. Two patients were diagnosed with active TB during the contact tracing and both were QFT, T-SPOT and TST positive. Four other contacts who developed TB all had a positive TST, T-SPOT and QFT result which was just above the cut-off level in two patients. One patient, who had a negative TST and QFT at time of contact investigation developed active TB with a different genotype of *Mtb* compared to the index patient and was thus probably infected later by another source, possibly during his work in a prison. None of the contacts were immunocompromised.

4. Discussion

Previous reports on borderline QFT results were unable to discriminate between *Mtb*-specific responses and random test variability. In order to address this diagnostic gap, the present study evaluated a large number of BCG-unvaccinated contacts in a Dutch outbreak setting who were simultaneously tested with TST, QFT and T-SPOT. Based on simultaneous positive TST and/or a positive T-SPOT result, we concluded that 69% of borderline QFT results just below the regular cut-off and 88% of low-positive QFT results were probably *Mtb*-specific responses, although this can be considered subjective as no true gold standard for LTBI exists. Two of sixteen contacts with a low-positive result developed active TB.

The main aim of this study was to investigate borderline QFT results and the key challenge was how LTBI can be diagnosed independent of QFT if there is no gold standard available. We reasoned that a TST ≥10 mm in BCG-unvaccinated contacts and/or a clear positive T-SPOT result would indicate a high probability of infection with *Mtb*, especially if occurring concurrently. Among contacts with a borderline QFT result, 62.1% had a positive TST and 41.3% had a positive T-SPOT result, mostly with a large number of spots and responses to both ESAT-6 and CFP-10 peptide antigen panels, which effectively excludes random variability. The gradual increase of the proportion of positive TST and T-SPOT results in higher QFT categories challenges the dichotomous regular cut-off. The reliability of TST and T-SPOT as alternative indicators of LTBI needs consideration, however. Both T-SPOT and TST have a high sensitivity for detecting an infection with *Mtb*, while the specificity of T-SPOT is superior [24]. Limitations of the TST

Table 1
Characteristics of contacts in cohort 1 by QuantiFERON result.

Characteristic	QFT low-negative (< 0.15 IU/mL)	QFT borderline (0.15–0.35 IU/mL)	QFT positive (≥0.35 IU/mL)	All	p value
	N = 675	N = 29	N = 81	N = 785	
Age (y)	43.1 ± 11.4	43.4 ± 10.7	44.1 ± 10.7	43.2 ± 11.3	0.766
range (y)	14.4 to 60.1[a]	17.9–59.9	18.7–59.8	14.4–60.1[a]	
Sex (female)	389/668 (58.2)	15/29 (51.7)	55/79 (69.6)	459/776 (59.1)	0.106
Immigrant (first generation)	20/666 (3.0)	2/27 (7.4)	1/81 (1.2)	23/774 (3.0)	0.282
Travel to TB-endemic country[b]	266/674 (39.5)	14/29 (48.3)	31/81 (38.3)	311/784 (39.7)	0.687
Professional risk	172/648 (26.5)	6/29 (20.7)	18/79 (22.8)	196/756 (25.9)	0.622
Immunocompromised	20/662 (3.0)	1/29 (3.4)	2/81 (2.5)	23/772 (3.0)	0.909
Cumulative shopping time (h)	24.7 ± 25.3	29.4 ± 20.7	31.7 ± 25.4	25.6 ± 25.3	0.056
TST result (mm)	4.5 ± 6.7	11.6 ± 8.4	18.3 ± 5.7	6.17 ± 7.9	< 0.0001
TST category					< 0.0001
0–4 mm	441/675 (65.3)	7/29 (24.1)	1/81 (1.2)	449/785 (57.2)	
5–9 mm	66/675 (9.8)	4/29 (13.8)	6/81 (7.4)	76/785 (9.7)	
10–14 mm	87/675 (12.9)	6/29 (20.7)	6/81 (7.4)	99/785 (12.6)	
≥15 mm	81/675 (12.0)	12/29 (41.4)	68/81 (84.0)	161/785 (20.5)	
T-SPOT.TB result (nr. of spots)	1.7 ± 4.6	14.3 ± 19.6	36.6 ± 36.5	5.9 ± 17.1	< 0.0001
T-SPOT.TB					< 0.0001
negative (≤4 spots)	583/649 (89.8)	14/29 (48.3)	9/81 (11.1)	606/759 (79.8)	
borderline (5–7 spots)	25/649 (3.9)	3/29 (10.3)	2/81 (2.5)	30/759 (4.0)	
positive (≥8 spots)	41/649 (6.3)	12/29 (41.3)	70/81 (86.4)	123/759 (16.2)	
8-10 spots	12/41 (29.3)	0/12 (0)	2/70 (2.9)	14/123 (11.3)	
11-20 spots	24/41 (58.5)	4/12 (33.3)	21/70 (30.0)	49/123 (39.8)	
21-50 spots	4/41 (9.8)	6/12 (50.0)	27/70 (38.6)	37/123 (30.1)	
≥50 spots	1/41 (2.4)	2/12 (16.7)	20/70 (28.6)	23/123 (18.7)	
ESAT-6 and/or CFP-10 positive					< 0.0001
ESAT-6 only	3/41 (7.3)	1/12 (8.3)	6/70 (8.6)	10/123 (8.1)	
CFP-10 only	36/41 (87.8)	4/12 (33.3)	16/70 (22.9)	56/123 (45.6)	
ESAT-6 and CFP-10 both	2/41 (4.9)	7/12 (58.3)	48/70 (68.6)	57/123 (46.3)	

Continuous variables are displayed as mean ± SD, categorical values are displayed as numerator over denominator (%).
Abbreviations: QFT, QuantiFERON-TB Gold In-Tube; TST, tuberculin skin test.
[a] After omission of one contact aged 85.5 y.
[b] Defined as country with TB incidence ≥50 cases of active tuberculosis/100,000 inhabitants.

are inter-observer variability and possible false positive results due to BCG-vaccination or exposure to non-tuberculous mycobacteria [25,26]. In our study, inter-observer variability was minimal because TST indurations were the average of measurements by two independent readers and a third person made the final reading if the inter-observer difference exceeded two mm [19]. BCG was not an issue as our study included only BCG-unvaccinated contacts. In theory, exposure to non-tuberculous mycobacteria could not be excluded as a source of false positive TST results but we expect this factor to be minimal in our setting [27]. Thus, the TST and T-SPOT data allowed us to discriminate between true *Mtb*-specific responses and random variation both at the group and the individual level with fair reliability.

An additional argument in favor of *Mtb*-specificity of most borderline QFT results was based on our hypothesis that the distribution of QFT results in uninfected contacts would be symmetrical relative to zero. The observed distribution of QFT results, however, was non-symmetrical with a clear excess of results in the borderline range. In three previous studies in different settings, a similar nonsymmetrical distribution was observed [14,15,28]. This consistency supports the idea of an overlap between QFT responses in *Mtb*-infected and

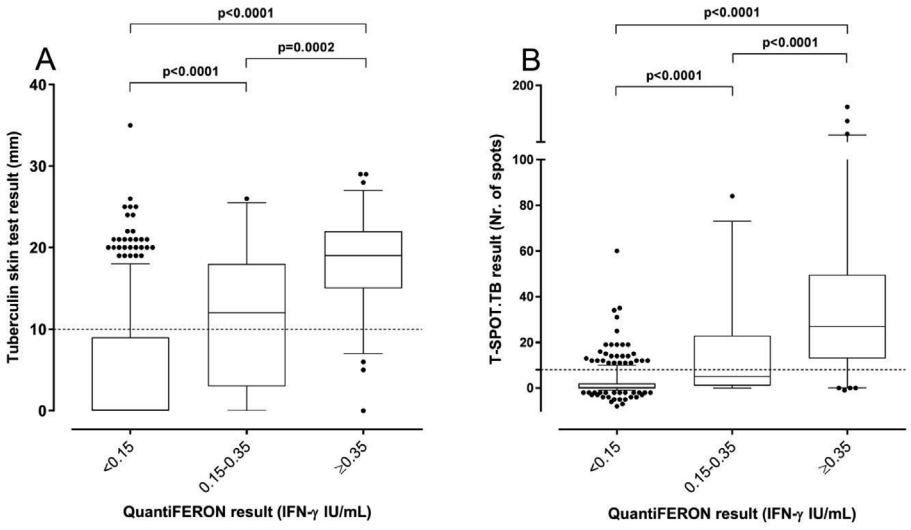

Fig. 3. Distribution of tuberculin skin test and T-SPOT results by QuantiFERON category. Tuberculin skin test results (TST) (A) and T-SPOT.*TB* (T-SPOT) (B) results by QuantiFERON-category. The upper and lower margins of each rectangle enclose the interquartile range; the middle line represents the median and the whiskers equal the 5–95% percentile range. The horizontal dotted line denotes the cut-off (being 8 spot forming units in T-SPOT and 10 mm in TST). Nonparametric comparison of two groups was done with the Mann-Whitney test, Kruskal-Wallis test was used to determine differences between the three QFT groups.

Table 2
Overview and interpretation of contacts with a borderline or low-positive QuantiFERON test result.

Category QFT	Nr.	Sex	Age (y)	TST (mm)	Cumulative shopping time (min)	Quantiferon result (IU/mL)				T-SPOT result[a] (Nr. of spots)				Interpretation[b]
						at time of investigation	at 6 mo	at 12 mo	at 24 mo	panel A (ESAT6)	panel B (CFP10)	maximum panel A/B	Test result	
Borderline (0.15–0.35 IU/mL)	1	M	54	600	0	0.15				0	0	0	neg	test variability
	2	F	47	600	0	0.15				0	0	0	neg	test variability
	3	F	60	2400	0	0.21				0	0	0	neg	test variability
	4	F	49	2400	0	0.24				0	0	0	neg	test variability
	5	M	43	1200	0	0.33				0	0	0	neg	test variability
	6	M	41	960	0	0.16				−4	1	1	neg	test variability
	7	F	43	N.A.	0	0.28				1	0	1	neg	test variability
	8	M	55	1200	6	0.22				0	**20**	**20**	**pos**	LTBI
	9	F	38	2400	6	0.18				4	**25**	**25**	**pos**	LTBI
	10	M	18	270	8	0.17				3	1	3	neg	test variability
	11	F	47	2400	8	0.31				*7*	1	*7*	*borderline*	test variability
	12	F	50	2400	**10**	0.21				4	*5*	*5*	*borderline*	LTBI
	13	M	48	2400	**10**	0.19				**14**	**18**	**18**	**pos**	LTBI
	14	F	26	2400	**11**	0.34				**21**	**9**	**21**	**pos**	LTBI
	15	M	32	N.A.	**12**	0.19				3	2	3	neg	LTBI
	16	M	33	4800	**12**	0.21				3	**33**	**33**	**pos**	LTBI
	17	M	46	2400	**13**	0.16				**19**	**25**	**25**	**pos**	LTBI
	18	M	29	120	**16**	0.15	N.A.	**0.53**	**0.61**	0	1	1	neg	LTBI
	19	F	56	15	**17**	0.19				4	3	4	neg	LTBI
	20[c]	M	46	N.A.	**17**	0.17				**18**	*5*	**18**	**pos**	LTBI
	21	M	57	270	**18**	0.15				−4	2	2	neg	LTBI
	22	F	60	1200	**18**	0.25	**1.27**	**0.67**	**2.13**	**28**	**14**	**28**	**pos**	LTBI
	23	F	43	960	**18**	0.26				**24**	**84**	**84**	**pos**	LTBI
	24	F	34	2400	**19**	0.31				3	4	4	neg	LTBI
	25	F	39	2400	**21**	0.2	0.09	0.02	N.A.	0	1	1	neg	LTBI
	26	M	57	1200	**21**	0.16				*7*	*7*	*7*	*borderline*	LTBI
	27	M	34	1200	**23**	0.22	0.32	N.A.	**0.67**	**13**	**27**	**27**	**pos**	LTBI
	28	F	37	4800	**25**	0.25				**20**	**62**	**62**	**pos**	LTBI
	29	F	38	2400	**26**	0.3				*5*	**14**	**14**	**pos**	LTBI
Low positive (0.35–0.70 IU/mL)	1	F	57	4800	0	**0.56**				−1	−1	−1	neg	test variability
	2	F	58	2400	7	**0.46**				3	3	3	neg	test variability
	3	N.A.	59	300	**12**	**0.38**				0	0	0	neg	LTBI
	4	F	28	600	**14**	**0.57**				*7*	*6*	*7*	*borderline*	LTBI
	5[d]	F	40	2400	**15**	**0.46**				**13**	**12**	**13**	**pos**	LTBI
	6	M	47	400	**15**	**0.54**				4	**14**	**14**	**pos**	LTBI
	7	M	28	2400	**15**	**0.35**	**0.65**	**0.45**	**0.43**	**15**	**15**	**15**	**pos**	LTBI
	8	F	45	2400	**15**	**0.43**				*5*	**28**	**28**	**pos**	LTBI
	9	F	55	2400	**17**	**0.45**	0.3	N.A.	0.13	*6*	**13**	**13**	**pos**	LTBI
	10	M	42	1200	**18**	**0.56**				**16**	**15**	**16**	**pos**	LTBI
	11	F	36	1200	**20**	**0.63**	N.A.	0.16	0.16	2	**9**	**9**	**pos**	LTBI
	12[d]	F	39	2400	**20**	**0.38**	N.A.	N.A.	**1.83**	**16**	**13**	**16**	**pos**	LTBI
	13	F	49	600	**20**	**0.38**	**4.09**	**10**	**1.47**	**72**	**77**	**77**	**pos**	LTBI
	14	M	54	2400	**21**	**0.39**				**35**	**25**	**35**	**pos**	LTBI
	15	F	38	N.A.	**22**	**0.66**				**13**	4	**13**	**pos**	LTBI
	16	F	23	2400	**25**	**0.37**	**3.6**	**0.85**	**1.44**	*7*	**18**	**18**	**pos**	LTBI

Abbreviations: M, male; F, female; INH, isoniazid; TST, tuberculin skin test; QFT, QuantiFERON-TB Gold In-Tube; N.A., not acquired; LTBI, latent tuberculosis infection.

All positive test results for TST (≥10 mm), Quantiferon (≥ 0.35 IU/mL) or T-SPOT (according to the more recent cut-off: ≥8 spots) are indicated in bold.
Borderline T-SPOT results (5 to 7 spots) are indicated in italic.

[a] T-SPOT result at time of contact investigation, available later T-SPOT results are not shown (T-SPOT follow up results of subjects 18, 22, 25 and 27 are shown in Supplementary Fig. 1).

[b] Contacts with at least a positive TST (≥10 mm) and/or a positive T-SPOT (≥8 spots) were interpreted as having a high likelihood of infection with *M. tuberculosis*.

[c] Immunocompromised.

[d] Developed active tuberculosis

-uninfected individuals. The relative proportion of *Mtb*-specific responses among individuals with a borderline QFT result will vary by setting, being lower in low-risk populations such as in serial screening of healthcare workers in a low incidence setting but higher in case of screening of close contacts or other high risk groups.

Nevertheless, one third of borderline results (9/29) and 12% (2/16) of low-positive QFT results could not be accounted for by TST or T-SPOT and most likely reflected test variability. Underlying causes of these responses could be technical or immunological such as cross-reactivity to *Mycobacterium marinum* or *M. kansasii* [29,30] or immunomodulation by microbial products which may induce increased IFN-γ production and even false-positive results [31]. The proportion of contacts of cohort 1 with an assumed false positive QFT result was just 2/785 (0.25%) indicating that this is a quantitatively small problem.

While our study showed that borderline QFT results are mostly *Mtb*-specific, the question is whether this finding is of clinical relevance. In a cohort such as described, mostly consisting of immunocompetent contacts, the finding of borderline QFN results is probably of limited

Table 3
Contacts diagnosed with active tuberculosis during 12-years follow-up.

Case	Age (y)	Sex	TST (mm)	QFT (IFN-γ IU/mL)	T-SPOT result	T-SPOT test result	Follow-up/treatment	Interval (months)	TB Type	Diagnosis based on	TB cluster
1	31	F	27	18.13	210	pos	N.A.	0[a]	pulmonary	chest X-ray	
2	49	M	17	2.15	81	pos	N.A.	0[a]	pulmonary	culture positive	365
3	59	M	15	9.87	137	pos	referred to pulmonologist for analysis	2.8	pulmonary	PCR on lung biopsy positive	
4	40	F	15	0.46	13	pos	chest X-ray (2 years)	3.8	pulmonary	culture positive	365
5	39	F	20	0.38	16	pos	INH prophylaxis[b]	71.0	extra-pulmonary	clinical: TB-uveitis	
6	53	M	16	2.00	50	pos	chest X-ray (2 years)	131.4	pulmonary	culture positive	365
7	30	F	0	−0.02	−1	neg	no follow up	3.3	pulmonary	culture positive	16

Abbreviations: TST, tuberculin skin test; QFT, QuantiFERON-TB Gold In-Tube; F, female; M, male; INH, isoniazid; N.A., not applicable.
[a] These two patients were diagnosed with active TB during the initial contact screening following abnormalities on chest X-ray.
[b] Prophylaxis discontinued due to elevated liver enzyme.

clinical significance. While lowering the cut-off has been considered and discarded for routine use, we think that a borderline QFT result in patients who are or will be immunocompromised should be regarded as a significant finding, justifying treatment for LTBI even in the absence of absolute proof. We recently diagnosed a patient with disseminated tuberculosis after negative QFT screening before starting infliximab, but in retrospect the QFT result was 0.22 IU/mL (manuscript submitted). Although none of the individuals with a borderline QFT result in our study cohort developed active TB, two of the seven patients who developed active TB during follow up had QFT results just above the regular cut-off level indicating that a low positive QFT response is relevant. In another study, 19/1664 (1.1%) of individuals with a borderline (defined as 0.2–0.35 IU/mL) compared to 77/28976 (0.3%) in those with a low-negative results and 70/1992 (3.5%) with a low-positive QFT result (defined as 0.35–0.99 IU/mL) developed active TB, most of whom within three months [28]. QFT results near the cut-off thus are not a 'safe zone' regarding the risk of active TB, although the risk seems to be directly related to the quantitative QFT result [3,14].

Follow-up individual IGRA results in our study were variable, as previously published [20]. The observed conversion rate was similar to that of a previous study in which one-fifth of results just below the cut-off converted [32]. Retesting may give a different and more 'convenient' test result but our follow up data show that QFT reversion does not exclude infection with *Mtb*, although it probably reflects a lower risk of progression. Based on just one borderline QFT result it is not possible to make a reliable interpretation without additional tests, which are justified if it is important to obtain a higher level of certainty. Borderline QFT results just below the cut-off are found in just a small minority of tested individuals, varying between 2.4 and 4.2% [15,28,32]. In practice, other information such as the clinical setting, a history of exposure or signs of LTBI on a chest X-ray contributes to the evaluation and clinical decision making. Banaei and Pai have proposed to define a formal borderline range of QFT results [33]. However, if it is clinically important, the TST and T-SPOT may help to obtain more certainty unless the patient is immunocompromised and immune-based tests are therefore less sensitive [17,34–36].

Among the limitations of our study are the retrospective design and the low number of borderline and low-positive results. The number of contacts who developed active tuberculosis was low and the study therefore lacked power to evaluate the risk among contacts with a borderline QFT results. Due to the study protocol it was not possible to retrieve the treatment decision for the study participants, which affects the individual risk of development of active TB. The main strength of our study was the availability of TST, QFT as well as T-SPOT results in a large homogeneous cohort of contacts, allowing the distinction between *Mtb* infection and test variability. We think that future studies of borderline QFT results will gain persuasiveness if the distinction between *Mtb* infection and test variability will be made based on additional TST and/or T-SPOT data. IFN-γ being just one cytokine, the potential of QFT might be augmented by measurement of alternative cytokines or other biomarkers [37–39].

Since 2015, the QuantiFERON-TB Gold Plus (QFT Plus) has replaced the previous In-Tube version. QFT Plus uses peptides of the same antigens ESAT-6 and CFP-10 but lacks TB7.7, while a second antigen-specific tube in addition contains shorter peptides of CFP-10 to stimulate CD8 T-cells [40]. The test was designed for higher sensitivity for active TB or recent infection but, thus far, the sensitivity of QFT Plus was not different from that of QFT [41–43]. A formal analysis of borderline QFT Plus result has not yet been published. However, a study among low risk health-care workers found that most participants with a discordance between QFT and the TB1 and/or TB2 tube of QFT Plus had a response between 0.2 and 0.7 IU/mL in one or both assays, thus including borderline responses [44]. This is another argument in favor of the idea that borderline results are mostly true antigen-specific responses, with the positive result in the other format as validation.

5. Conclusion

In conclusion, two-third of borderline QFT results and 88% of low-positive QFT results in tuberculosis contacts were *Mtb*-specific as corroborated by TST and T-SPOT data. Active tuberculosis was diagnosed in 12.5% of contacts with a low-positive QFT. We think that future studies of borderline QFT responses should address the discrimination between *Mtb*-specific responses and test variability. The clinical relevance, however, will be most distinct in patients who are or will be immunocompromised.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tube.2018.06.002.

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