Proof of concept that most borderline Quantiferon results are true antigen-specific responses

To the Editor:

Interferon-γ release assays (IGRAs) such as Quantiferon (QFT) that detect T-cell responses to antigens specific for *Mycobacterium tuberculosis* (MTB) have superior specificity to the tuberculin skin test (TST). While IGRAs are technically robust and the test result is a simple positive or negative, the interpretation nevertheless requires thorough understanding of the test's characteristics [1]. An area of debate is that of "borderline" results near the cut-off value, mentioned first in the setting of serial screening of health care workers [2]. While different authors used different ranges of test results under this denominator, borderline results were generally attributed to random assay variability [3, 4]. In reproducibility studies, conversions and reversions were often seen around the manufacturer-recommended cut-off, with the advice to "interpret such results cautiously" [5, 6].

In clinical practice, we observed borderline IGRA results predominantly in patients with evidence of tuberculosis (TB) infection, suggesting that borderline results reflected antigen-specific responses. The aims of this study were to investigate whether borderline QFT results below the regular cut-off occur in excess of random test variability and to compare patients with negative, borderline or positive results with regard to all IGRA-independent evidence of TB infection.

This proof-of-concept retrospective study with anonymous data collection was evaluated by the Medical Ethics Committee of Leiden University Medical Center (LUMC), a tertiary care teaching hospital, and waived from the requirement of informed consent (protocol G16.105). Quantiferon TB Gold in-tube® has been used at LUMC in routine practice since 2010 for clinical and outpatient departments, the occupational health service (OHS) and regional municipal health service (MHS). QFT was performed according to the manufacturer's instructions.

First, the distribution of all QFT results obtained between January 2010 and September 2016 was analysed to assess excess borderline results. Of 2985 valid QFT results, 2496 (83.6%) were negative and 489 (16.4%) were positive. There was a three-fold excess of borderline test results: 128 (5.1%) were $\geqslant$0.15 and <0.35 IU·mL^{-1}; this range defining borderline results in this study, compared to 42 (1.7%) in the corresponding inverse range between −0.35 and −0.15 IU·mL^{-1} ($p<0.0001$), while test results between −0.15 and 0.15 IU·mL^{-1} were distributed perfectly symmetrical relative to zero.

Secondly, patients with borderline results were compared to all patients with a low positive result ($\geqslant$0.35 and <0.7 IU·mL^{-1}) and a random selection (blinded selection by an independent person) of patients with low negative (<0.15 IU·mL^{-1}, n=90) or high positive ($\geqslant$0.70 IU·mL^{-1}, n=30) responses.

Clinical data were retrieved from the electronic dossiers between October 2016 and June 2017.

All of the patient population originated from the hospital setting (70%), MHS (20%) or OHS (10%). The indication for QFT was evaluation of possible active TB in two-third of patients in the hospital setting, while it was mostly detection of latent TB infection in the other two settings.

Results of the comparison between patients with low negative, borderline, and low or high positive results is shown in table 1. With increasing QFT results, there was a gradual increase in the proportion of immigrants or other risk groups, travel to TB-endemic countries, reported TB contacts and TST size.

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TABLE 1 Comparison between individuals by Quantiferon (QFT) test results

	QFT low negative (<0.15 IU·mL^{-1})	QFT borderline (0.15–0.35 IU·mL^{-1})	QFT low positive (0.35–0.70 IU·mL^{-1})	QFT high positive (⩾0.70 IU·mL^{-1})	All	p-value
Subjects n	90	107	66	30	293	
Age years	46.1±18.7	48.0±19.9	49.8±19.5	43.9±17.9	47.4±19.3	0.47
Males	41/90 (45.6)	52/107 (48.6)	29/66 (43.9)	15/30 (50.0)	137/293 (46.8)	0.91
Immigrant (first generation)	30/90 (33.3)	54/107 (50.5)	32/66 (48.5)	16/30 (53.3)	132/293 (45.1)	0.06
Incidence in country of origin ×10^5 per year	54.9±57.0	74.7±77.0	135.3±129.0	111.9±94.4	89.4±95.0	0.002
Travel to TB-endemic country	25/90 (27.8)	38/92 (41.3)	29/57 (50.9)	17/26 (65.4)	109/265 (41.1)	0.002
Risk group (any)	19/90 (21.1)	36/107 (33.6)	24/66 (36.4)	11/30 (36.7)	90/293 (30.7)	0.11
Comorbidities (any)	39/90 (43.3)	42/107 (39.3)	31/66 (47.0)	12/30 (40.0)	124/293 (42.3)	0.78
Immunocompromised (any)	17/90 (18.9)	23/107 (21.5)	16/66 (24.2)	5/30 (16.7)	61/293 (20.8)	0.80
Primary immunodeficiency	0/90 (0)	1/107 (0.9)	1/66 (1.5)	0/30 (0)	2/293 (0.7)	0.78
HIV	2/90 (2.2)	4/107 (3.7)	1/66 (1.5)	0/30 (0)	7/293 (2.4)	0.77
Glucocorticoids	12/90 (13.3)	12/107 (11.2)	11/66 (16.7)	4/30 (13.3)	39/293 (13.3)	0.77
Methotrexate	2/90 (2.2)	6/107 (5.6)	3/66 (4.5)	1/30 (3.3)	12/293 (4.1)	0.70
TNF-antagonist	1/90 (1.1)	1/107 (0.9)	0/66	0/30	2/293 (0.7)	1.0
Other	6/90 (6.7)	7/107 (6.5)	5/66 (7.6)	2/30 (6.7)	20/293 (6.8)	0.98
Reported exposure	17/90 (18.9)	28/107 (26.2)	17/66 (25.8)	9/30 (30.0)	71/293 (24.2)	0.52
History of TB infection	2/90 (2.2)	7/107 (6.5)	4/66 (6.1)	3/30 (10.0)	16/293 (5.5)	0.26
Active TB	1/90 (1.1)	3/107 (2.8)	1/66 (1.5)	1/30 (3.3)	6/293 (2.0)	0.72
LTBI	1/90 (1.1)	4/107 (3.7)	3/66 (4.5)	2/30 (6.7)	10/293 (3.4)	0.33
BCG vaccinated	33/90 (36.7)	47/107 (43.9)	27/66 (40.9)	17.30 (56.7)	124/293 (42.3)	0.28
<1 year of age	26/33 (78.8)	36/47 (76.6)	22/27 (81.5)	15/17 (88.2)	99/124 (79.8)	
⩾1 year of age	7/33 (21.2)	11/47 (23.4)	5/27 (18.5)	2/17 (11.8)	25/124 (20.2)	
At least one TST available	47/90 (52.2)	60/107 (56.1)	41/66 (62.1)	24/30 (80.0)	172/293 (58.7)	0.054
At time of QFT	39/90 (43.3)	48/107 (44.9)	34/66 (51.5)	21/30 (70.0)	141/293 (48.1)	
Past or later	15/90 (16.7)	27/107 (25.2)	20/66 (30.3)	8/30 (26.7)	70/293 (23.9)	
TST result at time of QFT mm	9.1±9.3	12.0±8.5	11.7±8.5	18.8±6.2	12.1±8.9	0.0007
TST highest of available TST mm	9.5±8.8	12.1±7.8	12.9±9.9	18.7±7.4	12.5±9.0	0.0005
TST highest value[#]						
Positive (⩾10 mm) at least once	27/47 (57.4)	42/60 (70.0)	30/41 (73.2)	22/24 (91.7)	121/172 (70.3)	0.027
Positive (⩾15 mm) at least once	15/47 (31.9)	20/60 (33.3)	18/41 (43.9)	18/24 (75.0)	71/172 (41.3)	0.002
Positive (⩾20 mm) at least once	8/47 (17.0)	10/60 (16.7)	10/41 (24.4)	14/24 (58.3)	42/172 (24.4)	0.0004
TST category (highest value)						0.007
0 to 4 mm	18/47 (38.3)	10/60 (16.7)	8/41 (19.5)	1/24 (4.2)	37/172 (21.5)	
5 to 9 mm	2/47 (4.3)	8/60 (13.3)	3/41 (7.3)	1/24 (4.2)	14/172 (8.1)	
10 to 14 mm	12/47 (25.5)	22/60 (36.7)	12/41 (29.3)	4/24 (16.7)	50/172 (29.1)	
⩾15 mm	15/47 (31.9)	20/60 (33.3)	18/41 (43.9)	18/24 (75.0)	71/172 (41.3)	
QFT test results IU·mL^{-1}						
NIL tube	0.07±0.15	0.12±0.5	0.26±1.0	0.23±0.6	0.15±0.6	0.16
PHA tube	7.07±2.1	7.61±1.2	7.34±1.8	7.54±1.4	7.37±1.7	0.14
TB minus NIL	0.00±0.1	0.23±0.1	0.50±0.1	3.76±3.08	0.58±1.5	<0.0001
Reason QFT						0.31
Suspected active TB	40/90 (44.4)	55/107 (51.4)	31/66 (47.0)	20/30 (66.7)	137/293 (46.8)	
Screening LTBI	50/90 (55.6)	52/107 (48.6)	35/66 (53.0)	10/30 (33.3)	156/293 (53.2)	
Microbiological findings of active TB (any)						
AFB positive	0/11 (0)	1/22 (4.5)	1/17 (5.9)	0/10 (0)	2/60 (3.3)	
PCR positive	0/7 (0)	1/14 (7.1)	2/12 (16.7)	0/5 (0)	3/38 (7.9)	
Culture positive	0/12 (0)	1/23 (4.3)	1/19 (5.3)	0/11 (0)	2/65 (3.1)	
Histology with granulomas	2/18 (11.1)[¶]	3/33 (9.1)[+]	1/19 (5.3)	0/6 (0)	6/76 (7.9)	
Final clinical diagnosis						<0.0001
No TB infection	83/90 (92.2)	78/107 (72.9)	5/66 (7.6)	0/30 (0)	166/293 (56.7)	
LTBI	7/90 (7.8)	24/107 (22.4)	56/66 (84.4)	26/30 (86.7)	113/293 (38.6)	
Active TB	0/90 (0)	2/107 (1.9)	5/66 (7.6)	4/30 (13.3)	11/293 (3.8)	
TB infection, not sure active or LTBI	0/90 (0)	3/107 (2.8)	0/66 (0)	0/30 (0)	3/293 (1.0)	

Continuous variables are displayed as mean±SD and categorical values are displayed as numerator over denominator (%). Differences between categorical data were evaluated with Chi-squared tests or Fishers exact probability test if appropriate. Continuous data were compared between groups using one-way ANOVA or nonparametric with Mann–Whitney U- or Kruskal–Wallis tests as appropriate. Using two-sided testing, differences were considered significant at p<0.05. Statistical analysis was performed using IBM SPSS Statistics version 23 and GraphPad Prism 7. [#]: in case of multiple tuberculin skin tests (TST's), the highest value was used; [¶]: the final diagnoses in these two patients were sarcoidosis and tertiary syphilis; [+]: the final diagnoses in these three patients were grey zone lymphoma, sarcoidosis as well as latent tuberculosis infection (LTBI) and peripheral multifocal chorioretinitis. TB: tuberculosis; TNF: tumour necrosis factor; BCG: bacille Calmette–Guérin; AFB: acid-fast bacilli.

Roughly one-fifth of all patients had an impaired immune status, which was independent of the QFT result. A history of active or latent TB was present in 16/293 (5.5%) individuals, seven of whom had a borderline QFT result. The proportion of patients with a positive TST result (⩾10 mm) varied from 57.4% to 91.7% in a gradient along the QFT categories. The final clinical diagnosis was not associated with the TST result (data not shown) but strongly associated with the QFT result.

These findings support that borderline QFT results mostly reflected MTB-specific responses rather than random assay variability. First, borderline QFT results below the cut-off occurred in three-fold excess over the number expected based on random assay variability. Secondly, patients with borderline results were similar to those with positive results with regard to all other available evidence of TB infection, except that TST size was larger in the subgroup with the highest QFT results. There was a gradient of increasing prevalence of risk factors and indicators of TB infection in association with the quantitative QFT result, with near identical findings in patients with borderline or low positive results. This "dose-response effect" suggests a causal relation. However, risk factors and indicators of TB infection, such as high TST responses, were present in a lower but still considerable proportion of patients with a low negative QFT result as well. This reflects that QFT is used predominantly in patients with a high *a priori* risk of infection and underscores that a negative QFT result does not rule out past or present TB infection. A previous study showed that prolonged incubation may increase the sensitivity of IGRA in patients with past TB infection [7]. Our study reflects the use of QFT in daily practice, with indications varying from patients without a diagnosis to confirmation in those with probable TB infection.

The limited specificity of the TST plus the lack of a validated gold standard has led to the proposition that latent TB infection can be defined as a persisting immunological response to MTB with IGRA as a surrogate marker [8]. That proposition would preclude analysis of the significance of borderline QFT results because these are formally negative. While this seems a deadlock, characteristics such as a documented history of TB infection, a TST result ⩾15 mm, a TST result ⩾10 mm in bacillus Calmette–Guérin-unvaccinated persons or vaccinated in the first year of life, or microbiological evidence will generally be agreed upon to indicate infection with MTB. In our study, such "convincing" non-IGRA parameters were found in high frequency in patients with borderline results, indicating past or present TB infection. This highlights that all available information should be taken into account regarding evaluation of TB infection.

Among the limitations of our study are the heterogeneous population of limited size, with few patients having microbiologically proven active TB. Further research should include larger homogenous groups including follow-up to study the associated risk of active TB. Comparing QFT with ELISPOT-based IGRA, for which a borderline zone for the interpretation of results is already used [9], will be especially interesting.

The practical implications of our findings depend on the risk of active TB in persons with borderline QFT results. If our findings can be confirmed, results near the cut-off could be transformed from a grey zone of uncertainty into strata characterised by a different cut-off depending on the risk of TB, analogous to the graded cut-off for the interpretation of the TST at 5, 10 or 15 mm depending on setting and immune status [10]. More studies are needed to determine cut-off values which optimise sensitivity and specificity by setting.

Of note, Quantiferon TB Gold PLUS® was recently introduced which includes a tube with additional CD8-peptides [11]; its sensitivity was comparable to that of QFT Gold in-tube [12–15]. It remains to be determined whether the formats differ with regard to detection or prediction of active TB.

In conclusion, there was a significant excess of borderline QFT results over random variability and an association of borderline results with relevant risk factors and/or evidence of TB infection. These findings justify further research using the quantitative QFT test results.

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