Cellular Immune responses during latent tuberculosis : immunodiagnosis and correlates of protection

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Chapter 5

Comparison of two interferon-gamma assays and tuberculin skin test for tracing TB contacts

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ABSTRACT

Background

The tuberculin skin test (TST) has low specificity. QuantiFERON®-TB Gold (QFT-G) and T-SPOT™.*TB* are based on interferon-γ responses to *Mycobacterium tuberculosis*-specific antigens. A novel in-tube format of QFT-G (QFT-GIT) offers logistical advantages.

Objective

To compare TST, QFT-GIT and T-SPOT.*TB* in Bacille Calmette-Guérin unvaccinated contacts and correlate results with measures of recent exposure.

Methods

When a supermarket employee with smear-positive TB had infected most close contacts, a contact investigation among >20,000 customers was performed. We recruited subjects randomly on the day of TST administration (N=469) and subjects with TST > 0 mm on the day of TST reading (N=316). QFT-GIT and T-SPOT.*TB* were performed. Demographic data and measures of exposure were collected. TST results were analyzed at cut-off 10 or 15 mm. Blood tests were interpreted following the manufacturer's criteria and by varying cut-off levels.

Results

Among 785 study participants, TST results were associated with age while positive interferon-γ responses were significantly associated with cumulative shopping time, most markedly for QFT-GIT. Among participants with a TST ≥ 15 mm, sensitivity of QFT-GIT and T-SPOT.*TB* was 42.2% and 51.3%, respectively. Inter-assay agreement was 89.6 % (κ= 0.59). By varying cut-off values, agreement between the interferon-γ assays was optimal at 93.6 % (κ= 0.71) using a cut-off of 0.20 IU/mL for QFT-GIT and 13 spots for T-SPOT.*TB*.

Conclusions

Blood test results were associated with exposure, while the TST was not. A possible lack of sensitivity of interferon-γ assays in detecting individuals with TST ≥15mm, despite negative BCG vaccination status, warrants further investigation into alternative cut-off values.

INTRODUCTION

Most cases of tuberculosis (TB) disease arise as reactivation TB in latently infected individuals. One-third of the world population is thought to harbour latent TB infection (LTBI) [1]. Five to 15% of immunocompetent persons with LTBI will ever develop TB disease. In countries with a low incidence of TB, the tracing and targeted treatment of individuals with LTBI constitutes a major pillar of TB control [2;3]. Until recently, the detection of LTBI was based exclusively on tuberculin skin testing that has low specificity following vaccination with Bacillus Calmette-Guérin (BCG) or exposure to environmental mycobacteria, due to cross-reactive immune responses. The treatment of LTBI is effective when treatment is sustained [4], However, effectiveness tends to be decreased when compliance is low [5]. These facts underscore the need for more accurate methods for detection of LTBI and targeting treatment.

The search for improved tools for detection of LTBI has led to the development of in-vitro assays based on interferon-gamma (IFN-γ) production in response to ESAT-6 and CFP-10, antigens that are highly specific for *Mycobacterium tuberculosis* [6-8]. Various formats of such IFN-γ release assays (IGRA) showed a high sensitivity and nearly complete specificity [9-14]. Two IGRA have thus far been marketed. QuantiFERON®-TB Gold (QFT-G, Cellestis, Carnegy, Australia) is a whole-blood assay using enzyme-linked immunosorbent assay (ELISA) for detection of IFN-γ responses [13;15;16]. It has been approved for use in Europe and received approval from the U.S. Food and Drug Administration in 2005. Recent guidelines that were issued by the Centers for Disease Control (CDC) recommend that QFT-G may be used in all circumstances in which the TST is currently used [17]. A novel in-tube version of QFT-G that contains a third *M. tuberculosis*-specific antigen (TB7.7) has been approved for use in Europe (further referred to as QFT-GIT). T-SPOT®.*TB* (Oxford Immunotec, Oxford, UK) is based on the enzyme-linked immunospot technique (ELISPOT) and has been marketed in Europe. Both tests are included in the UK guidelines issued by The National Institute for Health and Clinical Excellence, recommending a two-stage strategy of TST testing followed by an IGRA to confirm a positive TST result although there are no studies that have demonstrated the validity of this approach [18]. A recent review reported comparable specificity of QFT-GIT and T-SPOT.*TB* [19]. The sensitivity of these tests for detection of TB disease varied between studies [1;13;20]. With regard to LTBI, test results in low incidence settings were significantly correlated with measures of exposure while the TST was not [10;12;16;21-23]. However, the sensitivity for detection of presumed LTBI varied widely between studies [9;10;12;16;20-24]. Differences in study populations plus the lack of a gold standard for LTBI impeded the interpretation of these differences.

Recently, two publications reported on a direct comparison between the QFT-G and T-SPOT.*TB* [25;26]. In the first study among a heterogeneous population of 393 consecutive

hospitalized patients with suspected active TB disease or LTBI, including many immune-suppressed patients, T-SPOT.*TB* produced significantly more positive results and less indeterminate results than did QFT-G [25]. The second study, among 218 subjects suspected of active TB in Korea, showed higher sensitivity of T-SPOT.*TB* compared to TST and QFT-G, while QFT-G showed superior specificity over TST and T-SPOT.*TB* [26]. The clinical relevance of discordant blood test results was not known. However, the agreement between both blood tests was higher than between the TST and either assay. To assess the diagnostic value of these assays in various clinical-epidemiological settings further studies are needed. In the present study, we aimed to compare QFT-GIT and T-SPOT.*TB* results in relation to TST responses and measures of exposure among BCG unvaccinated and predominantly immune-competent contacts in a large-scale contact investigation in a population with an estimated background prevalence of LTBI in these age groups of 1.4% [27]. Part of the data was presented at the American Thoracic Society 2006 conference in San Diego, California [28, 29].

SUBJECTS AND METHODS

Setting

In November 2004, a 25-year old male, Dutch-born supermarket employee in the city of Zeist (approx. 60,000 inhabitants) in The Netherlands was diagnosed with sputum-positive cavitary TB. From 19 November to 2 December, 12 close contacts including his family and close friends (median age 25 years, range 13-80) were examined using TST and CXR. Of these 12, three (25%) were diagnosed with active TB and seven (58%) with LTBI. Subsequently, 80 occupational contacts were examined from 6 to 9 December. TBI was diagnosed in 39/67 (58%) current and in 8/13 (61%) former supermarket employees. One of the current employees had developed TB disease and the *Mycobacterium tuberculosis* isolate later turned out to have the same IS6110 restriction fragment length polymorphism (RFLP) pattern as the index case. [B.Koster, unpublished data]. From positive TST results in colleagues who had stopped working in that supermarket at different time points, it was deduced that the infectious period of the index case had lasted from February 2004 until identification in mid-November 2004. During this period, he had performed varying tasks in the supermarket that involved contact with customers. In view of a high rate of transmission, it was decided to investigate all customers who had visited the supermarket during the infectious period. The interval between last possible contact with the source patient and TST placement and blood sampling was 10 weeks.

A TST was offered to all customers except persons born before 1945, BCG vaccinated persons or those with a history of a positive TST or TB disease, following Dutch guidelines.

An anteroposterior CXR was performed on all individuals born before 1945 and on individuals with BCG vaccination, or with a history of TB or positive TST.

Study design

Nested within this large-scale contact investigation we aimed to recruit 500 subjects on the two days of TST administration by random selection (pre-TST inclusion group). In order to include sufficient numbers of subjects with probable LTBI, we aimed to also include 500 subjects on the reading days who had a TST result ≥ 1 mm (post-TST inclusion group). In the pre-TST group and post-TST group, blood was collected, respectively immediately after, and 72 ± 8 hours after the TST was administered. Written informed consent was obtained from all participants. The Ethical Review Board of Hospital Diakonessenhuis Utrecht/Zeist, The Netherlands approved the study (protocol nr. 2004.23).

Inclusion criteria

Eligible for inclusion were BCG unvaccinated subjects aged ≥ 17 years who had visited the supermarket at least once monthly within the period of infectiousness of the index case and in whom a TST was indicated.

Questionnaire

Demographic data and data reflecting the amount of exposure were obtained by questionnaire. The following demographic data were were included in the questionnaire: age, sex, country of origin (TB-endemic country with reported incidence of >50/100,000 new TB cases per year), BCG vaccination status, use of immunosuppressive drugs, work with risk groups for TB, travel to TB endemic regions, previous TST and previous TST results. Exposure at the supermarket was measured as frequency of visits to the supermarket in question (categories: ≤ 1 ×/month, > 1×/month and < 1×/week, 1 ×/week or > 1×/week), contact period (calculated as the number of months between the first and last supermarket visit within the presumed contagious period of the index case), estimated average duration of a supermarket visit (categories: <5 min, 5-15 min, 15-30 min, 30-60 min, >60 min per visit). From these three parameters, the cumulative shopping time was calculated by multiplication of the contact period, frequency of visits per month and average duration of a visit. For reasons of confidentiality it could not be assessed whether face to face exposure to the index case had actually occurred.

Tuberculin skin testing

TST was performed according to the Mantoux method using two TU of tuberculin RT-23 (Statens Serum Institute, Copenhagen, Denmark) was used according to standard protocol. This tuberculin is bioequivalent to the international standard of 5TU PPD-S. [30]. TST were administered and read by experienced staff from the Municipal Health Authority

(MHA). Indurations were measured at 72 ± 8 h by two independent readers, the average was used as final result. In case of a discrepancy exceeding 2 mm, a third person made the final reading.

Blood sampling and laboratory procedures

In total, 10 mL of blood was collected in three tubes; one 8 mL cell preparation tube (Vacutainer citrate CPT™, BD, Franklin Lanes, NJ, USA) for the isolation of peripheral blood mononuclear cells (PBMC) for use in T-SPOT.*TB*, and two heparinized tubes of 1 mL each for QFT-GIT.

QFT-GIT

In this study, QFT-GIT was used following the manufacturers instructions (www.cellestis.com/IRM/contentAU/gold/InTube_PackageInsert.pdf). This novel format provided two heparinized blood tubes per study subject, one containing a mixture of peptides of the *M. tuberculosis*-specific antigens ESAT-6, CFP-10 and TB7.7, and one negative control tube without antigens. The test format thus did not include a positive control tube, as was approved by the European authorities. After this study, in the course of 2005, positive control tubes have become available for use in settings where false-negative test results can be expected (hospital setting, immune-compromised persons). Test results were obtained using the software provided by the manufacturer. A positive result was defined as IFN-γ concentration in antigen stimulated tube minus that in the negative control tube ≥ 0.35 IU/mL.

T-Spot.TB

T-SPOT.*TB* (Oxford Immunotec LTD, Oxford, UK) was performed assisted by two technicians from Oxford Immunotec, following the manufacturers instructions. The number of spots was scored visually using a magnifying glass by two independent observers who did not have knowledge of TST results. In case of discrepancies, a third observer made the decisive spot count. Interpretation of results was according to the criteria defined by the manufacturer (www.oxfordimmunotec.com/downloads/PI.200 UK.pdf). A positive result was defined as ≥ 6 spots in either the ESAT-6 or the CFP-10 panel after subtracting the number of spots found in the negative control panel, where the negative control has 0 – 5 spots. In case ≥ 6 spots were seen the negative control panel, the ESAT-6 or the CFP-10 panel had to contain at least twice the number of spots found in the negative control panel to obtain a positive result.

Statistical Analysis

Statistical analyses were performed using SPSS version 11.5.0 (SPSS Benelux, Gorinchem, The Netherlands) and Stata v8 (Stata Corp, College Station, Tx). Proportions were

compared by the Pearson's chi-square test or Fisher's exact test as appropriate. Associations between test result and exposure were assessed by uni- and multivariate case-control analysis using logistic regression. In order to make maximum use of records with complete data, we combined the pre- and post-TST inclusion groups, including as cases all study subjects with a positive test result, and as controls all study subjects with a negative test result. Since this approach might introduce selection bias, we checked its validity in two ways. First, in the multivariate analysis, we adjusted for inclusion group, and assessed interactions between inclusion group and other variables in the model. Second, we compared the results of our univariate analyses with those of restricted analyses in which cases were only selected from subjects included post-TST and the subjects included pre-TST served as controls. This was done separately after in- and excluding subjects with a positive test result from the control group.

Analyses of associations between test result and exposure were restricted to subjects with complete data on exposure in the supermarket. The assumption of linearity was checked by plotting the log odds and by comparing model likelihoods with categorical and the scale variables using the LR test. Concordance between test results was assessed using κ coefficients. P values < 0.05 were considered significant. All reported P values were two-sided. TST results were analyzed in mm, as categorical value (Cat. 0: 0 mm; Cat. 1: 1-4 mm; Cat. 2: 5-9 mm; Cat. 3: 10-14 mm; Cat. 4: ≥ 15 mm) or as binary value using ≥ 15 mm as cut-off for a positive response following Dutch guidelines [31]. For the present study data were also analyzed using ≥ 5 mm and ≥ 10 mm as cut-offs.

RESULTS

Characteristics of the study population

Between January 31st and February 4th 2005, a total of 15,515 tuberculin skin tests were administered and 14,128 (92%) were read at 72 ± 8 h. In the present study, 878 subjects gave informed consent. Both a blood sample and the TST result were available for 785 unvaccinated subjects (Figure 1). Of 31 subjects with two blood samples obtained, only IGRA results of the first blood sample were used for the analyses.

Characteristics that were observed more frequently in subjects who were included in the post-TST group were origin from a TB-endemic country, a history of occupational exposure and travel to TB endemic regions (data not shown). We found no significant interaction in any of the models between day of inclusion and age or cumulative shopping time. For the remainder of the analysis, both groups were combined. Although it is tempting to extrapolate the results to the whole population screened by the MHA we caution against it because we selected subjects with any kind of induration in the post TST group and we excluded participants <17 years.

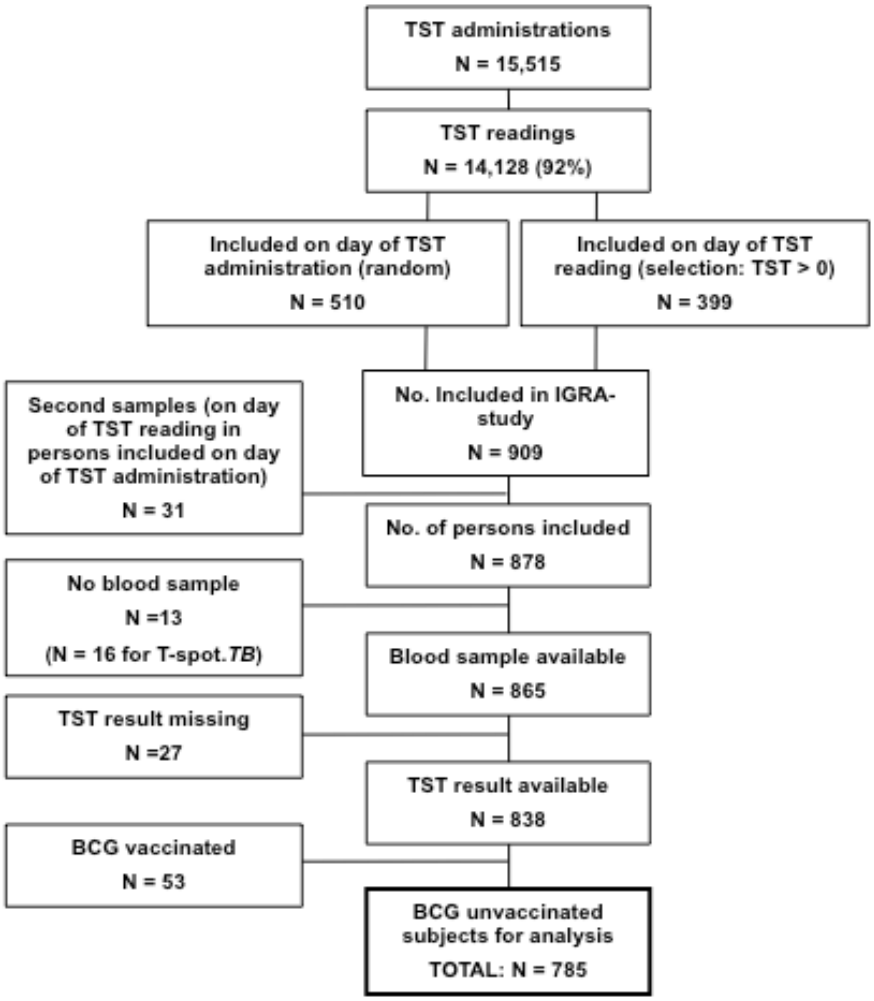

Figure 1. Flow diagram of study population.

TST results

Of 469 persons included in the pre-TST group, 90.6%, 1.3%, 1.9%, 1.9% and 4.3% were in TST category 0, 1, 2, 3 and 4, respectively. These values were not significantly different from the distribution of TST results among all 14,128 individuals of whom TST results were read in the complete contact investigation [B.Koster, unpublished data]. The corresponding percentages in the post-TST group (N = 316) were 2.8%, 2.8%, 21.1%, 28.5% and 44.6%. The distribution of TST results is shown in Figure 2A.

Complete data on exposure were available for 712 subjects. Age was the only characteristic associated with a TST result ≥ 15 mm, (OR for a positive TST result per step

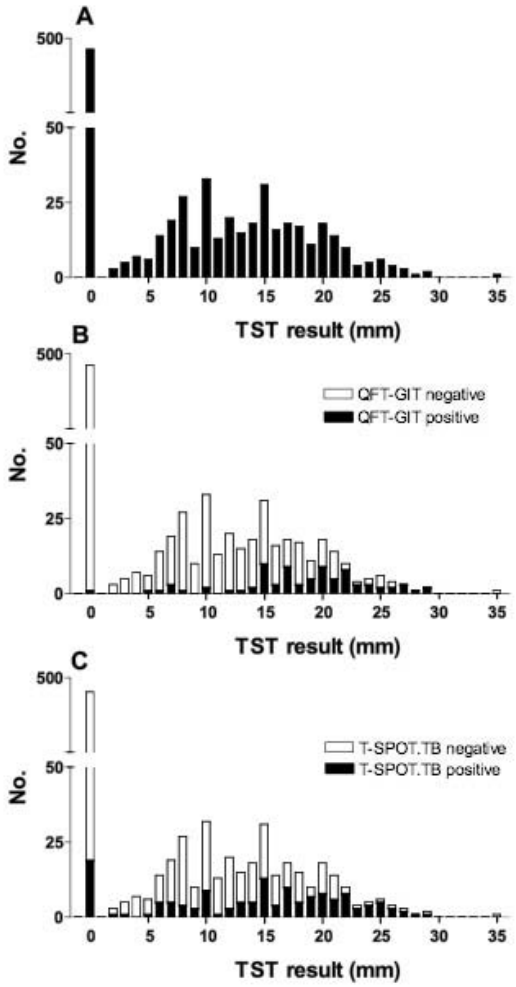

Figure 2. Distribution of TST results.

A. Distribution of all 785 TST results.

B. Distribution of positive QFT-GIT results in relation to TST result among 785 participants

C. Distribution of positive T-SPOT.*TB* results in relation to TST result among 759 participants (there were 3 missing blood samples and 23 indeterminate test results).

increase in 10-year age category: 1.40, 95% CI: 1.13-1.74, P = 0.002, Table 1). TST results were not associated with any measure of exposure to the index case at the supermarket (Table 1). When 10 mm was applied as the cut-off value for a positive TST (Table 1), we observed a similar association with age (OR 1.34, 95% CI: 1.08 -1.66, p=0.008) and no association with any measure of exposure. With a cut-off of 5 mm findings were similar (see Online-Only Repository), except for a significantly higher prevalence of a TST ≥ 5

Table 1. UNIVARIATE AND MULTIVARIATE ANALYSIS OF PREDICTORS OF A TST ≥10 OR ≥15 MM.

	TST ≥ 10 mm			TST ≥ 15 mm			TST ≥ 10 mm		TST ≥ 15 mm	
	Cases (%)	Controls (%)	P*	Cases (%)	Controls (%)	P*	Adj. OR (95% CI)	P*	Adj. OR (95% CI)	P*
Sex										
male	95 (41.0)	194 (41.0)	0.99	60(41.1)	229 (41.0)	0.98	1	0.66	1	0.98
female	137 (59.0)	279 (59)		86 (58.9)	330 (59.0)		0.90 (0.56-1.45)		1.01 (0.63-1.60)	
Age (y)										
<35	50 (21.4)	134 (28.0)	0.09	27 (18.4)	157 (27.8)	0.01	1.34 (1.08-1.66)	0.008	1.40 (1.13-1.74)	0.002
35-44	57 (24.4)	130 (27.2)		37 (25.2)	150 (26.6)					
45-54	80 (34.2)	142 (29.7)		47 (32.0)	175 (31.0)					
≥ 55	47 (20.1)	72 (15.1)		36 (24.5)	83 (14.7)					
Birth in high TB prevalence country										
no	225 (97.8)	462 (98.1)	0.82	144 (99.3)	543 (97.7)	0.16	1	0.2	1	0.07
yes	5 (2.2)	9 (1.9)		1 (0.7)	13 (2.3)		0.41 (0.11-1.61)		0.14 (0.02-1.17)	
Work in health care‡										
no	198 (87.6)	388 (84.2)	0.23	126 (88.1)	460 (84.6)	0.28	1	0.06	1	0.16
yes	28 (12.4)	73 (15.8)		17 (11.9)	84 (15.4)		0.53 (0.27-1.03)		0.62 (0.32-1.21)	
Travel to high prevalence countries										
No	135 (58.4)	283 (59.7)	0.19	87 (59.6)	331 (59.2)	0.93	1	0.73	1	0.22
≤ 3 months (cumulative)	76 (32.9)	167 (35.2)		49 (33.6)	194 (34.7)		0.85 (0.53-1.38)		0.98 (0.61-1.56)	
> 3 months (cumulative)	20 (8.7)	24 (5.1)		10 (6.9)	6 (6.1)		1.11 (0.46-2.67)		0.78 (0.33-1.83)	
History of TB in household										
no	226 (98.3)	453 (95.8)	0.07	141 (97.9)	538 (96.2)	0.30	1	0.051	1	0.27
yes	4 (1.7)	20 (4.2)		3 (2.1)	21 (3.8)		0.27 (0.07-1.00)		0.46 (0.11-1.83)	

Table 1. CONTINUED

	TST ≥ 10 mm			TST ≥ 15 mm			TST ≥ 10 mm		TST ≥ 15 mm	
	Cases (%)	Controls (%)	P*	Cases (%)	Controls (%)	P*	Adj. OR (95% CI)	P*	Adj. OR (95% CI)	P*
Duration of exposure (months)										
0-3	15 (6.4)	17 (3.6)	0.13	8 (5.4)	24 (4.3)	0.20				
4-6	8 (3.4)	24 (5.0)		3 (2.0)	29 (5.1)					
7-9	26 (11.1)	38 (8.0)		17 (11.6)	47 (8.3)					
≥ 10	185 (79.1)	399 (83.5)		119(81.0)	465 (82.3)					
Frequency of shopping										
≤ 1 ×/month	47 (20.1)	58 (12.1)	0.01	27 (18.4)	78 (13.8)	0.36				
> 1×/month and < 1×/week	43 (18.4)	119 (24.9)		27 (18.4)	135 (23.9)					
1 ×/week	63 (26.9)	154 (32.2)		46 (31.3)	171 (30.3)					
> 1×/week	81 (34.6)	147 (30.8)		47 (32.0)	181 (32.0)					
Average shopping time (min)										
1-15	67 (28.6)	131 (27.4)	0.33	46 (31.3)	152 (26.9)	0.12				
16-30	104 (44.4)	216 (45.2)		58 (39.5)	262 (46.4)					
31-60	54 (23.1)	123 (25.7)		36 (24.5)	141 (25.0)					
> 60	9 (3.9)	8 (1.7)		7 (4.8)	10 (1.8)					
Cumulative exposure time (min)										
1-300	59 (25.2)	103 (21.6)	0.07	36 (24.5)	126 (22.3)	0.51	1.12 (0.94-1.34)	0.20	1.05 (0.89-1.25)	0.54
301-600	35 (15.0)	94 (19.7)		23 (15.7)	106 (18.8)					
601-1200	53 (22.7)	124 (25.9)		34 (23.1)	143 (25.3)					
1201-2400	70 (29.9)	107 (22.4)		43 (29.3)	134 (23.7)					
> 2400	17 (7.3)	50 (10.5)		11 (7.5)	56 (9.9)					

Table 1. NOTES

Abbreviations: TB: tuberculosis; TST: tuberculin skin test. * P-values based on LR test in logistic regression. ‡ Work including direct patient contact

mm among subjects born in high-prevalence countries (OR 12.8, 95% CI: 1.87 - 87.0, p=0.009). We observed no significant interaction between inclusion group and any of the variables in these models. Restricted case-control analyses yielded similar associations; the odds ratios for associations with age and exposure in the supermarket rarely differed by more than 5% from those in the primary analyses (data not shown).

QFT-GIT and T-SPOT.TB

QFT-GIT results were obtained for all 785 participants, none of whom could have been determined to have indeterminate results because the tube with positive controls was not available. For T-SPOT.*TB*, 23 (2.9 %) of 782 blood samples yielded indeterminate test results due to insufficient responses to the positive control. None of these 23 subjects reported use of immunosuppressive drugs and 4 (17.4 %) had a TST result of ≥ 15 mm, compared with 164 of 759 (20.6 %) participants with valid T-SPOT.*TB* results (P= 0.628). The agreement between independent readers of T-SPOT.*TB* results was 94.9 % (κ= 0.84). The agreement between visual and automated readings was 97.5% (κ= 0.923). Overall, positive QFT-GIT responses were observed in 81/785 (10.3 %) subjects, compared with 142/759 (18.7 %) for T-SPOT.*TB* (P <0.001). A positive QFT-GIT result was observed in 0.2 %, 0 %, 7.9 %, 6.1 % and 42.2 % of subjects with a TST result of 0 mm, 1-4 mm, 5-9 mm, 10-14 mm and ≥15 mm, respectively (Figure 3A). The corresponding percentages of positive T-SPOT.*TB* results were higher in each TST category, being 4.6 %, 13.3 %, 23.7 %, 23.5 % and 51.3 %, respectively (Figure 3A).

Complete data on exposure were available for 712 subjects with complete QFT-GIT results, and for 691 subjects with complete TSPOT.*TB* results. QFT-GIT and T-SPOT.*TB* results were not associated with age, sex, occupational exposure or country of origin in univariate analysis (Table 2). The probability of a positive QFT-GIT increased significantly in association with the frequency of shopping as well as with the cumulative shopping time (Table 2). The probability of a positive T-SPOT.*TB* was significantly associated only with the monthly number of visits to the supermarket (Table 2). In multivariate analysis, adjusting for day of inclusion and all variables in Table 2 except duration of exposure, frequency of shopping and average shopping time per visit to the supermarket, both QFT-GIT and T-SPOT.*TB* were significantly associated with the cumulative shopping time. Per increase of category of the latter parameter, the average OR (95% CI) of a positive IGRA result was 1.48 (1.19-1.84, P <0.001) for QFT-GIT and 1.30 (1.10-1.53, P = 0.002) for T-SPOT.*TB*, implicating a 4.8 times (1.48^4) and 2.9 times (1.30^4) increased risk in the highest exposure category compared to the baseline category for QFT-GIT and T-SPOT.*TB,* respectively. Significant interactions between inclusion group and any of the variables in these models were not observed. Also here, restricted case-control analyses yielded similar associations, with small differences in odds rations compared to the primary analyses (data not shown).

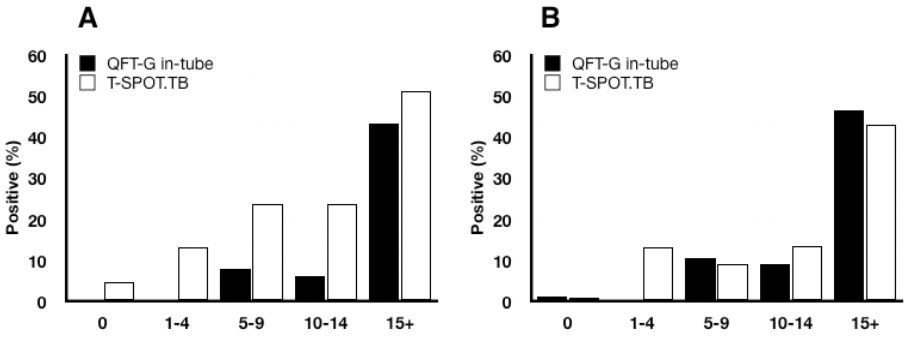

Figure 3: Proportion of positive results of QuantiFERON TB Gold and T-SPOT.*TB* by category of tuberculin skin test results among 785 BCG unvaccinated study participants (N=759 T-SPOT.*TB* results),
A. using the cut-off values for a positive test result as provided by the manufacturer.
B. using cut-off values for a positive test result that yielded the highest agreement between both tests (see also Table 6).

Of 23 participants who reported the use of immunosuppressive drugs, 3 (13 %) had a TST result of ≥ 15 mm, compared with 20.8 % of participants who did not report the use of immunosuppressive drugs (p=0.370). Positive QFT-GIT results were observed in 2/23 (8.7 %) and positive T-SPOT.*TB* results in 6/23 (26.1 %). These percentages were not significantly different from those observed in the complete study population (p=0.801 and p=0.374, respectively).

Using 5 mm as TST cut-off, the sensitivity and specificity of QFT-GIT were 80/333 (23.8%) and 448/449 (99.8%), respectively, and the agreement was 67.3 % (κ= 0.26, Table 3). The sensitivity and specificity of T-SPOT.*TB* compared to TST at ≥ 5 mm were 121/330 (36.7%) and 408/429 (95.1%), respectively, and the agreement was 69.7 % (κ= 0.34; Table 3).

Among 70 subjects with a TST result 5-9 mm, 5 (7.1%) had a positive QFT-GIT result, and 16 (22.9%) had a positive T-SPOT.*TB* result. Neither a positive QFT-GIT result nor a positive T-SPOT.*TB* result in this group was significantly associated with exposure in the supermarket (data not shown).

Using 10 mm as TST cut-off, the sensitivity and specificity of QFT-GIT were 74/260 (28.5%) and 518/525 (98.7%), respectively, and the agreement was 75.4 % (κ= 0.33, Table 3). The sensitivity and specificity of T-SPOT.*TB* compared to TST at ≥ 10 mm were 103/254 (40.6%) and 466/505 (92.3%), respectively and the agreement was 75.0 % (κ= 0.37; Table 3). Using the TST at cutoff ≥ 15 mm as a reference, the sensitivity and specificity of QFT-GIT were 68/161 (42.2%) and 611/624 (97.9%), respectively. The agreement between the TST at cutoff ≥ 15 mm and QFT-GIT was 86.5 % (κ= 0.49; Table 3). The

Table 2. UNIVARIATE AND MULTIVARIATE ANALYSIS OF PREDICTORS OF A POSITIVE INTERFERON-Γ TEST RESULT.

	QFT-GIT positive			T-SPOT.*TB* positive			QFT-GIT positive		T-SPOT.*TB* positive	
	Cases (%)	Controls (%)	P*	Cases (%)	Controls (%)	P*	Adj. OR (95% CI)	P*	Adj. OR (95% CI)	P*
Sex										
male	22 (30.6)	267 (42.2)	0.054	47 (37.6)	240 (42.9)	0.27	1	0.18	1	0.85
female	50 (69.4)	366 (57.8)		78 (62.4)	319 (57.1)		1.50 (0.83-2.71)		1.04 (0.66-1.65)	
Age (y)										
<35	14 (18.9)	170 (26.7)	0.52	27 (21.4)	151 (26.7)	0.60	1.10 (0.84-1.43)	0.50	1.07 (0.87-1.31)	0.52
35-44	22 (29.7)	165 (25.9)		35 (27.8)	143 (25.3)					
45-54	25 (33.8)	197 (30.9)		40 (31.8)	179 (31.7)					
≥ 55	13 (17.6)	106 (16.6)		24 (19.0)	92 (16.3)					
Birth in high TB prevalence country										
no	73 (98.7)	614 (97.9)	0.66	121 (97.6)	546 (97.9)	0.76			1	0.85
yes	1 (1.3)	13 (2.1)		3 (2.4)	11 (2.0)				1.89 (0.35-10.3)	
Work in health care‡										
no	64 (88.9)	522 (84.9)	0.35	105 (86.1)	467 (85.9)	0.95	1	0.24	1	0.71
yes	8 (11.1)	93 (15.1)		17 (13.9)	77 (14.2)		0.60 (0.26-1.40)		0.89 (0.47-1.67)	
Travel to high prevalence countries										
No	44 (60.3)	374 (59.2)	0.70	73 (58.4)	331 (59.2)	0.90	1	0.51	1	0.25
≤ 3 months (cumulative)	26 (35.6)	217 (34.3)		45 (36.0)	192 (34.4)		1.03 (0.59-1.81)		1.07 (0.68-1.68)	
> 3 months (cumulative)	3 (4.1)	41 (6.5)		7 (5.6)	36 (6.4)		0.46 (0.13-1.65)		0.65 (0.26-1.62)	
History of TB in household										
no	71 (98.6)	608 (96.4)	0.26	119 (96.8)	539 (96.4)	0.86	1	0.35	1	0.91
yes	1 (1.4)	23 (3.6)		4 (3.2)	20 (3.6)		0.36 (0.05-3.04)		0.93 (0.28-3.11)	

Table 2. CONTINUED

	QFT-GIT positive			T-SPOT.*TB* positive			QFT-GIT positive		T-SPOT.*TB* positive	
	Cases (%)	Controls (%)	P*	Cases (%)	Controls (%)	P*	Adj. OR (95% CI)	P*	Adj. OR (95% CI)	P*
Duration of exposure (months)										
0-3	0	32 (5.0)	0.09	4 (3.2)	27 (4.8)	0.17				
4-6	1 (1.4)	31 (4.9)		2 (1.6)	29 (5.1)					
7-9	4 (5.4)	60 (9.4)		10 (7.9)	53 (9.4)					
≥ 10	69 (93.2)	515 (80.7)		110 (87.3)	456 (80.7)					
Frequency of shopping										
≤ 1 ×/month	8 (10.8)	97 (15.2)	0.005	19 (15.1)	83 (14.7)	0.04				
> 1×/month and < 1×/week	7 (9.5)	155 (24.3)		17 (13.5)	139 (24.6)					
1 ×/week	28 (37.8)	189 (29.6)		42 (33.3)	168 (29.7)					
> 1×/week	31 (41.9)	197 (30.9)		48 (38.1)	175 (31.0)					
Average shopping time (min)										
1-15	20 (27.0)	178 (27.9)	0.17	34 (27.0)	161 (28.5)	0.12				
16-30	27 (36.5)	293 (45.9)		49 (39.9)	263 (46.6)					
31-60	23 (31.1)	154 (24.1)		37 (29.4)	130 (23.0)					
> 60	4 (5.4)	13 (2.0)		6 (4.8)	11 (1.9)					
Cumulative exposure time (min)										
1-300	8 (10.8)	154 (24.1)	0.007	21 (16.7)	137 (24.3)	0.17	1.48 (1.19-1.84)	<0.001	1.30 (1.10-1.53)	0.002
301-600	11 (14.9)	118 (18.5)		20 (15.9)	107 (18.9)					
601-1200	17 (23.0)	160 (25.1)		31 (24.6)	138 (24.4)					
1201-2400	30 (40.5)	147 (23.0)		39 (31.0)	132 (23.4)					
> 2400	8 (10.8)	59 (9.3)		15 (11.9)	51 (9.0)					

Table 3. AGREEMENT BETWEEN INTERFERON-γ ASSAYS AND TST RESULTS.

	TST		TST		TST	
QuantiFERON-TB Gold in-tube (N=785)	< 5 mm	≥ 5 mm	< 10 mm	≥ 10 mm	< 15 mm	≥ 15 mm
negative (N=704)	448* (63.6)	256 (36.4)	518 (73.6)	186 (26.4)	611 (86.8)	93 (13.2)
positive (N=81)	1 (1.2)	80 (98.8)	7 (8.6)	74 (91.4)	13 (16.0)	68 (84.0)
% Agreement	67.3		75.4		86.5	
OR (95% C.I.)	140.0 (19.4-1012.1)		29.4 (13.3-65.1)		34.4 (18.3-64.7)	
Kappa	0.26		0.33		0.49	
	TST		TST		TST	
T-SPOT.*TB* (N=759) †	< 5 mm	≥ 5 mm	< 10 mm	≥ 10 mm	< 15 mm	≥ 15 mm
negative (N=617)	408 (66.1)	209 (33.9)	466 (75.5)	151 (24.5)	541 (87.7)	76 (12.3)
positive (N=142)	21 (14.8)	121 (85.2)	39 (27.5)	103 (72.5)	62 (43.7)	80 (56.3)
% Agreement	69.7		75		81.8	
OR (95% C.I.)	11.25 (6.87-18.41)		8.15 (5.40-12.30)		9.19 (6.10-13.8)	
Kappa	0.34		0.37		0.42	

Notes: TST denotes tuberculin skin test. *Data are expressed as number (%) † For T-SPOT.*TB* there were 3 missing blood samples and 23 (2.9 %) indeterminate test results

Table 4. AGREEMENT BETWEEN QUANTIFERON TB GOLD IN-TUBE AND T-SPOT.*TB*

				T-SPOT.*TB*				
TST category	No.			neg	pos	% Agreement	OR (95% CI)	Kappa
Cat 0 (0 mm)	414	QFT-GIT	neg	394 (99.7)*	19 (100)	95.2	0	-0.005
			pos	1 (0.3)	0			
Cat 1 (1-4 mm)	15	QFT-GIT	neg	13 (100)	2 (100)	86.7	0	#
			pos	0	0			
Cat 2 (5-9 mm)	76	QFT-GIT	neg	56 (96.6)	14 (77.8)	78.9	8.0 (1.5-∞)	0.24
			pos	2 (3.4)	4 (22.2)			
Cat 3 (10-14 mm)	98	QFT-GIT	neg	74 (98.7)	18 (78.3)	74.1	20.0 (2.9-∞)	0.27
			pos	1 (1.3)	5 (21.7)			
Cat 4 (≥ 15 mm)	156	QFT-GIT	neg	71 (93.4)	17 (21.3)	85.9	52.6 (18.8-146.4)	0.72
			pos	5 (6.6)	63 (78.8)			
All	759†	QFT-GIT	neg	608 (98.5)	70 (49.3)	89.6	69.5 (33.3-145.0)	0.59
			pos	9 (1.5)	72 (50.7)			

Abbreviations: neg, negative; pos, positive; TST, tuberculin skin test; # denotes not applicable * Data are expressed as No. (%).

† For T-SPOT.*TB* there were 3 missing blood samples and 23 indeterminate results.

Table 5. MULTIVARIATE ANALYSIS OF DETERMINANTS A DISCORDANT INTERFERON-γ TEST RESULT (T-SPOT.*TB* positive/ QFT-GIT negative, n=70) compared to a concordant negative and a concordant positive control group.

	Compared to T-SPOT.*TB* negative/ QFT-GIT negative (n=608)		**Compared to T-SPOT.*TB* positive/ QFT-GIT positive (n=72)**	
	Adj. OR (95% CI)	p*	Adj. OR (95% CI)	p*
Age (10 years)	1.15 (0.87-1.51)	0.330	1.88 (1.14-3.11)	0.010
cumulative exposure time‡	1.16 (0.93-1.44)	0.177	0.86 (0.58-1.27)	0.450
TST result		<0.001		<0.001
0-4 mm	1		-	
5-9 mm	6.86 (3.04-15.5)		-	
0-9 mm	-		1	
10-14 mm	6.33 (2.95-13.6)		0.47 (0.09-2.54)	
15+ mm	6.05 (2.74-13.3)		0.02 (0.00-0.08)	
Immunocompromised†	3.94 (0.93-16.6)	0.089	0.85 (0.07-9.80)	0.894

Abbreviations: QFT-GIT: QuantiFERON-TB Gold in-tube; TB: tuberculosis; TST: tuberculin skin test.

* P-values based on LR test in logistic regression

† Compared to immunocompetent (reference category)

‡ Average increase in odds ratio per step increase in exposure category (see table 1).

sensitivity and specificity of T-SPOT.*TB* compared to TST at at ≥ 15 mm were 80/156 (51.3%) and 541/603 (89.7%), respectively. The agreement between the TST at ≥ 15 mm and T-SPOT.*TB* was 81.8 % (κ= 0.42; Table 3). Thus, agreement between each IGRA and TST increased with a higher TST cut-off. Furthermore, in a two stage approach with TST being used to screen contacts and those with a"positive TST result" being assayed by IGRA to define likely LTBI, an increasing proportion of positive IGRA test results is found for both QFT-GIT and T-SPOT.*TB* (Table 3).

T-SPOT.*TB versus* QFT-GIT

Among 759 persons with valid results of both IGRA, results were concordant negative in 608 (80.1%), concordant positive in 72 (9.5%) and discordant in 79 (10.4%, overall agreement 89.6%, κ=0.59, P<0.0001). Of the discordant results, 70 (88.6%) were T-SPOT. TB positive, and 9 (11.4%) QFT-GIT positive. The agreement between QFT-GIT and T-SPOT.*TB* increased with each TST category (Table 4).

We assessed the characteristics of subjects with positive T-SPOT.*TB* but negative QFT-GIT by comparing them in multivariate analyses with two control groups of the subjects with concordant-negative, and those with concordant-positive results, respectively (Table 5). Compared to concordant-negative control group, a discordant-positive T-SPOT.*TB* was significantly associated with the TST result (p<0.001) with similar ORs (6.0-6.9) for TST categories ≥5 mm. It was near-significantly associated with immunosuppression

Table 6. AGREEMENT BETWEEN T-SPOT.*TB* AND QUANTIFERON TB GOLD IN 759 SAMPLES AT VARYING CUT-OFF VALUES FOR A POSITIVE TEST

		T-SPOT.*TB* cut-off value (Nr. of spots)													
		≥ 4 Spots	≥ 5 Spots	≥ 6 Spots	≥ 7 Spots	≥ 8 Spots	≥ 9 Spots	≥ 10 Spots	≥ 11 Spots	≥ 12 Spots	≥ 13 Spots	≥ 14 Spots	≥ 15 Spots	≥ 16 Spots	≥ 17 Spots
QuantiFERON TB Gold cut-off value (IU/mL interferon-γ)	≥ 0.40	0.454 83.6 67	0.515 87.5 67	0.556 89.1 67	0.587 90.3 67	0.606 91.2 65	0.615 91.6 64	0.623 91.9 63	0.640 92.4 63	0.646 92.9 61	0.649 93.2 58	0.619 92.9 54	0.634 93.4 53	0.649 93.8 53	0.650 93.8 52
	≥ 0.35	0.480 85.2 72	0.543 88.0 72	0.585 89.7 72	0.617 90.8 72	0.638 91.7 70	0.647 92.1 69	0.656 92.5 68	0.673 93.0 68	0.680 93.3 66	0.685 93.7 63	0.657 93.3 59	0.673 93.9 58	0.675 94.0 57	0.662 93.9 55
	≥ 0.30	0.499 85.6 76	0.554 88.2 75	0.597 89.8 75	0.629 91.0 75	0.639 91.6 72	0.648 91.6 71	0.657 92.3 70	0.674 92.8 70	0.681 93.3 68	0.686 93.6 65	0.659 93.3 61	0.662 93.5 59	0.663 93.6 58	0.651 93.5 56
	≥ 0.25	0.513 85.9 79	0.569 88.3 78	0.612 90.0 78	0.644 91.2 78	0.655 91.9 75	0.664 92.2 74	0.674 92.6 73	0.691 93.1 73	0.699 93.5 71	0.704 92.9 68	0.678 93.5 64	0.682 93.7 62	0.683 93.9 61	0.672 93.7 59
	≥ 0.20	0.525 86.0 83	0.582 88.6 82	0.615 89.9 81	0.648 91.0 81	0.658 91.7 78	0.668 92.1 77	0.667 92.5 76	0.694 93.0 76	0.702 93.1 74	0.708 93.8 71	0.683 93.3 67	0.686 93.6 65	0.688 93.8 64	0.677 93.6 62
	≥ 0.15	0,535 85.9 89	0,583 88.1 87	0.616 89.6 86	0.64890.7 86	0.649 91.1 82	0.657 91.4 81	0.667 91.8 80	0.683 92.3 80	0.690 92.7 78	0.696 93.1 75	0.672 92.8 71	0.676 93.0 69	0.678 93.1 68	0.667 93.0 66
	≥ 0.10	0.573 86.5 100	0.605 88.2 96	0.621 89.2 93	0.643 90.0 92	0.644 90.4 88	0.643 90.6 86	0.652 90.9 85	0.667 91.4 85	0.664 91.6 82	0.669 92.0 79	0.647 91.6 75	0.650 91.8 73	0.652 92.0 72	0.643 91.6 70

NOTE. Values in each cell represent kappa value, % agreement and number of tests that were positive in both assays, respectively. Light grey shadowed cells represent the optimal values for either test at varying cut-offs of the other test. The agreement was optimal in the dark grey cell.
Manufacturers cut-off values are ≥ 0.35 IU/mL for QFT-GIT and ≥ 6 spots for T_SPOT.*TB* (see on line supplement).

(OR 3.94, 95% CI: 0.93-16.6), but not with cumulative exposure time. A significant univariate association with increasing age disappeared after adjustment for TST result (data not shown). Assuming a causal relationship, 4.3% of all discordant-positive T-SPOT.*TB* results were directly attributable to immune suppression (population attributable fraction, 95% CI: 0-5.4%). Compared to the concordant-positive control group, a discordant-positive T-SPOT.*TB* was significantly associated with increasing age (average OR per 10 year increase 1.88, 96% CI: 1.14-3.11) and, inversely, with TST result ($p<0.001$), but not with immunosuppression or exposure.

The observed discrepancies between QFT-GIT and T-SPOT.*TB* prompted us to reanalyze the inter-assay agreement at varying cut-off values of both assays (Table 6). Among all subjects of whom both IGRA results were available, agreement between both IGRA was maximized at IFN-γ ≥0.20 IU/mL for QFT-GIT and ≥13 spots for T-SPOT.*TB*. At these optimum cut-off values the absolute number of results that were positive in both assays was very similar to the number of concordant positive results when using the manufacturers cut-off values, but with a different distribution in relation to the TST categories (Figure 3B).

DISCUSSION

This study describes a direct comparison between the TST and two commercially available IGRA, QFT-GIT and T-SPOT.*TB*, for detection of LTBI in a large contact investigation. The setting of our study was unique as more than 20,000 mainly BCG unvaccinated individuals from an area with low TB endemicity were potentially exposed to *M. tuberculosis* repeatedly for as long as ten months. In our study, in which BCG vaccinated subjects were excluded, a TST ≥ 15 mm cutoff was regarded to reliably indicate LTBI. Among participants in the pre-TST group, the rate of positive TST results was 4.3% reflecting the infection risk of the contact investigation at large [B.Koster, unpublished data.] TST results ≥ 15 mm were significantly associated with age but not with measures of exposure at the supermarket, suggesting that positive TST responses reflected largely delayed-type hypersensitivity due to remote infection with *M. tuberculosis* acquired before the source case at the supermarket became infectious. Using the cut-off of 10 mm resulted in similar but less pronounced associations, likely reflecting bias due to the less specific outcome measure, but did not affect our conclusions.

In contrast, results of QFT-GIT and those of T-SPOT.*TB* were not associated with age but were significantly associated with the cumulative shopping time in the supermarket, which was most marked for QFT-GIT.

In this large contact investigation it was not possible to document actual face-to-face contact with the source case and we therefore used the number of months that a customer

frequented the supermarket during the infectious period of the source case, the shopping frequency, and average time of each shopping visit as proxy indicators. Even though we used only the cumulative exposure time as variable in our multivariate models, we observed similar patterns of association with IGRA responses for various individual proxy indicators in the univariate analysis, suggesting that our findings were robust to the way exposure was estimated. For reasons of study efficiency we enriched our sample by including not only a random sample of supermarket customers who reported for skin testing, but also a non-random sample of customers who had a TST reaction >0 mm. This could in theory affect the observed associations with age or exposure. We corrected for this by adjustment for inclusion group in the multivariate analysis. Moreover, we found no significant interactions, i.e. the size of observed associations did not differ between both inclusion groups.

The observed association of IGRA results with exposure is in accordance with previous studies using either ELISPOT or whole blood based IGRA [10;12;16;21-23]. Our results confirm these findings and in addition demonstrate that IGRA, in particular QFT-GIT, correlate better with the level of exposure than the TST even in a BCG unvaccinated population.

Sensitivity of IGRA for detection of LTBI

In recent CDC guidelines [17], several cautions and potential limitations of QFT-G were discussed among which the determination of the sensitivity of IGRA for detection of LTBI was a key issue, a concern that had previously been expressed [32;33]. Our study provides important new data in this regard. In our study, the high agreement between both IGRA that were performed independently at different laboratories, as well as the significant association of IGRA results with exposure argue against technical problems with the IGRA as an explanation of the low sensitivity. False-positive TST results were also unlikely as the study population was BCG unvaccinated. Moreover, a cut-off value of 15 mm has a specificity for LTBI exceeding 97% in the Dutch population suggesting that cross-reactive TST responses due to previous nontuberculous mycobacterial infections rarely exceed 15 mm [35]. The lower sensitivity of IGRA compared to TST must therefore be related to intrinsic differences between blood and skin tests. A positive TST result following infection with *M. tuberculosis* often remains positive during a lifetime ("once positive, no longer useful"), waning being infrequent below the age of 55 [36].

In persons who were actually infected at the supermarket, the infection could have been acquired as long ago as one year before the study because the source case had been contagious since February 2004 and the large-scale contact investigation was carried out at the end of January 2005. While there are no definitive data of the kinetics of IGRA responses, we think that the decay kinetics of IGRA responses in relation to the interval between infection and blood sampling provides a hypothesis for the observed difference in sensitivity of IGRA between studies, as has been suggested earlier [16;24]. In this notion,

IGRA are highly sensitive for detection of recent infection but test responses can revert to negative if the antigen is cleared when the infection is adequately controlled and activated T cells are no longer required. Memory T cells may remain undetected during the short incubation period of 16-24 h of IGRA, whereas the TST measures infiltration of the skin by immune cells 72 h after injection of tuberculin.

In support of decreasing IGRA responses over time was the observation that results of an ELISPOT-based IGRA reverted to negative in patients treated successfully for TB disease [37;38]. Another study reported increased ELISPOT responses after four weeks of treatment for LTBI followed by a decrease [39]. Follow-up with IGRA of treated and untreated TST-positive individuals in our study is currently ongoing and may provide further clarification of this issue.

Clinical significance of IGRA test result

While IGRA are now considered more specific and show a better correlation with exposure than the TST, it has not been demonstrated whether they provide a valid basis for therapeutic decisions regarding treatment. The risk of TB disease in the presence of a positive test result has not been established. Notably, positive IGRA results were observed in a significant proportion of recently exposed contacts with a negative TST result [15;22;23;40]. The clinical significance of this finding merits further study if IGRA are to replace the TST and be used for therapeutic decisions [41].

If a positive IGRA result reflects an ongoing immune response against *M. tuberculosis*, it is possible that IGRA will have a higher prognostic value with regard to the risk of progression to TB disease than the TST. This would allow better targeting of preventive treatment of LTBI cases found in outbreak investigations. Thus far, only one study reported an increased risk of TB disease within 2 y among ESAT-6 responsive contacts [42]. More follow-up studies of the natural kinetics of IGRA in both immunocompetent and immunocompromised hosts and the development of TB disease following infection are needed.

We therefore agree with Mazurek et al. that negative results of IGRA must be interpreted with caution and should always be regarded in the light of all other available clinical and epidemiological data [17].

Discordances between QFT-GIT and T-SPOT.TB results

The agreement between QFT-GIT and T-SPOT.*TB* was 89.5 % (κ= 0.59). Nevertheless, there were important discrepancies between the results of both IGRA. A positive result of T-SPOT.*TB* in combination with a negative result of QFT-GIT was observed eight-fold more frequently than the reverse discrepant combination. The difference in percentage of positive results of both assays varied from 4.6 % to 14.5% in different TST categories, the difference being most pronounced with reaction sizes < 5 mm. The reported percentage

positive ELISPOT results in association with a negative TST in contact investigations was even higher in several earlier studies[22,23]. Among contacts with a TST result < 5 mm, 30/205 (14.6%) were positive in ELISPOT [40]. Finally, positive T-SPOT.*TB* results were observed in a comparable frequency in association with negative TST results in a heterogeneous cohort of patients suspected of TB disease or LTBI [25]. The consistency of these findings suggests that this is an inherent characteristic of ELISPOT.

With regard to QFT-G, positive results were observed in association with a TST result < 10 mm in 62/421 (15%) highly exposed health-care workers in India [15], in 13/372 (3.5%) of U.S. jail inmates [24] and a similar proportion of the above-mentioned heterogeneous cohort [25]. As yet, no cases of TB disease have been reported in persons with such discrepant results and the clinical significance is therefore unclear, but this becomes essential information if the TST would be replaced by IGRA and there will be no discrepancy but just a positive or negative IGRA result to act on.

Our data suggested that the specificity of T-SPOT.*TB* could be improved by increasing the cut-off value, while the sensitivity of QFT-G could be improved by decreasing the cut-off. By bi-directional variation of the cut-off values of both IGRA, the inter-assay agreement was found to be optimal at cut-off values of IFN-γ ≥ 0.20 IU/mL for QFT-GIT and ≥ 13 spots for T-SPOT.*TB*. At these optimized cut-off points, the proportion of positive test results in each TST category was comparable for both tests (Figure 3B) while these were significantly different when results were based on the manufacturers cut-off values (Figure 3A). In general, cut-off points are determined by the aims of a study, which are different for comparative studies, prevalence assessments or those concerning patient management. Further study is needed to evaluate whether cut-off values different from those advocated by the manufacturers may provide a better basis for decision making in specific clinical or epidemiological settings. Furthermore, it remains speculative what the results of our study would be if indeterminate QFT-GIT results were known.

CONCLUSIONS

In conclusion, in this study among 785 BCG non-vaccinated Dutch adults who had been exposed to a patient with smear-positive TB, IGRA results related to measures of the level of exposure better than did the TST. In relation to each other, QFT-GIT was more closely associated with exposure than was T-SPOT.*TB*. However, a possible lack of sensitivity for both assays in detecting individuals with TST ≥15mm, despite negative BCG vaccination status, requires further investigation. Optimum agreement between both IGRA was reached after lowering the cut-off value for QFT-GIT and increasing the cut-off value for T-SPOT.*TB*. Despite the higher correlation between T-SPOT.*TB* and QFT-GIT than between the TST and either assay, the discrepancies between both IGRA await clarification.

Important subjects for future research are the sensitivity of IGRA in relation to the interval since infection, the evaluation of different cut-off levels and the predictive value of an IGRA result for development of TB disease.

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