Immunodiagnosis of latent tuberculosis : new answers to an old question?

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INTERFERON-γ RELEASE ASSAYS IN IMMIGRANT CONTACTS AND EFFECT OF REMOTE EXPOSURE TO *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Objective

To assess the association between remote exposure to tuberculosis (TB) and results of the tuberculin skin test (TST), and two interferon-gamma release assays (IGRA), QuantiFERON-TB Gold in-tube (QFT-GIT) and T-SPOT.*TB,* in immigrant contacts of sputum smear-positive TB patients.

Methods

Immigrants aged ≥16 years who were a close contact of a smear-positive TB patient were included. QFT-GIT and T-SPOT.*TB* were performed if TST induration was ≥5 mm. Associations between test results and origin from an endemic country were assessed.

Results

Out of 433 close contacts, 322 (74%) had TST ≥5 mm and of these, 282 (88%) had valid test results of all assays. Positive QFT-GIT results were obtained for 152/282 (54%) and positive T-SPOT.*TB* for 168/282 (60%). After adjustment for age, gender and recent contact, positive IGRA results and TST results ≥10 mm were more frequent among immigrants who originated from Africa, in particular sub-Saharan Africa.

Conclusion

When IGRA are used to determine latent TB infection among foreign-born individuals, positive findings not only relate to recent TB infection, but also reflect prior TB exposure in the country of origin. This late reactivity will limit their usefulness in contact investigations among immigrants originating from endemic areas.

INTRODUCTION

The incidence of tuberculosis (TB) in low-endemic areas, like Europe, declined over the past decades (1-3). Yet, TB rates among foreign-born individuals living in these areas remain high and account for more than 50% of new TB cases (1-3). Possible explanations include limited testing and treatment of latent tuberculosis infection (LTBI) since the tuberculin skin test (TST) is not routinely used in this subpopulation. Until recently, routine practice in the Netherlands among foreign-born contacts consisted of screening for active TB by chest radiography only.

One of the limitations of the TST is that it cannot distinguish recent from remote infections. In the latter, the benefit of preventive therapy may not outweigh the risk of side effects, since the risk of breakdown to disease is low.

T-SPOT.*TB*® and QuantiFERON-TB® Gold in-tube (QFT-GIT), interferon-γ release assays (IGRA), have been developed which measure T-cell responses to the antigens ESAT-6 and CFP-10 (and TB 7.7 for QFT-GIT as well) that are specific for *M. tuberculosis* (4;5). These assays offer more specific screening for LTBI in BCG-vaccinated individuals. However it is unclear to what extent these assays distinguish recent from remote infections (6).

Due to the lack of a gold standard for the diagnosis of LTBI, surrogate measures have been used to evaluate IGRA, such as a gradient of recent exposure (7-16). While some individual studies concluded that IGRA better correlate with an exposure gradient (7-18), a meta-analysis concluded that the sensitivity of the TST and IGRA was similar for individuals with different gradients of exposure (19). So far most studies assessed only the correlation of IGRA with recent exposure, without taking into account the possible effect of past infections, and none used both QFT-GIT, T-SPOT.*TB* and TST in contact investigations among immigrants.

We assessed whether QFT-GIT, T-SPOT.*TB* and TST responses were influenced by remote exposure to TB among immigrants with recent contact with a sputum-smear positive TB patient.

STUDY POPULATION AND METHODS

Study population

Between April 2005 and July 2007 immunocompetent close contacts of sputum-smear positive TB patients aged ≥16 years who were born in a high TB endemic country and visited one of the 15 participating municipal health services (MHSs) were invited to participate. Furthermore we included second generation immigrants if they were BCG-vaccinated and at least one of their parents was born in a TB endemic country. Close contacts were individuals who had frequent (at least 3 times a week) and/or intensive contact (contact within a small closed space or physically nearby) with the index patient (20). Excluded were individuals with: diabetes mellitus, HIV/AIDS, a mental retardation, those diagnosed with TB during the contact investigation, those given preventive therapy as decided by the physician, and those not expected to adhere to the follow-up of this study.

TST

After written informed consent, participants received a chest X-ray and a TST using 2 TU RT23 (Statens Serum Institute, Copenhagen, Denmark). If the TST induration, measured after 48-72 hours, was ≥5 mm, blood samples were taken. IGRA were performed only for contacts with TST indurations ≥5 mm since we included only healthy, immuno-competent participants who are unlikely to have false negative TST results. Therefore the risk of LTBI among contacts with TST <5 mm was considered negligible. If <5 mm the TST was repeated 3 months later and only followed by IGRA testing if ≥5 mm. Known past TST responders did not undergo a TST, but blood was drawn during their first visit. Participants with TST ≥5 mm were interviewed by MHS staff regarding medical history, BCG-vaccination status and known remote exposure to TB patients.

IGRA

Both IGRA were performed in one laboratory. QFT-GIT was performed following the manufacturers instructions (http://www.cellestis.com) (two-tube format). At the start of the study the incubation of QFT-GIT tubes was allowed by the manufacturer up until 72 hours, which was later reduced to 16-24 h. Samples collected on a Friday were incubated until the following Monday, all other samples were incubated about 24 hours. QFT-GIT results were expressed as positive or negative, using the cut-off value of ≥ 0.35 IU/ml.

T-SPOT.*TB* was performed following the manufacturers instructions (http://www.oxfordimmunotec.com). When blood was obtained on Fridays, cells were isolated and frozen at minus 152°C until testing. The number of spots was scored visually

using a magnifying glass by two independent observers. In case of discrepancies, both observers reread the wells until agreement was reached. Interpretation of the results was according the latest manufacturers instructions.

Ethical approval for this study was obtained from the Netherlands Central Committee on Research Involving Human Subjects (CCMO, P04.1214C).

Statistical analysis

Concordance between the different assays was assessed using κ coefficients. The McNemar test was used to determine interassay agreement. Categorical variables were compared using the Chi-square test. Associations between test results and remote exposure, defined as birth in a country outside Europe and North America, were assessed by logistic regression. The analysis was limited to subjects who were tested with both IGRA and TST. TST indurations ≥10 mm were considered positive in the analysis of risk factors associated with a positive TST. This analysis was also performed with a TST cut-off of 15 mm, in order to increase the specificity of TST (comparing TST 5-14 mm with TST ≥15). To quantify the influence of previous exposure, the attributable fraction (AF) was calculated. The AF represents the proportion of cases that is attributable to a certain risk factor, i.e. would not have occurred if that risk factor would be completely eliminated. In this study the AF represents the proportion of contacts with a positive test that is attributable to birth in an endemic country. The AF is calculated as (risk ratio -1) / risk ratio (21). This risk ratio was based on the adjusted model, comparing the risk of a positive test in individuals born in South America, Asia, sub-Saharan Africa, or Other Africa (exposure group) to those born in Europe or North America (reference group). Statistical analyses were performed using SPSS 14.0 for Windows (Chicago, IL, USA).

RESULTS

Within the study period 380 contact investigations around contagious TB patients were executed, including 812 foreign-born contacts aged ≥16 years (Figure 1). Among these 812, 97 contacts were excluded because they did not meet the inclusion criteria (n=66), were diagnosed with active tuberculosis during the contact investigation (n=14) or were prescribed preventive treatment (n=17). Another 282 contacts could not be included since they did not return for TST reading (n=11), were not asked to participate in the study (n=167) or did not give informed consent (n=104). Compared to contacts who had not been approached or did not provide informed consent (n=282), those who participated (n=433) were significantly more often born in a country in South America and less often in non-sub-Saharan African countries compared to Asia, and were less often the partner or sibling of the index case than a colleague or schoolmate. Characteristics of participants and non-participants are listed in Table 1.

TST results were <5 mm in 94/433, ≥5 mm in 322/433, and 17 contacts were known TST positive. Valid outcomes of QFT-GIT and T-SPOT.*TB* for participants with TST ≥5 mm were available for 282 individuals (Figure 1). The majority of participants (163/282=57.8%) had a TST induration of ≥15 mm, while 26.9% had an induration of 10-14 mm and 15.2% of 5-9 mm. Overall, QFT-GIT was positive in 53.9% while 59.6% were positive in the T-SPOT.*TB*. The T-SPOT.*TB* was performed with fresh material in 211/282 (74.8%) and with frozen and thawed cells in 71/282 (25.2%) (maximum interval between freezing and thawing was 207 days with an average of 95 days). The percentage of positive T-SPOT.*TB* results for samples that were fresh (58.3%) did not differ significantly from the percentage for frozen samples (63.4%, p=0.450). The agreement between the TST and both IGRA was poor using a TST cut-off value of 15 mm, decreasing further when 10 mm was used as cut-off (Table 2A). In contrast, there was a strong correlation between QFT-GIT and T-SPOT.*TB* (Table 2B) even though the outcome of both tests differed significantly (p=0.023). T-SPOT.*TB* was more often positive than QFT-GIT, especially in individuals with smaller TST indurations (Figure 2).

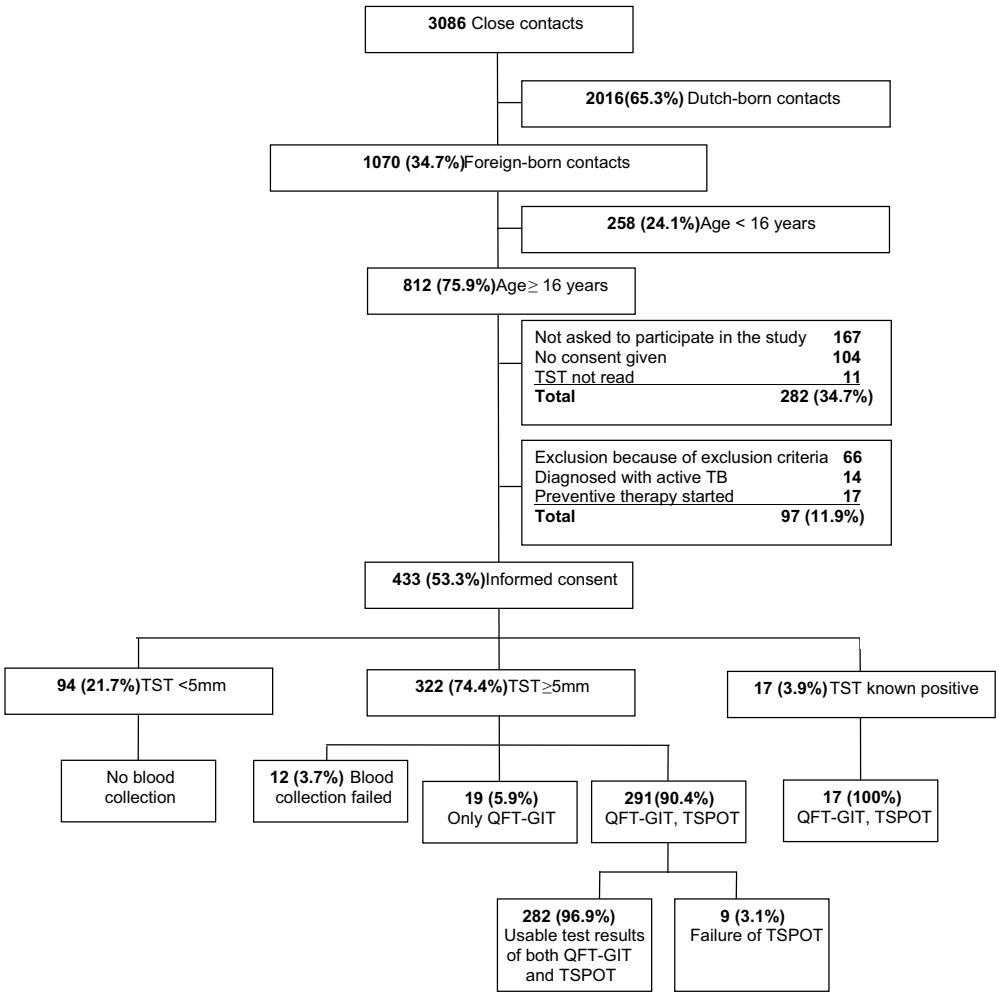

Figure 1. Flow diagram of the study population.
IGRA = Interferon-γ Release Assay
TB = tuberculosis
TST = tuberculin skin test
QFT-GIT = QuantiFERON-TB Gold in-tube

Table 1. Comparison of characteristics between eligible participants who participate and those who did not participate

	Consent / Total eligible	OR adjusted (95% CI)	p-value for chi square test
Total	**433/715 (60.6)**		
Gender			
Male	238/402 (59.2)	1	
Female	188/300 (62.7)	1.16 (0.85-1.57)	0.353
Unknown	7/13 (53.8)		
Age			
16-24	94/159 (59.1)	1	
25-34	104/197 (52.8)	0.77 (0.51-1.18)	0.233
35-44	131/198 (66.2)	1.35 (0.88-2.08)	0.171
45+	104/161 (64.6)	1.26 (0.80-1.98)	0.314
Continent of birth			
Europe, North America	36/68 (52.9)	0.64 (0.37-1.10)	0.109
South America	35/41 (85.4)	3.33 (1.35-8.22)	0.009
Asia	156/245 (63.7)	1	
Other Africa	121/225 (53.8)	0.66 (0.46-0.96)	0.030
Sub-Saharan Africa	76/108 (70.4)	1.36 (0.83-2.21)	0.223
Unknown	9/28 (32.1)		
Incidence in country of origin			
<50/100.000	130/220 (59.1)	1	0.331
≥50/100.000	294/467 (63.0)	1.18 (0.85-1.63)	
Unknown	9/28 (32.1)		
Recent contact			
Household contact	137/207 (66.2)	1	0.193
Non-household contact	253/416 (60.8)	0.79 (0.56-1.12)	
Unknown	43/92 (46.7)		
Frequency of contact			
Daily (>3x/wk)	327/536 (61.0)	1	0.153
Weekly	37/52 (71.2)	1.58 (0.84-2.94)	
Unknown	69/127 (54.3)		
Relation to index patient			
Partner	27/57 (47.4)	0.46 (0.23-0.90)	0.023
Brother /sister	19/41 (46.3)	0.44 (0.21-0.93)	0.032
Father / mother	32/43 (74.4)	1.48 (0.66-3.32)	0.344
Other family	126/202 (62.4)	0.84 (0.50-1.41)	0.516
Friend / relative	47/73 (64.4)	0.92 (0.48-1.75)	0.797
Colleague / schoolmate	61/92 (66.3)	1	
Other	79/140 (56.4)	0.66 (0.38-1.14)	0.658
Unknown	42/67 (62.7)		

Definition of abbreviations: OR = odds ratio; CI = Confidence interval

Table 2A. Agreement between interferon-γ release assays and tuberculin skin test results of immigrant close contacts

	Tuberculin skin test result		Tuberculin skin test result	
	5-14 mm	≥ 15 mm	5-9 mm	≥ 10 mm
QFT-GIT result				
Negative (n=130)	84 (64.6)	46 (35.4)	33 (25.4)	97 (74.6)
Positive (n=152)	35 (23.0)	117 (77.0)	10 (6.6)	142 (93.4)
Agreement, %	71.3		62.1	
κ	0.418		0.198	
T-SPOT.*TB* result				
Negative (n=114)	74 (64.9)	40 (35.1)	29 (25.4)	85 (74.6)
Positive (n=168)	45 (26.8)	123 (73.2)	14 (8.3)	154 (91.7)
Agreement, %	69.9		64.9	
κ	0.379		0.190	

Definition of abbreviations: QFT-GIT = QuantiFERON-TB Gold in-tube
Data are expressed as number (%).

Table 2B. Agreement between QuantiFERON-TB Gold in-tube and T-SPOT.*TB* results of immigrant close contacts

	T-SPOT.*TB* result	
N=282	Negative	Positive
QFT-GIT result		
Negative	100 (76.9)	30 (23.1)
Positive	14 (9.2)	138 (90.8)
Agreement, %	84.4	
κ	0.683	

Definition of abbreviations: QFT-GIT = QuantiFERON-TB Gold in-tube
Data are expressed as number (%).

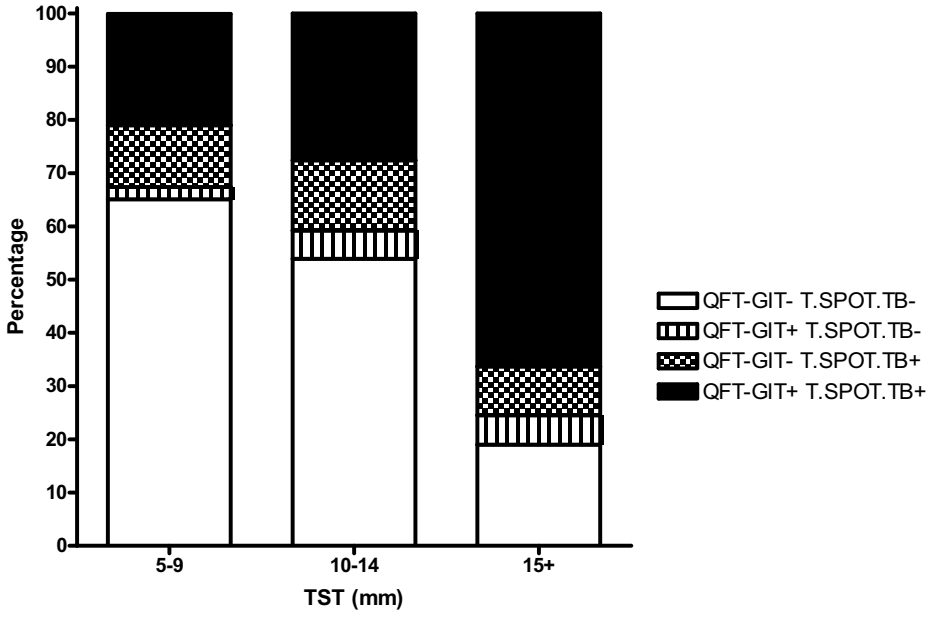

Figure 2. Percentage of concordant and discordant IGRA results per TST category
QFT-GIT = QuantiFERON-TB Gold in-tube
TST = tuberculin skin test
Mm = millimeter

TST results did not differ between household and non-household contacts (Table 3). After adjustment for age, gender and the degree of recent contact (being a household contact or not), individuals originating from sub-Saharan Africa more often had positive TST results than those from Asia. When repeating the analysis with a cut-off of 15 mm for TST, similar results were obtained, with slightly higher risk estimations. Positive QFT-GIT and T-SPOT.*TB* results were more frequent in older individuals. Furthermore, univariate analysis showed that previous participation in a contact investigation was strongly associated with a positive QFT-GIT (OR=3.36; 95% CI 1.43-9.21) and T-SPOT.*TB* result (OR=2.80; 95% CI 1.10-7.12) as was a history of past TB. Individuals with a history of daily smoking had more often a negative T-SPOT.*TB* than never smokers (OR=0.57; 95% CI 0.35-0.93). This effect was not significant for QFT-GIT (OR=0.64; 95% CI 0.39-1.03).
In the multivariate analysis the independent risk of a positive IGRA result for different regions of origin was assessed; after adjustment for age, gender and recent exposure (household or non-household contact), origin from sub-Saharan

Africa or other African countries were associated with a positive outcome of both IGRA (Table 4 and Table 5). Participation in previous contact investigation did not significantly improve the multivariate model.

The AF for remote exposure of a positive test was, after adjustment, (3.22-1)/3.22=0.69 (95% CI 0.17-0.88) for QFT-GIT, (4.41-1)/4.41=0.77 (95% CI 0.40-0.91) for TSPOT.*TB* and (0.73-1)/0.73=-0.37 (95% CI -3.88-0.62) for TST at a cut-off of 10 mm.

Table 3. Risk factors for a positive TST (≥ 10 mm) compared to those with a TST reaction of 5-9 mm and the adjusted risk for country of origin for a positive TST among 282 immigrant contacts in the Netherlands with valid TST and IGRA results

	TST positive*	OR (95% CI)	p-value	OR adjusted (95% CI)	p-value
Total	**239/282 (84.8)**				
Gender					
Male	140/164 (85.4)	1	0.736	1	0.854
Female	99/118 (83.9)	0.89 (0.46-1.72)		1.07 (0.53-2.16)	
Age					
16-24	38/48 (79.2)	1	0.211†	1	0.323
25-34	56/70 (80.0)	1.05 (0.42-2.62)		1.02 (0.39-2.67)	
35-44	81/91 (89.0)	2.13 (0.82-5.55)		2.16 (0.78-5.99)	
45+	64/73 (87.7)	1.87 (0.70-5.02)		1.67 (0.60-4.66)	
Continent of birth					
Europe, North America	20/23 (87.0)	1.41 (0.38-5.26)	0.031†	1.69 (0.44-6.45)	0.018
South America	13/19 (68.4)	0.46 (0.15-1.37)		0.48 (0.15-1.48)	
Asia	85/103 (82.5)	1		1	
Other Africa	70/84 (83.3)	1.06 (0.49-2.28)		1.17 (0.51-2.69)	
Sub-Saharan Africa	49/51 (96.1)	5.19 (1.16-23.31)		6.00 (1.32-27.24)	
Unknown	2/2 (100)				
BCG scar present					
No	34/37 (91.9)	1	0.133		
Yes	193/233 (82.8)	0.43 (0.13-1.45)			
Unknown	12/12 (100)				
Recent contact					
Household contact	79/94 (84.0)	1	0.900	1	0.981
Non-household contact	131/157 (83.4)	0.96 (0.48-1.92)		1.01 (0.49-2.09)	
Unknown	29/31 (93.5)				
TB in the past					
No	231/273 (84.6)	1	0.198		
Yes	5/5 (100)	NA			
Unknown	3/4 (75.0)				
Previous in contact investigation					
No	213/251 (84.9)	1	0.769		
Yes	24/29 (82.8)	0.86 (0.31-2.38)			
Unknown	2/2 (100)				
Ever smoked daily					
No	128/146 (87.7)	1	0.192		
Yes	105/128 (82.0)	0.64 (0.33-1.25)			
Unknown	6/8 (75.0)				

** Data are expressed as N/N (%)*
† Chi-square for trend
Definition of abbreviations: OR = odds ratio; CI = Confidence interval; n.s = not significant; NA= not applicable

Table 4. Risk factors for a positive QuantiFERON-TB Gold in-tube and the adjusted risk for country of origin for a positive QuantiFERON-TB Gold in-tube among 282 immigrant contacts in the Netherlands

	QFT-GIT positive*	OR (95% CI)	p-value	OR adjusted (95% CI)	p-value
Total	**152/282 (53.9)**				
Gender					
Male	88/164 (53.7)	1	0.923	1	0.446
Female	64/118 (54.2)	1.02 (0.64-1.64)		1.23 (0.72-2.11)	
Age					
16-24	20/48 (41.7)	1	0.015†	1	0.175
25-34	37/70 (52.9)	1.57 (0.75-3.29)		1.42 (0.64-3.16)	
35-44	47/91 (51.6)	1.50 (0.74-3.03)		1.48 (0.69-3.20)	
45+	48/73 (65.8)	2.69 (1.27-5.69)		2.44 (1.09-5.49)	
Continent of birth					
Europe, North America	6/23 (26.1)	0.39 (0.14-1.07)		0.48 (0.17-1.36)	
South America	7/19 (36.8)	0.64 (0.23-1.76)		0.64 (0.23-1.83)	
Asia	49/103 (47.6)	1	<0.001†	1	0.001
Other Africa	54/84 (64.3)	1.98 (1.10-3.58)		2.20 (1.13-4.30)	
Sub-Saharan Africa	36/51 (70.6)	2.65 (1.29-5.41)		2.97 (1.40-6.27)	
Unknown	0/2 (0)				
BCG scar present					
No	23/37 (62.2)	1	0.225		
Yes	120/233 (51.5)	0.65 (0.32-1.32)			
Unknown	9/12 (75.0)				
Recent contact					
Household contact	54/94 (57.4)	1	0.137	1	0.171
Non-household contact	75/157 (47.8)	0.68 (0.41-1.13)		0.68 (0.39-1.18)	
Unknown	23/31 (74.2)				
TB in the past					
No	147/273 (96.8)	1	0.783		
Yes	3/5 (60.0)	1.13 (0.21-7.82)			
Unknown	2/4 (50.0)				
Previous in contact investigation					
No	129/251 (51.4)	1	0.003		
Yes	23/29 (79.3)	3.62 (1.43-9.21)			
Unknown	0/2 (0)				
Ever smoked daily					
No	86/146 (58.9)	1	0.062		
Yes	61/128 (47.7)	0.64 (0.39-1.03)			
Unknown	5/8 (62.5)				

** Data are expressed as N/N (%)*
† Chi-square for trend
Definition of abbreviations: OR = odds ratio; CI = Confidence interval; n.s = not significant

Table 5. Risk factors for a positive T-SPOT.*TB* and the adjusted risk for country of origin for a positive T-SPOT.*TB* among 282 immigrant contacts in the Netherlands

	TSPOT positive*	OR (95% CI)	p-value	OR adjusted (95% CI)	p-value
Total	**168/282 (59.6)**				
Gender					
Male	94/164 (57.3)	1	0.362	1	0.206
Female	74/118 (62.7)	1.25 (0.77-2.03)		1.42 (0.82-2.46)	
Age					
16-24	24/48 (50.0)	1	0.032†	1	0.314
25-34	41/70 (58.6)	1.41 (0.68-2.96)		1.30 (0.59-2.89)	
35-44	51/91 (56.0)	1.28 (0.63-2.57)		1.42 (0.66-3.05)	
45+	52/73 (71.2)	2.48 (1.16-5.29)		2.14 (0.94-4.84)	
Continent of birth					
Europe, North America	6/23 (26.1)	0.29 (0.10-0.78)		0.35 (0.13-0.99)	
South America	10/19 (52.6)	0.90 (0.34-2.39)		0.91 (0.33-2.51)	
Asia	57/103 (55.3)	1	0.001†	1	<0.001
Other Africa	58/84 (69.0)	1.80 (0.98-3.29)		2.43 (1.22-4.86)	
Sub-Saharan Africa	37/51 (72.5)	2.13 (1.03-4.41)		2.40 (1.13-5.10)	
Unknown	0/2 (0)				
BCG scar present					
No	24/37 (64.9)	1	0.452		
Yes	136/233 (58.4)	0.76 (0.67-1.57)			
Unknown	8/12 (66.7)				
Recent contact					
Household contact	59/94 (62.8)	1	0.252	1	0.292
Non-household contact	87/157 (55.4)	0.74 (0.44-1.24)		0.74 (0.42-1.30)	
Unknown	22/31 (71.0)				
TB in the past					
No	161/273 (59.0)		0.022		
Yes	5/5 (100)	NA			
Unknown	2/4 (50.0)				
Previous in contact investigation					
No	145/251 (57.8)	1	0.020		
Yes	23/29 (79.3)	2.80 (1.10-7.12)			
Unknown	0/2 (0)				
Ever smoked daily					
No	97/146 (66.4)	1	0.025		
Yes	68/128 (53.1)	0.57 (0.35-0.93)			
Unknown	3/8 (37.5)				

** Data are expressed as N/N (%)*
† Chi-square for trend
Definition of abbreviations: OR = odds ratio; CI = Confidence interval; n.s = not significant

DISCUSSION

The main finding of this study is that among foreign-born individuals tested in a contact investigation, a positive IGRA not only relates to possible recent TB infection but also to exposure to TB in their country of origin. Such persistently positive IGRA responses following past infection may limit the usefulness of IGRA to identify those foreign-born individuals in a contact investigation who may benefit from preventive treatment.

Several limitations need to be addressed. Firstly, we were unable to include all eligible individuals. Since individuals who did not participate were slightly more often a partner, brother or sister of the index patient or originating from non-sub-Saharan African countries, our results are slightly less representative for these groups. We found that 54-60% of immigrant contacts with TST ≥5 mm were positive in one of the IGRA which corresponds with 40-45% of all contacts. This percentage was similar to that reported in other contact studies using IGRA among close contacts from endemic countries (7;11;23;24). Thus, although we cannot completely exclude that selection of cases occurred, it is unlikely to have affected the overall representativeness of our findings. Secondly, the fact that samples obtained on Fridays were frozen and the T-SPOT.*TB* was performed later may have affected our results. However, one study reported that it is possible to perform this assay on frozen cells and we did not observe a difference in the percentage of positive results between fresh and frozen T-SPOT.*TB* assays (25). Thirdly, we did not perform IGRA among contacts with TST <5mm. More research will be needed to evaluate the IGRA in this specific group as well as in immuno-compromised individuals. This may have resulted in an underestimation of the association between age, recent contact and positive test results. We sampled blood for IGRA at the day of TST reading. It has been argued that TST can boost IGRA testing, hampering its interpretation. Previously we showed that boosting of IGRA testing does not occur when blood for the IGRA is collected on the day of TST reading (26). Other studies showed that boosting of IGRA occurred when the assays were performed several weeks after the TST (27-29).

QFT-GIT and T-SPOT.*TB*, considered more specific to detect TB infection than the TST, were both independently associated with increasing age and origin from either sub-Saharan Africa or other African countries. This is in line with a study in Norway where up to 29% of immigrants from high endemic countries were

QFT-GIT positive at entry, also implying the presence of remote infections (30). The AF suggests that positive TST results were not attributable to exposure in the country of origin. The AF for TST found in our study might be influenced by false positive TST results induced by BCG-vaccination or infections with other non-tuberculous Mycobacteria (NTM) and therefore underestimate the influence of previous exposure on TST. However, since the IGRA are considered to be influenced neither by BCG-vaccination nor by most NTM infections, the AF estimates for both IGRA more reliable reflect the influence of previous exposure than the one for TST. In 69-77% of close contacts with positive IGRA results these were attributable to previous exposure in the country of origin. One limitation of the AF is that if several causal factors coexist, the sum of AFs of each adds up to more than 100%. Therefore a part of the proportion of positive results that was attributable to previous exposure can be the result of another causal factor that was not measured in our study. Furthermore, we cannot exclude the possibility that the proxy we used for previous exposure to tuberculosis in the country of origin may be related to other factors than increased exposure alone. Since only a number of our participants were born in Europe or North America this may have affected the power of our AF estimates. The AF found in our study may not be applicable for populations that differ greatly in composition from that described in this study.

Speculating on the immunological explanation of this finding, it is likely that immigrants have been exposed repeatedly in their country of birth. This boosting of the immune response to *M. tuberculosis* antigens may have resulted in a persistent pool of circulating responsive cells, which can be activated even within the short incubation period of the IGRA. It needs to be investigated whether this implies that these individuals have an excellent protection against TB or that they are still in danger of progression to active TB. Long term follow-up studies are needed to determine the predictive value of IGRA for breakdown to active TB. The contacts in this study are therefore being followed for two years to determine the predictive value of the IGRA in this cohort.

Previous studies performed among close contacts in different settings showed a better correlation both for QFT and for T-SPOT.*TB* with recent exposure (closeness of contact) as compared to TST (7-18). They were performed mainly among contacts with a relatively low risk of previous infection, while our study was restricted to close contacts with a high risk of recent exposure.

CONCLUSIONS

In conclusion, IGRA responses among recently TB exposed immigrant close contacts were influenced by remote exposure to TB in the country of origin. Especially immigrants originating from (sub-Saharan) Africa more often had positive IGRA results, which could not be explained by differences in recent exposure. Positive IGRA results among immigrants should therefore not indiscriminately be considered to reflect recent infection.

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REFERENCE LIST

(1) Falzon D, Ait-Belghiti F. What is tuberculosis surveillance in the European Union telling us? Clin Infect Dis 2007 May 15;44(10):1261-7.
(2) Cain KP, Haley CA, Armstrong LR, Garman KN, Wells CD, Iademarco MF, et al. Tuberculosis among foreign-born persons in the United States: achieving tuberculosis elimination. Am J Respir Crit Care Med 2007 Jan 1;175(1):75-9.
(3) Khan K, Wang J, Hu W, Bierman A, Li Y, Gardam M. Tuberculosis infection in the United States: national trends over three decades. Am J Respir Crit Care Med 2008 Feb 15;177(4):455-60.
(4) Brock I, Weldingh K, Leyten EM, Arend SM, Ravn P, Andersen P. Specific T-cell epitopes for immunoassay-based diagnosis of Mycobacterium tuberculosis infection. J Clin Microbiol 2004 Jun;42(6):2379-87.
(5) Arend SM, van Meijgaarden KE, de Boer K, de Palou EC, van Soolingen D, Ottenhoff TH, et al. Tuberculin skin testing and in vitro T cell responses to ESAT-6 and culture filtrate protein 10 after infection with Mycobacterium marinum or M. kansasii. J Infect Dis 2002 Dec 15;186(12):1797-807.

(6) Andersen P, Doherty TM, Pai M, Weldingh K. The prognosis of latent tuberculosis: can disease be predicted? Trends Mol Med 2007 May;13(5):175-82.

(7) Brodie D, Lederer DJ, Gallardo JS, Trivedi SH, Burzynski JN, Schluger NW. Use of an interferon-gamma release assay to diagnose latent tuberculosis infection in foreign-born patients. Chest 2008 Apr;133(4):869-74.

(8) Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P, et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. Lancet 2003 Apr 5;361(9364):1168-73.

(9) Lalvani A, Pathan AA, Durkan H, Wilkinson KA, Whelan A, Deeks JJ, et al. Enhanced contact tracing and spatial tracking of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. Lancet 2001 Jun 23;357(9273):2017-21.

(10) Richeldi L, Ewer K, Losi M, Bergamini BM, Roversi P, Deeks J, et al. T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. Am J Respir Crit Care Med 2004 Aug 1;170(3):288-95.

(11) Hill PC, Brookes RH, Fox A, Fielding K, Jeffries DJ, Jackson-Sillah D, et al. Large-scale evaluation of enzyme-linked immunospot assay and skin test for diagnosis of Mycobacterium tuberculosis infection against a gradient of exposure in The Gambia. Clin Infect Dis 2004 Apr 1;38(7):966-73.

(12) Zellweger JP, Zellweger A, Ansermet S, de Senarclens B, Wrighton-Smith P. Contact tracing using a new T-cell-based test: better correlation with tuberculosis exposure than the tuberculin skin test. Int J Tuberc Lung Dis 2005 Nov;9(11):1242-7.

(13) Brock I, Weldingh K, Lillebaek T, Follmann F, Andersen P. Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. Am J Respir Crit Care Med 2004 Jul 1;170(1):65-9.

(14) Higuchi K, Harada N, Mori T, Sekiya Y. Use of QuantiFERON-TB Gold to investigate tuberculosis contacts in a high school. Respirology 2007 Jan;12(1):88-92.

(15) Kang YA, Lee HW, Yoon HI, Cho B, Han SK, Shim YS, et al. Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA 2005 Jun 8;293(22):2756-61.

(16) Nakaoka H, Lawson L, Squire S.B, Coulter B, Ravn P, Brock I, et al. Risk for tuberculosis among children. Emerg Infect Dis 2006;12(9):1383-8.

(17) Arend SM, Thijsen SF, Leyten EM, Bouwman JJ, Franken WP, Koster BF, et al. Comparison of two interferon-gamma assays and tuberculin skin test for tracing tuberculosis contacts. Am J Respir Crit Care Med 2007 Mar 15;175(6):618-27.

(18) Adetifa IM, Lugos MD, Hammond A, Jeffries D, Donkor S, Adegbola RA, et al. Comparison of two interferon gamma release assays in the diagnosis of Mycobacterium tuberculosis infection and disease in The Gambia. BMC Infect Dis 2007;7:122.

(19) Menzies D, Pai M, Comstock G. Meta-analysis: New Tests for the Diagnosis of Latent Tuberculosis Infection: Areas of Uncertainty and Recommendations for Research. Ann Intern Med 2007 Mar 6;146(5):340-54.

(20) Veen J. Microepidemics of tuberculosis: the stone-in-the-pond principle. Tuber Lung Dis 1992 Apr;73(2):73-6.

(21) Rothman KJ, Greenland S. Modern Epidemiology. Second Edition. Philidelphia: Lippincott Williams & Wilkins; 1998.

(22) Steenland K, Armstrong B. An overview of methods for calculating the burden of disease due to specific risk factors. Epidemiology 2006 Sep;17(5):512-9.

(23) Janssens JP, Roux-Lombard P, Perneger T, Metzger M, Vivien R, Rochat T. Quantitative scoring of an interferon-gamma assay for differentiating active from latent tuberculosis. Eur Respir J 2007 Oct;30(4):722-8.

(24) Dominguez J, Ruiz-Manzano J, Souza-Galvao M, Latorre I, Mila C, Blanco S, et al. Comparison of two commercially available gamma interferon blood tests for immunodiagnosis of tuberculosis. Clin Vaccine Immunol 2008 Jan;15(1):168-71.

(25) Smith JG, Joseph HR, Green T, Field JA, Wooters M, Kaufhold RM, et al. Establishing acceptance criteria for cell-mediated-immunity assays using frozen peripheral blood mononuclear cells stored under optimal and suboptimal conditions. Clin Vaccine Immunol 2007 May;14(5):527-37.

(26) Leyten EM, Prins C, Bossink AW, Thijsen S, Ottenhoff TH, van Dissel JT, et al. Effect of tuberculin skin testing on a Mycobacterium tuberculosis-specific interferon-gamma assay. Eur Respir J 2007 Jun;29(6):1212-6.

(27) Richeldi L, Ewer K, Losi M, Roversi P, Fabbri LM, Lalvani A. Repeated tuberculin testing does not induce false positive ELISPOT results. Thorax 2006 Feb;61(2):180.

(28) Naseer A, Naqvi S, Kampmann B. Evidence for boosting Mycobacterium tuberculosis-specific IFN-gamma responses at 6 weeks following tuberculin skin testing. Eur Respir J 2007 Jun;29(6):1282-3.

(29) Vilaplana C, Ruiz-Manzano J, Gil O, Cuchillo F, Montane E, Singh M, et al. The Tuberculin Skin Test Increases the Responses Measured by T Cell Interferon-Gamma Release Assays. Scand J Immunol 2008 Apr 4.

(30) Winje BA, Oftung F, Korsvold GE, Mannsaker T, Jeppesen AS, Harstad I, et al. Screening for tuberculosis infection among newly arrived asylum seekers: comparison of QuantiFERON(R)TB Gold with tuberculin skin test. BMC Infect Dis 2008 May 14;8(1):65.