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

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PREDICTIVE VALUE OF IGRA AND TST FOR DEVELOPMENT OF ACTIVE TUBERCULOSIS AMONG RECENTLY EXPOSED IMMIGRANTS

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ABSTRACT

Rationale

Interferon-gamma release assays (IGRA) have shown to be more specific than the tuberculin skin test (TST) for detection of Mycobacterium tuberculosis infection, but little is known about their predictive value for progression to tuberculosis (TB) disease.

Objective

To determine the positive predictive value (PPV) for TB disease of two IGRA, QuantiFERON-TB Gold in-tube (QFT-GIT) and T-SPOT.TB, compared to the TST in immigrants contacts.

Methods

Immigrant close contacts of sputum smear-positive TB patients were included when aged ≥16 years and the TST result was ≥5 mm at zero or three months after diagnosis of the index patient. Contacts were followed during two years for development of TB disease.

Measurements and Main Results

Of 339 immigrant contacts with TST ≥5 mm, 324 and 299 had valid results of QFT-GIT and T-SPOT.TB, respectively. Nine contacts developed active TB. The PPV for progression to TB during this period was 9/288=3.1% (95% CI; 1.3-5.0) for TST ≥10 mm, 7/184=3.8% (95% CI; 1.7-5.9) for TST ≥15 mm, 5/178=2.8% (95% CI; 1.0-4.6) for QFT-GIT and 6/181=3.3% (95% CI; 1.3-5.3) for T-SPOT.TB. Sensitivity was 100% (9/9), 88% (7/8), 63% (5/8) and 75% (6/8), respectively.

Conclusions

Progression to TB disease was not predicted better by QFT-GIT or T-SPOT.TB compared to TST in immigrant close contacts. Based on the high incidence rate of TB progression, preventive measures could be considered in this population.

INTRODUCTION

Until some years ago the diagnosis of a latent tuberculosis (TB) infection (LTBI) relied only on the tuberculin skin test (TST). Early studies have shown that TST responders have a higher risk of developing TB disease compared to those with no induration (1-3). However, one of the limitations of the TST is that its specificity is not optimal, and false-positive reactions may occur among individuals with BCG-vaccination and those infected with nontuberculous mycobacteria (NTM).
Interferon-gamma release assays (IGRA) have emerged as an alternative for the TST. Currently two commercial IGRA are available: QuantiFERON-TB® Gold in-tube (Cellestis, Carnegie, Australia) and T-SPOT.*TB*® (Oxford Immunotec, Abingdon, UK). These IGRA measure an immune response to *M. tuberculosis*-specific antigens, which are absent in *M. bovis* BCG and most NTM. Consequently, IGRA results are not affected by previous BCG-vaccination and infection with most NTM (4). Furthermore, repeated testing does not influence later test results, in contrast to the boosting effect that can be observed when the TST is repeated over time (5). Several countries have incorporated IGRA as a diagnostic test for LTBI in their guidelines and recommend its use as a confirmative test after a positive TST (6, 7), or as an alternative to the TST (7-9). However, firm evidence is needed that positive IGRA results correlate with subsequent development of TB disease and can therefore be the basis for preventive measures (4, 10).

So far, few prospective studies assessed progression of TB among contacts of infectious pulmonary TB patients in relation to IGRA results (11-14). A study among mainly German-born contacts, showed that the QuantiFERON-TB Gold in-tube (QFT-GIT) was a more accurate indicator for progression to active disease than the TST at a cut-off of 5 mm (12). Two other studies, performed in Gambia (14) and Turkey (11), found that the ELISPOT (an in-house version of the T-SPOT.*TB*) and the TST both missed some of the contacts who progressed to TB disease, suggesting that the ELISPOT did not predict disease progression better than the TST in these settings. It is unclear if these different outcomes can be attributed to the different IGRA used, the type of contacts included in these studies, or to differences in the infection prevalence.

In this study, we assessed the positive predictive value for TB disease of QFT-GIT and T-SPOT.*TB* compared with TST in immigrant individuals in The Netherlands who were recently exposed to infectious pulmonary TB patients. To our knowledge this is the first longitudinal study that describes the predictive value of both

commercially available IGRA in a population with high risk of recent infection, a high lifetime risk of previous infection and a low risk of re-infection after inclusion.

METHODS

Participants

Between April 2005 and July 2007, close contacts of sputum-smear positive pulmonary TB patients when at least 16 years old and born in a TB endemic country (first generation immigrant) (see list, appendix A) were recruited shortly after the diagnosis of the index patient. Furthermore we included Dutch-born individuals when at least one of their parents was born in a TB endemic country (second generation immigrants) and they were BCG-vaccinated, since their TST results may be false positive due to their BCG-status. Recruitment took place at 15 municipal health services (MHSs) throughout the Netherlands. We excluded contacts with known conditions associated with an increased risk of progression to disease (including diabetes and HIV infection) and individuals who were given preventive treatment.

Data collection

Screening of close contacts in a contact investigation is performed in two rounds in the Netherlands. The first round, performed soon after the diagnosis of the index patient, aims to find infected or diseased contacts as early as possible. The second round is performed at least eight weeks after the last infectious contact with the index patient, to find additional infected or diseased contacts who converted in the meantime. At the time of recruitment, all contacts underwent a chest X-ray (CXR) to exclude the presence of active TB disease. Additionally a TST was administered (2 TU, PPD RT23 in Tween-80, Statens Serum Institute, Copenhagen, Denmark) and read after 48-72 hours. Contacts with TST results ≥5 mm were interviewed and blood was obtained for T-SPOT.*TB* and QFT-GIT. If TST was <5 mm in the first round it was repeated at the second round and only followed by IGRA testing if ≥5 mm. Individuals who underwent their first TST during the second round of the contact investigation were tested once. Known past TST responders (TST ≥10 mm) did not undergo a TST, but were only tested with IGRA. Characteristics of the cohort and factors related to positive test outcomes are described elsewhere (15).

Contacts with TST results ≥5 mm were invited for four follow-up visits at 6, 12, 18 and 24 months after inclusion and were interviewed for signs and symptoms suggestive of TB disease. Most MHSs offered CXR screening during these visits. Contacts who did not show up for their follow-up visit after several invitations were, if possible, interviewed by telephone.

Ethics

Ethical approval for this study was obtained from the Netherlands Central Committee on Research Involving Human Subjects (CCMO, P04.1214C) and all participants provided oral and written informed consent. Contacts with possible LTBI in our study did not received preventive treatment since at the time of the study the routine practice in the Netherlands was to screen these immigrants only for active TB disease. The justification for this policy is that among adults with a high likelihood of remote (instead of recent) infection and the possibility of false positive TST results due to previous BCG-vaccination the benefit of preventive therapy may not outweigh the risks related to the chemotherapy (16).

Incident TB cases

Contacts diagnosed with TB disease at least 3 months after the diagnosis of the index patient were considered to be incident cases. Co-prevalent TB cases, defined as contacts diagnosed with TB disease within 3 months after the diagnosis of the index patient, were excluded from the analysis. The diagnosis of TB disease was based on CXR, symptoms, smear and/or culture results.

Laboratory procedures

Both IGRA were performed according to the instructions of the manufacturers (17) (18), and tested in a single laboratory (Leiden University Medical Center, The Netherlands), as described earlier (15). For QFT-GIT a positive test was defined as ≥0.35 IU/ml. Interpretation of T-SPOT.*TB* results was according to the latest criteria defined by the manufacturer.

When available, *M. tuberculosis* isolates from the incident cases and their index patients were subjected to *IS6110* restriction fragment length polymorphism (RFLP) typing (19) and in case of less than 5 bands additionally sub-typed using the polymorphic GC-rich sequence as a probe (20), to determine if the RFLP patterns were identical. Molecular typing was done at the National Institute of Public Health and the Environment. Computer-assisted analysis of IS*6110*-PGRS RFLP was done using Bionumerics software, version 4.0 for Windows (Applied Maths, Sint-Maartens-Latem, Belgium) and in addition visually checked.

Predictive values

In our primary analysis we determined the positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of the different tests for progression to disease in our total cohort of contacts who by definition had a TST ≥5 mm. The PPV was calculated as: number of incident TB cases with a positive test outcome / total number of contacts with a positive test outcome. Since the cumulative number of TB cases, and therefore the PPV, is dependent on the duration of follow-up, and not all of our contacts could be followed for 2 years we performed a secondary analysis to determine test parameters for progression to disease within the first 12 months of follow-up. Because not all contacts attended the follow-up visits we performed an even more strict sensitivity analysis in which we determined the test parameters for progression to disease within the first 12 months only including contacts who attended the follow-up for at least 12 months.

Follow-up time

Since we were interested in the prediction of incident cases, the date of start of follow-up was defined as 3 months after the diagnosis of the index patient, or the date of blood collection for those who had IGRA testing >3 months after the diagnosis of their index patient. Follow-up time was calculated from the date of start of follow-up up to 24 months, the date of TB diagnosis, the date of death or emigration out of the Netherlands, whatever occurred first.

To ascertain that we did not miss any incident cases, we performed a search in the Netherlands Tuberculosis Register (NTR) and assessed if any of the included contacts was registered with TB up to August 1st 2008. Since the NTR is an anonymous register the search was based on the date of birth, gender and country of birth and MHSs were asked to confirm if the matches between the study database and the NTR database were indeed the same person. Although we excluded contacts with TST <5 mm from follow-up, since these individuals had a negligible risk of developing TB disease (1), the same search strategy in the NTR was performed to assess if any of them was registered with TB afterwards.

Statistical analysis

Poisson regression was used to estimate incidence rates and 95% confidence intervals (CI) for progression to TB per 1000 person-years. For the primary analysis we constructed Kaplan-Meier curves. The equality of the survival distributions were compared by the Gehan-Breslow-Wilcoxon-test that weighs the time points

by the number of cases. Statistical analyses were conducted using SPSS version 16.0 for Windows (SPSS, Inc., Chicago, IL).
pulsed for 16 hours with ^{3}H-TdR, and TdR incorporation was measured.

RESULTS

Participants and test results

During the study period, 380 contact investigations were conducted at the participating MHSs. Of 812 immigrant close contacts aged ≥16 years, 433 (53%) fulfilled the inclusion criteria and gave informed consent (Figure 1). Details on the comparison between contacts who were included and those who were not asked or refused participation are described elsewhere (15). Out of 433, 339 (78%) contacts were eligible for follow-up since they either had TST results ≥5 mm (n=322) or were known positive TST responders in the past (n=17). TST results were ≥10 mm in 288/339 (85%), and ≥15 mm in 184/322 (57%). Blood collection for IGRA failed in 12 contacts. At recruitment 178 (54%) of 327 remaining individuals had a positive QFT-GIT result. For 28 individuals no valid T-SPOT.*TB* result was available due to insufficient blood collection (n=19), inconclusive test result (n=5) or technical failure (n=4). T-SPOT.*TB* was positive in 181 (61%) of the remaining 299 individuals. Characteristics of the study population are given in Table 1.

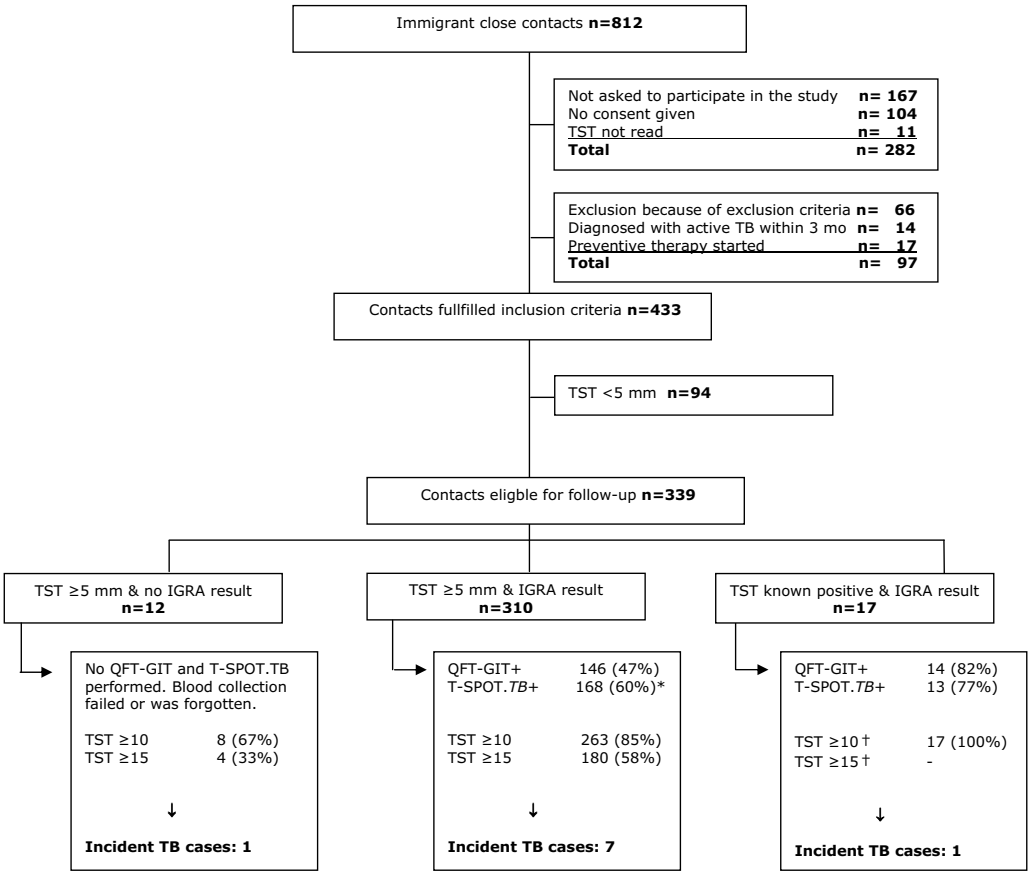


Figure 1. Cohort profile of recruited contacts
Definition of abbreviations: TST= tuberculin skin test; QFT-GIT=QuantiFERON TB Gold in tube; TB=tuberculosis pos= positive; neg= negative; n.r.=no result, y=years, IQR=inter quartile range.
** No T-SPOT.TB result of 28 individuals because of technical failure (n=4), inconclusive test results (n=5) or insufficient blood collected to perform the test (n=19).*
† Known positive TST results were all considered to be at least 10 mm (the regular cut-off for a positive TST result in the Netherlands), but were excluded from the analysis that used 15 mm as a cut-off since no exact indurations were known.

Table 1. Description of the study population; immigrant close contacts with a TST result of at least 5 mm (n=339)

	Study population	
	N	%
Total	**339**	**100**
Gender		
Male	189	55.8
Female	147	43.4
Unknown	3	0.9
Age (years)		
16-24	53	15.6
25-34	80	23.6
35-44	115	33.9
≥ 45	91	26.8
Continent of birth		
Europe, North America	27	8.0
South America	27	8.0
Asia	123	36.3
Other Africa	98	28.9
Sub-Saharan Africa	59	17.4
Unknown	5	1.5
Recent close contact		
Non-household contact	185	54.6
Household contact	115	33.9
Unknown	39	11.5
BCG scar		
Yes	274	80.8
No	43	12.7
Unknown	22	6.5
QFT-GIT result		
Negative	149	44.0
Positive	178	52.5
Not done	12	3.5
T-SPOT.*TB* result		
Negative	118	34.8
Positive	181	53.4
Not done/no valid result*	40	11.8
TST result (mm)		
5-9	51	15.0
10-14	87	25.7
≥ 15	184	54.3
Known TST responder	17	5.0

Definition of abbreviations: TST= tuberculin skin test; QFT-GIT=QuantiFERON TB Gold in tube.
** No T-SPOT.TB result because blood collection failed (n=12), technical failure (n=4), inconclusive test results (n=5) or insufficient blood collected to perform the test (n=19)*

Incident cases

Nine contacts developed TB disease >3 months after the diagnosis of the index patient. None of the participants with TST <5 mm and none of the participants who did not attend all follow-up visits matched with any of the TB-cases notified in the Netherlands Tuberculosis Register. One incident case was not tested with TST at recruitment and in another incident case blood collection for IGRA had failed. All eight IGRA tested patients had TST results ≥10 mm and seven (88%) had results ≥15 mm. T-SPOT.*TB* was positive in 6/8 (75%) (all >30 spots), while QFT-GIT was positive in 5/8 (63%) TB patients (4/5 were >10 IU/ml). The two patients with negative T-SPOT.*TB* results were also negative in the QFT-GIT. Six patients (including those with a negative QFT-GIT result) were confirmed by culture, and RFLP fingerprinting showed that all isolates were identical to those of the corresponding index case.

None of the three incident patients with at least one negative IGRA result at recruitment were known to be HIV positive or to have any other immune suppressive disorder. Furthermore none of them reported to have traveled to a TB endemic country or have been exposed to another TB case in the period between their inclusion and diagnosis. All had IGRA results far below the threshold of a positive test (QFT-GIT results: -0.24, 0.02 and 0.04 IU/ml; T-SPOT.*TB* results: 0 and 1 spot). Two of these three patients had extra-pulmonary TB. Contacts were tested with IGRA between 4 to 200 days after the diagnosis of the index case (median 37 days, IQR; 15-117). The three contacts who developed TB and who had at least one negative IGRA results were tested relatively early, at 5, 19 (positive in T-SPOT.*TB*) and 34 days after diagnosis of the index patient.

Survival analysis

No significant difference was observed between the incidence of TB in contacts who were IGRA positive and those who were IGRA negative (QFT-GIT; Gehan-Breslow-Wilcoxon-test p-value=0.718, T-SPOT.TB; p-value=0.443) (Figure 2). Using a cut-off of 15 mm, the difference between the incidence of TB among contacts who were TST positive or negative did not reach statistical significance (p-value=0.081). All contacts who progressed to disease had a TST result ≥10 mm (p-value not determined due to 0 observations in TST-negative group).

Figure 2. Kaplan-Meier curves showing the proportion of TB free contacts with a positive or negative result in QFT-GIT (a), T-SPOT.TB (b), TST at a cut-off of 10mm (c), TST at a cut-off of 15mm(d).

Persons at risk, figure 2a

Follow-up time (years)	**0**	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**	**1.50**	**1.75**
TST ≥10 mm	288	282	279	278	265	216	187	134
TST 5-9 mm	51	51	51	51	48	39	32	30

Persons at risk, figure 2b

Follow-up time (years)	**0**	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**	**1.50**	**1.75**
TST ≥15 mm	184	180	178	177	166	132	111	92
TST 5-14 mm	138	136	135	135	131	107	97	87

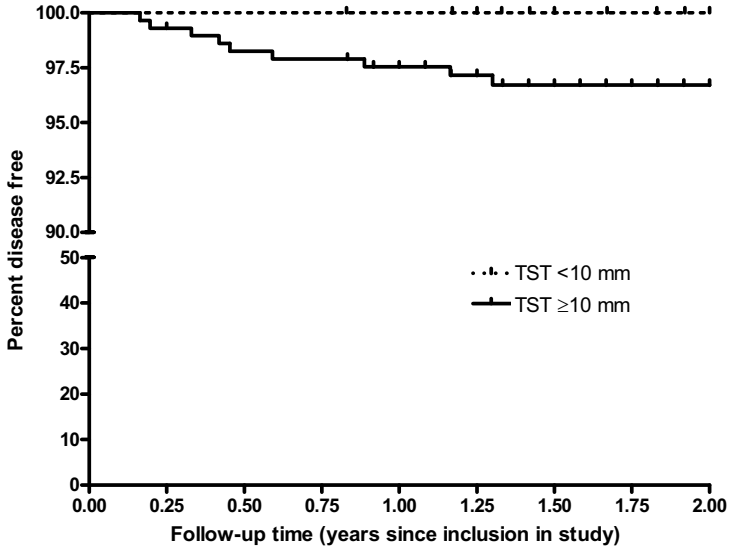
Persons at risk, figure 2c

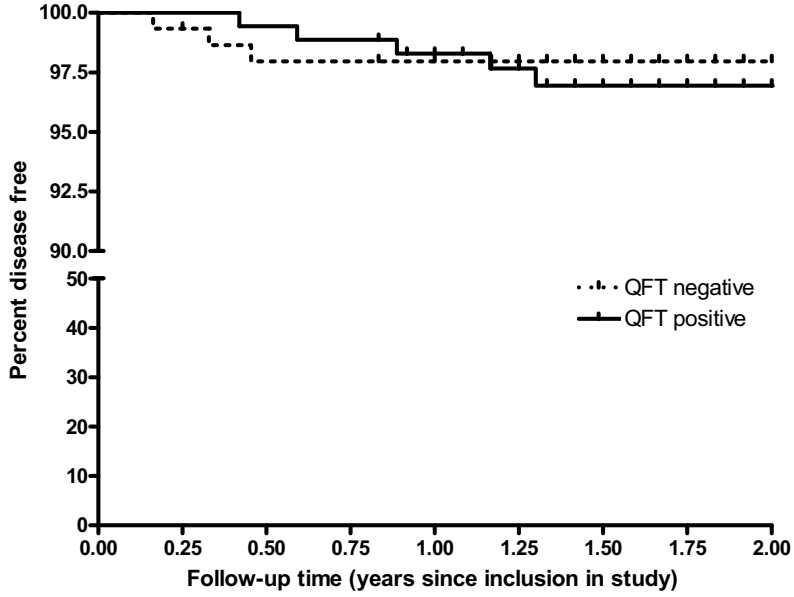
Follow-up time (years)	**0**	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**	**1.50**	**1.75**
QFT-GIT positive	178	176	175	174	163	135	116	95
QFT-GIT negative	149	146	144	144	140	113	96	87

Persons at risk, figure 2d

Follow-up time (years)	**0**	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**	**1.50**	**1.75**
T-SPOT.*TB* positive	181	178	176	175	165	140	121	101
T-SPOT.*TB* negative	118	116	115	115	111	84	71	64

† Follow-up time was calculated from the date of start of follow-up (the date 3 months after the diagnosis of the index patient, or the date of blood collection for those who had IGRA testing >3 months after the diagnosis of their index patient) up to 24 months, the date of TB diagnosis, the time of emigration or death of the subject, or at the 1st of August 2008, whichever data came first.


100.0
97.5
95.0
92.5
90.0
50
40
30
20
10
0
Percent disease free
TST <10 mm
TST ≥10 mm
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
Follow-up time (years since inclusion in study)


100.0
97.5
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92.5
90.0
50
40
30
20
10
0
Percent disease free
QFT negative
QFT positive
0.00
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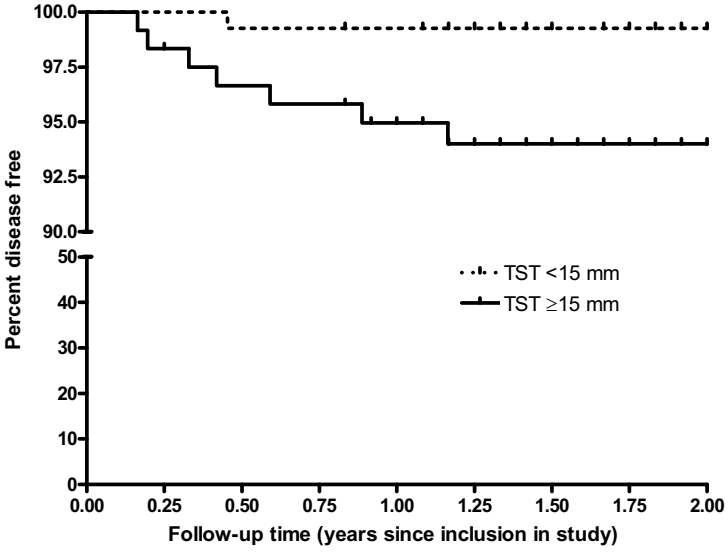
0.75
1.00
1.25
1.50
1.75
2.00
Follow-up time (years since inclusion in study)


100.0
97.5
95.0
92.5
90.0
50
40
30
20
10
0
Percent disease free
TST <15 mm
TST ≥15 mm
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
Follow-up time (years since inclusion in study)

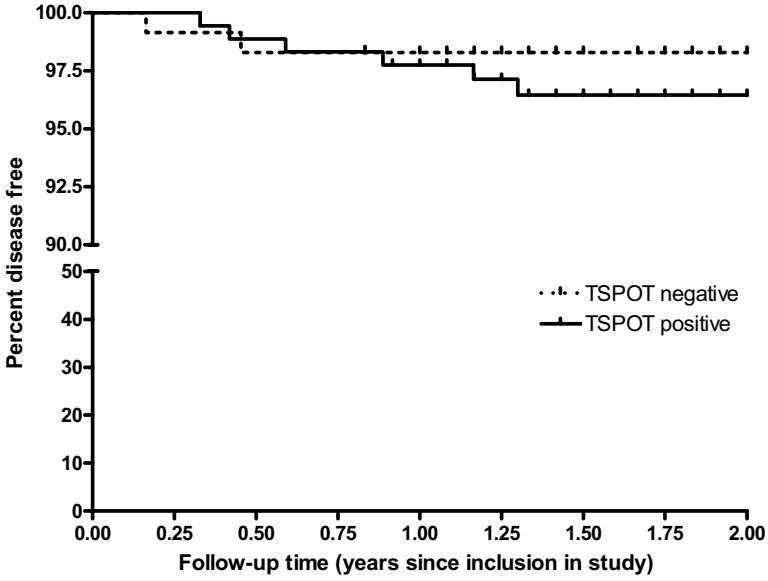

100.0
97.5
95.0
92.5
90.0
50
40
30
20
10
0
Percent disease free
TSPOT negative
TSPOT positive
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
Follow-up time (years since inclusion in study)

Table 2. Characteristics of contacts that developed TB disease during follow-up

Case	Sex	Age group (y)	Region of birth	HH contact	TST (mm) 1st round	TST (mm) 2nd round	QFT-GIT	T-SPOT. *TB*	Reactive panel (T-SPOT. TB)‡	Time until IGRA testing (d) ‖	Time to TB (mo) **	Type of TB	Cu	Case finding††
1	M	16-24	Asia	Yes	15	ND	Neg	Neg	None	34	4	ETB	Pos†	Passive
2	M	16-24	sS-Africa	Yes	ND	21	Pos	Pos	A&B	137	4	PTB	Neg	Active
3	F	25-34	sS-Africa	No	18	ND	ND	ND	ND	-	5	PTB &ETB	Pos†	Passive
4	F	35-44	sS-Africa	Yes	17	ND	Neg	Pos	B	19	6	ETB	Pos†	Active
5	M	16-24	O-Africa	No	10	ND	Neg	Neg	None	5	8	PTB	Pos†	Passive
6	F	16-24	O-Africa	Yes	15	ND	Pos	Pos	A&B	8	9	PTB	Neg	Active
7	F	≥45	S-America	Yes	25	ND	Pos	Pos	B	44	12	PTB	Neg	Passive
8	M	35-44	S-America	Yes	ND	18	Pos	Pos	A&B	92	13	PTB	Pos†	Active
9	F	35-44	S-America	Yes	*ND	*ND	Pos	Pos	A&B	28	18	PTB	Pos†§	Passive

Definition of abbreviations: TST= tuberculin skin test; NL=Netherlands; HH=household; QFT-GIT=QuantiFERON TB Gold in tube; F=female; M=male; pos=positive; neg=negative; ND=not done; S=South; sS=sub-Saharan; O=other; d=days; mo=months; y=years; NA=not applicable; FU=follow-up; Cu=culture.

** TST known positive, due to previous TB episode*
† RFLP pattern of isolate was identical to that of the index case.
‡ A=ESAT-6, B=CFP-10.
§ Isolate was not identical to the RFLP pattern of that of the previous TB episode of this case.
‖ Number of days between diagnosis of the index patient and blood collection for IGRA determination
*** Number of months between diagnosis of the index patient and diagnosis of TB of the contact*
†† Passive case finding=cases reported spontaneously, active case finding=cases were found during a screenings visit

Predictive values

In the primary analysis, the 339 contacts were followed for a median follow-up time of 1.83 year (IQR=1.30-2.00). This corresponded with an incidence rate of 16/1000 person-years (95% CI; 7.3-30.5). The PPV of a TST result ≥10 mm for progression to TB was 3.1% (95% CI; 1.3-5.0), 3.8% (95% CI; 1.7-5.9) for TST ≥15 mm, 2.8% (95% CI; 1.0-4.6) for QFT-GIT and 3.3% (95% CI; 1.3-5.3) for T-SPOT.*TB*, and sensitivity was 100%, 88%, 63% and 75%, respectively (Table 3). Specificity of the tests in this group of contacts with TST ≥5 mm (or known positive result), was highest for QFT (46%), followed by TST (cut-off 15 mm) (44%), T-SPOT.*TB* (36%) and lowest for TST (cut-off 10 mm) (13%).

Five contacts were excluded from the secondary analysis, since their follow-up started less than 12 months before August 1st 2008. The incidence rate during the first 12 months was 21/1000 persons-years (95% CI; 8.6-44.2). Similar to the analysis using the whole follow-up period, the PPV in the first 12 months was not better for QFT-GIT (1.7%, 95% CI; 0.3-3.1) or T-SPOT.TB (2.2%, 95% CI; 0.6-3.9%) than that of the TST using a cut-off of 10 mm (2.5%, 95% CI; 0.8-4.1) or 15 mm (3.3%, 95% CI; 1.4-5.2).

Restricting the analysis to contacts who attended at least the follow-up visits up to the first 12 months, the IGRA did not have a higher PPV compared to the TST either at a cut-off of 10 or 15 mm, although all PPVs were slightly increased.

Table 3. Sensitivity, specificity and predictive values for development of tuberculosis disease for QuantiFERON-TB Gold in tube, T-SPOT. TB and TST among immigrant contacts

Time point	Number of contacts	Number of incident TB cases	Incident TB cases		Other contacts		Sens (%)*	Spec (%)*	PPV (%)*	NPV (%)*
			Test + (n)	Test - (n)	Test + (n)	Test - (n)				
PRIMAIRY ANALYSIS										
All contacts with TST ≥5 mm. Test parameters determined with follow-up until 1st August 2008.										
TST ≥10 mm	339	9‡	9	0	279	51	100	15	3.1	100
TST ≥15 mm,	322	8‡	7	1	177	137	88	44	3.8	99.3
QFT-GIT	327	8	5	3	173	146	63	46	2.8	98.0
T-SPOT.*TB*	299	8	6	2	175	116	75	40	3.3	98.3
SECONDARY ANALYSIS										
Contacts with TST ≥5 mm, who started follow-up at least 12 months before August 1st 2008.										
Test parameters are determined after 12 months follow-up.										
TST ≥10 mm	334	7	7	0	278	49	100	15	2.5	100
TST ≥15 mm,	317	7	6	1	176	134	86	43	3.3	99.3
QFT-GIT	323	6†	3	3	173	144	50	45	1.7	98.0
T-SPOT.*TB*	295	6†	4	2	175	114	67	39	2.2	98.3
SENSITIVITY ANALYSIS										
Contacts with TST ≥5 mm, who were actively followed for at least 12 months.										
Test parameters determined after 12 months follow-up.										
TST ≥10 mm	196	7	7	0	165	31	100	16	4.1	100
TST ≥15 mm,	184	7	6	1	107	77	86	42	5.3	98.7
QFT-GIT	195	6†	3	3	112	83	50	43	2.6	96.5
T-SPOT.*TB*	180	6†	4	2	114	66	67	37	3.4	97.1

Definition of abbreviations: TST= tuberculin skin test; QFT-GIT=QuantiFERON TB Gold in tube; TB=tuberculosis += positive; - = negative; sens=sensitivity; spec=specificity; PPV=positive predictive value; NPV=negative predictive value.

** Sensitivity, specificity, positive and negative predictive value for development of tuberculosis disease are determined in immigrant close contacts with TST ≥5 mm.*

† IGRA was not performed in 1 incident TB case

‡ TST was known positive (≥10 mm) in 1 incident TB case and not determined at recruitment of the study. This case was excluded form the analysis of TST ≥15 mm.

DISCUSSION

In this prospective cohort study including recently exposed immigrant close contacts with TST results ≥5 mm who were followed without preventive treatment, we found that the positive predictive value of QFT-GIT and T-SPOT.*TB* for subsequent development of TB disease during the first two years after a contact investigation was not superior to that of the TST irrespective of the TST cut-off (10 or 15 mm). Our results differ from those in other populations in low incidence settings (12), that showed that the QFT-GIT may be a good predictor for development of active TB. In our study over half of the tested immigrant close contacts were QFT-GIT or T-SPOT.*TB* positive. If we assume that contacts with TST <5 mm, whom we excluded from IGRA testing in our study, would have had a negative IGRA result if IGRA had been performed, still 42-46% of all contacts would be IGRA positive. This high proportion of positive tests found among recently exposed immigrant contacts is probably not only attributable to recently acquired infections (15).

The incidence of TB disease among close contacts with TST ≥5 mm, during 3-24 months after the diagnosis of the index case was 16/1000 and is relatively high compared to estimations of others who assessed close contacts, ranging between 3.2-12.5/1000 (11, 14, 21, 22).

So far, four other contact studies assessed progression to disease in contacts tested with an IGRA (11-14), be it in different populations. Diel *et al*. (12) found 6 TB patients among 41 QFT-GIT positive contacts. The PPV of the QFT-GIT in this study (14.6%) was significantly higher than when a TST cut-off of 5 mm was used (PPV=2.3%, p=<0.003), although not at a cut-off of 10 mm (PPV=5.6%, p=0.10). In contrast, two studies assessing the ELISPOT, in household contacts in Gambia (14) or in child contacts in Turkey (11), reported a similar prediction of TB cases by ELISPOT compared to the TST. Similar to our findings, in the latter two studies (11, 14) the ELISPOT missed some of the contacts who progressed to disease. It is unclear if discrepancies between these studies may be explained by differences in the type of IGRA that was used. Direct comparison between the QFT-GIT and T-SPOT.*TB* showed that discrepancies occur, and T-SPOT.*TB* seemed to be slightly more sensitive than QFT-GIT (15, 23-26). Probably of more importance are the differences in the populations studied and the TB incidence in these countries.

Three incident cases had a negative IGRA result. We investigated possible reasons for the negative results in these three cases, and found that re-infection and co-morbidity were unlikely explanations. We performed the IGRA only once and only

after TST was ≥5 mm, usually shortly after the diagnosis of the index patient. Although the interval between infection and conversion to a positive test result may be shorter for IGRA than for the TST (27), it is nevertheless possible that we tested our contacts too early when the IGRA was not yet positive in contacts who later progressed to disease. On the contrary, reversions of previously positive IGRA results have also been reported (28, 29). More studies are needed to determine the optimal moment for IGRA testing after infection to develop new diagnostic algorithms for LTBI.

While the immigrant contacts in our study were all recently exposed, we observed previously that positive IGRA results may also be associated with remote infection (15). The implementation of IGRA in clinical practice in high TB endemic settings or among individuals with a high likelihood of previous exposure as recommended by some (30) is therefore debatable (31, 32). Over half of the tested close contacts had positive TST, QFT-GIT or T-SPOT.*TB* results in our study. Nevertheless the incidence rate among the recently exposed immigrant contacts was high. Based on these results it may be recommendable to incorporate the use of preventive therapy or other preventive measures in the Dutch setting for the screening of immigrant close contacts of sputum smear-positive TB cases, as is already the practice in many other low incidence countries (6-8, 16). The choice of the diagnostic test to be used may be based on their cost-effectiveness, including an assessment of the costs and hazards of preventive treatment in persons with an increased risk of side effect.

Our study had some shortcomings. Firstly, we determined the IGRA and followed contacts actively only when TST ≥5 mm and we determined the positive predictive values in the subgroup of contacts with a TST of at least this size. The exclusion of contacts with TST results <5 mm may have influenced our PPV estimations only to a minor extent. Few contact studies reported the percentage of positive IGRA results among contacts with TST <5 mm but found this to be less than 10% (12, 23, 24, 33, 34). Moreover, their risk of progression to disease is negligible (1) and we did not observe any case of TB in this subgroup upon checking the Netherlands Tuberculosis Register. Assuming that none of the immigrants with TST <5 mm would be IGRA positive and that no TB cases occurred in this group, by definition this would result in the same PPV as estimated here. Secondly, we did not have complete follow-up data for a considerable proportion of our study population. Although it is likely that contacts who stopped attending the follow-up visits were less likely to have developed TB disease, since they would otherwise have been notified to the national register, we do not know this with certainty. Therefore, in

the secondary analysis, we assessed the PPV for progression to disease within the first 12 months in the complete cohort as well as in the subgroup who attended the visits up to at least this time. Since the same pattern of PPVs was found as in the primary analysis, non-participation in the follow-up visits will have had limited effect on our study findings. Thirdly, 17 contacts were excluded because they received preventive chemotherapy, but they might have had a higher risk of disease progression than those included. The exclusion of these individuals may have resulted in an underestimation of the incidence. Their effect on the PPV estimation is unclear.

In conclusion, we observed a high incidence rate of TB disease among immigrant close contacts during the subsequent two years of follow-up. The positive predictive value of both IGRA for progression to TB disease among immigrant close contacts was not better than that of the TST. The incidence found among the study population justifies active preventive measures in this group.

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Appendix A

List of birth countries considered *not* to be high endemic for this study

Australia
Austria
Belgium
Canada
Czech Republic
Cyprus
Denmark
Estonia
Finland
Germany
Greece
Hungary
Iceland
Ireland
Israel
Italy
Japan
Latvia
Lithuania
Luxembourg
Malta
Monaco
New Zealand
Norway
Poland
Portugal
Slovakia
Slovenia
Spain
Sweden
Suriname *(if the individual has not received a BCG vaccination in Suriname during childhood)*
Switzerland
United Kingdom
USA