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

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COMPARISON OF MANTOUX AND QUANTIFERON-TB GOLD TEST FOR DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION IN ARMY PERSONNEL

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ABSTRACT

The tuberculin skin test (TST) was compared with QuantiFERON-TB Gold in-tube (QFT-GIT) in non-BCG vaccinated military personnel. Among subjects with a positive TST, 44.4% of recruits had a positive QFT-GIT compared with 11.5% after mission abroad, suggesting that most TST conversions in the latter group were caused by non-tuberculous mycobacteria.

Military personnel can be sent to high tuberculosis (TB) endemic countries and is therefore at higher risk of TB infection. Non-Bacillus Calmette-Guérin (BCG) vaccinated recruits entering the army are screened with the tuberculin skin test (TST) for detection of latent TB infection (LTBI), while BCG vaccinated recruits are screened using chest radiography. Screening is repeated following each return from a mission. If TST conversion (TST ≥ 10 mm) is documented, prophylactic isoniazide (INH) is prescribed for 6 months.
Earlier studies using skin testing with sensitins from atypical mycobacteria such as *M. avium* or *M. scrophulaceum* indicated that about half of positive TST reactions in military personnel following return from mission were false-positive (3). New diagnostics, like QuantiFERON-TB Gold in-tube (QFT-GIT), have been developed using TB specific antigens ESAT-6, CFP-10 and TB7.7 (1;7;15). The advantages over the TST of these new assays are higher specificity, excluding false-positive results due to BCG or environmental mycobacteria, logistic simplicity and need of only one patient visit (9;14;15;17). In this study we used QFT-GIT for screening military personnel.

This prospective, cross-sectional observational study aimed to compare the TST with QFT-GIT in Dutch Armed Forces personnel. We aimed to recruit 750 employees who would be screened for TB infection six weeks after return from a military mission to a TB endemic area, and 150 recruits (new employees of the Dutch Armed Forces) as controls. In order to include a sufficient number of subjects with a positive TST among those who had been on mission, part of the subjects were randomly included on the day of TST administration and part was included on the day of TST reading if the TST result was > 0 mm. The Ethical Review Board of the Leiden University Medical Center approved the study protocol (Protocol number P04-027). All participants provided written informed consent. The TST and QFT-GIT were carried out as described previously (2). Analyses were performed using SPSS (Version 12.0.1; Apache Software Foundation). Differences between the study groups were evaluated using Pearson Chi-Square and Linear-by-Linear associations for univariate analyses. Results were considered significant if the p-value was < 0.05. Multivariate analyses were performed using logistic regression. The agreement between TST and QFT-GIT was investigated using kappa statistics (16).

Between October 8th 2004 and February 3rd 2006, 909 subjects were included, of whom 171 were recruits and 674 had recently returned from mission, 34 were tested routinely for other reasons and of 30 participants these data are missing. Demographic characteristics are reported in Table 1.

TST results were available for 676/746 (90.6%) subjects. The TST was not performed in 163 subjects, 128 of whom were BCG vaccinated while 35 were known with previous TST conversion. Using ≥ 10 mm or ≥ 15 mm as TST cut-off, 139/676 (20.6%) and 51/676 (7.5%) subjects had a positive TST, respectively (table 2).

Analyzed by TST category, the distribution of TST results among subjects returning from mission and recruits was significantly different with an equally distributed higher percentage in each TST category >0 among subjects returning from mission (Table 1, P<0.001).

In univariate analysis, duration of the mission and birth in a high TB endemic region were predictive of a positive TST (data not shown). After adjusting for the day of inclusion in the study in a multivariate analysis, reported contact with the local population was the only parameter that was significantly associated with a positive TST.

Positive QFT-GIT results were obtained in 33/909 (3.6 %) of all participants. Among recruits, 5/171 results were positive (2.9 %), of whom two had previously been treated for TB. Of the remaining three, one was foreign born. Among subjects returning from mission, 28/738 (3.8 %) had a positive QFT-GIT result, which was not different from the percentage among recruits. Nine of those 28 (32.1%) reported past treatment of LTBI one to 14 years previously, compared to 1.6% (14/682) of the QFT-GIT negative participants. Three (10.7%) reported contact with smear-positive TB before the mission, compared to 6.7% (55/821) of the QFT-GIT negative group. As 12 of 28 (42.9%) positive QFT-GIT results were thus explained by the medical history, the actual risk of recent infection during mission was at most 16/725 (2.2%), or lower if LTBI had been acquired between enrollment in the Armed Forces and the mission. After adjusting for day of blood sampling in the multivariate analysis no parameters were associated with the QFT-GIT result (data not shown).

Results for both TST and QFT-GIT were available in 676 subjects, 20.6% of whom had a positive TST at cut off ≥ 10 mm and 3.1% of whom had a positive QFT-GIT result (Table 2). The overall agreement between TST and QFT-GIT was 82% (κ= 0.19). When using 15 mm as the cut off, the agreement was 92% (κ= 0.24, Table 2).

Table 1. Characteristics of the study population

		Recruits (N= 171)	After mission or other* (N= 738)	P-value
Gender	male	156/170 (91.8)	665/734 (90.6)	n.s.
Age, mean ± SD (y)		19.6 ± 2.8	30.3 ± 9.6	0.000
Country of Birth	The Netherlands	158/171 (92.4)	692/737 (93.9)	n.s
BCG vaccinated	yes	15/171 (8.8)	93/738 (12.6)	n.s
Visit Tropics	yes	6/169 (3.6)	211/733 (28.8)	0.000
Timing of blood sample in relation to TST	administration	146 (85.4)	384 (52.0)	0.000
	reading	7 (4.1)	209 (28.3)	
	no TST	18 (10.5)	145 (19.6)	
TST at cutoff 10 mm	positive	9 (6.2)	130 (24.5)	0.000
TST result, mean ± SD (mm)		0.95 ± 3.9	3.4 ± 6.1	0.000
TST result categorical, range (mm)	0	134 (92.4)	362 (68.2)	0.000
	1 to 4	0 (0)	8 (1.5)	
	5 to 9	2 (1.4)	31 (5.8)	
	10 to 14	5 (3.4)	83 (15.6)	
	≥15	4 (2.8)	47 (8.9)	
QuantiFERON TB Gold (QFT-GIT) in tube result	positive	5/171 (2.9)	28/738 (3.8)	n.s
QFT-GIT result among TST positive subjects	positive	4/9 (44.4)	15/130 (11.5)	0.000

Analysis based on available data; some data are missing due to incomplete questionaires
Data are expressed as Number (%) unless indicated otherwise
BCG = Bacillus Calmette-Guérin
TST = Tuberculin Skin Test
QFT-GIT = QuantiFERON TB Gold in-tube
* N=34 medical personnel of Armed Forces eligible for routine screening

Table 2. Overall agreement between tuberculin skin test and QuantiFERON TB Gold in-tube test result

	TST < 10 mm	TST ≥ 10 mm	TST < 15 mm	TST ≥ 15 mm
QFT-GIT negative (N=655)	535 (81.7)	120 (18.3)	614 (93.7)	41 (6.3)
QFT-GIT positive (N=21)	2 (9.5)	19 (90.5)	11 (52.4)	10 (47.6)
All (N=676)	537 (79.4)	139 (20.6)	625 (92.5)	51 (7.5)
% Agreement	82.0		92.3	
κ	0.19		0.24	

TST = Tuberculin Skin Test

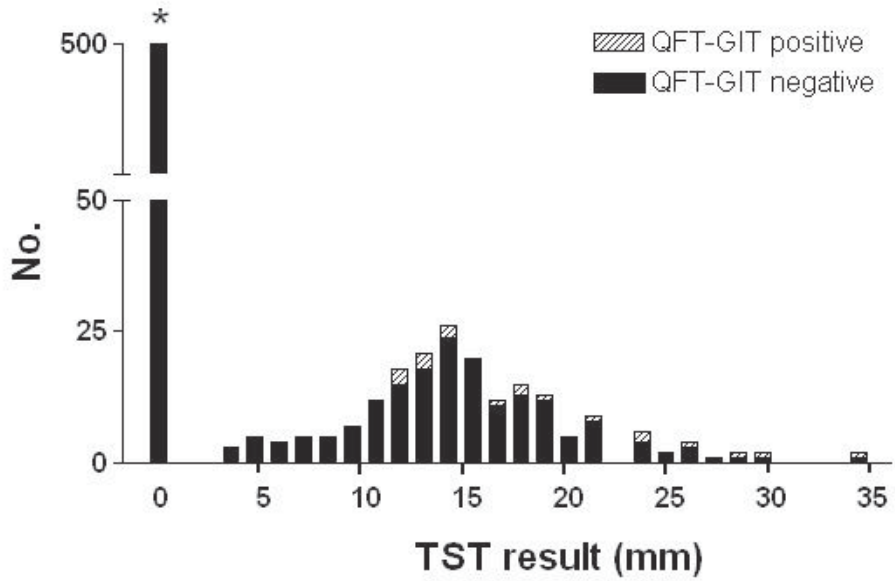
QFT-GIT = QuantiFERON TB Gold in-tube

Table 3. Distribution of concordant and discordant results for tuberculin skin test and QuantiFERON TB Gold in-tube

			TST/QFT-GIT result (% within category)				
			concordant		discordant		concordant vs discordant
		Nr.	neg/neg	pos/pos	pos/neg	neg/pos	P value
Gender	Male	607	79.4	2.6	17.6	0.3	0.535
	Female	66	77.3	4.5	18.2	0.0	
Birth Country	<10	660	79.7	2.6	17.4	0.3	0.297
(TB incidence/y)	10-49	2	100.0	0.0	0.0	0.0	
	50-99	4	50.0	0.0	50.0	0.0	
	100-199	5	40.0	20.0	40.0	0.0	
	≥ 200	5	60.0	20.0	20.0	0.0	
Tropics	No	531	80.0	2.4	17.1	0.4	0.363
	Yes	141	76.6	4.3	19.1	0.0	
Contact TB	No	648	79.8	2.5	17.4	0.3	0.226
	Yes	28	64.3	10.7	25.0	0.0	
Treated TB or LTBI	No	669	79.2	2.5	17.9	0.3	0.247
	Yes	7	71.4	28.6	0.0	0.0	
History of positive TST	No	645	81.9	1.9	16.1	0.3	0.000
	Yes	27	25.9	22.2	51.9	0.0	
Mission	No	18	38.9	11.1	50.0	0.0	0.000
	Yes	498	77.1	2.6	19.9	0.4	
	Recrute	145	93.8	2.8	3.4	0.0	
Location of mission	Bosnia	36	44.4	5.6	50.0	0.0	0.000
	Kyrgystan	21	76.2	0.0	23.8	0.0	
	Iraq	165	83.6	2.4	12.7	1.2	
	Afghanistan	269	77.7	2.6	19.7	0.0	
Duration mission (mo)	0-4	308	81.2	2.3	16.2	0.0	0.032
	4-6	176	72.2	2.3	25.0	0.6	
	>6	16	56.3	12.5	31.3	0.0	

TB = Tuberculosis
LTBI = Latent Tuberculosis Infection
TST = Tuberculin Skin Test
QFT-GIT = QuantiFERON TB Gold in-tube

Figure 1.



Distribution of QuantiFERON TB Gold in-tube results in military personnel
Asterisk indicates two persons with TST = 0 mm and positive QFT-GIT result.

Among subjects with a TST result ≥ 10 mm, 44.4 % (4/9) of the recruits had a positive QFT-GIT result, compared to 11.5 % (15/130) of participants returning from mission (P<0.001, Table 1). Among subjects with a TST result ≥ 15 mm, 25% of the recruits had concordant positive test results compared to 19% of subjects after mission (P<0.001). Discordance analysis (Table 3) showed that discordant results were more frequent among subjects returning from mission compared to recruits.

In this study, the low overall agreement between the TST and QFT-GIT of 82% (κ = 0.19) was explained by a high number of discordant TST positive, QFT-GIT negative results. At cut-off ≥10 mm, subjects returning from mission had a 4-fold higher rate of positive TST, whereas the rate of positive QFT-GIT was about 4-fold lower compared with new recruits. Notably, among participants with a positive TST 44.4 % of recruits had a positive QFT-GIT result compared with 11.5% of subjects returning from mission, confirming that most TST conversions after mission were false positive results caused by exposure to non-tuberculous mycobacteria (3). Assuming that most positive TST results among recruits truly indicated LTBI, the 11.5% positive QFT-GIT results among subjects returning from mission reflected 100/44.4 $\times$ 11.5%, thus 25% with true LTBI. This would implicate that 75% of

observed positive TST results among these BCG unvaccinated subjects were probably false positive.

A limited number of studies used QFT-GIT in comparison with TST (2;4-6;8;12;13). It appeared that the agreement between the TST and QFT-GIT result was strongly dependent on the clinical-epidemiological setting, with the prevalence of TB and BCG vaccination status among the studied population as important determinants (5;8;12). Agreement was higher in studies of persons at significant risk of infection and lower values were found among BCG vaccinated subjects.

The U.S. Centers for Disease Control and Prevention recommend that QFT-G may be used instead of the TST in all circumstances in which the TST is currently used (11). U.K. guidelines issued by The National Institute for Health and Clinical Excellence (NICE) in stead recommend a two-stage strategy of TST followed by an interferon gamma release assay to confirm a positive TST result (10). In the military setting of large scale screening and low *a priori* risk of LTBI, logistic problems could argue against the use of blood test for general screening. With a two-stage approach, however, it must be taken into account that the rate of TST reading can be low.

In conclusion, a positive TST was significantly less frequently associated with a positive QFT-GIT result among subjects returning from mission than among recruits. This suggests that false-positive TST results are frequent after mission. In this setting, QFT-GIT could guide more targeted treatment of individuals with actual LTBI and risk of TB disease.

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