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

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FOLLOW-UP STUDY OF TB EXPOSED SUPERMARKET CUSTOMERS WITH A NEGATIVE TUBERCULIN SKIN TEST IN ASSOCIATION WITH A POSITIVE INTERFERON- γ RELEASE ASSAY

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ABSTRACT

We report follow-up of 29 subjects with a negative tuberculin skin test (TST) in association with a positive interferon-gamma release assay (IGRA), mainly due to responses to CFP-10 in T-SPOT.*TB*, during a contact investigation. One year later, 12/29 (41%) had converted to a positive TST in association with negative IGRA result.

In 2004, a Dutch supermarket employee was diagnosed with extensive smear-positive pulmonary tuberculosis (TB). When the contact investigation revealed that he had infected the majority of his close contacts but also a significant proportion of casual contacts, a large-scale investigation was performed among all (>20,000) supermarket customers in February 2005. This initial report identified more than 400 additional persons with a positive tuberculin skin test (TST) (unpublished data and (2)). In a subset of these contacts, the TST was directly compared with two *Mycobacterium tuberculosis* (MTB) specific interferon-gamma release assays (IGRA); QuantiFERON-TB Gold in-tube (QFT-GIT, Cellestis, Carnegie, Australia) and T-SPOT. TB (Oxford Immunotec, Abingdon, UK), the results of which have been published (2). The advantage of IGRA over the TST is the use of the MTB-specific antigens ESAT-6 and CFP-10 (and additionally TB7.7 in QFT-GIT), as has been described extensively elsewhere (1;12;15;17;20). In contrast, the TST is based on PPD (purified protein derivative) which consists of a crude mixture of antigens with broad cross-reactivity and therefore leads to the occurrence of false-positive responses after vaccination with *Bacillus Calmette Guérin* (BCG) or exposure to nontuberculous mycobacteria. Among 505 individuals with a negative TST (defined as <10 mm) in the comparative study, 42 (8.3%) had a positive IGRA result. The significance of this finding was not clear and prompted further investigation. In order to study the subgroup with negative TST and positive IGRA results, we repeated all three tests one year after the initial contact investigation.

Participants of the contact investigation in February 2005 who had a negative TST and positive IGRA result (N=42) were invited in January 2006 for the study presented here (2). Participants with a positive TST result at the 2006 study would be offered chest radiography at the Municipal Health Authority. The Ethical Review Board of the Leiden University Medical Center approved the study protocol (Protocol number P05.53) and all participants provided informed consent. The TST, T-SPOT. TB and QFT-GIT were performed following the respective manufacturers' instructions as described in the previous study (2). In this study, data for TST and IGRA obtained at both time points (February 2005 and January 2006) was obtained for 29 participants.

Using a ≥ 10 mm cut-off to define a positive TST, we determined that 17 of 29 participants (58.6%) had converted to TST positive one year after the initial contact investigation, while 12 of 29 (41.4%) individuals remained TST negative (Table 1 and Figure 1a). When TST conversion was defined as ≥ 10 mm with an increase of at least 6 mm, 17/29 (59%) had TST conversion.

Of 28 persons with a positive T-SPOT.*TB* result during the contact investigation in 2005, 23 (82%) had reverted to T-SPOT.*TB* negative (Table 1). One person (3.4%) remained negative in the T-SPOT.*TB* assay.

Consistent negative QFT-GIT results were found in 27/29 (93.1%) participants. One person (3.4%) reverted from a positive to a negative result and one (3.4%) person remained positive (Table 1). The cumulative shopping time in the group with conflicting results, as a measure of exposure to the index patient in the supermarket, was not significantly different from the groups with concordant positive or negative TST and IGRA results (2).

Of 12 persons without TST conversion (Table 1), all had a negative QFT-GIT result at both time points while in 11/12 subjects T-SPOT.*TB* result had changed from positive to negative. In 10/12 persons, positive T-SPOT.*TB* results in 2005 were only caused by a response to panel B (CFP-10) (Figure 1B).

Of 17 individuals with TST conversion (Table 1), 15 had a consistent negative QFT-GIT result, 1 person converted from positive to negative and 1 person remained positive in QFT-GIT. T-SPOT.*TB* results reverted from positive to negative in 12/17 persons, 4 remained positive and 1 remained negative. In 2005, 14/16 maximum spot counts were found in response to panel B and 2/16 to panel A (Figure 1b).

Table 1. Characteristics of the study population and results of the assays at both time points.

Group (Nr. of subjects)	Age (y)	Tuberculin Skin Test			T-SPOT.TB			QFT-GIT (IU/ml)	
		2005	2006	2006	2005	2006	2006	2005	2006
		mm	result	mm	result	spots	result	spots	result
Group 1 (N=9)	41 ± 13	0	Neg	0	Neg	10 (8-35)	Pos	1 (0-3)	Neg
Group 2 (N=1)	51	0	Neg	0	Neg	7	Pos	8	Pos
Group 3 (N=2)	47 ± 18	0	Neg	8 ± 0.7	Neg	13 (12-14)	Pos	0 (0-0)	Neg
Group 4 (N=11)	42 ± 11	3 ± 4	Neg	15 ± 3.4	Pos	10 (6-60)	Pos	1 (0-3)	Neg
Group 5 (N=4)	40 ± 13	9 ± 0	Neg	18 ± 2.5	Pos	8 (6-25)	Pos	15 (12-23)	Pos
Group 6 (N=1)	42	9	Neg	19	Pos	12	Pos	2	Neg
Group 7 (N=1)	58	0	Neg	19	Pos	-1	Neg	0	Neg

Age is indicated as average ± SD
TST average ± SD
T-Spot.TB spots are median (range)
Cut off point T-SPOT.TB is 6 spots above NIL.
Cut off for QFT-GIT is 0.35 IU/ml above NIL.

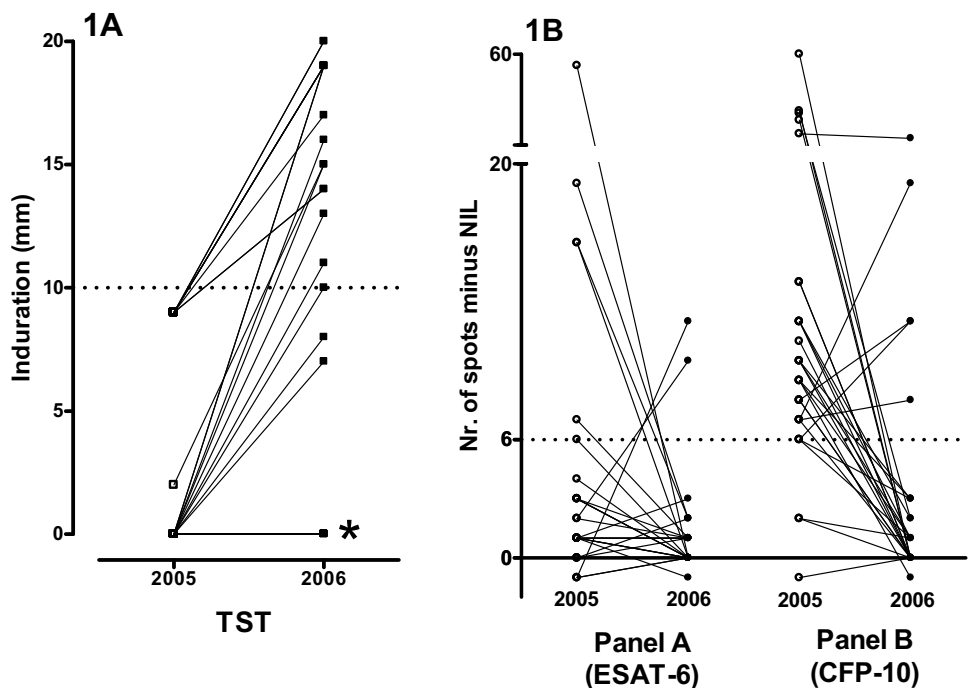


Figure 1
Figure 1a. Tuberculin skin test (TST) responses in 2005 and 2006.
** Indicates 10 subjects with TST result of 0 mm at both time points*
Dotted line: cut-off value for positive test

Figure 1b. Time course of individual spot counts for panel A (left) and panel B (right) of the TSPOT.TB assay in 2005 and 2006.
Dotted line: cut-off value for positive test

In this study, we report on individuals who presented a negative TST in association with a positive IGRA result (predominantly T-SPOT.TB positive) following an outbreak in February 2005 (2). These subjects had not received treatment for latent TB infection. One year later (January 2006), more than half of these persons converted to TST positive while the majority became IGRA negative. Given the epidemiological settings in the Netherlands, it was unlikely that the observed TST conversions were due to re-exposure to MTB, boosting of Mantoux reactivity of past TB, or reactivity to nontuberculous mycobacteria. None of the subjects were BCG vaccinated. Our study is the first to report on follow-up of both IGRA formats and TST in subjects with discrepant test results during a contact investigation. The association of a negative TST with a positive IGRA result has been reported in several studies, with varying percentages in different settings (7;11;16;19;21). In two of the studies (16;21), TST and one of the two IGRA were repeated showing

reversion to a negative IGRA in some subjects. A novel finding in our study is that more than half of the subjects had TST conversion but IGRA reversion. Of note, this phenomenon was observed in only 2-3% of the original study population, but the biological and clinical significance of this novel finding may be of high importance. Treatment based on IGRA would identify this group as eligible for treatment whereas treatment based on TST result would not. The actual risk of progression to TB disease in this subgroup is unknown and may not be the same as for individuals with an initial positive TST result.

We think that it is unlikely that all or most of the positive T-SPOT.*TB* results obtained in 2005 were false-positive results, since we documented TST conversion one year later in more than half of the subjects. An alternative explanation could be transient TB infection without clinical relevance in this group, a concept that has been mentioned earlier (7;14;19). If the risk of progression to TB disease would indeed be extremely low, as suggested by the hypothesis of transient TB infection, no treatment would be indicated.

A striking observation was that most positive T-SPOT.*TB* results during the initial screening in 2005 were in response to Panel B, i.e., to CFP-10 and not to ESAT-6. Thus, the reactivity to CFP-10 reverted to negative in most of the participants testing negative one year later. In previous studies it has been postulated that the response to CFP-10 antigen might be indicative of active replication of the bacteria, whereas responses to ESAT-6 antigen may persist as an immunological 'scar' (4;7;9).

In contrast to the initially positive T-SPOT.*TB* results, QFT-GIT results were almost uniformly negative in our subjects. QFT-GIT also includes CFP-10 but differences in assay formats might be a relevant factor. QFT-GIT is based on whole blood and thus is dependent on the number of antigen-specific T cells that are present per volume of blood as well as on the amount of IFN- γ that is produced by each cell upon antigenic stimulation, whereas ELISPOT uses a defined number of cells. A transient TB infection may induce a relatively low number of antigen-responsive T cells and/or cells producing only a limited amount of IFN- γ , which may explain the observed inter-assay difference. It has repeatedly been suggested that T-SPOT.*TB* might be more sensitive than QFT-GIT for detection of LTBI (8;10;13). This could be an advantage especially in immunocompromised patients, in children and in the elderly (5;6;8;18). In immunocompetent persons, however, it could be argued that a higher sensitivity might be associated with detection of infection without clinical relevance. Further studies are needed to address which assay

has the highest relevance for therapeutic decision making, especially since there seems to be no direct correlation between IFN-g production and protection against active disease (3).

In conclusion, this study reports on TB contacts with late TST conversion in association with reversion from positive to negative T-SPOT.*TB* within a period of one year. Further studies are needed to get to a better understanding of this phenomenon, which is of high practical importance when IGRA results will be used for therapeutic decision making.

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