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False-negative interferon-γ release assay results in active tuberculosis: a TBNET study

To the Editor:

Tuberculosis is one of the leading causes of morbidity and mortality worldwide [1]. Rapid identification of contagious tuberculosis patients and effective treatment are necessary to prevent the spread of *Mycobacterium tuberculosis*, the causative bacterium of the disease. Although interferon-γ release assays (IGRAs) have been developed for the diagnosis of latent infection with *M. tuberculosis*, these assays are sometimes used as adjunctive tests in the diagnostic workup for active tuberculosis, despite poor specificity [2].

A systematic review and meta-analysis [2] found a pooled sensitivity for the diagnosis of culture-proven active tuberculosis of 81% and 92% of the QuantiFERON Gold in-tube test (QFT-GIT) (Qiagen, Dusseldorf, Germany) and the T-SPOT.*TB* test (Oxford Immunotec, Oxford, UK), respectively. Thus, approximately 8–19% of patients have a negative IGRA result when presenting with active tuberculosis.

Several risk factors were associated with negative IGRA results including immunodeficiency, young or advanced age, a negative tuberculin skin test (TST) result, extrapulmonary tuberculosis, disseminated tuberculosis, concomitant tuberculosis treatment and smoking. However, these studies were limited by an observational design implemented in single centres and most of them did not include large numbers of patients with culture-confirmed tuberculosis.

An international, multicentre, retrospective, cross-sectional study was performed by the Tuberculosis Network European Trials Group (TBNET) (www.tb-net.org) to identify risk factors associated with false-negative IGRA results in patients with active tuberculosis.

Clinical data and laboratory results from patients enrolled at 25 participating centres with a confirmed diagnosis of active tuberculosis (*i.e.* positive *M. tuberculosis* culture and/or positive *M. tuberculosis*-specific nucleic acid amplification assay) who had a routine IGRA investigation by the T-SPOT.*TB* test or the QFT-GIT, as part of the diagnostic evaluation between April 2006 and May 2011, were retrospectively recorded on a standardised anonymous questionnaire. For each patient with a negative IGRA test result, two tuberculosis patients with a positive IGRA result admitted directly before and after the patient with a negative IGRA result were included as controls. Immunocompromised patients were defined as patients with at least one of the following medical conditions: HIV infection, treatment with immunosuppressive

drugs, diabetes mellitus, rheumatoid arthritis, malignancy and/or history of solid-organ or stem cell transplantation.

Logistic regression analysis was carried out to assess potential independent variables associated with a negative IGRA test result in patients with active tuberculosis. All variables with a p-value <0.1 were included in the multivariate analysis. A p-value <0.05 was considered to be statistically significant. Statistical analyses were conducted using Stata 9.0 (StataCorp, College Station, TX, USA).

Data were collected from 771 patients. Of these, 107 patients were excluded because of missing clinical data (n=55), missing nucleic acid amplification testing and/or culture confirmation of tuberculosis (n=21), indeterminate test results (n=17), missing control patients (n=9), identified false data entry (n=3) or patient data duplication (n=1).

For the final analysis, 221 tuberculosis patients with a negative IGRA test result and 442 control tuberculosis patients with a positive IGRA test result (total of 664 patients) were included. 32 tuberculosis patients had a negative T-SPOT.*TB*, 182 tuberculosis patients had a negative QFT-GIT, and seven tuberculosis patients had both a negative T-SPOT.*TB* and a negative QFT-GIT test result. The median age (interquartile range) of the patients was 41 (28–56) years in the QFT-GIT and 41 (30–53) years in the T-SPOT.*TB* group. 26.3% and 27.3% TB patients of the QFT-GIT and T-SPOT.*TB* test groups, respectively, were immunocompromised.

Age resulted the only significant variable associated with a negative QFT-GIT test in patients with tuberculosis in multivariate analysis (OR 1.04, 95% CI 1.02–1.07; p<0.0001) (table 1). Mean±SD age of the cases was 46.94±17.76 years and mean age of the control patients 41.16±16.24 (p<0.001). Immunocompromised patients did not have a significantly increased risk of a false-negative QFT-GIT test (OR 1.38, 95% CI 0.91–2.09; p=0.13). None of the different diseases with immunodeficiency when analysed independently were associated with an increased risk of a false-negative IGRA test result. In contrast to the QFT-GIT, age was not recognised as a predictor for a negative T-SPOT.*TB* test results in the univariate analysis (OR 1.02, 95% CI 0.99–1.05; p=0.06). Similar to the QFT-GIT, immunocompromised patients did not have a significant higher probability of a false negative T-SPOT.*TB* test (OR 1.33, 95% CI 0.62–2.82; p=0.47).

Older age was previously recognised as a risk factor for false-negative IGRA test results and the interferon (IFN)-γ concentration obtained in reaction to the 6-kDa early secretory antigenic target (ESAT-6) or the 10-kDa culture filtrate protein seems (CFP-10) to decrease gradually with age [3]. This can explain the difference between the tests in terms of statistical significance of the variable age as a risk factor for a negative result. The T-SPOT.*TB* test requires a specific number of peripheral blood mononuclear cells in the assay so that smaller amounts of IFN-γ can be detected, whereas the QFT-GIT uses whole blood without any standardisations of the number of mononuclear cells.

There is also evidence that false-negative IGRA results can be observed more often in younger children. However, because there were no children aged <5 years enrolled in this study, we were unable to address this possible relationship.

In contrast to previous investigations, immunodeficiency, concomitant tuberculosis treatment, disseminated tuberculosis, extrapulmonary tuberculosis and smoking could not be identified as risk factors for false-negative IGRA test results.

Apart from the association with older age, it remains unclear why some individuals with active tuberculosis have unidentifiable *M. tuberculosis*-specific adaptive immune responses at the time of tuberculosis diagnosis. Results from previous studies have suggested different aetiologies for false-negative IGRA test results that were not evaluated in this study.

During tuberculosis, progression the natural cytokine balance is altered while the bacterial load increases, potentially influencing the performance of IGRA tests [4, 5]. Decreased *M. tuberculosis*-specific immune responses, especially IFN-γ production [4–7], have been attributed to the immunomodulatory action of $CD4^+CD25^+FoxP3^+$ regulatory T (Treg)-cells, which expand in the course of active tuberculosis. To support speculatively this experimental hypothesis, TST reactions are reversely related to the frequency of Treg-cells in the peripheral blood [8].

Genetic variability of a specific major histocompatibility complex class II allele, *i.e.* human leukocyte antigen (HLA)-DRB1*0701, may cause less binding with *M. tuberculosis*-specific antigens ESAT-6 and CFP-10. When antigens are less presented to T-cells, there may be a failing immune response [9]. It was shown that also HLA-DRB1*0701 is significantly associated with a false-negative IGRA test result (OR 5.09) [10].

Another explanation for a negative IGRA result in active tuberculosis is compartmentalisation of T-cells [4, 11] during the course of active tuberculosis. IGRAs measure IFN-γ production by peripheral blood

TABLE 1 Logistic regression analysis of potential independent variables associated with negative interferon-γ release assay (IGRA) results in patients with active tuberculosis

Variables	QuantiFERON Gold In-Tube				T-SPOT.TB			
	Univariate		Multivariate		Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age years	1.02 (1.01–1.03)	<0.0001	1.04 (1.02–1.07)	<0.001	1.02 (0.99–1.05)	0.06		
Male sex	1.36 (0.91–2.03)	0.13			1.05 (0.50–2.19)	0.91		
Cigarette smoking[#]	1.14 (0.77–1.68)	0.53			0.52 (0.26–1.04)	0.06		
Immunodeficiency[¶]	1.38 (0.91–2.09)	0.13			1.33 (0.62–2.82)	0.47		
History of active tuberculosis	1.38 (0.70–2.72)	0.36			1.01 (0.37–2.79)	0.98		
Pulmonary tuberculosis	0.80 (0.47–1.37)	0.41			0.71 (0.23–2.21)	0.55		
Tuberculin skin test								
Positive	0.52 (0.28–0.98)	0.04	1.90 (0.65–5.60)	0.24	1.28 (0.38–4.39)	0.69		
Negative	1.92 (1.02–3.61)	0.04	0.53 (0.18–1.55)	0.24	0.78 (0.23–2.66)	0.69		
IGRA								
M. tuberculosis antigens	[+]	[+]	[+]	[+]	0.69[§] (0.58–0.82) 0.53[f] (0.40–0.71)	<0.0001[§] <0.0001[f]	0.43[§] (0.18–1.04) 0.31[f] (0.09–1.06)	0.06[§] 0.06[f]
Positive control	0.96 (0.93–0.99)	0.006	0.98 (0.94–1.01)	0.18	0.48 (0.27–0.84)	0.01	1.88 (0.20–17.33)	0.58
Negative control	1.01 (0.55–1.86)	0.98			0.85 (0.62–1.15)	0.29		
Exposure to antituberculosis therapy at time of IGRA	0.98(0.67–1.45)	0.93			1.10 (0.47–2.57)	0.83		

[#]: past or present. [¶]: *e.g.* HIV infection, malignancy, transplantation, diabetes mellitus or rheumatoid arthritis. [+]: 6-kDa early secretory antigen target (ESAT-6), 10-kDa culture filtrate protein (CFP-10) and TB7.7; values ≤0.34 predict data perfectly (the output of the logistic regression analysis did not define any specific odds ratios because there is a complete overlap between a specific outcome and the values of the interferon-γ responses lower than 0.35). [§]: ESAT-6. [f]: CFP-10.

T-cells, whereas the predominant production of IFN-γ in active tuberculosis occurs at the site of infection [12–14]. It has been observed that a substantial number of tuberculosis patients with negative TST test results at the time of presentation develop positive TST results on antituberculosis therapy.

Our study has limitations. IGRA test results were analysed in retrospect and quantitative test results were not collected. Apart from demographic parameters, immunological mechanisms or genetic causes of false-negative IGRA results could not be studied. Nevertheless, this is the largest study to evaluate false-negative IGRA responses in active tuberculosis to date.

In conclusion, apart from advanced age, we could not identify risk factors for false-negative IGRA results in patients with active tuberculosis. As IGRAs cannot distinguish latent *M. tuberculosis* infection from tuberculosis, there is need to improve immunodiagnostic methods to distinguish different stages of *M. tuberculosis* infection [15].

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TB outbreak investigation can be enhanced by using whole genome sequencing, IGRA and social network analysis http://ow.ly/AzxfH

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