The Role of Interferon-Gamma Release Assays in the Diagnosis of Active Tuberculosis

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ABSTRACT: **Background:** Interferon-gamma release tests are appealing alternatives to the tuberculin skin test (TST) for latent tuberculosis infection.

Objectives: To determine the yield of the Quantiferon TB Gold test (QFT-G) in the diagnosis of active tuberculosis disease, with a focus on elderly patients, human immunodeficiency virus (HIV) co-infection, and extra-pulmonary tuberculosis (EPTB).

Methods: The QFT-G test was performed in 98 patients suspected of having active tuberculosis. The results were evaluated for each subgroup of patients and compared to the results of the TST.

Results: Active tuberculosis was diagnosed in 92 of the 98 patients. Sixteen (17.3%) were elderly patients (over age 70), 15 (16%) were co-infected with HIV, and 14 (15%) had EPTB. QFT-G was positive in 49 patients (53%) and indeterminate in 4. The results were not significantly affected by HIV co-infection ($P = 0.17$), old age ($P = 0.4$), or the presence of EPTB ($P = 0.4$). There was a good correlation between the TST and the QFT-G test ($P < 0.001$). In EPTB and in the elderly, the QFT-G test appears to be better than the TST.

Conclusions: The QFT-G test is suboptimal in its ability to detect active tuberculosis and should not be used to exclude it.

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KEY WORDS: interferon-gamma release assay (IGRA), active tuberculosis, human immunodeficiency virus (HIV), elderly, extra-pulmonary tuberculosis (EPTB)

Interferon-gamma release assays are in vitro immunodiagnostic tests that measure T cell-mediated IFNγ response to the *Mycobacterium tuberculosis* specific antigen: the early secreted antigenic target and the culture filtrate protein 10. These genes are present in the *M. tuberculosis* complex (MTB complex) genome, but are absent from the BCG vaccine strains and from most of the non-tuberculosis Mycobacterium species [1].

The IGRAs are available in two commercial forms: the Quantiferon TB Gold in tube (QFT-G assay, Cellestis, Australia), which measures the amount of IFNγ secreted by T cells after incubating the blood with ESAT and CFP, and the T Spot TB assay (Oxford Immunotech, UK), which measures the quantity of secreting T cells after in vitro stimulation of tuberculosis-specific antigens [2].

IGRAs have an excellent specificity yield for the diagnosis of latent tuberculosis infection, unaffected by BCG (Bacillus Calmette-Guérin) vaccination and are used in place of (or in combination with) the tuberculin skin test for LTBI diagnosis [3,4].

The value of IGRA in evaluating active tuberculosis is unclear. Their sensitivity varies according to the type of IGRA test used and the population tested, and they do not differentiate between latent and active tuberculosis infections [5,6]. The general consensus is not to use IGRA for excluding active TB, and the gold standard remains bacteriological cultures or typical pathological findings.

The aim of the present study was to evaluate the yield of QFT-G test in active tuberculosis disease with a focus on elderly patients, HIV co-infection, and extra-pulmonary tuberculosis, and to compare these results with the TST results.

PATIENTS AND METHODS

The protocol of this study was approved by the Ethical Review Committee of Shmuel Harofe Hospital, and patients' confidentiality was respected. Patients attending the Pulmonary and Tuberculosis Department of our hospital who where undiagnosed but likely to have tuberculosis were asked to participate in this study and those who consented were then enrolled. After providing informed written consent, all patients participating in this study underwent blood collection for QFT-G (before treatment institution) in addition to their routine blood examinations and HIV serology. The TST was then carried out.

Sputum samples were collected from each patient for microscopy staining examination using the Ziehl-Neelsen method (AFB+) and for culture results using the Lowenstein-

IFNγ = interferon-gamma
IGRA = interferon-gamma release assay

ESAT = early secreted antigenic target
CFP = culture filtrate protein-10
LTBI = latent tuberculosis infection
QFT-G = Quantiferon TB Gold test
TST = tuberculin skin test

Jensen medium, the standard procedure for these patients. Pulmonary tuberculosis was diagnosed through the identification of *Mycobacterium tuberculosis* in at least one sputum sample and EPTB was diagnosed through typical fluid analysis and pathological findings. Patients without bacteriological or histopathological evidence of tuberculosis were then excluded from the study. Patients were considered elderly if they were over 70 years old.

LABORATORY ASSAY

Blood samples were collected in three QFT-G evacuated tubes and precoated with *M. tuberculosis* antigens, and phytohemagglutinin for positive control, or no antigen for negative control. The tubes were incubated for 16–24 hours at 37ºC, and plasma was collected after centrifugation and stored at -4ºC until assayed. QFT-G enzyme-linked immunosorbent assay was carried out and interpreted using the software provided by the manufacturer. A positive test was defined as IFNγ secretion > 0.35 IU after subtracting the control, a negative test if IFNγ secretion was < 0.05 IU ml).

TUBERCULIN SKIN TEST

The TST was carried out after the QFT-G test, by using 5 tuberculin units of purified protein derivative administered intradermally in the forearm. Positivity of TST was defined as an induration of > 10 mm (or > 5 mm in HIV-infected patients) occurring 72 hours after injection

STATISTICAL ANALYSIS

Data were collected and analyzed by a frequency table. Categorical variables were compared using either the chi-square or Fisher exact test. $P \leq 0.05$ was considered statistically significant. SPSS version 16 (SPSS, Chicago, USA, 2008) was used for the analysis

RESULTS

Of the 98 patients enrolled in the study, 6 were excluded as their culture results showed a non-tuberculosis Mycobacterium species (four *M. kansasii* and two *M. avium intracellulare*). Among the remaining 92 patients, 76 (82.6%) were males and 16 (17.4%) were females. The mean age was 45 years (range 18–94) and only 16 patients (17.3%) were older than 70. A survey of ethnicity showed that 60 patients (67%) were born in East Africa (of these, some were refugees from Eritrea and Sudan, and some were Israelis born in Ethiopia), 21 Israeli patients (22%) were born in the former Soviet Union, and 11 (12%) were Israeli born. HIV co-infection was found in 15 patients (16%). All cases were characterized by a low CD4 T cell number (< 200 cells/mm³) and high viral load (> 100,000 copies/ml). Fourteen patients (16%) presented with EPTB, which was then proved through pathological confirmation. These included peripheral lymphadenopathy in 11 patients, peritoneal involvement in 6, bone involvement in 3 and meningitis in 1. In all the pulmonary cases, the tuberculosis culture was positive. In four patients the Mycobacterium was resistant to at least rifampicin and isoniazid (multiple drug-resistant tuberculosis).

EPTB = extra-pulmonary tuberculosis

Table 1. Patients' characteristics

	All patients (n=92) (%)	QGT-G positive (n=49) (%)	QGT-G negative (n=39) (%)	QGT-G indeterminate (n=4) (%)	*P* value
Age/gender					
Mean age (yrs)	45	41	50	4	NS
Males	60 (65)	32 (53)	24 (40)	4 (6)	NS
Country of origin					
East Africa	60 (65)				
FSU	21 (23)				
Israel	11(12)				
HIV co-infection	15 (16)	6 (40)	9 (60)	0	NS
EPTB	14 (15)	9 (64)	5 (34)	0	NS
Elderly	16 (17.3)	7 (43)	8 (50)	1 (6)	NS
Clinical features					
Smear positive	51 (55)	26 (51)	22 (43)	3 (6)	NS
Cough	75 (81.5)	39 (52)	31 (41)	4 (5)	NS
Hemoptysis	12 (13)	5 (42)	7 (58)	0	NS
Pain	18 (19.5)	13 (72)	3 (16)	2 (11)	0.04
Fever	37 (34)	25 (67)	12 (32)	0	0.05
Old TB	14 (15)	10 (71)	3 (21)	1 (7)	0.001

FSU = former Soviet Union, HIV = human immunodeficiency virus, EPTB = extra-pulmonary tuberculosis, QFT-G = Quantiferon-Gold Test

The QFT-G test showed positive results in 49 patients (53.3%) and was indeterminate in 4 (4.3%). The indeterminate results were not related to HIV co-infection or to advanced age (above 70 years). The sensitivity of the QFT-G test was not statistically influenced by HIV co-infection (40% versus 60%, $P = 0.17$), age (43% vs. 50%, $P = 0.4$), or EPTB (64% vs. 34%, $P = 0.48$) [Table 1].

The QFT-G test was significantly positive in patients with a previous history of tuberculosis ($P = 0.001$), systemic symptoms such as fever (> 38ºC) ($P = 0.057$) or every type of pain ($P = 0.014$).

Positive TST results were observed in 47 patients (51%). The correlation between the two tests was significant, with an agreement of 62 subjects (70%) ($P < 0.001$). The 14 patients with positive QFT-G test in the presence of a negative TST were most likely to be elderly and to have EPTB. In the HIV co-infection population, both tests were suboptimal.

DISCUSSION

A review of the IGRA literature demonstrates the superiority of IGRA over TST in detecting LTBI, with a specificity of 98%–100% and a sensitivity of 70%–90% [3]. After 2 years, the progression rate of a positive test to active tuberculosis exceeds

that of TST (8–15% vs. 2–3%), and its high negative predictive value is an important indicator for a tuberculosis control program; therefore, it may limit therapy to contact people with a positive test [4,7].

The role of IGRA in detecting active tuberculosis disease is less clear. Its sensitivity has been shown in published studies to be between 55% and 88% [8-10], which is fairly similar to the results of our study. This may be related to the fact that interferon secretion is usually diminished during the early stage of the disease and to the decreased immunological defense of the studied population (HIV, elderly). It is important to note that the IGRA is a T cell-based test whose numbers are compromised in immune suppressed patients, and that the secretion of IFNγ is poorer in extra-pulmonary than in pulmonary tuberculosis. Hence, in our study, the sensitivity of the QFT-G test for HIV-TB was only 40% (and of TST 33%), which is lower than the sensitivity reported in published studies, which ranges from 65% to 90% [11-13]. This can be explained by the large number of patients with very low CD4 cell number (< 200 cells). With these conditions, it has been reported that the use of a lower cutoff point of concentration (< 0.25 IU/ml instead 0.35 IU/ml) will lead to better sensitivity and specificity, and that by controlling the number of T cells the T spot cell test should be more effective than the QFT-G test [11,14-16]. However, the reevaluation of our results by including a lower cutoff point for the QFT-G test did not bring about any change in the final results.

Since we found a moderate correlation between the QFT-G and the TST (70%), it is unlikely that the addition of one of these tests will yield better results than any other prior negative test. This result is confirmed by Mazurek et al. [17], who compared the sensitivity of QFT-G and TST in 69 patients with tuberculosis confirmed by cultures and found a similar sensitivity in both tests, with the exception of HIV-infected patients who were 13.5 times more likely to have false negative TST results.

Very few studies have investigated the clinical effectiveness of IGRA among elderly patients. Tuberculosis in the elderly is often missed, characterized by a fatal outcome (six times more likely than in the younger population), and a high proportion of diagnoses are only given at autopsy. Not only are the clinical and radiological features atypical among these patients who suffer from multiple co-morbidities, but the bacteriological diagnosis is often missed by the inability of the elderly to produce sputum [18]. Furthermore, efforts should be made to diagnose the disease accurately, and to this end the IGRA may be of interest. Kobashi and colleagues [19] reported a high sensitivity of the QFT-G test (77–80%) and its superiority to the TST (16–27%). The population in the present study was young, with a mean age of 45 years, and only 16 patients were over the age of 70. Even though the sensitivity of the QFT-G test was suboptimal (43%), results were still higher than the TST (40%), though not statistically significant overall.

The sensitivity of the QFT-G test was higher (64%) than that of the TST (42%) in the EPTB group. This is important in the context of a difficult diagnosis that is dependent on invasive procedures and is often delayed. This finding was also reported by Kobashi and team [20] who found a high positive rate of the QFT-G test (86%) in comparison to the TST (57%) in 35 patients with confirmed EPTB, and by Oznur and co-authors [21] who reported a sensitivity of 76% in 21 patients with EPTB in comparison to 62% positive in the TST.

In conclusion, the QFT-G test was shown to be suboptimal in its ability to detect active tuberculosis in immune competent and immune suppressed patients. Its sensitivity appears better than that of the TST; however, it is unable to exclude a diagnosis of tuberculosis and it is costly. While the IGRA is useful in detecting LTBI, culture results remain the gold standard for the diagnosis of active tuberculosis. It is important that the role of QFT-G in EPTB be tested in larger studies.

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Capsule

Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination

Active multiple sclerosis lesions show inflammatory changes suggestive of a combined attack by autoreactive T and B lymphocytes against brain white matter. These pathogenic immune cells derive from progenitors that are normal, innocuous components of the healthy immune repertoire but become autoaggressive upon pathological activation. The stimuli triggering this autoimmune conversion have been commonly attributed to environmental factors, in particular microbial infection. However, using the relapsing-remitting mouse model of spontaneously developing experimental autoimmune encephalomyelitis, Berer and team show that the commensal gut flora – in the absence of pathogenic agents – is essential in triggering immune processes, leading to a relapsing-remitting autoimmune disease driven by myelin-specific CD4+ T cells. The authors show further that recruitment and activation of autoantibody-producing B cells from the endogenous immune repertoire depends on availability of the target autoantigen, myelin oligodendrocyte glycoprotein (MOG), and commensal microbiota. These observations identify a sequence of events triggering organ-specific autoimmune disease and these processes may offer novel therapeutic targets.

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Eitan Israeli

Capsule

A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma

So far, no common environmental and/or phenotypic factor has been associated with melanoma and renal cell carcinoma (RCC). The known risk factors for melanoma include sun exposure, pigmentation and nevus phenotypes; risk factors associated with RCC include smoking, obesity and hypertension. A recent study of coexisting melanoma and RCC in the same patients supports a genetic predisposition underlying the association between these two cancers. The microphthalmia-associated transcription factor (MITF) has been proposed to act as a melanoma oncogene; it also stimulates the transcription of hypoxia inducible factor (HIF1A), whose pathway is targeted by kidney cancer susceptibility genes. Bertolotto and co-workers therefore proposed that MITF might have a role in conferring a genetic predisposition to co-occurring melanoma and RCC, and they identified a germline missense substitution in MITF (Mi-E318K) that occurred at a significantly higher frequency in genetically enriched patients affected with melanoma, RCC or both cancers, when compared with controls. Overall, Mi-E318K carriers had a higher than fivefold increased risk of developing melanoma, RCC or both cancers. Codon 318 is located in a small-ubiquitin-like modifier (SUMO) consensus site (ΨKXE) and Mi-E318K severely impaired SUMOylation of MITF. Mi-E318K enhanced MITF protein binding to the HIF1A promoter and increased its transcriptional activity compared to wild-type MITF. Further, the authors observed a global increase in Mi-E318K-occupied loci. In an RCC cell line, gene expression profiling identified a Mi-E318K signature related to cell growth, proliferation and inflammation. Lastly, the mutant protein enhanced melanocytic and renal cell clonogenicity, migration and invasion, consistent with a gain-of-function role in tumorigenesis. Our data provide insights into the link between SUMOylation, transcription and cancer.

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Eitan Israeli

"It is a truism that almost any sect, cult, or religion will legislate its creed into law if it acquires the political power to do so"

Robert A. Heinlein (1907-1988) , American science-fiction writer