Utility of QuantiFeron -TB® Gold test in diagnosis of tuberculosis in HIV-infected patients

Utilitatea testului QuantiFeron -TB® Gold în diagnosticul tuberculozei la pacienții infectați cu HIV

Magdalena Dinu[1*], Mihaela Iosipenco[1], Carmen Craciun[1], Cristian Baicus[2], Ruxandra Neagu Draghicenoiu[1], Lucian Gavrilă[3]

1. National Institute for Infectious Diseases"Prof. Dr. Matei Bals", Bucharest
2. Clinical Hospital Colentina, Bucharest
3. Faculty of Biology, Bucureşti University

Abstract

HIV is a risk factor for passage from latent tuberculosis to an active form of the disease. In HIV seropositive patients after infection with Mycobacterium tuberculosis, the number of T_{CD4+} decreases drastically for a short period of time and the HIV viral loads (VL) increase. Theoretically, because of the decreased number of T_{CD4+} and T_{CD8+} in HIV infection, QuantiFeron test (QFT) is not useful for diagnosis of BK co-infection. When the number of CD4+/CD8+ cells is low, the level of IFN-gamma released by lymphocyte stimulation is too low to be meaningfully evaluated by an ELISA test. The aim of our study was to determine the influence of immunodepression in HIV infected patients on the utility of QuantiFERON®-TB test in diagnosis of co-infection with Mycobacterium tuberculosis. No correlation between HIV viral load and TCD4+/TCD8+ count was revealed in our study. The percent of indeterminate results increased along with increases of viral load. A good response for QuantiFeron TB-Gold test was identified in patients with a TCD4 count higher than 95/µl. Since the test result was indeterminate for more than 10% of patients even in subjects with CD4 count higher than 95/µl, its potential as rule-out test for active TB disease is limited.

Keywords: *QuantiFeron-TB Gold test, viral load, TCD4+/TCD8+count*

Rezumat

Infecția cu HIV este un factor de risc pentru trecerea de la tuberculoza latentă la forma activă de boală. La pacienții seropozitivi HIV, după infecția cu Mycobacterium tuberculosis, numărul de limfocite T CD4+ scade dramatic pentru o perioadă scurtă de timp, iar încărcătura virală creşte. Teoretic, datorită numărului scăzut de limfocite TCD4+ şi TCD8+ din infecția cu HIV, testul QuantiFeron (QFT) nu este util pentru diagnosticul co-infecției cu bacilul Koch. Când numărul de limfocite CD4+/CD8+ este redus, nivelul de interferon gamma eliberat

[*]**Corresponding author:** Magdalena Dinu, St. Grozovici Calistrat, No. 1, Bucharest, zip code: 021105
Tel: +40 724 040 391; E-mail: magda_dinu2003@yahoo.co.uk

în urma stimulării limfocitelor este prea scăzut pentru a fi evaluat printr-un test ELISA. Scopul studiului nostru a fost de a determina influența imunodepresiei la pacienții infectați cu HIV asupra utilității testului QuantiFeron TB Gold în diagnosticul co-infecției cu Mycobacterium tuberculosis. În studiul nostru nu a fost evidențiată nici o corelație între numărul de limfocite CD4+ sau CD8+ şi încărcătura virală. Procentul de rezultate nedeterminate la testul QFT a crescut odată cu creşterea încărcăturilor virale. Un răspuns bun la testul QFT a fost identificat la pacienții cu un număr de limfocite TCD4+ mai mare de 95/µl. Deoarece rezultatele testului au fost nedeterminate la mai mult de 10% din pacienți, chiar şi la pacienți cu număr de limfocite TCD4+ mai mare de 95/µl, potențialul său de test de excludere a tuberculozei active este limitat.

Cuvinte-cheie: *test QuantiFeron-TB Gold, încărcătură virală, număr de limfocite TCD4+/TCD8+*

Introduction

In HIV infection, contaminations with opportunistic agents are very frequent, because the cells involved in immune system functions are altered. Very common co-infections involve protozoa, fungi, mycobacteria, other bacteria, viruses.

Until 2001, the only test used to diagnose latent tuberculosis infection (LTBI) was the tuberculin skin test (TST). Tuberculin skin testing has been used for years as an aid in diagnosing latent tuberculosis infection (LTBI) and includes measurement of the delayed type hypersensitivity response 48-72 hours after intradermal injection of purified protein derivative (PPD). However, in 2001, a new in vitro test (QuantiFERON®-TB or QFTT; manufactured by Cellestis Limited, Carnegie, Victoria, Australia) based on cell-mediated immunity was approved by the Food and Drug Administration. The test is based on the quantification of interferon-gamma (IFN-γ) released from sensitized lymphocytes in heparinized whole blood incubated overnight with synthetic polypeptide antigens ESAT-6 and CFP-10, without similarities with peptide from BCG strains and from non-tuberculosis bacteria (with the exception of *M. kansasii, M. szulgai* and *M. marinum)*. Therefore, the test is not influenced by the BCG vaccination (2). IFN-gamma is a lymphokine belonging to a subset of the cytokines family, secreted by Th1 cells, Tc cells, dendritic cells and NK cells. It has antiviral, immunoregulatory and anti-tumor properties. TST and QFTT do not measure the same components of the immunologic response and are not interchangeable. Assessment of the accuracy of these tests is limited by lack of a standard for confirming LTBI.

Theoretically, because of the decreased number of TCD4+ and TCD8+, this test is not recommended when the patient is severely immunodepressed (1, 2), because the level of IFN–gamma obtained by lymphocyte stimulation is too low to be evaluated by an ELISA test.

CD4 is the most common receptor for HIV, but some studies revealed the capacity of HIV to change the tropism *in vivo* for TCD8+ lymphocytes, that could be transiently infected by HIV (4, 5).

In previous studies (3) high HIV viral load was reported to be correlated with low TCD4 count, because TCD4+ lymphocytes are the most drastically affected by HIV (3).

HIV pandemic has modified the tuberculosis (TB) epidemiology in many countries (6). HIV is a risk factor for passage from latent tuberculosis to an active form of this disease (7). After the infection of a HIV seropositive patient with *Mycobacterium tuberculosis*, theoretically the number of TCD4+ decreases drastically for a short period of time, and an increase of HIV-1 viral load was also reported (8). A previous study considered the CD4:CD8 ratio to be a predictor for HIV-TB co-infection (9).

The aim of our study was to determine the influence of immunodepression in HIV infected patients upon the utility of QuantiFERON®-TB test in the diagnosis of *Mycobacterium tuberculosis* co-infection.

Materials and methods

Our group consisted of 289 HIV infected patients, who were monitored for antiretroviral treatment, and in whom medical evaluation indicated a possible co-infection with *Mycobacterium tuberculosis* (signs and symptoms suggesting a TB disease, chest radiograph and examination of sputum or other clinical samples for the presence of *M. tuberculosis*). HIV RNA viral load and TCD4, TCD8 counts were performed in fasting EDTA blood samples. The patients had no hematological disorders, specific malignancies, diabetes, silicosis and clinical renal failure.

Samples used for viral load determination were obtained in the same day or in the very near days with QuantiFeron testing. All viral load tests used are based on end-point (Abbott LCx HIV RNA quantitative and Roche Cobas Amplicor HIV-1 Monitor Test) or Real-Time RT-PCR (Roche TaqMan HIV-1 RNA Test and Abbott HIV-1 RNA m2000sp). Viral load tests were carried out according with manufacturer's specification.

Blood samples were analyzed for absolute number of TCD4 and TCD8 lymphocytes, by flow-citometry (Becton Dickinson TriTest CD4/CD8/CD3 with TruCount). Blood samples were obtained in the same day with QuantiFeron testing.

Patient peripheral mononuclear cells respond to specific antigen stimulation by secreting cytokines (IL-2, IFN-γ, etc) when they are incubated with mixtures of synthetic peptides: early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10). ESAT-6, CFP-10 and TB7.7(p4) are secreted by all *M. tuberculosis* and pathogenic *M. bovis* strains. Because genes encoding these proteins are absent in all Bacille Calmette-Guérin (BCG) vaccine strains and in commonly encountered non-tuberculous mycobacteria (NTM), except *M. kansasii*, *M. szulgai*, and *M. marinum* (*1*), QFT-G is expected to be more specific for *M. tuberculosis* than tests that use tuberculin purified protein derivative (PPD) as the antigen.

Test kits include three mixtures of synthetic peptides representing ESAT-6, CFP-10 and TB7.7(P4) as test antigens, phytohemaglutinin (a mitogen used as a positive assay control), and saline (used as a nil sample to measure the background level of IFN-γ). After 16-24 hours incubation at 37C, the concentration of IFN-g in the plasma is determined by ELISA using the reagents included in the test kit. The amount of IFN-γ released is determined by subtracting the amount in the nil from the amount in the ESAT-6, CFP-10, TB7.7(p4) or mitogen-stimulated plasma. QFT-G test results can be calculated by using the software provided by the manufacturer. ELISA test was performed in agreement with manufacturer's recommendation. The test contains an internal control - human recombinant IFN-γ and results are reported by comparison with this standard. Standard human recombinant IFN-γ is used in dilution to generate a standard curve, applied to calculate the IFN-γ plasma concentration.

The result is communicated in IU/ml and

Table 1. Interpretation of QuantiFERON®-TB Gold test results

Nil (UI/mL)	TB Antigen minus Nil (UI/mL)	Mitogen minus Nil(UI/mL)	QuantiFERON-TB Gold IT Result	Report / Interpretation
≤8.0	≥0.35 and ≥25% of Nil value	Any	Positive	*M. tuberculosis* infection likely
	≤ 0.35 OR ≥ 0.35 and < 25% of Nil value	≥ 0.5	Negative	*M. tuberculosis* infection NOT likely
		< 0.5	Indeterminate	Result are indeterminate for TB antigen responsiveness

Table 2. Description of the studied group according to QuantiFeron TB Gold results and the TCD4 lymphocytes count

	QuantiFeron TB Gold results			Total [**n** (%)]
	Positive	**Indeterminate**	**Negative**	
CD4 < 95/µl [**n** (%)]	**8** (7.6)	**46** (43.8)	**51** (48.6)	**105** (36.3%)
CD4 > 95/µl [**n** (%)]	**50** (27.2)	**19** (10.3)	**115** (62.5)	**184** (63.7%)
Total [**n** (%)]	**58** (20.1)	**65** (22.5)	**166** (57.4)	**289** (100%)

compared with standard values of test. The final result is qualitative: positive /negative /indeterminate.

QFT results are based on the quantity of IFN- γ released in response to stimulating ESAT-G, CFP-10 and TB7.7(p4) as compared with mitogen. QFT results indicative of *M. tuberculosis* infection include the criteria described in *Table 1*. The magnitude of the mesuread IFN gamma level cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease.

Statistical analysis

The results obtained were analyzed with statistical test *Spearman Rank correlation* and *Mann-Whitney U*.

Results and discussions

Our group of patients contained 289 subjects with HIV-1 infection, most of them with F subtype. The description of the studied group according to QuantiFeron (QFT) results and TCD4 counts is presented in *Table 2*.

We analyzed whether there were any correlations between values obtained for viral load (HIV RNA VL), TCD4/CD8 count and the results for QFT.

Spearman rank correlation for viral load and TCD4 count was r = –0.4013, which indicates a low negative correlation between these two sets of values (95% CI= –0.5012 to –0.2909, p<0.0001).

HIV infection is frequently correlated with a small number of TCD4+ lymphocytes for superior values of viral load, because TCD4 lymphocytes are target cells for HIV. No correlation between these two markers of HIV infection was noticed in our study.

We compared the results of QuantiFeron test with HIV viral loads comprised in the dynamical range of tests. All the results for viral load are divided in three separate parts: first category comprises undetectable viral loads, the second category 2 log-4 log RNA HIV-1 copies/ml and the third category 4 log- 7 log RNA HIV-1 copies /ml.

The percent of indeterminate results increased along with increases of viral load: for undetectable HIV viral load - 15% indeterminate results (IR) for QuantiFeron test were obtained, for the second category - 17 % IR, and for the third category - 28% IR.

Mann-Whitney U association for QFT negative and positive results are as follows: for QFT – HIV viral load p=0.771 and QFT-TCD4 p=0.06; indeterminate results were not considered.

Threshold values for TCD4 in indeterminate results cases was: CD4=15/µl, with specificity Sp=95%; CD4=95/µl, with sensibility Sn=74% and Sp=73%; CD4=243/µl with Sn=95%. These results suggest that QFT test will be most frequently indeterminate for TCD4 count lower than 15 cells/µl, while in patient with TCD4 count higher than 243/µl QFT test will render have valid results. The area under the ROC curve (AUC) of TCD4 for QFT indeterminate prediction is AUC=0.788, CI 95%=[0.726, 0.849]. Low TCD4 levels were noticed in most of the patients with indeterminate results of QFT test. An explanation for this could be that IFN-γ response is insufficient when the number of these lymphocytes is inadequate. In the studied group, the average TCD4 count was 278/µl in patients with positive QFT results, 353/µl in pa-

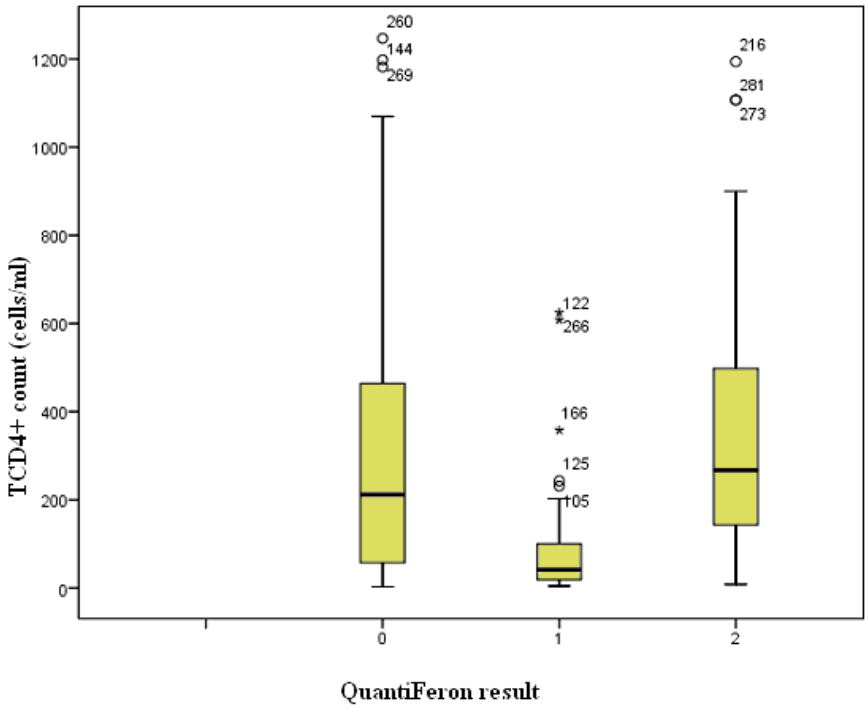

Figure 1. T_{CD4} count - QuantiFeron result association
(QF: negative = column 0, positive = column 2 and indeterminate results = column 1)

tients with negative results and 86/µl in those with indeterminate results (*Figure 1*).

HIV infected patients are very often predisposed to opportunistic infections; one of these is *Mycobacterium tuberculosis* infection, with a high prevalence in HIV individuals. It is estimated that one third of HIV/AIDS population is co-infected with *M. tuberculosis* (4), incidence of tuberculosis in HIV patients is 7-12% of all new MTB infection cases (8), and higher results are observed in African countries (20-67%) (11, 12).

Our investigation about the correlation between TCD4/TCD8 counts and QFT results is closely related with other previous study outcomes (10). There is an inverse correlation between TCD4+ and TCD8+ counts which explains an IFN-gamma response from HIV-1 infected persons, because they have a sufficient quantity of one type of cells (TCD8+ or TCD4+) to obtain a good IFN-γ response. Unlike the relationship between TCD4+ and the indeterminate results for QFT, a low prediction is observed regarding the CD8+-QFT relationship (the area under ROC curve of TCD8+ for QuantiFeron indeterminate prediction is AUC = 0.619, CI 95% = (0.543, 0.695).

The utility of QuantiFeron testing even in HIV infected patients was reported elsewhere (1). Still, theoretically, this test is not recommended in immunocompromised patients (13). No correlation between HIV viral load and TCD4+/TCD8+ count was revealed in our study, and a good response for QuantiFeron TB-Gold test was identified if the patient had a TCD4 count higher than 95/µl. A positive result is strongly suggestive for a latent tuberculosis form. However, since the test was indeterminate for more than 10% of patients even in subjects with CD4 count higher than 95/µl, its potential as rule-out test for active TB disease is limited, as it was reported in a previous study (14). Interpretative caution must be considered in those severe immunodeficiency cases in which the total number of lymphocytes is

drastically decreased and the result obtained for QuantiFeron-TB Gold test (indeterminate/negative) is not relevant.

In the future, the possibility to improve the sensibility of this test could answer to the need for a HIV-tuberculosis diagnosis in immunosuppressed patients.

Conclusions

In our group, we observed a negative correlation between TCD4 count and HIV viral load and between TCD4 and TCD8 count. These results are in accordance to those available in the literature.

In our group, 22.5% of the patients had an indeterminate result upon QuantiFeron-TB Gold testing. 36.3% from our patients had a TCD4 count lower than 95/μl; among these, 43.8% had an indeterminate QuantiFeron-TB Gold result; 10.3% patients with a TCD4 count higher than 95/μl had an indeterminate QuantiFeron-TB Gold result.

There is a direct proportionality between increase of HIV VL and the percentage of QuantiFeron-TB Gold indeterminate results.

Because of the high specificity for *M. tuberculosis*, the application of this test improves the quality of patient monitoring, but an upgrade is necessary in order to obtain better results even in patients with imunosuppresion.

As more than half of the patients severe immunocompromised have either positive or negative QuantiFeron-TB Gold results, the clinical utility of QuantiFeron-TB Gold testing in these patients should be evaluated. This study will be continued by one in which the correlation between QuantiFeron-TB Gold results and the presence of active tuberculosis will be assessed in patients with a TCD4 count lower than 95/μl.

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