Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Interferon γ in patients with HIV/AIDS and suspicion or latent tuberculosis infection

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ARTICLE INFO

Article history:
Received 10 August 2012
Received in revised form 15 October 2012
Accepted 15 December 2012
Available online 20 February 2013

Keywords:
AIDS
HIV
IGRA
Interferon γ
Latent tuberculosis infection

ABSTRACT

Objective: To assess the usefulness of IGRA test (QuantiFERON® -Cell mediated immune) compared with the tuberculin skin test. **Methods:** A cross-sectional study was carried out in Mexico, 25 infected patients with HIV-AIDS and the suspicion or with latent tuberculous infection (LTBI) who were >18 years of age and without treatment for tuberculosis (TB), were enrolled in the study. **Results:** Median cluster of differentiation (CD4) count was 364 cells/μL and median HIV viral load was 50 copies/mL. Overall, 20 patients (80%) had at least one positive diagnostic test for LTBI: four (16%) had a positive tuberculin skin test and 19 (76%), a positive QuantiFERON® -tuberculosis. **Conclusions:** No agreement is found between the two diagnostic tests: k = -0.004, 95% confidence interval (-0.2219, 0.2210). Additional longitudinal studies among HIV-infected populations with high prevalence of TB are needed to further assess the usefulness of IGRAs in this patient population.

1. Introduction

Tuberculosis (TB) is one of the most important causes of disease and death worldwide, mainly in Africa and Asia. In the year 2006, 9 million new cases of TB were presented, of which 0.7 million comprised patients who were co-infected with human immunodeficiency virus (HIV). Of 1.7 million TB-related deaths, 0.2 million were patients with HIV infection[1].

A report of the National Center for the Prevention and Control of HIV/acquired immunodeficiency syndrome (AIDS) (CENSIDA, its acronym in Spanish) in Mexico noted that Mexico occupies 42nd place worldwide in number of cases and the 16th place in Latin America. To March 31, 2009 in the National Registry of AIDS Cases in Mexico, 130 969 cases had been accounted for in accumulated manner, of whom 82% were males and 18%, females. In addition, it has been estimated that in Mexico there are ca. 200 000 persons with HIV/AIDS[2].

Prevention of active TB (ATB) by means of latent tuberculosis infection (LTBI) treatment is one of the greatest strategic elements to eliminate TB, particularly in the US[3]. It has been calculated that ca. 5%-10% with the latent form of tuberculosos (LTBI) will progress to active disease during their lifetime, and subjects co-infected with HIV and TB exhibit an active disease-progression degree of 3.5% to16.2%[4]. This will increase up to 20% or more in persons who are tuberculin skin test (TST)-positive[5]. However, this test has limitations, yielding false-positive results when the individual has the antecedent of the Bacillus Calmette-

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Guerin vaccination in the last 15 years and exposure to environmental non-tuberculous mycobacteria (NTM)[6]. Likewise, the low sensitivity and specificity of this test have been reported in patients with HIV and pulmonary TB with anergy up to 24.6% and associated with low Cluster of differentiation (CD4) count[7].

In recent years, a new generation of diagnostic tests for LTBI, the T cell-based IGRAs, have been developed: QuantiFERON® -Cell mediated immune (QFT-CMI) (Cellestis, Carnegie, Australia)[8,9]. IGRAs measure the amount of IFN γ released from sensitized T-cells after exposure to *Mycobacterium tuberculosis* antigens[10]. Studies examining the diagnostic sensitivity of IGRAs in HIV-seropostitive patients suggest that the assay possesses higher sensitivity than the TST[11].

The purpose of our study was to assess the usefulness of IGRAs, specifically QTF-CMI, among HIV-infected persons and to determine the degree of concordance between two diagnostic tests for LTBI (TST and QFT-CMI).

2. Materials and methods

2.1. Study population and setting

A cross-sectional study was carried out at the Infectology Hospital and at the Immunology and Infectology Medical Research Unit of the National Medical Centre La Raza of the Mexican Social Security Institute in Mexico City. The study included patients with HIV-AIDS and the suspicion or with LTBI who were >18 years of age and without treatment for TB. Information was gathered from March through December, 2006. Of the 34 patients selected for the study, 25 accepted to participate in the study after signing informed consent. The study protocol was reviewed and approved by the Institutional Ethics Committee. Patients were selected during their consultancy appointments, the latter including clinical evaluation, viral load control, treatment compliance, and appraisal of potential side effects. Clinical information comprising age, gender, year of diagnosis of HIV, the diagnosis that defined AIDS, and other risk factors for TB such as exposure to an index case with positive sputum, were gathered by means of review of the patients' clinical files. We excluded patients with type 2 diabetes, with previous severe reaction to TST or with active opportunistic infections.

Case study definitions were the following:

Patients with LTBI: the patient with HIV/AIDS infected with TB without radiological and clinical manifestations and with positive TST, who could potentially develop ATB within an undetermined time.

Patient with the suspicion of LTBI: patient with HIV/AIDS with radiological or clinical manifestations and with negative TST who have the antecedent of exposure to TB or who reside in a country with high TB endemicity.

2.2. T-cell interferon-gamma release assay

The QFT test was carried out according to the manufacturer's instructions. Briefly, 1 mL of the heparinized whole blood of each subject was placed in three wells of a 24-well culture plate. We added mitogen, purfied protein derivative (PPD) antigen skin test, and negative control to appropriate wells. The plates were incubated for 16 -24 h at 37 ℃ in a humid atmosphere; later, the plasma supernatant was removed and frozen until use. The amount of IFN γ produced was determined employing enzyme-linked immunosorbent assay, and liberation of IFN γ in the saline control tube was subtracted from that of Phytohemagglutin (PHA)- and TB (PPD)-stimulated tubes. Samples with ⩾ 0.35 IU/mL of IFN γ following stimulation with TB-specific antigen (PPD) were considered positive, while samples with <0.35 IU/mL were considered negative.

Alter blood was drawn for the IGRA, a TST was conducted utilizing the Mantoux method[12]; 0.1 mL [5 tuberculin units of PPD (Tubersol; Sanofi Pasteur, Inc., Swiftwater, PA, USA)] was administered intradermally (i.d.). TST was measured at between 48 and 72 h by a trained reader. Induration of ⩾5 mm was considered positive[9].

On the other hand, there are four classical criteria for the study of contacts of tuberculosis patients (COMBE test): epidemiological, clinical, tuberculin and radiological. If the analysis of these criteria suggests the possibility of a case of tuberculosis, can do a better study of diagnostic aids such as isolation of the bacillus, biopsy, serodiagnosis and other tests more technology.

2.3. Statistical analysis

Agreement was assessed using k, where k >0.75 represents excellent agreement, k values ranging from 0.4-0.75 represent fair-to-good agreement, and k <0.4 represents poor agreement, beyond chance[13]. Median values were compared utilizing the Mann-Whitney U test. We also employed central-trend, bivariate-analysis, and Pearson-correlation measurements.

3. Results

A total of 25 HIV-infected persons were enrolled in the study. Baseline characteristics of the patient population are displayed in Table 1. Twenty three patients were receiving highly effective antiretroviral therapy (HAART), and the most frequent combinations were the following: six patients (2 ITRN + 1 ITRnN); three patients (2 IRTnN + 1 IP); three patients (2 ITRN+ 1 IPr), and one patient (1 ITRN + 2 IP) (data not shown).

Thirteen patients had normal chest teleradiography (median IFN γ 2.5 UI/mL) and 12 patients with indistinct radiographic patterns suggesting no TBA (Table 2).

Overall, 20 (80%) of 25 patients had at least one positive diagnostic test for LTBI. Among the 25 patients who had their TST read, four (16%) were positive, and 19 of the 25 patients (76%) had a positive QFT.

Only three patients had positive test results for both

diagnostic tests (TST and QFT). There was no agreement between TST and QFT [k = 0.004, 95% CI −(0.2219, 0.2210)].

No association was found between concentrations of IFN γ and stage of disease in patients with HIV/AIDS utilizing the Mann-Whitney U test. There was also no correlation to analyze the lymphocyte subsets CD4, CD8, CD3 and viral load in IFN γ, using the Pearson correlation test.

Table 1

Baseline characteristics of population (n = 25).

Patient characteristics		Numbers (percentages)
Age in years, median (range)		34 (19 – 65)
Gender	Masculine	24 (96%)
	Feminine	1 (4%)
HIV history	CD4 cell counts, median (range)	364 (7 -842 cells/ μL)
	Viral load, median (range)	12×10^3 (50 -10^6 copies/mL)
	Duration in years of HIV diagnosis, median (range)	6 (1-18)
	Patients on HAART	23 (92%)
	History of opportunistic infections	16 (64%)
	Malnutrition	6 (24%)

Table 2

Teleradiography-ray analysis and correlation with positivity for COMBE, TST and mean values of IFN γ (n = 25).

Radiographic pattern	n	Positive COMBE test (%)	Positive TST (%)	Median IFN γ (UI/mL)
Nonspecific interstitial infiltrate suggesting no ATB	10	1(10)	3(30)	4.3
Calcified pulmonary nodule	2	2(100)	0(0)	8.0
Pulmonary overdistencion	1	0(0)	0(0)	5.0
Normal	12	2(17)	1(8)	1.3

4. Discussion

The most striking finding in our study comprised the very poor agreement found between the two different tests, with a k value of −0.004. Other studies also have also found poor concordance between TST and QFT[14]. Overall prevalence of a positive test with TST was 16%, and with QFT, was 76%. However, only three individuals tested positive for both tests.

In this study, we found a high percentage of patients with HIV/AIDS and positive IFN γ (76%), higher than percentages reported in other related studies, in which prevalences was 4.6% in European countries, 13% in countries with high endemicity, 16% when there was an epidemiological antecedent of exposure, and 60% to 70% in contacts with patients with active TB and in those who reside in high-endemicity zones[15].

Prior publications report that subpopulations low in CD4 (<200 cells) are associated with anergy on being tested for TST (*in vivo* and *in vitro*)[7,15,16]. There is little information in the literature on IFN γ on individuals with HIV/AIDS; in our study, five patients had low CD4 values; of these, four (80%) had IFN γ values that were >5 IU/mL, and of these, three had IFN γ values >10 IU/mL. Thus, our values are different from those published in the literature[8,17].

In this study, we analyzed variables such as history of exposure to TB. We found that in patients who were positive COMBE (TB contact), median IFN γ value was 3.1 IU/mL, in comparison with patients with negative COMBE, in whom this was 3.8 IU/mL, although without statistical significance, which can be related with the number of patients studied.

Likewise, we analyzed variables such as age, gender, CD4 count, and RNA-HIV viral load, and found no correlation with IFN γ levels, this similar to that reported in the literature[8,15,18].

In patients with HIV/AIDS, radiological manifestations are modified by the degree of immune compromise; patterns can be diverse, such as superior-lobule pleural cavities, infiltrate, nodules, and pleural leakage. Some series report a lesser incidence of cavitary disease, a greater frequency of mean pulmonary infiltrate, and less in comparison with clinical reactivation pictures in immunocompetent population[19]. In this study, the main radiological finding was bilateral diffuse interstitial infiltrate.

This study does not resolve the question of whether QFT testing should be performed simultaneously with, or instead of, TST testing in HIV-infected patients. On the one hand, the significant risk of LTBI reactivation in HIV-infected subjects presents a case for simultaneous screening with both TST and QFT to minimize false-negative tests and missed opportunities for LTBI therapy[20]. Until further data are available on the implication of discordant TST and QFT results, a strategy of simultaneous TST and QFT testing, when feasible, would maximize potential LTBI diagnoses in HIV-infected subjects.

On the other hand, simultaneous QFT and TST may be unrealistic in many settings. TST limitations, which include poor performance in HIV-infected populations[21,22] and personnel requirements for appropriate placement and interpretation, render a one-visit blood test attractive for LTBI diagnosis.

In this study, PPD-stimulated IFN γ levels exhibited a median of 3.8 IU/mL (10 times the positive cut-off value). Patients with positive TST and Combe presented the highest levels of IFN γ Given that tests with IFN γ are more sensitive than TST, the cost-benefit of diagnosis of LTBI

should be appraised in patients with HIV/AIDS, and QFT implementation as sole test in this population, application of a timely prophylactic treatment, and performance of follow-up studies with newIFN γ determinations after 2 years of prophylactic-treatment application should be proposed.

This study is limited by its small number of subjects and its cross-sectional design, which preclude prospective evaluation of TB incidence.

In conclusion, our findings indicate poor agreement between the TST and IGRA assays. Additional studies, preferably with a longitudinal design, in HIV-infected individuals are required to determine the true role that IGRA will play in the diagnosis of LTBI in these patients.

Conflict of interest statement

We declare that we have no conflict of interest.

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