Author's response to reviews

Title: IFN-gamma response on T-cell based assays in HIV-infected patients for detection of tuberculosis infection

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Version: 2 Date: 30 September 2010

Author's response to reviews: see over
ANSWER TO REVIEWERS

Title: IFN-gamma response on T-cell based assays in HIV-infected patients for detection of tuberculosis infection.


Manuscript number: MS: 1370713695417924

ANSWER TO REVIEWER 1: D Palmero.

The paper “IFN-# response on T-cell based assays in HIV-infected patients for detection of tuberculosis infection” by Latorre et al describes the responses to TST and to the two IFN-# tests available in the market in 75 HIV positive patients recruited in two hospitals located at Cataluña, Spain during a period of 34 months. Twenty of the patients had CD4+ count lower than 200 while the rest were over this number. The aim of the study was to describe comparatively the sensitivity of the three methods to detect LTBI. There is not a gold standard to define LTBI, so the real sensitivity (proportion of proven infected patients among all the patients studied) and the positive predictive value in this population are hard to define.

It is well known that TST has a higher proportion of false positive cases due to BCG vaccination and NTM infection while IFN-# tests are more specific because due to the antigens used in both tests (CFP10, ESAT 6 in quantiferon and T-spot with the addition of TB7.7 in the quantiferon gold in tube assay) that are not represented in BCG and in most of the NTM (with exceptions like M. kansasii, M. marinum and M. szulgai). It means an advantage in specificity and NPV. Sensitivity of TST is usually greater than IGRA’s.

Also is well known that in HIV infected people with low CD4+ count the values of any of the three tests is unpredictable, with a clear trend to negative or indeterminate results using both specific antigens or mytogen (see Luetkemeyer AF, Am J Respir Crit Care Med. 2007 for QF vs TST, for example).

The relative originality of this study is the comparison between both available tests vs. TST. Some of the conclusions are very obvious (possible false positive in BCG vaccinated population for the TST and not for IFN-# assays) and the other cross comparisons between the three methods don’t arrived to significant conclusions different from the bibliography, as is mentioned in the paper. Probably applying the same methodology in a higher population, with a higher proportion of severely immunodepressed patients the authors will have more opportunities to obtain valid (positive or negative) results.

In my opinion the study doesn’t contribute to solve the conundrum of the LTI in severely immunodepressed HIV patients: are they MTB infected or not?

In the last years, promising studies have been conducted in HIV infected individuals, in order to assess the utility of IFN-γ assays for the detection of LTBI and active TB. Until now, an association between positive results by the IFN-γ tests and the presence of risk factors to LTBI has been described. Furthermore, in HIV infected patients, that have a high incidence of NTM infections, IFN-γ tests increase the specificity in diagnosing LTBI. However, some aspects remain controversial and additional data are required.
Regarding the indeterminate results, in most studies T-SPOT.TB and in-house ELISPOT appear relatively unimpaired by low CD4 cell counts. On the contrary, using QFN-G-IT a strong correlation between low CD4 T-cell count and a low mitogen response was detected. It has been described that patients with low CD4 cell count had more indeterminate QFN-G-results. Luetkemeyer et al found that patients with a CD4 count less than 100 cells/mm\(^3\) had a relative risk ratio of indeterminate results of 4.24 compared to those with CD4 count of 100 or more. Jones et al noticed that all indeterminate results occurred in patients with CD4 counts <200 cells/mm\(^3\). Furthermore, Raby et al observed that with falling CD4 cell count there was a decrease in positive QFN-G-IT results with a relative increase of negative and indeterminate results. This was particularly marked at counts less than 100 cells/mm\(^3\); in Brock et al study, the 24% (4/17) of the patients with CD4 cell count <100 cells/mm\(^3\) had indeterminate result compared to only 2.8% (16/573) of patients with CD4 cell count >100 cells/mm\(^3\), and Aabye et al reported that the number of indeterminate results using QFN-G-IT significantly increase with the decrease of the CD4 cells count.

However, although indeterminate results have been more frequently described for QFN tests than for T-SPOT.TB, Stephan et al reported the opposite: T-SPOT.TB provided significant more indeterminate results than QFN-G (8 vs 1 in 256 patients); Karam et al found that the proportion of patients with a positive result for an in-house ELISPOT test decreased significantly with declining CD4 counts; and Talati et al described a higher number of indeterminate results by T-SPOT.TB than by QFN-G-IT, being a CD4 count less than 200 cells/\(\mu\)l associated with indeterminate results with the T-SPOT.TB, but not with the QFN-G-IT.

In addition, only 3 studies have been performed comparing the TST with the two commercial available IFN-\(\gamma\) tests, being one of them developed in a high TB-incidence country. Therefore, more studies comparing T-SPOT.TB and QFN-G-IT with TST in the same HIV population are required. On the one hand, to add new data to the information already available from previous studies, is necessary in order to better understand the role of IFN-\(\gamma\) assays in the diagnosis of LTBI in this kind of population. And, on the other hand, to analyze the impact of the degree of immunosuppression on the antigen-specific T-cell responses.

Our study has the originality that compares both T-SPOT.TB and QFN-G-IT assays with TST in the same study population in a non-high TB incidence country. Although, we are aware that the number of patients included in this study is limited to state definite conclusions, from our point of view, the results reported in this study are consistent enough to add valuable data about the utility of the IFN-\(\gamma\) tests in the diagnosis of LTBI in HIV infected patients, and the influence of the number of CD4 cell counts in the results of the different techniques.

In our study, we evaluate TST, T-SPOT.TB and QFN-G-IT assays in 75 HIV infected patients being screened for LTBI, finding a low rate of positive results with the three assays, detecting, intriguingly, poor concordances between three diagnostic tests. From the 7 cases with a positive T-SPOT.TB result and the 5 cases with positive QFN-G-IT results, both tests were positive simultaneously in only 2 cases. Diagnostic agreement between tests was moderate (\(k=0.40-0.65\)). In addition, we have found negative IFN-\(\gamma\) assays results among 2 non BCG-vaccinated HIV-infected individuals with a positive TST.

Therefore, the use of IFN-\(\gamma\) assays in combination with TST could be a helpful method for diagnosing LTBI in HIV population. Our study suggests that IFN-\(\gamma\) assays are influenced with level of immunosuppression. Further studies are required for understanding the meaning of the discrepancies between both IFN-\(\gamma\) tests.

On the other hand, we found that number of responder T cells to specific \textit{M. tuberculosis} antigens detected by T-SPOT.TB and the IFN-\(\gamma\) released in QFN-G-IT was lower in HIV-
positive patients with CD4 cell counts <200 than >200 cells/µl but not statistically significant. In addition, we studied the PHA T cell responses on QFN-G-IT according CD4 T cells and the differences between <200 and >200 cell counts were nearly significant (P=0.052).

The proportion of positive results obtained by T-SPOT.TB and QFN-G-IT were lower in HIV patients with a CD4 cell count <200 than above >200 cells/µl. In patients with a CD4 cell count below 200, we only obtained only one (5%) positive result with T-SPOT.TB in one patient with 39 CD4 cells/µl, but QFN-G-IT and TST were negative in all cases. These results suggest that T-SPOT.TB is less affected by the immunosuppression.

Finally, although, is well known that IFN-γ tests are less influenced by BCG vaccination, our study reinforce this information and give more data for a best management of the LTBI screening in the HIV BCG vaccinated patients.

According with the comments of the reviewer we have revised the manuscript indicating the limitations, featuring our results and avoiding overstating the conclusions.
ANSWER TO REVIEWER 2: Adithya Cattamanchi

In this study, Latorre and colleagues evaluate TST, QFT-GIT and TSPOT responses in 75 HIV-infected patients being screened for LTBI. They found a low number of positive results with all tests (<10 with all tests) and poor to moderate correlation between assays (kappa 0.4-0.6). The main issue with the study is that the conclusions are overstated given the relatively small number of patients evaluated. Specific comments are below.

MAJOR COMPULSORY REVISIONS

1. The conclusions in the abstract do not seem justified by the results presented. With only 8 BCG vaccinated patients, the difference between positive TST (4/8 positive) and IGRA (0/8 positive) results are unlikely to be statistically significant. It is also unclear why the authors feel that using IGRAs in combination with TST is beneficial. Lastly, the authors state that TSPOT appears more sensitive than TST or QFT-GIT in persons with advanced immunosuppression. However, only 1 positive result was obtained in this group, which could be by chance.

We agree with the reviewer that the conclusions stated in the study maybe are overstated given the relatively small number of patients included.

However, regarding the effect of the BCG vaccination on the TST and IGRA tests we have obtained that the difference between the proportion of TST and IFN-\(\gamma\) assays (T-SPOT.TB and QFN-G-IT) positive results in BCG vaccinated patients are statistically significant (\(p=0.046\)).

On the other hand, the conclusion that the use of IGRAs in combination with TST is beneficial is subtended by our results. In our study, we have described a low rate of positive results with the three assays, detecting, intriguingly, poor concordances between three diagnostic tests. From the 7 cases with a positive T-SPOT.TB result and the 5 cases with positive QFN-G-IT results, both tests were positive simultaneously in only 2 cases. In addition, we have found negative IFN-\(\gamma\) assays but positive TST results among 2 non BCG-vaccinated HIV-infected individuals (Page: 13; Lines 10-21).

Although routine testing with TST and IFN-\(\gamma\) assays is not usually recommended, results from both tests may be are useful when the first test (TST or IFN-\(\gamma\) assay) is negative in patients with high risk for progression to active TB, like HIV infected individuals (Mazurek M MMWR Recomm Rep 2010).

The conclusion regarding the higher sensitivity of the T-SPOT.TB than TST or QFN-GIT in persons with advanced immunosuppression is also based in our results. First of all, we studied the PHA T cell responses on QFN-G-IT according CD4 T cells and the differences between <200 and >200 cell counts were nearly significant. On the other hand, it was impossible to assess the number of responder T cells after PHA stimulation on T-SPOT.TB due to saturation in the control positive well. And secondly, the T-SPOT.TB was the only one test able to give a positive result in a patient with less than 200 cells/\(\mu l\) (39 cells/\(\mu l\)).

However, following reviewer’s suggestion, we have modified the text as follows in order to be more cautious (Page: 12; Line: 10-15):

"Regarding the differences in the IFN-\(\gamma\) assays results in HIV-positive individuals with a CD4 cell count <200 respect those with a CD4 cell count >200, T-SPOT.TB seems to be less affected by an advance immunosuppression because we obtained at least 1/20 (5%) positive
result in individuals with a CD4 cell count <200 (39 cells/µl), but none for QFN-G-IT, although the number of positive results is limited to drawn robust conclusions.”

According to reviewer’s comments, we have modified the abstract and the manuscript for avoiding overstated conclusions.

2. The Methods section does not describe any inclusion and/or exclusion criteria. What patients were eligible for the study and were any excluded? Was active TB ruled out by symptoms and/or tests at study entry?

The inclusion criteria were: HIV patients screened for LTBI for the three tests (TST, TSPOT-TB and QFN-GIT). In those individuals considered LTBI, active TB was ruled out basing on clinical and radiological examination. None of them had active TB. In other to clarify this, we have explained it in Study setting and patient recruitment section (Page: 5; Line: 12-14). In addition, all patients that presented a previous documented positive TST were excluded from the study (Page: 5; Line: 15-16).

3. Were patients consecutively recruited or was this convenience sample?

In our study, patients were consecutively recruited. They attended to the Hospital Universitari Germans Trias i Pujol or to the Hospital Universitari Múltia Terrassa for screening of LTBI. For a better understanding of the manuscript we have rewritten it in the Study setting and patient recruitment section (Page: 5; Line: 5-6).

4. Were any IGRA results indeterminate due to high background response in the Nil control well?

We have found only two indeterminate results due to an insufficient response to PHA and M. tuberculosis antigens. This finding is written in the Results section (Page: 8; Line: 10-12). We did not obtain any T-SPOT.TB or QFN-G-IT indeterminate result due to a high background response in the control negative well.

5. The authors state: “The difference between the results obtained by TST in non BCG-vaccinated and BCG-vaccinated was statistically significant (p=0.006).” But was the difference between the proportion of positive TST (4/8) and IGRA (0/8) results statistically significant?

We appreciate this reviewer’s contribution. So, following this suggestion we have determined this statistical information, finding that the difference between the proportion of TST and IFN-γ assays (T-SPOT.TB and QFN-G-IT) positive results are statistically significant (p=0.046). We have indicated this p value in the Results section (Page: 8; Line: 21).

6. The authors state: “T-SPOT.TB and QFN-G-IT sensitivities were lower in HIV patients with a CD4 cell count <200 than above >200 cells/µl.” The word “sensitivity” should not be used as this implies assay results are being compared to a gold standard. Instead, it is more accurate to say the proportion of positive results was lower in HIV patients with a CD4 cell count <200 than above >200 cells/µl.

We totally agree with the reviewer that this term is incorrectly used in the absence of a gold standard test. In order to clarify this, we have rewritten it in the text “proportion of positive results” instead of the word “sensitivity”, following reviewer’s comments (Page: 9; Line: 12).
7. In paragraphs 5 and 6, the authors discuss indeterminate results and CD4 count. Many of the studies cited in these paragraphs evaluated patients with active TB. The proportion of indeterminate results was 6% or less in the majority (12/16 studies I am aware of) of studies evaluating both tests in HIV-infected patients without active TB (ie, those being screened for LTBI, as was the case in this study). In contrast, studies of HIV-infected patients with active TB generally report a much higher proportion of indeterminate results. For clarity, the authors should focus on comparisons with a similar study population (HIV-infected patients without active TB).

In order to improve this part of the discussion about indeterminate results, we have modified the paragraph clarifying which studies are from LTBI individuals and which are from active TB patients. Furthermore, we clarify that high numbers of indeterminate results are obtained on active TB patients (Page: 11; Lines: 16-24).

8. The authors state: “However, T-SPOT.TB appears less affected by an advance immunosuppression because we obtained 1/20 (5%) positive result in individuals with a CD4 cell count <200, but none for QFN-G-IT.” As stated previously, this statement does not seem well justified based on obtaining 1/20 vs. 0/20 positive results.

We agree with the reviewer. Our number of patients included in the study with an advanced immunosuppression is limited; as a result, we obtain a low number of positive results, and this is insufficient to draw robust conclusions. In order to be more accurate we have rewritten the statement as follows (Page: 12; Line: 10):

“Regarding the differences in the IFN-γ assays results in HIV-positive individuals with a CD4 cell count <200 respect those with a CD4 cell count >200, T-SPOT.TB seems to be less affected by an advance immunosuppression because we obtained at least 1/20 (5%) positive result in individuals with a CD4 cell count <200 (39 cells/µl), but none for QFN-G-IT, although the number of positive results is limited to draw robust conclusions.”

9. The limitations section should acknowledge that the study was underpowered to detect differences between any of the tests.

Following reviewer’s recommendations, we have added this limitation in the Discussion (Page: 13; Line: 25-27).

MINOR ESSENTIAL REVISIONS

1. When describing interpretation of the TSPOT assay, the authors state that: “The result was considered indeterminate if the response to both antigen panels were negative and if the number of spots in the control positive well was higher than 20. The immunoresponse was considered adequate if the number of spots in the negative control was less than 10.” In the first sentence, I think the authors mean “if the number of spots in the control positive well was LESS than 20.” The second sentence should be clarified to state that the assay was considered indeterminate if the number of spots in the negative control was greater than 10.

In the first sentence there was a mistake that we have corrected as the reviewer suggest us. On the other hand, we have modified the second sentence in order to clarify that an indeterminate result is also considered when the number of spots in the negative control is greater than 10 (Page: 7; Lines: 13-15).
DISCRETIONARY REVISIONS

1. In the introduction, the authors state few studies have compared all 3 tests in HIV-infected patients (there are 4 studies I am aware of; the authors should also reference a previous study conducted by their group: Rivas I, Latorre I, Sanvisens A, Dominguez J, Tor J, Prat C, et al. Prospective evaluation of latent tuberculosis with interferon-gamma release assays in drug and alcohol abusers. Epidemiol Infect 2009;137(9):1342-7. The findings from these studies or at least what is different about the present study should be mentioned in the Introduction.

We did not include it in the manuscript due to that its objective was to analyze both IFN-γ assays and TST in current alcohol and drug abusers. Notwithstanding, a percentage of this population was HIV infected. So, in the current revised study, we wanted to analyze the three assays in a higher number of participants, in order to better understand IFN-γ clinical tests performance. Following reviewer’s suggestions, the reference has been included in the introduction (Page: 4; Line: 16) and included and commented in the discussion (Page: 10; Line: 16).