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Effect of HIV-infection on QuantiFERON-plus accuracy in patients with active tuberculosis and latent infection

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Abstract

Objective: HIV-infection increases the risk to progress to active-tuberculosis (TB). Detection of latent TB infection (LTBI) is needed to eventually propose preventive-therapy and reduce TB reservoir. QuantiFERON-TB Plus (QFT-Plus)-test identifies LTBI. Currently, only two studies on QFT-Plus accuracy in HIV-infected-population are available in high TB-endemic-countries. Therefore we aimed to evaluate the effect of HIV-infection on QFT-Plus accuracy to detect LTBI in a low TB-endemic-country.

Methods: We enrolled 465 participants, among the 167 HIV-infected-persons: 32 with active-TB (HIV-TB), 45 remote-LTBI (HIV-LTBI) and 90 at low *M. tuberculosis* (Mtb)-infection risk. Among the 298 HIV-uninfected-persons: 170 with active-TB, 76 recent-LTBI, 34 remote-LTBI and 18 with low Mtb-infection risk.

Results: QFT-Plus sensitivity was similar in TB regardless of HIV-status. CD4-count did not influence the distribution of IFN- γ values in HIV-TB and HIV-LTBI. Moreover HIV-LTBI and HIV-uninfected remote LTBI had a similar proportion of results in the uncertain range (IFN γ

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Declaration of Competing Interest

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Supplementary materials

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0.2–0.7 IU/ml) differently from those LTBI-persons reporting recent-exposure ($p = 0.016$). Cytometry results demonstrated that CD8-response was similar in HIV-infected- and -uninfected-persons whereas CD4-response was impaired in HIV-infected-persons ($p = 0.011$).

Conclusions: HIV-infection does not affect QFT-Plus response in active-TB, whereas the time of exposure influences the proportion of uncertain-results in LTBI.

Keywords

Latent tuberculosis; HIV; IGRA; QuantiFERON-TB Gold Plus; M.tuberculosis

Introduction

It is estimated that one fourth of world population is infected with *M. tuberculosis* (Mtb) ^{1–5} and that only the 5–10% of Mtb-infected individuals will progress to tuberculosis (TB) disease during life time.^{2,5} In 2017, 1 million (9%) of the worldwide TB cases were related to HIV-infected persons leading to 300,000 deaths.⁵ HIV-infection is responsible of an increased risk of progression to active disease, representing one of the main cause for active-TB disease, even in the absence of a deep CD4 T-cell impairment and even if under antiretroviral therapy (ART).^{3,6,7} Latent TB infection (LTBI) should be investigated in each HIV-infected person and the preventive TB treatment should be eventually offered.^{5,8} T-cells from individuals that have been infected with Mtb will release interferon-gamma (IFN- γ) when stimulated with Mtb antigens and the majority of these Mtb specific T-cells represent a recall of the memory response.^{9–12} The Interferon- γ release assays (IGRAs) used to diagnose LTBI measure the immune reactivity to Mtb antigens. Recently, a new IGRA, the QuantiFERON-TB Gold Plus (QFT-Plus)^{13–20} has been proposed. Compared to the previous version, it includes an additional tube (TB2) containing peptides stimulating both CD4⁺ and CD8⁺ T-cells.^{15,20} Several studies on the CD8 Mtb-specific response have inspired the development of the QFT-Plus.^{12,21–26} Recently, it has been also demonstrated that TB1- and TB2- antigens induce IP-10, suggesting it as an additional LTBI biomarker.^{27,28} The literature comparing QFT-Plus with the QFT-gold in tube (QFT-GIT) reports a high agreement in LTBI detection.^{29–34} Only few studies in high TB endemic countries are available on the QFT-Plus accuracy in HIV-infected individuals.^{35,36} Therefore, aim of the present study is to evaluate the impact of HIV-infection on the accuracy of QFT-Plus in patients with active-TB and LTBI in a low TB endemic country. According to the manufacturer's instructions, the QFT results interpretation is based on a cut-off of 0.35 IU/mL.²⁰ Studies on the healthcare workers demonstrated that results falling near the cut-off have a high conversion or reversion rate.^{37–42} These results fall in a zone of uncertainty ranging between 0.20- and 0.70 IU/MI.^{37,38,40,43} Recently it has been reported that LTBI patients with immune-mediated inflammatory diseases have a low IFN- γ response to QFT-P and a high proportion of results ranging in the grey zone. Moreover, a study conducted in a low TB endemic country such as Netherland, demonstrated an association between the results falling in the gray-zone range and relevant risk factors and/or evidence of Mtb infection.⁴⁴ The same authors reported also a case of a pregnant woman, with a QFT result falling in the negative uncertainty range before starting a tumor necrosis gactor (TNF) inhibitor (infliximab) therapy that developed active TB after starting it. According to the

authors, a borderline QFT result in individuals with higher risk to develop active TB, should be considered as a TB-specific response that justifies the TB preventive therapy.⁴⁵ In a high TB incidence country the TB preventive therapy is not offered to all LTBI individuals, with the exception of persons living with HIV (PLWH). PLWH have a higher risk to develop active TB disease, therefore in this vulnerable population it is crucial to diagnose LTBI and offer TB preventive therapy in both high and low TB endemic country.⁸ For this reason, as secondary aim, we investigated the distribution of QFT-P results according to the uncertain range in the fragile population of PLWH. Finally, for the first time to our knowledge, we aimed to characterize by cytometry in PLWH the response to QFT-P assay to specifically measure the CD4⁺ and CD8⁺ T-cell response comparing it to individuals without HIV infection.

Methods

Population characteristics and study design

This study was approved by the Ethical Committee of L. Spallanzani National Institute of Infectious Diseases (INMI) in Rome, (approval number 72/2015) and Villa Marelli Institute, Niguarda Ca' Granda Hospital in Milan (approval number 110–022019). Written informed consent was obtained. Research was performed following the STROBE-statement guidelines for observational studies.^{46,47} The study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).⁴⁸ Patients were prospectively enrolled (April 2015/January 2019).

International guidelines for the management of HIV infection recommend to perform the screening for LTBI in those with recent HIV diagnosis infection.^{2,3,6} Therefore, we enrolled PLWH already in ART that performed the screening for TB after starting the antiviral therapy or ART-naïve undergoing LTBI screening as routine test after newly diagnosed with HIV infection.

Among the HIV-uninfected individuals, we enrolled individuals undergoing screening because contacts of active TB patients defined as “recent LTBI”, or we enrolled the individuals known to have had LTBI and never underwent to TB preventive therapy defined as “remote LTBI”.

PLWH with low risk of TB infection performed only QFT-plus as routine LTBI screening. For all the other individuals enrolled with or without HIV infection, we performed the QFT-Plus assay in our laboratory for research purposes and we used our generated results for the present study. Therefore, patients were prospectively enrolled either those included for research purposes, or for clinical routine purposes as the PLWH newly diagnosed or the HIV-uninfected recent contacts that underwent routine LTBI screening. Among them, we identified those with LTBI based on the positivity to QFT-plus.

Microbiological-confirmed TB cases were based on a positivity to at least one of these tests: Mtb culture (sputum, bronchial lavage, pleural fluid, abscesses); Mtb-specific RNA amplification (TRCReady M.TB, Tosoh, Japan) and/or Mtb -specific NAT (Home-made PCR (IS6110) GeneXpert, Cepheid; Genotype MTBDRPlus Hain Life-science); histo-

pathological findings and presence of acid fast bacilli in tissues. Clinical TB diagnosis was based on clinical and radiologic criteria, including appropriate response to TB-specific therapy. TB patients were enrolled no later than the first week of TB-treatment. In the absence of clinical, microbiological and radiological signs of active-TB, LTBI definition was based on a positive QFT-Plus (Qiagen, Hilden, Germany) score. LTBI individuals were enrolled before starting preventive-therapy. Mtb-exposure was classified as recent for individuals reporting a contact with a smear positive active-TB patient no more than 3 months before. Individuals not reporting contacts with active-TB patients in the 3 years before the screening and with unknown TB contact, were defined as remotely exposed. As control we enrolled HIV-infected and-uninfected individuals with low risk of Mtb-exposure; LTBI was excluded on the basis of IGRA results and absence of clinical and radiological signs of active-TB. The low TB risk individuals were persons not reporting contact with an active TB patient. To note that in the study we have included 106 LTBI,¹⁴ 18 healthy donors and 66 active-TB patients, already described in previous reports.^{14,17}

QFT-Plus assay

QFT-Plus kits were donated by Qiagen and used according to manufacturer's instructions. Levels of IFN- γ were quantified by ELISA and analyzed by a QFT-Plus Analysis Software.²⁰

Intracellular staining assay

Intracellular staining was performed, concomitantly to QFT-Plus, in 13 HIV-TB, 12 HIV-LTBI, 12 TB, 5 remote LTBI and 5 recent LTBI. As reported,¹⁵ we isolated peripheral blood mononuclear cells (PBMC) and calculated frequencies of CD4 and CD8 T-cells producing IFN- γ , TNF- α and IL-2, acquiring at least 200,000 lymphocytes at FACS CANTO II (BD, Biosciences). Cytometry data were analyzed by FloJo software. Cytokine background found in the Nil tube was subtracted from each condition. We assigned a negative score when the background was higher than half of antigen-specific response. A frequency of cytokine producing T-cells of at least 0.03% was considered as positive response. Data analysis has been concomitantly performed in blind by EP and TC. Concordance of the analyses was 90% and agreement was achieved by discussion.

Statistical analysis

For quantitative measures median and interquartile ranges (IQRs) were reported and Kruskal-Wallis or Mann-Whitney U test were used for comparison across groups. Chi square or Fisher exact test was used for comparison among categorical variables. Logistic regression models were used to evaluate association among QFT-Plus results and diagnosis, odds ratio (OR) their confidence intervals (CI) and p-value were reported. In the HIV-positive subgroup, we used Spearman's correlation to evaluate the relationship between antigen-specific response to TB1 and TB2 as well as between each antigen-specific response and CD4 cells/mm.³

Data analysis was performed using STATA (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC) and Prism 6 (Graphpad Software 6.0, San Diego, USA).

Results

Population characteristics

We enrolled 465 participants: 167 HIV-infected and 298 HIV-uninfected. Among the HIV-infected individuals, 32 had active-TB (HIV-TB), 45 had LTBI (HIV-LTBI) and 90 had a low risk of Mtb infection (HIV-low TB risk). Among the HIV-uninfected individuals, 170 had active-TB disease (TB), 76 were recent LTBI (recent LTBI), 34 were remote LTBI (remote LTBI) and 18 were individuals with low risk of Mtb infection (low TB risk) (Table 1).

Among the PLWH, the majority were from Western Europe (54%) and male (82%). We found significant differences regarding the Bacillus Calmette et Guerin (BCG) vaccination status ($p < 0.001$) and the origin ($p < 0.001$); to note that majority of HIV-TB and HIV-LTBI subjects came from high endemic TB countries where the vaccination is mandatory at the birth. We found significant differences also comparing the ART intake, the number of HIV-RNA copies and the CD4-count (Table 1, supplementary Table S1). Among the HIV-uninfected individuals, the majority were from Western Europe (44%), 53.4% were male; we observed significant differences regarding the BCG-vaccination and the origin (Table 1).

Sensitivity of the QFT-Plus assay in active-TB patients

Sensitivity of the QFT-Plus, which is a routine test for LTBI, was evaluated in active-TB as a surrogate of LTBI because it is not possible to isolate Mtb in LTBI individuals. Sensitivity for TB was 80% and it was not significantly different from that found in HIV-TB patients either including or not the indeterminate results (68.7% or 73%). As expected, we found a higher number of indeterminate results in the HIV-infected patients compared to HIV-uninfected ($p = 0.024$) (Table 2). Response to TB1 or TB2 did not significantly differ between HIV-TB and TB patients ($p = 0.158$ and $p = 0.216$ respectively). Performing a two-sample test to compare the proportion of the total TB1 response with the total TB2 response in each group, we did not observe significant differences (HIV-TB: $p = 0.6$; TB: $p = 0.4$; data not shown), therefore the tubes had a similar sensitivity both in HIV-infected and -uninfected TB patients.

Stratifying the QFT-Plus results according to the ability to respond to both TB1 and TB2 (“TB1 and TB2”), only to TB1 (“only TB1”) or only to TB2 (“only TB2”) (Table 2), we found that the majority of patients simultaneously responded to both stimulations (HIV-TB 90.9, TB 91.9%), as reported in the HIV-uninfected individuals^{15,17} and we did not find significant differences between HIV-TB and TB.

HIV-infection does not have any impact on the distribution of results according to the “uncertain range” of QFT-Plus in active-TB patients

Positivity to the QFT-Plus assay is based on IFN- γ values ≥ 0.35 IU/mL.⁴⁹ Several studies have highlighted the variability of the results falling close to the assay cut-off,^{37,50,51} identifying an “uncertain range” (0.2–0.7 IU/ml). To evaluate the distribution of the IFN- γ values in the “uncertain range”, we reported our results stratifying the results as: <0.2 IU/mL; 0.2–0.34 IU/mL; 0.35–0.7 IU/mL; >0.7 IU/mL (Table 3). Evaluating the QFT-

Plus positive scores, we found that the majority of the positive responses were out of the “uncertain range” (>0.7 IU/mL) both in HIV-TB (TB1 81.8%; TB2 81.8%) and TB (TB1 83.1%; TB2 86.8%). Regrouping the data as “certain” and “uncertain” results, we found a similar number of “uncertain” and “certain” results in the HIV-infected individuals and in the HIV-uninfected individuals (Table 3). Similarly, performing the analysis of the data from active-TB patients scored negative to QFT-Plus, we found a higher number of results falling in the negative “certain range” in both HIV-infected and –uninfected groups (Table 3). Moreover, HIV-TB patients did not have a higher risk to have uncertain results vs certain results compared to active TB patients, as shown by the OR evaluation (Table 3).

Based on previous studies demonstrating that CD4 counts have an impact on the IGRA response in HIV-infection,^{52–54} we analyzed the effect of immune suppression on QFT-Plus in HIV-TB. To verify the presence of a correlation between the IFN γ production expressed as International Unit (IU)/ml and the number of CD4 cells/mm³, we performed a Spearman correlation and we did not find any significant differences (Fig. 1A). Similarly, applying a univariable logistic regression, we did not find nor linear trend between the TB1- and TB2-response and CD4-count increment. (Supplementary Table S2), nor significant differences. Finally, we did not find any significant association neither between the TB1- or TB2-response and ART status (Supplementary Table S2).

QFT-Plus results in LTBI individuals

Evaluating the sensitivity to total TB1- and total TB2-stimulation, we found that both recent LTBI and remote LTBI individuals had a risk similar to HIV-LTBI to have a positive total TB1 or total TB2 response (Table 4). To understand the impact of HIV-infection on the QFT-Plus results in LTBI, we stratified the QFT-Plus results according to the ability of individuals to respond to both TB1 and TB2, only to TB1 or only to TB2 (Table 4). We found that recent LTBI individuals had a significant higher OR to have a TB1 and TB2 response compared to HIV-LTBI (OR: 4.5; $p = 0.018$), whereas no differences was observed in remote LTBI compared to HIV-LTBI.

Analyzing the distribution of the results according to the “uncertain range” (Table 5) we found that the majority of HIV-LTBI and remote LTBI individuals had values >0.7 IU/mL (HIV-LTBI: TB1-response 73.3% and TB2-responses 73.3%; remote LTBI: TB1-response 76.5% and TB2-response 88.2%). Differently, HIV-LTBI had a lower number of positive results >0.7 IU/mL compared to HIV-uninfected recent LTBI (TB1-response: HIV-LTBI: 73.3%, recent LTBI 93.4%, $p = 0.005$; TB2-response: HIV-LTBI 73.3%, recent LTBI 92.1%, $p = 0.008$). Regrouping the data as “certain” and “uncertain” results, we found a higher number of “uncertain” results in the HIV-infected individuals and a higher number of “certain” results in the HIV-uninfected individuals; however only comparing the recent LTBI with the HIV-LTBI individuals we observed significant differences (TB1 $p = 0.010$; TB2 $p = 0.016$). Consequently we found a lower significant risk to have uncertain results only for recent LTBI compared with HIV-LTBI (TB1: OR 0.22, $p = 0.008$, TB2: OR 0.22, $p = 0.018$).

Distribution of discordant QFT-Plus results

The proportion of discordant QFT-Plus results in HIV-infected and -uninfected population with active-TB and LTBI were not significantly different (Table 6). However, we observed that the HIV- LTBI had the highest proportion of discordant results, TB1 positive and TB2 negative or vice versa, (20%). Calculating the OR to have a discordant results, we found that HIV-uninfected recent LTBI had the lower probability to have discordant results compared to HIV- LTBI (OR 0.22, $p = 0.018$).

Then, we analyzed the distribution of concordant and discordant results according to the “uncertain range”,^{37,50,51} (Table 7). We found that the majority or the half of discordant results fell in the “uncertain range” in all groups (HIV-TB: 100%, $p = 0.043$, TB: 81.8%, $p < 0.001$; HIV-LTBI: 88.9%, $p < 0.001$; recent LTBI: 50% $p = 0.02$; remote LTBI: 80% $p = 0.007$).

Time of Mtb exposure impacts the IFN- γ production in LTBI individuals

We evaluated the QFT-Plus results also by quantitative means (Fig. 2). We found that the HIV-LTBI individuals produce lower IFN- γ compared to LTBI, in response to TB1 (HIV-LTBI: median 2.2 IQR 0.7–4.7; remote LTBI: median 5.12, IQR 0.8025–10, recent LTBI: median 4.055, IQR 1.46–10 $p = 0.005$) and TB2 (HIV-LTBI: median 2.1 IQR 0.6–6.1; remote LTBI: median 4.675, IQR 0.94–10, $p = 0.03$; recent LTBI: median 4.345, IQR 1.757–10, $p = 0.003$). Differently in active-TB, HIV-infection did not affect the amount of IFN- γ response to TB1 (HIV-TB: median 1.0 IQR 0.09–4.9; TB: median 1.4 IQR 0.3–5; $p = 0.3$) or TB2 (HIV-TB: median 1.0 IQR 0.1–5.1; TB: median 1.9 IQR 0.5–5.9; $p = 0.2$).

Comparing the IFN- γ level in response to TB1 and TB2 within the same group, we did not find significant differences. No response to QFT-Plus was found in the control groups with low risk of Mtb-exposure. Two HIV-infected individuals with a negative result falling in the uncertain zone (Fig. 2) were from Italy, therefore without TB-risk associated to the origin.

We also investigated the correlation between the amount of IFN γ produced in response to TB1 and/or TB2 stimulation (Fig. 3). Interestingly we found a positive and significant correlation in all groups between TB1 and TB2 IFN γ amount (HIV-TB $r = 0.9657$, $p < 0.0001$; active TB: $r = 0.944$, $p < 0.0001$; HIV-LTBI: $r = 0.9513$, $p < 0.0001$; remote LTBI: $r = 0.9287$, $p < 0.0001$, recent LTBI: $r = 0.7655$ $p < 0.0001$). To verify the presence of a correlation between the IFN γ production expressed as International Unit (IU)/ml and the number of CD4 cells/mm³, we performed a Spearman correlation and we did not find any significant differences (Fig. 1B).

Antigen-specific response within the CD4⁺ or CD8⁺ T-cells: proportion of QFT-Plus-responders by flow cytometry

To characterize the antigen-specific response to TB1 or TB2 in a smaller cohort of patients, we evaluated by cytometry, the IFN- γ , TNF- α and IL-2 production in CD4⁺ and CD8⁺ T-cells (Figs. 4 and 5). We performed the analysis comparing the results of all HIV-infected individuals with the results of all HIV-uninfected individuals. Regarding the CD4⁺ T-cells, we found a higher proportion of IFN- γ , TNF- α and IL-2 responders to TB1 and TB2 in

HIV-uninfected individuals compared to HIV-infected (comparison including remote LTBI TB1: TNF α p = 0.015, IL2 p = 0.001; TB2: TNF α p = 0.034, IL2 p = 0.021 (Fig. 4A,B); comparison including recent LTBI TB1: TNF α p = 0.008, IL2 p = 0.001 TB2: IFN γ p = 0.011, TNF α p = 0.018, IL2 p = 0.004 (Fig. 5A–B). Differently, the analysis of CD8⁺ T-cells response did not show any significant difference among the groups (Fig. 4C–D; 5C–D).

Discussion

We evaluated the accuracy of QFT-Plus in HIV-infected and -uninfected individuals at different TB-stages in a low TB endemic country. We found that HIV-infection does not have an impact on the detection of positive results to QFT-Plus in active-TB. Evaluating the quantitative values, among the remote LTBI, we found similar results falling in the uncertain range independently of HIV infection. Conversely, in the HIV-uninfected recent LTBI individuals we found a significant lower number of results falling in the uncertain range. In this study the CD4-count did not influence the IFN γ (IU/ml) release. However, the cytometry results demonstrated that the CD4-response to QFT-Plus was impaired in HIV-infected- persons whereas the CD8-response was similar in HIV-infected-and -uninfected-persons. Collectively, these results suggest that the observed similar accuracy of QFT-Plus in HIV-infected and uninfected individuals is due to the CD8 compensation for the impaired CD4-response related to HIV infection.

The analysis of the QFT-Plus results demonstrated a similar sensitivity of the assay in HIV-TB and TB patients and a comparable ability to respond concomitantly to both TB1 and TB2, as shown in HIV-infected individuals in Zambia³⁵ and in uninfected active-TB patients in Italy.¹⁷ Moreover, the “only TB2” and “only TB1” response was observed with a similar frequency both in HIV-infected and HIV-uninfected TB patients and this was confirmed by cytometry. A recent meta-analysis on QFT-Plus highlighted a higher sensitivity of TB2-stimulation compared to TB1, in HIV-uninfected active TB patients.³⁴ In our study, we observed a higher but not significant sensitivity of TB2-stimulation compared to TB1, in active TB patients independently of HIV-infection. Moreover, the sensibility to TB2-stimulation was similar in HIV-infected and –uninfected active-TB patients. Based on this results, in the population studied, HIV-infection does not influence the TB1 or TB2 response to QFT-Plus in active-TB.

As previously reported,³⁵ HIV-infection did not influence the magnitude of the response to TB1- and TB2- stimulation in HIV-TB patients. Although active-TB patients had a low CD4 T-count, the high Mtb-load associated with TB-disease induced a strong immune response similar to that found in the HIV-uninfected patients. Differently, HIV-infection affects the IFN- γ production in individuals with LTBI, as demonstrated in Ethiopia in HIV-infected pregnant women.³⁶ In Zambia, it has been demonstrated a higher sensitivity of QFT-Plus compared to QFT-GT in HIV-TB adults.³⁵ Interestingly, the authors reported also a higher, but not significant, sensitivity of the QFT-Plus compared to QFT-GIT, in individuals with 100 CD4⁺ T-cells/mm³. In our study the CD4 T-cell counts did not affect neither the number of QFT-Plus responders nor the amount of the IFN γ produced in the HIV-TB population. In line with previous results,³⁵ we observed that the intake or not of ART did not affect the number of QFT-Plus responders.

Several reports discussed about the “true negative and positive values” of QFT-Plus.^{37,50,51} A study based on serial QFT evaluations,⁵⁰ suggests that values less than 0.2 IU/mL, should be considered as “true negative values”, whereas if at least one value is within the “uncertain range” (0.2–07 IU/mL) the conversions are uncertain and the results are partially explained by technical assay variability. In our study the majority of TB patients, both HIV-infected and –uninfected, displayed positive results out of the “uncertain range”, as expected.¹⁷ The analysis in LTBI patients demonstrated that the determining factor for the distribution of IFN γ values out or in the uncertain range, is the time of exposure to Mtb. Indeed, the QFT-Plus results from the HIV-LTBI individuals, all classified as with a remote exposure, did not significantly differ in terms of number of “certain and uncertain” when compared to the results from remote LTBI HIV-uninfected. To note that the majority of the QFT-Plus results from HIV-LTBI, HIV-TB and remote LTBI HIV-uninfected individuals were “discordant results” falling inside the uncertain range; differently in recent LTBI only 50% of discordant results were within the uncertain range, indicating that recent TB contacts are associated with “certain results”. On the other side, considering that the majority of the HIV-LTBI individuals enrolled had, as main factor for TB risk, an origin from high TB endemic countries, it is unlikely that the discordant results were false positive scores.

We observed that HIV-LTBI and HIV-uninfected remote LTBI had a similar QFT-Plus response to TB1 or TB2, similar distribution of uncertain and discordant results, a positive correlation between IFN γ in response to TB1 and TB2 stimulation, and that the CD4 count did not influence the IFN γ production of HIV-LTBI. Collectively these data, although limited, may suggest that the HIV infection does not impact the LTBI screening performed by QFT-Plus. Moreover it may be important to investigate about the time of Mtb exposure as influential factor for the quantitative QFT-Plus response. Indeed, based on the clinical practice in low TB-endemic countries, it is reasonable to assume that the PLWH undergoing the routine LTBI screening have likely had an Mtb remote exposure rather than a recent one.

We characterized by cytometry the involvement of CD4⁺ and CD8⁺ T-cells in the QFT-Plus response and demonstrated that the HIV-infection affected the CD4⁺ T-cell response; differently the CD8⁺T-cell response was similar in HIV-infected and HIV-uninfected individuals. These data are particularly important to show that the role of TB2 stimulation in the assay was not impaired in PLWH. Probably, the CD8-specific response compensates for the CD4-response impairment related to HIV-infection, determining a similar sensitivity of QFT-Plus in HIV-infected and uninfected individuals.

In conclusion, we assessed the impact of HIV-infection on the accuracy of QFT-Plus in patients with active-TB and LTBI in a low TB endemic country. We showed a similar sensitivity in active TB in HIV-infected and -uninfected population, and that CD4 count did not influence the distribution of IFN- γ values in HIV-TB and HIV-LTBI patients. In those with remote LTBI, HIV infection did not have any impact on the quantitative QFT-Plus results falling in the “uncertain range”. Finally, the cytometry results demonstrated that HIV infection reduced the CD4⁺ T-cells response but did not impact the CD8⁺ T-cells response which likely compensates for the CD4-response impairment related to HIV-infection. Further studies are needed to confirm these results worldwide.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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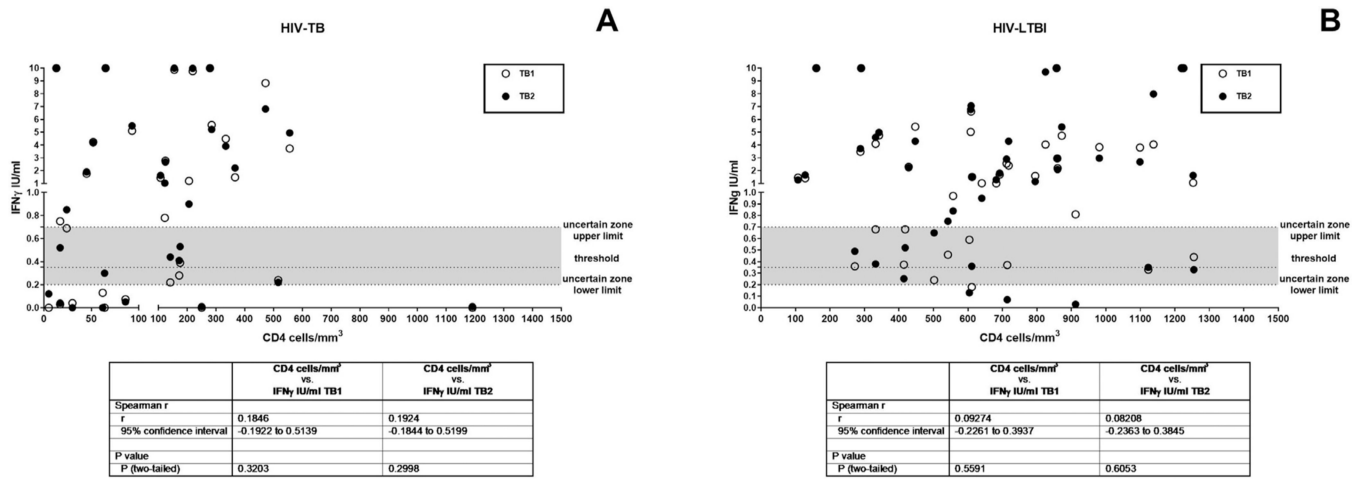


Fig. 1. Correlation between the IFN γ production and the number of CD4 cells/mm³. Spearman correlation was performed. A) HIV-TB; B) HIV-LTBI. IFN: interferon; IU: international unit; white circles for TB1 stimulation; black circles for TB2 stimulation. CD4 count is not available for 4 individuals (1 HIV-TB and 3 HIV-LTBI).

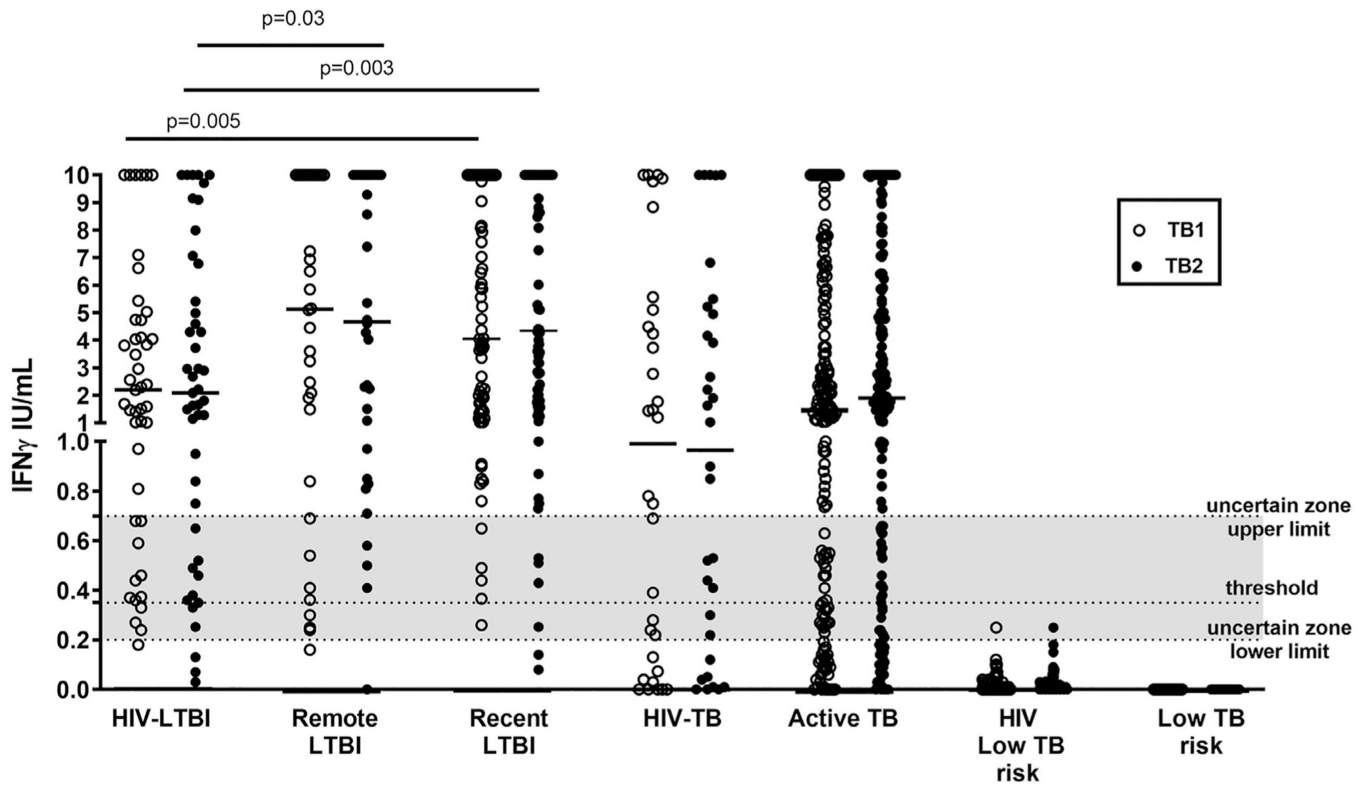


Fig. 2. Quantitative IFN- γ response to stimulation with QFT-Plus antigen TB1 and TB2 in HIV-infected and -uninfected individuals with active-TB, LTBI and with low TB risk. Horizontal lines indicate the median production. The data are presented as IU/mL. Footnotes: IFN: interferon; IU: international unit; white circles for TB1 stimulation; black circles for TB2 stimulation. The grey part of the graph represents the uncertain range of QFT-Plus results.

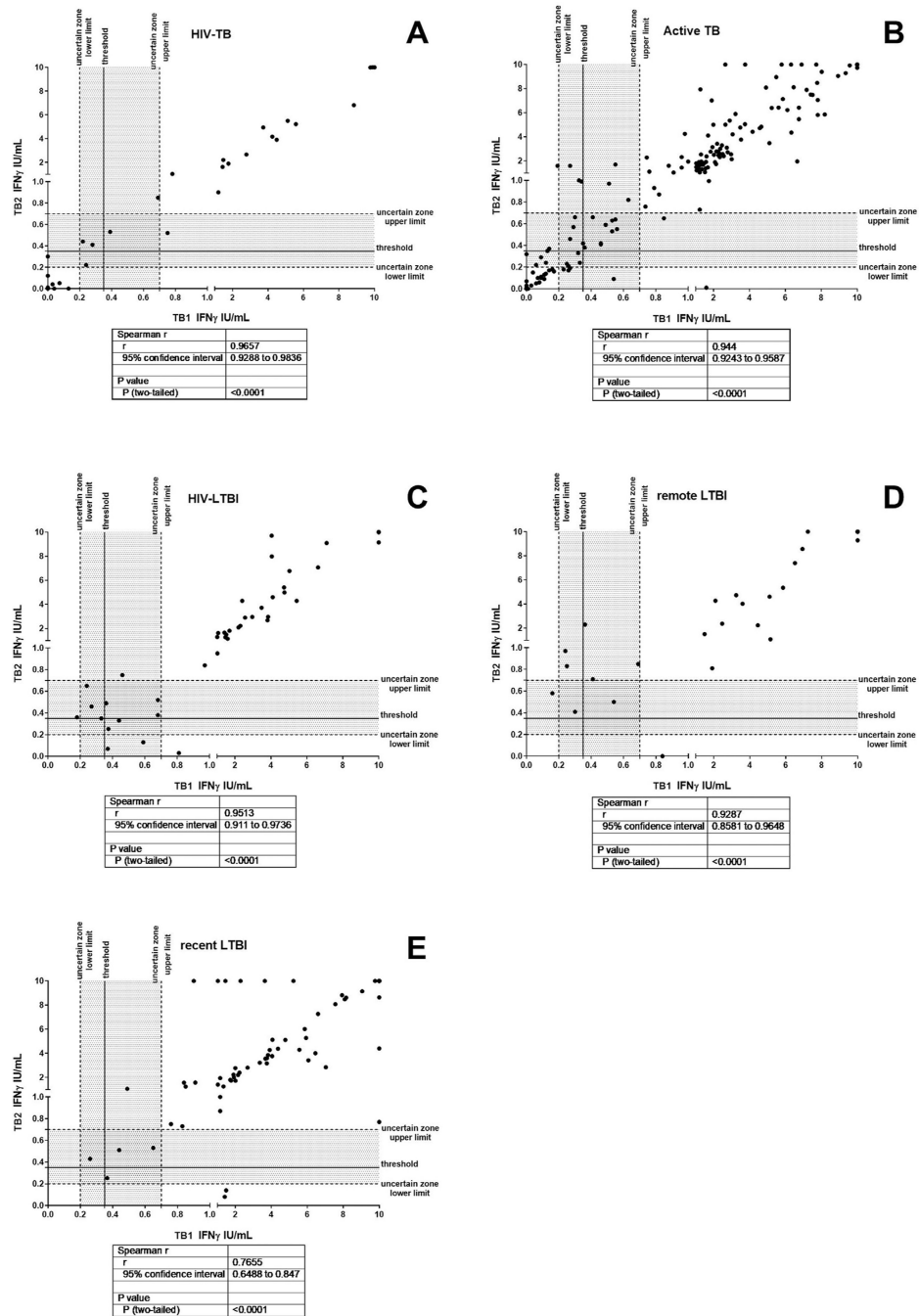


Fig. 3. Correlation between the IFN γ production in response to TB1 and TB2 stimulation, Spearman correlation was performed. A) HIV-TB; B) active TB; C) HIV-LTBI; D) remote LTBI; E) recent LTBI. Footnotes: IFN: interferon; IU: international unit; the grey part of the graph represents the uncertain range of QFT-Plus results.

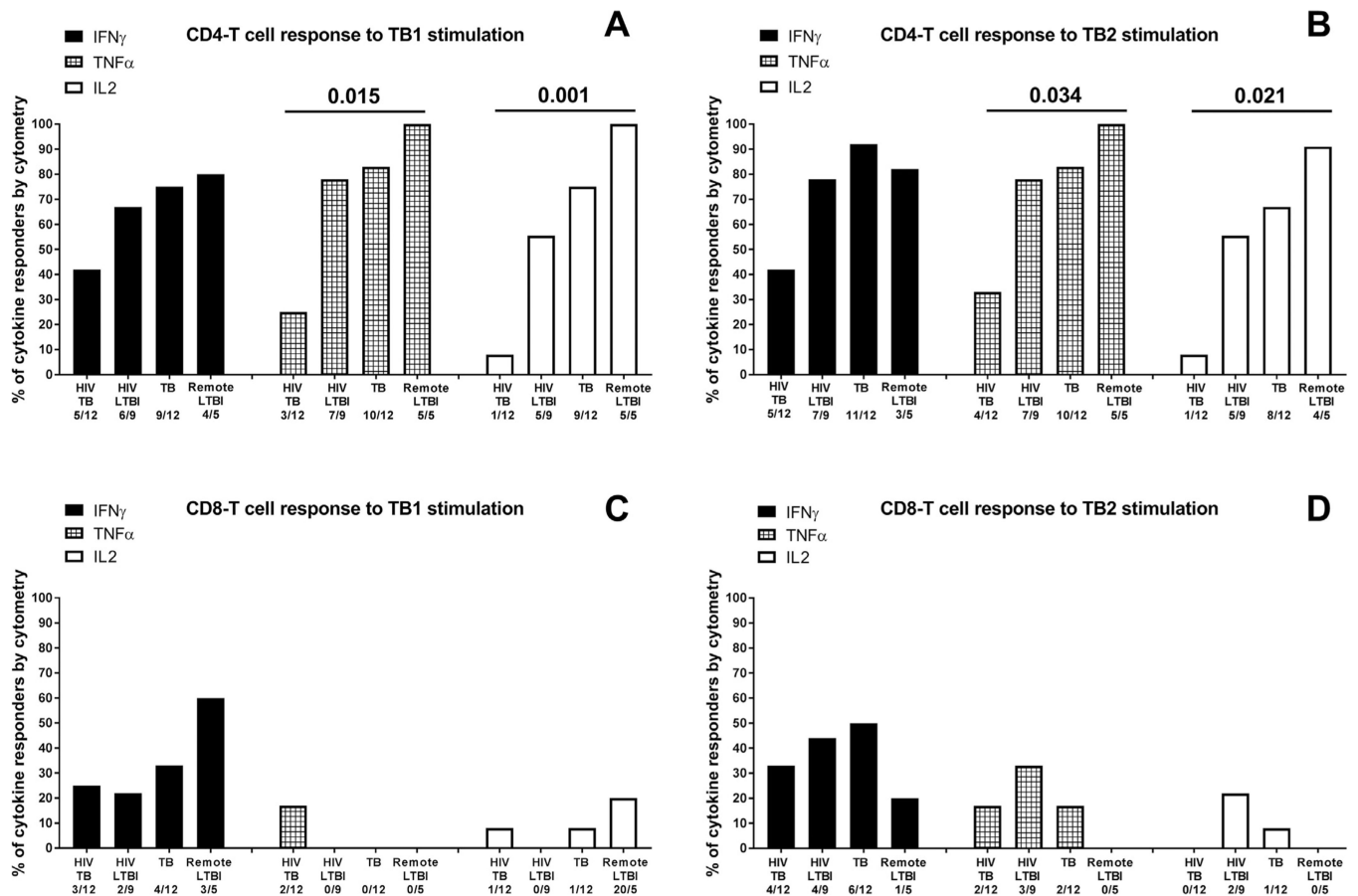


Fig. 4. Proportion of responders to TB1 and TB2 stimulation of the QFT-Plus test evaluated by flow cytometry.

Responders were defined based on IFN- γ or TNF- α or IL-2 production by CD4⁺ and CD8⁺ T-cells. A) CD4 T-cell response and CD8⁺ T-cell response to TB1 and TB2 stimulation considering only the remote LTBI in the in the HIV- uninfected LTBI group. The “x axis” reports the number of responders over total, for each group and for each cytokine. The statistical analysis has been performed comparing all the HIV-infected individuals with all the HIV-uninfected individuals using the Fisher exact test. The data are presented as proportion of responders for each cytokine. Footnotes: IFN: interferon; TNF: tumor necrosis factor; IL: interleukin.

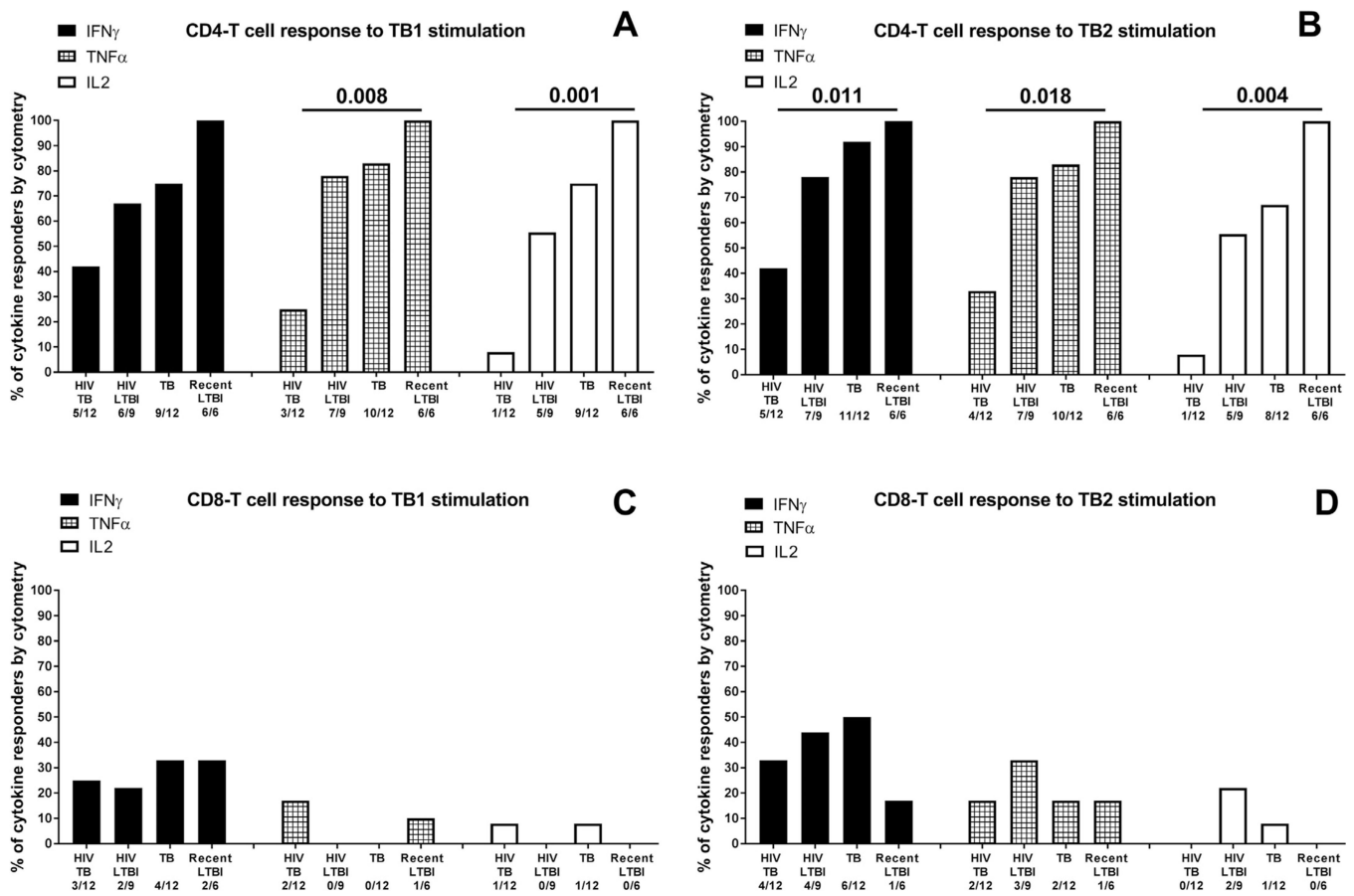


Fig. 5. Proportion of responders to TB1 and TB2 stimulation of the QFT-Plus test evaluated by flow cytometry.

Responders were defined based on IFN- γ or TNF- α or IL-2 production by CD4⁺ and CD8⁺ T-cells. CD4 T-cell response and CD8⁺ T-cell response to TB1 and TB2 stimulation considering only the recent LTBI in the HIV-uninfected LTBI group. The “x axis” reports the number of responders over total, for each group and for each cytokine. The statistical analysis has been performed comparing all the HIV-infected individuals with all the HIV-uninfected individuals using the Fisher exact test. The data are presented as proportion of responders for each cytokine. Footnotes: IFN: interferon; TNF: tumor necrosis factor; IL: interleukin.

Table 1

Demographic, epidemiological and clinical features of the enrolled patients.

	HIV-infected patients				HIV-uninfected patients				P	TOTAL	TOTAL	P
	HIV-TB	HIV-LTBI	HIV-Low TB risk	TOTAL	TB	LTBI recent	LTBI remote	Low TB risk				
N	32	45	90	167	170	76	34	18	298			
Sex male, N (%)	24 (75.0)	39 (86.7)	74 (82.2)	137 (82.0)	100 (58.8)	37 (48.7)	14 (41.2)	8 (44.4)	159 (53.4)	#	0.148	
Age, median (IQR)	40 (32–51)	36 (27–41)	37 (29–49)	37 (28–48)	37 (29–47)	39 (25–54)	44 (32–56)	39 (33–46)	38 (28–49)	§	0.461	
BCG-vaccinated, N (%)	23 (71.9)	35 (77.8)	19 (21.1)	77 (46.1)	116 (68.2)	38 (50.0)	15 (44.1)	1 (5.5)	170 (57.0)	#	<0.001	
TB diagnosis, N (%)												
Microbiological confirmation	25 (78)	-	-	-	132 (78)	-	-	-	-	-	-	-
Clinical diagnosis	5(16)	-	-	-	38 (22)	-	-	-	-	-	-	-
ART, N (%)	18 (56.2)	28 (62.2)	27 (30.0)	73 (43.7)	<0.001*	-	-	-	-	-	-	-
HIV RNA log10 copies/ml, median (IQR)	4.91 (3.60–5.41)	1.84 (1.59–4.29)	4.65 (3.92–5.26)	4.47 (2.22–5.18)	<0.001*	-	-	-	-	-	-	-
CD4 /mm³, Median (IQR) [†]	124 (52–279)	626 (418–860)	418 (253–631)	417 (208–646)	<0.001*	-	-	-	-	-	-	-
Origin, N (%)												
West Europe	9 (28.1)	10 (22.2)	71 (78.9)	90 (53.9)	55 (32.3)	39 (51.3)	19 (55.9)	18 (100)	131 (44.0)			
East Europe	7 (21.9)	6 (13.3)	7 (7.8)	20 (12.0)	65 (38.2)	21 (27.6)	8 (23.5)	0	94 (31.5)			
Asia	1 (3.1)	2 (4.4)	0	3 (1.8)	23 (13.5)	8 (10.5)	2 (5.9)	0	33 (11.1)			
Africa	8 (25.0)	7 (15.6)	4 (4.4)	19 (11.4)	14 (8.4)	5 (6.7)	5 (14.7)	0	24 (8.0)			
South America	7 (21.9)	20 (44.4)	8 (8.9)	35 (21.0)	13 (7.6)	3 (3.9)	0(0)	0	16 (5.4)			

TB: tuberculosis; BCG: Bacillus Calmette et Guérin; N: number; IQR: interquartile range.

§ Kruskal Wallis.

Fisher test.

* Chi Square test.

† CD4 count is not available for 5 patients (1 HIV-TB, 3 HIV-LTBI, 1 HIV-Low TB risk).

Table 2

QFT-Plus results in patients with active-TB with and without HIV-infection.

	QFT-Plus Results	HIV-TB (N = 32) N (%)	TB (N = 170) N (%)	TB vs HIV-TB OR (95% CI)	P
Sensitivity of the test	TB1 or TB2 positive	22 (68.7)	136 (80.0)	1.82 (0.79–4.20)	0.161
	Indeterminate	2 (6.2)	0 (0)	NA	0.024*
	Negative	8 (25.0)	34 (20.0)	0.75 (0.31–1.81)	0.524
Sensitivity of each test tube	Total TB1	20 (62.5)	127 (74.7)	1.77 (0.80–3.92)	0.158
	Total TB2	22 (68.7)	134 (78.8)	1.69 (0.73–3.89)	0.216
Type of response among QFT-Plus positive patients	TB1 and TB2	20 (90.9)	125 (91.9)	1.14 (0.23–5.51)	0.874
	only TB1	0 (0)	2 (1.5)	NA	0.100*
	only TB2	2 (9.1)	9 (6.6)	0.71 (0.14–3.52)	0.674

TB: tuberculosis; TB1: peptides of TB1 tube; TB2: peptides of TB2 tube; N: number; OR: Odds Ratio; CI: confidence Interval; NA: not available because one category has frequency = 0.

* If OR was not available, we compared proportion using Fisher Test.

Table 3

Distribution of IFN- γ values in response to TB1 and TB2 of QFT-Plus assay in TB patients with and without HIV-infection according to the “uncertain zone range”.

	Subjects scored positive to QFT-Plus				Subjects scored negative to QFT-Plus							
	TB1- response		TB2- response		TB1- response		TB2- response					
Results in range IFN- γ IU/mL	HIV-TB TB (N = 22) N (%)	TB (N = 136) N (%)	# P	HIV-TB TB (N = 22) N (%)	TB (N = 136) N (%)	# P	HIV-TB TB (N = 8) N (%)	TB (N = 34) N (%)	# P			
Negative certain <0.2	0 (0)	3 (2.2)	1.000	0 (0)	2 (1.5)	1.000	7 (87.5)	27 (79.4)	1.000	6 (75.0)	26 (76.5)	1.000
Negative uncertain 0.2–0.34	2 (9.1)	6 (4.4)	0.308	0 (0)	0 (0)	NA	1 (12.5)	7 (20.6)	2 (25.0)	2 (25.0)	8 (23.5)	
Positive uncertain 0.35–0.7	2 (9.1)	14 (10.3)	1.000	4 (18.2)	16 (11.8)	0.486	-	-	-	-	-	
Positive certain > 0.7	18 (81.8)	113 (83.1)	1.000	18 (81.8)	118 (86.8)	0.514	-	-	-	-	-	
Total certain <0.2 or > 0.7	18 (81.8)	116 (85.3)	0.748	18 (81.8)	120 (88.2)	0.486	-	-	-	-	-	
Total uncertain 0.2–0.7	4 (18.2)	20 (14.7)		4 (18.2)	16 (11.8)		-	-	-	-	-	
Risk of uncertain results vs certain for TB vs HIV-TB			P			P	OR (95% CI)		P	OR (95% CI)		P
	0.77 (0.24–2.53)		0.674	0.60 (0.18–2.00)		0.405	1.81 (0.19–17.29)		0.604	0.92 (0.15–5.50)		0.930

TB: tuberculosis; TB1: peptides of TB1 tube; TB2: peptides of TB2 tube; N: number; OR: Odds Ratio; CI: confidence Interval; NA: not available because one category has frequency = 0.

If OR was not available, we compared proportion using Fisher Test.

Table 4

QFT-Plus results in patients with LTBI with and without HIV-infection.

	QFT-Plus Results		HIV-LTBI (N = 45)		LTBI recent (N = 76)		LTBI remote (N = 34)		P
	N (%)	N (%)	N (%)	N (%)	vs HIV-LTBI OR (95% CI)	P	N (%)	vs HIV-LTBI OR (95% CI)	
Sensitivity of each test tube									
Total TB1	41 (91.1)	75 (98.7)	7.32 (0.79–67.65)	0.079	30 (88.2)	0.73 (0.16–3.16)	0.676		
Total TB2	40 (88.9)	73 (96.0)	3.04 (0.69–13.39)	0.141	33 (97.0)	4.12 (0.45–37.08)	0.206		
Type of response among QFT-Plus									
TB1 and TB2	36 (80.0)	72 (94.7)	4.5 (1.30–15.61)	0.018	29 (85.3)	1.45 (0.43–4.80)	0.543		
only TB1	5 (11.1)	3 (3.9)	0.33 (0.07–1.15)	0.141	1 (2.9)	0.24 (0.03–2.18)	0.206		
only TB2	4 (8.9)	1 (1.3)	0.14 (0.01–1.26)	0.079	4 (11.8)	1.37 (0.32–5.91)	0.676		

TB1: peptides of TB1 tube; TB2: peptides of TB2 tube; N: number; OR: Odds Ratio; CI: confidence Interval. LTBI subjects have been enrolled to be QFT-Plus positive, therefore it was not possible to calculate the sensitivity of the test.

Table 5

Distribution of IFN- γ values in response to TB1 and TB2 of QFT-Plus assay in LTBI patients with and without HIV-infection according to the “uncertain zone range”.

Results in range IFN- γ IU/mL	TB1-Positive response				TB2-positive response			
	HIV-LTBI (N = 45) N (%)	LTBI Recent (N = 76) N (%)	HIV-LTBI vs LTBI recent # p	HIV-LTBI vs LTBI remote # p	HIV-LTBI (N = 45) N (%)	LTBI Recent (N = 76) N (%)	HIV-LTBI vs LTBI recent # p	HIV-LTBI vs LTBI remote # p
Negative certain < 0.2	1 (2.2)	0 (0)	0.372	0.100	3 (6.7)	2 (2.6)	0.359	1 (2.9)
Negative uncertain 0.2–0.34	3 (6.7)	1 (1.3)	0.144	0.100	2 (4.4)	1 (1.3)	0.555	0 (0)
Positive uncertain 0.35–0.7	8 (17.8)	4 (5.3)	0.054	0.540	7 (15.6)	3 (3.9)	0.038	3 (8.8)
Positive certain > 0.7	33 (73.3)	71 (93.4)	0.005	0.799	33 (73.3)	70 (92.1)	0.008	30 (88.2)
Total certain <0.2 or > 0.7	34 (75.6)	71 (93.4)	0.010	0.790	36 (80.0)	72 (94.7)	0.016	31 (91.2)
Total uncertain 0.2–0.7	11 (24.4)	5 (6.6)			9 (20.0)	4 (5.3)		3 (8.8)
Risk of uncertain results vs certain for LTBI vs HIV-LTBI		OR (95% CI)	P	P		OR (95% CI)	P	OR (95% CI)
		0.22 (0.07–0.68)	0.008	0.686		0.22 (0.06–0.77)	0.018	0.39 (0.09–1.56)
								0.181

TB: tuberculosis; TB1: peptides of TB1 tube; TB2: peptides of TB2 tube; N: number; OR: Odds Ratio; CI: confidence Interval.

Fisher test. LTBI subjects have been enrolled to be QFT-Plus positive.

Distribution of discordant and concordant response to TB1 and TB2 stimulation according to the diagnosis among all subjects resulted QFT-Plus positive.

Table 6

Diagnosis	Concordant		Discordant		# p	OR for a discordant result among diagnosis		
	N (%)	N (%)	N (%)	N		OR	95% CI	P
HIV-LTBI remote	36 (80.0)	9 (20.0)	45	0.081	1			
HIV-TB	20 (90.9)	2 (9.1)	22		0.40	0.08–2.03	0.270	
LTBI recent	72 (97.7)	4 (5.3)	76		0.22	0.06–0.77	0.018	
LTBI remote	29 (85.3)	5 (14.7)	34		0.69	0.21–2.28	0.543	
TB	125 (91.9)	11 (8.1)	136		0.35	0.13–0.91	0.032	
Total	282 (90.1)	31 (9.9)	313		0.40	0.08–2.03	0.270	

LTBI: latent tuberculosis infection; TB: tuberculosis; TB1: peptides of TB1 tube; TB2: peptides of TB2 tube; N: number.

Fisher test; OR: Odds Ratio; CI: confidence interval.

Table 7

Distribution of discordant and concordant IFN- γ response to TB1 and TB2 stimulation according to the “uncertain” and “certain range”.

TB1-TB2 results IFN- γ IU/mL	HIV-TB*			TB			HIV-LTBI			LTBI recent			LTBI remote		
	Concordant N(%)	Discordant N(%)	# p	Concordant N(%)	Discordant N(%)	# p	Concordant N(%)	Discordant N(%)	# p	Concordant N(%)	Discordant N(%)	# p	Concordant N(%)	Discordant N(%)	# p
§ Certain <0.2 or >0.7	17 (85)	0 (0)	0.043	111 (88.8)	2 (18.2)	<0.001	32 (88.9)	1 (11.1)	< 0.001	69 (95.8)	2 (50.0)	0.020	25 (86.2)	1 (20.0)	
§§ Uncertain 0.2-0.7	3 (15)	2 (100)		14 (11.2)	9 (81.8)		4 (11.1)	8 (88.9)		3 (4.2)	2 (50.0)		4 (13.8)	4 (80.0)	0.007
Total	20 (100)	2 (100)		125 (100)	11 (100)		36 (100)	9 (100)		72 (100)	4 (100)		29 (100)	5 (100)	

LTBI: latent tuberculosis infection; TB: tuberculosis; N: number.

Fisher test.

§ if TB1 and TB2 results fall in the certain range.

§§ if at least one result falls in the uncertain range.

* indeterminate results have been excluded.