The diagnostic performance of TB-IGRA on HIV infected patients with active tuberculosis is associated with CD4+ T-cell counts

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Abstract

Background To investigate the factors associated with the diagnostic performance of interferon-gamma release assay (IGRA) on HIV infected patients with active tuberculosis (TB). **Methods** We retrospectively collected and analyzed the data of HIV infected patients with active tuberculosis hospitalized in Xixi Hospital of Hangzhou and Ruian people's hospital of Wenzhou from January 1, 2016 to September 1, 2019. TB-IGRA was performed on all patients. For evaluating the diagnostic performance of TB-IGRA, all patients were divided into TB-IGRA positive group and TB-IGRA negative group. **Results** Among 184 patients, 133 patients were TB-IGRA positive and 51 patients were TB-IGRA negative. Performed by logistic regression analysis, we found that CD4⁺ T cell counts were an independent risk factor for false negative of TB-IGRA (OR=1.006, 95%CI 1.003-1.010, P<0.001). Additionally, the false negative rates of TB-IGRA were 68.75%, 27.77%, 20.58% and 14.63%, respectively, in the four groups whose CD4⁺ T cell counts were <20/ μ L (group 1, n=32), 20-50/ μ L (group 2, n=36), 51-100/ μ L (group 3, n=34) and >100/ μ L (group 4, n=82). The group 1 had the highest false negative rate, compared with other groups (P<0.001). However, there was no significant difference of the false negative rate between group 2 and 3 (P=0.483), group 3 and 4 (P=0.623). **Conclusions** The low level of CD4⁺ T cell counts could increase the false negative rate of TB-IGRA in HIV infected patients with active tuberculosis. And TB-IGRA may have unreliable diagnostic performance on HIV patients with active tuberculosis, especially when CD4⁺ T cell counts were <20/ μ L.

Background

Global data in 2018 showed that tuberculosis (TB) caused 10.0 million cases and 1.2 million deaths, and 64% of notified TB patients had a documented human immunodeficiency virus (HIV) test result. Number of 477461 TB cases among HIV-positive people were reported; of these, 86% were on antiretroviral therapy (ART) [1]. The HIV infected patients with advanced immunodeficiency had a higher risk of active TB[2]. Moreover, TB is one of the most common diseases resulting in morbidity and mortality in the world[1, 3].

The immunodiagnosis of Mycobacterium tuberculosis (MTB) infection was a new adjuvant method. The interferon-gamma release assay (IGRA) was one of the most important advances of immunodiagnosis, and has been widely applied and accepted in the clinic[4, 5]. Enzyme-linked immunosorbent spot (ELISPOT) assay and enzyme-linked immunosorbent assay (ELISA), both through detecting released IFN- γ in culture supernatants, are two forms of the IGRA. In general, ELISPOT and ELISA were performed with the immunogenic and specific MTB antigens including early secreted antigenic target (ESAT-6) and culture filtrate protein 10 kDa (CFP-10)[6, 7].

The false negative of IGRA had been found in some TB patients especially combined HIV infection[8]. In addition, older age, non-Hispanic white race/ethnicity, being tested with T-SPOT.TB, albumin-globulin ratio, CD4 + T cell counts and CD8 + T cell counts had been thought as the influencing factors of TB-IGRA diagnostic performance in previous studies[8, 9]. In HIV infected patients, diagnostic performance of TB-IGRA(QFT-G-IT immune responses) was negatively correlated with circulating CD4 + T cell counts, and

was influenced by the degree of immunodeficiency[10]. The determined diagnosis for HIV infected patients with active TB is important. However, the in-depth studies of the diagnostic performance of IGRA on HIV infected patients with active TB has not been found. The objectives of this study were to investigate the factors associated with the diagnostic performance of IGRA on HIV infected patients with active TB, and to determine the relationship between the different hierarchy CD4 + T cell counts and the diagnostic performance of IGRA.

Methods

Study population

All patients (> 16 years of age) diagnosed as HIV infected patients with naïve active TB in the infectious department of Xixi Hospital of Hangzhou and Ruian people's hospital of Wenzhou in China, who were enrolled from January 1, 2016 to September 1, 2019, were retrospectively included into the study. HIV infection was confirmed in accordance with Centers for Disease Control and Prevention (CDC) definitions. History of AIDS diagnosis was determined according to the AIDS management guidelines[11].

The patients diagnosed with active TB was based on laboratory data (sputum smear microscopy and/or identification of *M. tuberculosis* in sputum/blood culture and/or GeneXpert for bronchial lavage). And the diagnosis of pulmonary TB with culture, smear and GeneXpert negativity was based on the combination of signs, symptoms, computed tomography (CT), good response to against TB treatment and the clinician's opinion. In addition, the diagnosis of extra pulmonary TB with clinically suspected but negative sputum results and normal CT was based on Fine Needle Aspiration Cytology and Pathology. All patients received anti-tuberculosis treatment and had written informed consent.

The exclusion criteria were as follows: (1) patients who did not undergo TB-IGRA examination; (2) history of administration of agents with activity against TB treatment; (3) evidence of nontuberculous mycobacteria infection; (4) patients with combined cirrhosis; (5) patients with other immune deficiencies (including malignancy, congenital immunodeficiency and treated with immunosuppressants); and (6) patients with immune reconstitution inflammatory syndrome (IRIS).

Clinical biochemical parameters and TB-IGRA assay

Laboratory data including white blood cells (WBC), neutrophil counts, lymphocyte counts, red blood cell counts (RBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin (ALB), CD4 + T cell counts, CD8 + T cell counts, CD4 + T cell to CD8 + T cell ratio, platelet counts (PLT), platelet distribution width (PDW), alanine transaminase (ALT) and aspartate transaminase (AST) were examined in a central laboratory in our hospital within 12 hours of patient admission.

MTB IFN- γ release assay according to the manufacturer's instructions using the Wantai quantitative diagnostic kit (WT-IGRA, Beijing Wantai Biological Pharmacy Corporation Ltd). First, BD (American BD company) heparin lithium anticoagulation blood collection tubes were used to extract more than 5 ml of

venous blood, anticoagulant dissolved by immediately gently inverting and mixing more than 3 times. Whole blood was collected and dispensed into 3 different culture tubes. "T" was a test culture tube in which TB specific antigens ESAT-6 and CFP-10 were added; "N" was a bottom control culture tube; "P" was a positive control culture tube with non-specific stimulating antigen phytohemagglutinin (PHA). 1 ml of venous blood was dispensed to each culture tube and mixed upside down for more than 3 times before dispensing. After the dispensing, the culture tubes were gently inverted 5 times, and immediately cultured in a 37 °C incubator (22 ± 2 h). After standing culture (22 ± 2 h), the culture tubes were taken out, and the supernatant was collected by centrifugation. Secondly, the content of IFN- γ in the supernatant was quantitatively detected by double antibody sandwich method. The positive or negative result of MTB infection was determined based on the amount of IFN- γ specifically raised in the stimulation culture system. In theory, each subject can respond to the non-specific stimulating antigen PHA.

Statistical analysis

Discrete data were presented as counts (percentage), and continuous data were presented as the mean $\pm$ standard deviation for normally distributed, medians and interquartile ranges for non-normally distributed. The differences between continuous data were compared using the Mann-Whitney U-test (nonparametric) or the Student t test (normally distributed). The differences between categorical data were evaluated using the χ^2 test with Continuity correction. P values were evaluated by Bonferroni correction. For predicting the risk factors of false negative rates, we performed univariate and multivariate analyses by logistic regression analysis. Receiver operating characteristic curve (ROC) analysis was conducted to present a varying cut-off values of CD4 + T cell counts for positive TB-IGRA and negative TB-IGRA. All statistical analysis was performed using SPSS, version 19 (SPSS, Armonk, NY, USA), and $P < 0.05$ was considered statistically significant.

Results

Clinical data pertaining to HIV infected patients with active TB between TB-IGRA positive and TB-IGRA negative groups

184 patients were divided into TB-IGRA positive ($n = 133$) and TB-IGRA negative ($n = 51$) groups. The sensitivity of the TB-IGRA was 72.28% among the 184 patients. And clinical characteristics and baseline demographics of the 184 patients between TB-IGRA positive and TB-IGRA negative groups were shown in Table 1. Through analysis of the TB-IGRA positive and negative results, we found that significant difference of CD4 + T cell counts ($P \leq 0.001$), CD8 + T cell counts ($P = 0.001$), CD4 + T cell to CD8 + T cell ratio ($P \leq 0.001$), ALB ($P = 0.005$), ESR ($P = 0.047$), Lymphocyte counts ($P = 0.001$) and AST ($P = 0.004$) between two groups.

Table 1
Clinical data analyses in HIV-infected patients with active TB between positive TB-IGRA and negative TB-IGRA.

	Positive TB-IGRA	Negative TB-IGRA	<i>p</i> -value
Subjects (<i>n</i>)	133	51	NA
Male/female	119/14	45/6	0.809
Median Age, year	41(16–86)	39(23–74)	0.548
Median CD4 + T cell counts, / μ L	108(0-797)	25(0-700)	< 0.001
Median CD8 + T cell counts, / μ L	541(40-2078)	354(74-1805)	0.001
CD4 + to CD8 + ratio	0.33(0-3.92)	0.05(0-0.67)	< 0.001
CRP, mg/dl	46.00(0.40-209.80)	35.00(0.39–203.00)	0.532
ALB, g/l	31.6(14.8–50.5)	27.5(16.3–49.4)	0.005
ESR, mm/h	62.90 $\pm$ 31.26	74.73 $\pm$ 35.91	0.047
WBC,10 ⁹ /L	5.54(0-12.21)	5.66(1.80–33.00)	0.894
Neutrophil counts,10 ⁹ /L	3.81(0.35–61.20)	4.07(1.12–29.60)	0.233
Lymphocyte counts,10 ⁹ /L	1.02(0-2.99)	0.68(0.07–3.94)	0.001
RBC,10 ¹² /L	3.65 $\pm$ 0.82	3.54 $\pm$ 0.79	0.391
PLT,10 ⁹ /L	242.50(7.90–266.00)	255.00(3.50–477.00)	0.628
PDW, fl	11.10(7.60–106.00)	11.10(7.20–21.60)	0.936
ALT, U/L	21(4-667)	22(7-190)	0.326
AST, U/L	27(8-542)	40(10–139)	0.004
Patients on HARRT, n (%)	62	22	0.671
HIV-1 viral load, copies/mL			0.282
≥ 100 copies/mL	82	27	
< 100 copies/mL	51	24	
TB disease			
Pulmonary	72	25	0.104
Extrapulmonary	15	12	

Data are presented as the mean $\pm$ standard deviation for normally distributed, medians and interquartile ranges for non-normally distributed.

	Positive TB-IGRA	Negative TB-IGRA	<i>p</i> -value
Pulmonary and extrapulmonary	46	14	
Acid-fast staining method(n)	38	13	0.840
Pathology(n)	11	2	0.520
Data are presented as the mean ± standard deviation for normally distributed, medians and interquartile ranges for non-normally distributed.			

Univariate and multivariate analyses of the diagnostic performance of TB-IGRA on HIV infected patients with active TB

Performed by logistic regression analysis, we determined the value of CD4 + T cell counts in the diagnostic performance of TB-IGRA. Via univariate analysis, CD4 + T cell counts, CD8 + T cell counts, CD4 + T cell to CD8 + T cell ratio, ALB, ESR, Lymphocyte counts and AST were included to the logistic regression analysis model. Finally, we found that only CD4 + T cell counts were an independent risk factor for false negative of TB-IGRA (Table 2).

Table 2

Univariate and multivariate analyses of the diagnostic performance of TB-IGRA on HIV infected patients with active tuberculosis.

	Odds ratio	95% CI	<i>P</i> value
Univariate analysis			
CD4 + cell count, /μL	1.006	1.003–1.010	0.001
CD8 + cell count, /μL	1.002	1.001–1.003	0.004
CD4 + to CD8 + ratio	40.189	3.925-411.482	0.002
ALB, g/l	1.070	1.018–1.124	0.008
ESR, mm/h	0.989	0.979–0.999	0.031
Lymphocyte count, 10 ⁹ /L	2.379	1.271–4.452	0.007
AST, U/L	0.999	0.994–1.004	0.671
Multivariate analysis			
CD4 + cell count, /μL	1.006	1.003–1.010	0.001
Data are presented by univariate and multivariate analyses using logistic regression analysis.			
Figure legends.			

Low CD4 + T cell counts in HIV infected patients with active TB were associated with false negative of TB-IGRA

We found that CD4 + T cell counts were significantly lower in TB-IGRA negative group (Fig. 1A, $P < 0.001$), compared with TB-IGRA positive group. Additionally, we obtained an optimal cut-off value (28.5 cells/ μL) of CD4 + T cell counts via ROC analysis. For examining whether declined hierarchy of CD4 + T cell counts were associated with the diagnostic performance of TB-IGRA on HIV infected patients with active TB, patients were divided into three groups based on the levels of CD4 + T cell counts: $<20/\mu\text{L}$ ($n = 32$), $20-100/\mu\text{L}$ ($n = 70$) and $> 100/\mu\text{L}$ ($n = 82$). And false negative rates of TB-IGRA were 68.75%, 24.29% and 14.63%, respectively, in the three groups (Fig. 1B, $P < 0.001$). Furthermore, patients were divided into two groups based on the levels of CD4 + T cell counts: $\leq 50/\mu\text{L}$ ($n = 68$) and $> 50/\mu\text{L}$ ($n = 116$). False negative rates of TB-IGRA of the two groups were 47.05% and 16.38%, (Fig. 1C, $P < 0.001$). In addition, patients were also divided into four groups based on the levels of CD4 + T cell counts: $<20/\mu\text{L}$ (group 1, $n = 32$), $20-50/\mu\text{L}$ (group 2, $n = 36$), $51-100/\mu\text{L}$ (group 3, $n = 34$) and $> 100/\mu\text{L}$ (group 4, $n = 82$). The group 1 had the highest false negative rate, compared with other groups (Fig. 1D, $P < 0.001$). However, there was no significant difference of the false negative rate between group 2 and 3 ($P = 0.483$), group 3 and 4 ($P = 0.623$).

Discussion

TB is the leading cause of death among patients co-infected with HIV. And due to a high frequency of smear-negative and high rates of extrapulmonary TB in HIV co-infected people, TB is difficult to diagnostic in these patients[12]. In the past two decades, the immune diagnosis of MTB infection provided more rapid and accurate results than traditional laboratory techniques in the fight against TB. However, the diagnostic performance of TB-IGRA on HIV infected patients with active tuberculosis was not clear enough.

In this study, the sensitivity of the TB-IGRA was 72.28% in HIV infected patients with active TB, and that was higher than the reported in the studies of Prabhavathi et al[13], Sauzullo et al[14] and Takwoingi et al[15] (54.55%;66%; 67.3%, respectively), but lower than the reported in the studies of Danel et al[16] and Aichelburg et al[17] (88.0%; 90.9%, respectively). Thus, multicenter and large sample study was needed for the accurate sensitivity of IGRA in HIV infected patients with active TB, and it may be different in studies with a small sample.

Similar results were reported by Yu et al that TB-IGRA was affected by the low level of CD4 + T cell counts[18]. However, hierarchy of CD4 + T cell counts was not used for further explanation. In our study, we found that the group of CD4 + T cell counts $< 20/\mu\text{L}$ in HIV infected patients with active TB had the highest false negative rate of TB-IGRA (68.75%). And there were significant differences compared with the groups whose CD4 + T cell counts were $20-100/\mu\text{L}$ and $> 100/\mu\text{L}$; $20-50/\mu\text{L}$, $51-100/\mu\text{L}$ and $> 100/\mu\text{L}$. Moreover, there were no significant differences between groups of $20-100/\mu\text{L}$ and $> 100/\mu\text{L}$; $20-50/\mu\text{L}$, $51-100/\mu\text{L}$ and $> 100/\mu\text{L}$. These data suggest that IGRA may have unreliable diagnostic performance for advanced HIV patients with active TB whose CD4 + T cell counts were $< 20/\mu\text{L}$. But IGRA may had the value of assisted diagnosis for advanced HIV patients with active TB whose CD4 + T cell counts were $> 20/\mu\text{L}$.

Dheda et al, Cattamanchi et al and Cai et al suggested that IGRA positive rates was not affected by CD4 + T cell depletion in HIV infected patients with active tuberculosis [19–21]. But they used an ELISPOT-based IGRA that was not same as our methods. The study of Ahamed et al suggested that there was no significant difference of CD4 + T cell counts between QFT-G positive and negative subjects, but they also suggested that the sensitivity of the assay was impaired when CD4 + T cell count < 200 cells/ μ L in HIV patients with active TB [22]. Our results strongly supported Leidl et al who showed QuantiFERON-TB Gold In-Tube (QFT-G-IT) positive rates were correlated with T cell stratification in patients with HIV infection and active TB [10]. But we made more detailed in T-cell stratification.

There were some limitations in our study. The diagnosis of active TB in patients including smear and culture negative results was made only by clinical and radiology. However, the positive rate of smear and culture is low for diagnosis of active TB and we found good response to anti-TB treatment from enrolled patients by following up. Another limitation of our study is the lack of report about specificity of TB-IGRA since we did not get the related tests of HIV positive but TB negative individuals.

Conclusions

The low level of CD4 + T cell counts could increase the false negative rate of TB-IGRA in HIV infected patients with active tuberculosis. And TB-IGRA may have unreliable diagnostic performance on HIV patients with active tuberculosis, especially when CD4 + T cell counts were < 20/ μ L.

Abbreviations

IGRA, interferon-gamma release assay; TB, tuberculosis; HIV, human immunodeficiency virus; ART, antiretroviral therapy; MTB, Mycobacterium tuberculosis; WBC, white blood cell; RBC, red blood cell counts; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ALB, albumin; PLT, platelet counts; PDW, platelet distribution width; ALT, alanine transaminase; AST, aspartate transaminase.

Declarations

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the first author on reasonable request.

Authors' contributions

M.Y.W. and J.Y. designed research; performed research; analysed data and wrote the manuscript. J.T.C. collected the data and modified the manuscript. X.T.D cleaned and analysed the data. Y.Z. and S.R.L. contributed to project conception, data analysis interpretation, and manuscript preparation. Z.D.Z; J.C.S. contributed to data analysis interpretation and manuscript preparation. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All patients provided written informed consent and this study was approved by Ethics Committee of Xixi Hospital of Hangzhou in China. All procedures and methods were performed in accordance with the relevant international guidelines and regulations in order to reduce physical discomfort of the subjects.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

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