Methods and Applications

Auxiliary Diagnostic Value of the Interferon Gamma-Induced Protein 10 mRNA Release Assay for Tuberculosis in People Living with HIV/AIDS — Beijing Municipality, China, 2022–2024

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ABSTRACT

Introduction: Diagnosing tuberculosis (TB) in HIV-infected individuals presents significant challenges due to difficulties in obtaining specimens containing adequate quantities of *Mycobacterium tuberculosis* (*Mtb*). This study aimed to evaluate the diagnostic performance of the *IP-10* mRNA assay independently and in combination with established diagnostic tests for *Mtb* detection.

Methods: The study cohort comprised 111 HIV-infected individuals who presented with TB at Beijing Youan Hospital from 2022 to 2024. Participants were categorized into confirmed TB, probable TB, or non-TB groups according to the diagnostic criteria for tuberculosis (WS288-2017). The performance of the *IP-10* mRNA release assay was evaluated by the STARD guidelines on blood samples collected after enrollment.

Results: The *IP-10* mRNA release assay demonstrated significantly higher sensitivity than interferon-γ release assays (IGRAs) and culture methods for confirming pulmonary tuberculosis (PTB) diagnosis while maintaining comparable specificity. Receiver operating characteristic (ROC) analysis revealed that the diagnostic performance of the *IP-10* mRNA release assay used in parallel with Xpert MTB/RIF significantly exceeded that of the *IP-10* mRNA release assay alone (0.731 *vs*. 0.687, P=0.02). Among HIV-infected individuals, the *IP-10* mRNA release assay showed superior performance compared to IGRAs for diagnosing extrapulmonary tuberculosis.

Conclusions: The *IP-10* mRNA release assay exhibited excellent diagnostic performance and demonstrates substantial potential as an auxiliary tool for diagnosing TB in HIV-infected individuals. The combined application of IP-10.TB and Xpert MTB/RIF further enhance diagnostic efficacy.

Mycobacterium tuberculosis (*Mtb*) infection represents one of the most prevalent opportunistic infections and a leading cause of mortality among people living with human immunodeficiency virus (HIV, PLWH) (*1*–*2*). The Global Tuberculosis Report 2024 (*3*) documented 10.8 million new tuberculosis (TB) cases in 2023, with 662,000 cases involving HIV/*Mtb* coinfection. Notably, HIV/*Mtb* coinfection accounts for 161,000 of the 1.25 million global TB deaths (12.9%). Due to the profound immunodeficiency induced by HIV, *Mtb* coinfection typically progresses rapidly and substantially increases the incidence of disseminated tuberculosis and associated mortality. Therefore, HIV/*Mtb* coinfection has emerged as a critical global public health concern.

Mtb infection significantly amplifies HIV replication, exacerbates chronic immune activation, and accelerates the disease progression. At the same time, HIV infection substantially increases the risk of both endogenous reactivation and exogenous reinfection of TB (*4*). These two diseases create a synergistic relationship that leads to rapid clinical deterioration. Therefore, prompt and accurate diagnosis followed by effective treatment remains critical for patients with HIV/*Mtb* coinfection. Primary laboratory diagnostic approaches include sputum smears, cultures, nucleic acid amplification tests (NAATs), and immunological assays. Traditional *Mtb* culture remains the gold standard for TB diagnosis. However, this method requires extended detection periods and demonstrates limited diagnostic sensitivity. Sputum smears offer cost-effective, simple, and rapid testing but are susceptible to high false-positive rates. In HIV-infected individuals, *Mtb* infection frequently presents with atypical clinical manifestations and reduced sputum bacillary loads (*5*).

Among severely immunocompromised patients, the incidence of disseminated or extrapulmonary tuberculosis (EPTB) — including central nervous system tuberculosis, lymph node tuberculosis, tuberculous pleurisy, and skeletal tuberculosis — increases significantly. The combination of low sputum *Mtb* loads and smear-negative results may substantially reduce the sensitivity of traditional pathogen detection methods and NAATs (*6*).

Multiple strategies have been developed to enhance early diagnosis rates in HIV-infected individuals (*7*). Among these, the supplementary application of immunological testing has become a primary research focus. The interferon-gamma (IFN-γ) release assays (IGRAs), such as Wantai TB-IGRA, represent laboratory diagnostic methods based on cellular immunity (*8*) that partially address the challenge of low bacillary loads in TB patients. IGRAs have been widely implemented for auxiliary TB diagnosis and the screening of *Mtb* infection in high-risk populations. However, these assays demonstrate suboptimal diagnostic sensitivity in HIV-infected individuals (*9*). The interferon gamma-induced protein 10 (*IP-10*) has recently emerged as a promising diagnostic biomarker. Following stimulation with TB-specific peptides, *IP-10* expression levels significantly exceed those of IFN-γ. Studies have demonstrated that *IP-10* transcription increases approximately 100-fold within 2.5–8 hours after TB antigen stimulation. The elevated expression levels and stability of *IP-10* mRNA contribute to enhanced diagnostic sensitivity (*10*). Previous research has shown that *IP-10* mRNA-based assays demonstrate comparable effectiveness to IGRAs for tuberculosis diagnosis (*11*). However, the application of *IP-10* mRNA-based assays in immunocompromised populations remains insufficiently explored. Therefore, this study aimed to evaluate the diagnostic performance of *IP-10* mRNA release assays in HIV/*Mtb*-coinfected individuals and explore potential alternative methods for auxiliary TB diagnosis in HIV-infected populations.

METHODS

This prospective cohort study was conducted at Beijing Youan Hospital, Capital Medical University, Beijing, China. Between August 2022 and December 2024, we consecutively enrolled 111 HIV-infected individuals with suspected tuberculosis (Figure 1). Fasting venous blood samples were collected from all participants for *IP-10* mRNA release assays and IGRAs. Inclusion criteria comprised of 1) a confirmed positive HIV-1 test results; 2) clinical suspicion of TB; and 3) the absence of prior anti-TB treatment history. Exclusion criteria included 1) the age under 18 years, 2) incomplete medical documentation, and 3) unavailable acid-fast staining smears or culture results that precluded definitive diagnosis.

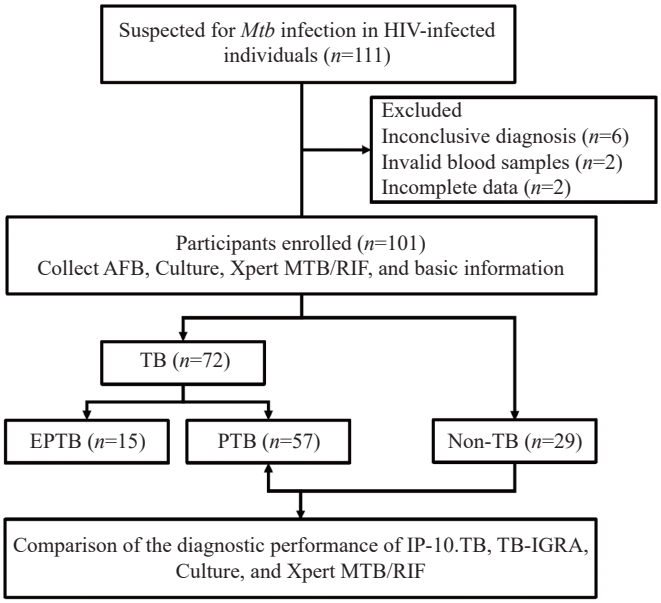

FIGURE 1. Flowchart of inclusion and grouping for the study participants.
Abbreviation: *Mtb=Mycobacterium tuberculosis*; AFB=acid-fast bacilli; TB=tuberculosis; PTB=pulmonary tuberculosis; EPTB=extrapulmonary tuberculosis.

The Research Ethics Committee of Beijing Youan Hospital approved this study and related experiments (No. 2022-115). We obtained written informed consent in accordance with the Declaration of Helsinki and conducted the study with strict adherence to approved guidelines and regulations. Study reporting followed the Standards for Reporting Diagnostic Accuracy Studies (STARD) guidelines.

Final diagnoses were determined through a comprehensive evaluation of clinical presentation, pathogenicity tests, imaging studies, and pathological findings. Participants were categorized into three groups following the Chinese guidelines for the diagnosis and treatment of human immunodeficiency virus infection/acquired immunodeficiency syndrome (*1*) and the diagnostic criteria for tuberculosis (WS288-2017) (*12*). The groups were 1) HIV with definite TB, which included positive *Mtb* culture results, nucleic acid detection in sputum or bronchoalveolar lavage fluid (BALF), or histopathological evidence; 2) HIV with probable TB, which were typical chest imaging manifestations of tuberculosis with characteristic clinical symptoms, positive tuberculin skin test, positive IGRAs results, or positive histopathology for EPTB but negative results for AFB smears, cultures, and nucleic acid tests in sputum or BALF; and 3) HIV with non-TB, which included individuals with no history of TB or TB contact who were ultimately not diagnosed with active TB. Participants with *Mtb* infections at any anatomical site other than the lungs (e.g., pleura, meninges, or bones) were classified as EPTB.

We collected demographic information and laboratory results from all participants. Approximately 4–5 mL of fasting venous blood was obtained from each participant for the simultaneous *IP-10* mRNA release assays and TB-IGRA testing. Using the final clinical diagnosis as the reference standard, we calculated and statistically analyzed the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and agreement for both detection methods. All participant samples underwent sputum smear microscopy, sputum culture, and nucleic acid testing to evaluate the diagnostic performance of these assays.

The *IP-10* mRNA release assay was conducted following the manufacturer's protocol for the IP-10.TB kit (CLR001A48, Suzhou Chuanglan Biotechnology Co., Ltd., China). Heparin-anticoagulated blood samples were distributed equally among three tubes: the negative control tube (N tube), the *Mtb* antigen tube (T tube), and the positive antigen tube (P tube). After gentle inversion to ensure thorough mixing, samples were incubated at 37 °C for 6 hours. Following incubation, RNA extraction from whole blood samples was performed, followed by reverse transcription to generate cDNA. Real-time quantitative PCR (qPCR) was conducted using an ABI 7500 qPCR system (Applied Biosystems™ 7500, Thermo Fisher, America). Target *IP-10* gene expression levels in the N, T, and P tubes were calculated using the ΔCt method, where ΔCt=Ct (target gene) – Ct (reference gene). Results were interpreted according to the following criteria: a positive result when the relative expression of *IP-10* in the T tube minus the relative expression of *IP-10* in the N tube was ≤–1.04; a negative result when the relative expression of *IP-10* in the T tube minus the relative expression of *IP-10* in the N tube was >–1.04, and the relative expression of *IP-10* in the P tube minus the relative expression of *IP-10* in the N tube was ≤–1.2; and an indeterminate results when the relative expression of *IP-10* in the T tube minus the relative expression of *IP-10* in the N tube was >–1.04 and the relative expression of *IP-10* in the P tube minus the relative expression of *IP-10* in the N tube was >–1.2.

IGRAs were performed using the Wantai TB-IGRA kit (TB-IGRA, Beijing Wantai Biological Pharmacy Enterprise) according to the manufacturer's specifications. Effector T cells underwent *in vitro* stimulation with *Mycobacterium tuberculosis*-specific antigens to induce IFN-γ secretion. Samples were then incubated at 37 °C for 22.0±2.0 hours. Following incubation, plasma samples were analyzed using ELISA to quantify secreted IFN-γ concentrations. Results were classified as positive when the difference between the test tube (T) and negative control tube (N) values (T - N) was ≥14 pg/mL and ≥N/4.

Data were collected and organized using Microsoft Excel. All statistical analyses were performed using Jamovi software (version 2.6.17), R version 4.4.0 (R Core Team R (2024), Vienna, Austria) and MedCalc Statistical Software version 23.0.8 (MedCalc Software, Ostend, Belgium). Measurement data with skewed distributions are expressed as median (interquartile range, IQR) [M (P25, P75)] and were compared using the Mann−Whitney U test. The categorical data are presented as frequencies and percentages (%) and were compared using Pearson's chi-square test. McNemar's test was employed for paired data comparisons. The sensitivity, specificity, positive predictive value (PPV),

and negative predictive value (NPV) were calculated for each diagnostic parameter. To evaluate diagnostic performance, receiver operating characteristic (ROC) curves were generated for each test. Differences in area under the curve (AUC) values between tests were assessed using the DeLong test, with statistical significance set at $P<0.05$. Agreement between methods was evaluated using the Kappa test, where Kappa≤0.4 indicated poor agreement, 0.4<Kappa<0.75 indicated moderate agreement, and Kappa≥0.75 indicated strong agreement.

RESULTS

The study enrolled 111 HIV-infected individuals with suspected TB (Figure 1). After excluding cases with incomplete diagnoses and invalid samples, 101 participants were included in the final analysis. Based on the definitive diagnosis criteria, 72 individuals were diagnosed with TB, while 29 individuals were classified as non-TB patients. The TB group comprised 57 patients with pulmonary tuberculosis (PTB) and 15 with EPTB. Among the pulmonary tuberculosis cases, 22 presented with hematogenous disseminated tuberculosis. In the non-TB group, the final diagnoses included 19 cases of nontuberculous mycobacterial infection, eight cases of bacterial pneumonia, one case of pulmonary aspergillosis, and one case of mycoplasmal pneumonia. The $CD4^+$ T-cell count was significantly elevated in the TB group compared to the non-TB group ($P=0.004$). No significant differences were observed in age, sex, or viral load between the TB and non-TB groups. Detailed demographic and clinical characteristics are presented in Table 1.

We analyzed the diagnostic performance of the *IP-10* mRNA release assay and TB-IGRA in 57 PTB patients and 29 non-TB patients. Based on clinical diagnostic criteria, the *IP-10* mRNA release assay demonstrated a diagnostic sensitivity of 68.4% [95% confidence interval (*CI*): 55.5%–79.0%] and a specificity of 69.0% (95% *CI*: 50.8%–82.7%). Contrastingly, TB-IGRA showed a diagnostic sensitivity of 36.8% (95% *CI*: 25.5%–49.8%) and a specificity of 89.7% (95% *CI*: 73.6%–96.4%). The difference in the diagnostic sensitivity between these two methods was statistically significant ($P<0.01$),

TABLE 1. Demographic and clinical characteristics of the study population [*N* (%)].

Characteristics	TB (*n*=72)	Non-TB (*n*=29)	*P*
Gender			
Male	68 (94.4)	27 (93.1)	>0.999
Female	4 (5.6)	2 (6.9)	
Age [median (P25, P75)]	36.5 (30.0, 47.0)	34 (30.0, 42.5)	0.589
$CD4^+$ T-cell count (cells/μL)	85 (29.25, 168.75)	31 (5.5, 103.5)	0.004
Viral load (copies/mL)			
>1,000	38 (52.8)	19 (65.5)	0.096
≤1,000	34 (47.2)	10 (34.5)	
Pulmonary tuberculosis	57 (79.2)	-	
Hematogenous disseminated PTB	22 (38.6)		
Extrapulmonary tuberculosis	15 (20.8)	-	
Tuberculous meningitis	10 (66.7)		
Tuberculous lymphadenitis	3 (20.0)		
Intestinal tuberculosis	1 (6.7)		
Tuberculous pleurisy	1 (6.7)		
Other diagnosis	-		
NTM infection		19 (65.5)	
Bacterial pneumonia		8 (27.6)	
Pulmonary aspergillosis		1 (3.4)	
Mycoplasmal pneumonia		1 (3.4)	

Abbreviation: TB=tuberculosis; PTB=pulmonary tuberculosis; Non-TB=other non-tuberculosis infectious diseases; NTM=non-tuberculous mycobacteria.

while no significant differences were observed in the specificity (P=0.07). The positive predictive value (PPV) and negative predictive value (NPV) of the *IP-10* mRNA assay were 81.3% (95% *CI*: 68.1%–89.8%) and 52.6% (95% *CI*: 37.3%–67.5%), respectively. For TB-IGRA, the PPV and NPV were 89.7% (95% *CI*: 73.6%–96.4%) and 87.5% (95% *CI*: 69.0%–95.7%), respectively. Among the non-TB group, the negative detection rates for nontuberculous mycobacterial (NTM) infection, bacterial pneumonia, pulmonary aspergillosis, and mycoplasmal pneumonia were 52.6% (10/19), 87.5% (7/8), 100% (1/1), and 100% (1/1), respectively (Table 2).

We evaluated the concordance between the two diagnostic methods using 86 valid samples from patients with suspected pulmonary tuberculosis. Both the *IP-10* mRNA release assay and TB-IGRA yielded positive results in 20 participants and negative results in 34 participants. The Kappa statistic for agreement was 0.292 (P=0.001), indicating poor agreement between the two diagnostic methods (Table 3).

The sensitivity, specificity, PPV, and NPV for *Mtb* culture were 24.6%, 100.0%, 100.0%, and 40.3%, respectively. The corresponding values for Xpert MTB/RIF were 52.6%, 96.6%, 96.8%, and 50.9%, respectively. The detection rate of IP-10.TB was significantly higher than that of TB-IGRA and *Mtb* cultures (P=0.012), although the difference with Xpert MTB/RIF was not statistically significant (P>0.05). Combined detection using IP-10.TB and TB-IGRA yielded a sensitivity, specificity, PPV, and NPV of 71.9%, 69.0%, 82.0%, and 55.6%, respectively, while parallel testing of IP-10.TB and Xpert MTB/RIF demonstrated corresponding values of 77.2%, 69.0%,

TABLE 2. Diagnostic performance of the IP-10.TB assay and other diagnostic methods for PTB in HIV-infected individuals.

Test	PTB (*n*=57)	Non-PTB (*n*=29)	Sensitivity/% (95% *CI*)	Specificity/% (95% *CI*)	PPV/% (95% *CI*)	NPV/% (95% *CI*)	AUC (95% *CI*)
IP-10.TB							
Positive	39	9	68.4 (55.5, 79.0)	69.0 (50.8, 82.7)	81.3 (68.1, 89.8)	52.6 (37.3, 67.5)	0.687 (58.2, 79.2)
Negative	18	20					
TB-IGRA							
Positive	21	3	36.8 (25.5, 49.8)	89.7 (73.6, 96.4)	87.5 (69.0, 95.7)	41.9 (30.5, 54.3)	0.632 (54.8, 71.7)
Negative	36	26					
Culture							
Positive	14	0	24.6 (15.2, 37.1)	100.0 (88.3, 100.0)	100.0 (78.5, 100.0)	40.3 (29.7, 51.8)	0.623 (56.6, 67.9)
Negative	43	29					
Xpert MTB/RIF							
Positive	30	1	52.6 (39.9, 65.0)	96.6 (82.8, 99.8)	96.8 (83.8, 99.8)	50.9 (38.1, 63.6)	0.746 (67.2, 82.0)
Negative	27	28					
IP-10.TB or TB-IGRA							
Positive	41	9	71.9 (59.2, 81.9)	69.0 (50.8, 82.7)	82.0 (69.2, 90.2)	55.6 (39.6, 70.5)	0.704 (60.1, 80.8)
Negative	16	20					
IP-10.TB or Xpert MTB/RIF							
Positive	44	9	77.2 (64.8, 86.2)	69.0 (50.8, 82.7)	83.0 (70.8, 90.8)	60.6 (43.7, 75.3)	0.731 (62.9, 83.3)
Negative	13	20					

Abbreviation: TB=tuberculosis; PTB=pulmonary tuberculosis; Non-PTB=other non-tuberculosis infectious diseases affecting lung tissue.

TABLE 3. IP-10.TB and TB-IGRA test concordance evaluation.

IP-10.TB	TB-IGRA		Kappa value	*P*
	Positive (*n*)	Negative (*n*)		
Positive (*n*)	20	28	0.292	0.001
Negative (*n*)	4	34		

Abbreviation: TB=tuberculosis.

83.0%, and 60.6%, respectively. The detailed results are presented in Table 2.

Further analysis was conducted by generating ROC curves (Figure 2), which enabled comparisons of the AUC values of IP-10.TB with those of other detection methods (DeLong test). AUC values for the parallel testing of IP-10.TB and Xpert MTB/RIF were significantly greater than those for IP-10.TB alone (0.731 *vs.* 0.687, *P*=0.02).

As the immunodeficient and immunocompromised populations continue to expand, the incidence of EPTB and disseminated tuberculosis has correspondingly increased. Among patients diagnosed with extrapulmonary tuberculosis, the detection rates for IP-10.TB and TB-IGRA were 60.0% and 13.3%, respectively (*P*=0.016). For patients with hematogenous disseminated tuberculosis, the positive detection rates were 72.7% and 45.5%, respectively (*P*>0.05) (Table 4).

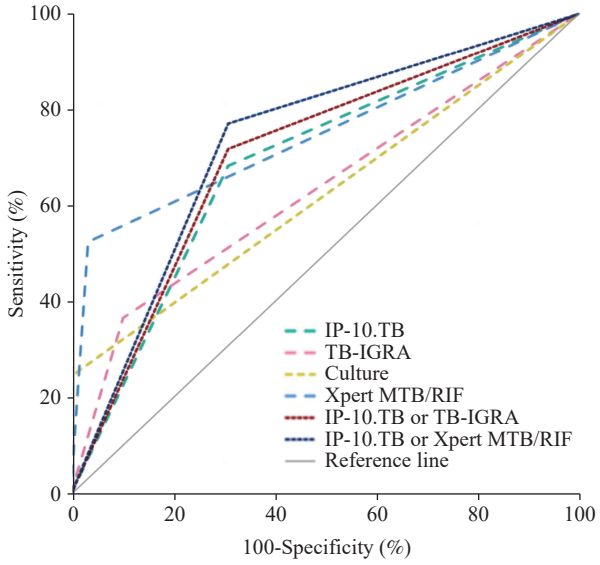

FIGURE 2. ROC curve-based PTB detection results using four individual assays and two combined diagnostic approaches.
Abbreviation: AFB=acid-fast bacilli; TB=tuberculosis; PTB=pulmonary tuberculosis; EPTB=extrapulmonary tuberculosis; ROC=Receiver operating characteristic.

DISCUSSION

Tuberculosis represents a chronic infectious disease whose clinical presentation becomes increasingly complex and atypical in HIV-infected individuals (*1*). Laboratory detection of *Mtb* often proves challenging, necessitating the integration of multiple diagnostic techniques to directly or indirectly identify evidence of *Mtb* infection — a critical step for ensuring timely diagnosis and treatment. Meta-analysis has demonstrated that the aggregated sensitivity of the *IP-10* assay reaches 85% (95% *CI*: 80%–88%), with a specificity of 89% (95% *CI*: 84%–92%) (*13*), indicating the substantial potential of *IP-10* as a biomarker for auxiliary TB diagnosis. However, evidence supporting effective diagnosis using this method in HIV-infected individuals, particularly those with severe immunosuppression, remains extremely limited.

In this study, we evaluated the diagnostic performance of the *IP-10* mRNA release assay in 101 HIV-infected individuals suspected of having TB, categorizing them into 57 cases of pulmonary TB and 29 cases of non-TB based on final diagnosis. When compared with the commonly used immunological assay TB-IGRA, our results demonstrated the sensitivity and specificity of IP-10.TB for diagnosing PTB were 68.4% and 69.0%, respectively. Notably, the sensitivity of IP-10.TB was significantly superior to that of TB-IGRA (*P*<0.01), while no statistically significant difference in specificity was observed (*P*=0.07). Meta-analysis has revealed that the aggregated sensitivity of the *IP-10* assay reaches 85% (95% *CI*: 80%–88%), with specificity at 89% (95% *CI*: 84%–92%) (*13*), indicating the substantial

TABLE 4. Diagnostic performance of the IP-10.TB assay in HIV-infected individuals with extrapulmonary tuberculosis.

	Positive cases (*n*)	Detection rate (%)	*P*
EPTB (*n*=15)			
IP-10.TB	9	60.0	0.016
TB-IGRA	2	13.3	
Hematogenous disseminated tuberculosis (*n*=22)			
IP-10.TB	16	72.7	0.070
TB-IGRA	10	45.5	

Abbreviation: EPTB=Extrapulmonary tuberculosis.

potential of *IP-10* as a biomarker for auxiliary TB diagnosis. However, evidence supporting effective diagnosis using this method in HIV-infected individuals, particularly those with severe immunosuppression, remains extremely limited.

Our findings demonstrate that the *IP-10* is predominantly secreted by monocytes and myeloid dendritic cells (*14*), with its release process being minimally affected by $CD4^+$ T cell depletion. Furthermore, previous multicenter studies have indicated that the sensitivity of *IP-10* mRNA as a biomarker remains largely uninfluenced by $CD4^+$ T cell counts (*15*). Our results align with these conclusions, demonstrating that IP-10.TB exhibits high diagnostic sensitivity even in severely immunosuppressed populations. This enhanced performance can be attributed to the fundamental differences in detection targets between TB-IGRA and IP-10.TB. The former detects IFN-γ at the protein level, whereas the latter quantifies the transient expression of *IP-10* mRNA, a downstream molecule in the IFN-γ signaling pathway (*16*), thereby substantially improving detection sensitivity.

In our study, *IP-10* mRNA release assays demonstrated lower specificity compared to TB-IGRA, although this difference was not statistically significant. This reduced specificity may be attributed to the fact that most participants had $CD4^+$ T-cell counts below 200 cells/μL, and this compromised immunity likely increased the prevalence of latent *Mtb* infection (*17*). Additionally, the limited sample size of the non-TB group may have further amplified this difference. Furthermore, false-positive cases in the non-TB group consisted mainly of NTM-infected individuals, with a negative detection rate of 52.6% (10/19). The ESAT-6, CFP-10, and PPE antigens utilized in IP-10.TB are derived from the region of difference-1 (RD-1) of *Mtb*. This region exhibits no cross-reactivity with antigens from BCG or the vast majority of NTM strains, except for a few species such as *M. kansasii*, *M. marinum*, and *M. szulgai* (*18*). Therefore, it effectively avoids interference from most NTM strains. However, when infected with NTM strains containing ESAT-6, CFP-10, or PPE antigens, these antigenic components may stimulate the host immune system, inducing a T-cell immune response similar to that elicited by *Mtb* infection. This phenomenon may result in positive *IP-10* release assay results, thereby causing false-positive outcomes. False positives may also result from differences in the TB-specific antigens employed: TB-IGRA utilizes ESAT-6 and CFP-10 antigens, whereas IP-10.TB employs ESAT-6, CFP-10, and PPE antigens. However, the precise mechanisms underlying these differences require further investigation. In the present study, concordance between IP-10.TB and TB-IGRA results were poor, which may be attributed to three factors. Firstly, the differences in assay targets and their expression levels (mRNA versus protein). Secondly, a greater variability in HIV-infected individuals with severe immunosuppression. Thirdly, the lower sensitivity of TB-IGRA. Consequently, IP-10.TB may represent an attractive alternative diagnostic method for HIV-infected individuals.

Compared with traditional IGRAs, the IP-10.TB method requires a shorter incubation time, with results available in as little as six hours. Moreover, the linear detection range of PCR technology is broader than that of ELISA. By leveraging the amplification curves to calculate the results, the variability in result interpretation is reduced, enhancing the objectivity of the detection results. The findings of this study suggest that IP-10.TB has great potential for use in the early diagnosis of tuberculosis in HIV-infected individuals. By optimizing and integrating specific tests, patients with tuberculosis can be identified earlier, thus facilitating early initiation of treatment and potentially reducing the mortality rate associated with the disease.

This study also has some limitations. First, it was a single-center, small-sample study, and the extrapolation of conclusions are challenging. Moreover, the enrolled hospitalized patients were predominantly severely immunocompromised, which may have resulted in selection bias. Lastly, it might be more objective and convincing to use a third alternative method to validate the inconsistency between the *IP-10* mRNA release assay and the TB-IGRA test results. This part of our work is in progress.

Therefore, multicenter studies with larger and more diverse samples are necessary to validate the diagnostic performance of *IP-10* mRNA release assay. Additionally, although IP-10.TB has a high sensitivity, it is not effective in differentiating between latent infection with *Mtb* and active tuberculosis, and cannot predict the transition from latent infection to active tuberculosis. Besides, the IP-10.TB results could not reflect the relationship with the *Mtb* bacterial load, thus limiting its application in evaluating the efficacy of anti-tuberculosis treatment.

IP-10 mRNA release assays have significant clinical value and potential as an auxiliary tool for diagnosing TB in HIV-infected individuals. Furthermore, the combined use of IP-10.TB and Xpert MTB/RIF can

increase the diagnostic efficacy of TB. In the cases of EPTB with insufficient diagnostic evidence, *IP-10* mRNA release assays provide complementary and auxiliary diagnostic benefits.

Conflicts of interest: No conflicts of interest.

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