Journal of Clinical Laboratory Analysis

WILEY

RESEARCH ARTICLE OPEN ACCESS

The Diagnostic Value of Interleukin-2 and Interferon-γ Induced by Fusion Protein (ESAT-6/CFP-10/Rv1985c) for Active *Mycobacterium tuberculosis* Infection

Zhipeng Zhao[1] | Runqing Li[1] | Xiuying Zhao[1] | Yujie Wang[2] | Minggui Lin[3] | Qian Wei[1] | Xiaochen Li[1] | Pan Xiong[1]

[1]Laboratory Medicine Department of Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, China | [2]Medical Data Science Center of Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, China | [3]Infectious Disease Department of Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing, China

Correspondence: Runqing Li (lrqa00335@btch.edu.cn)

Received: 27 May 2024 | **Revised:** 10 February 2025 | **Accepted:** 14 February 2025

Keywords: active tuberculosis (ATB) | extra-pulmonary tuberculosis | interferon-γ (IFN-γ) | Interleukin-2 (IL-2) | tuberculosis

ABSTRACT

Objective: This study aimed to evaluate the diagnostic ability of interleukin 2 (IL-2) and interferon gamma (IFN-γ) release assay induced by the fusion protein (ESAT-6/CFP-10/Rv1985c) for detecting active tuberculosis (ATB) in clinically visiting patients.

Methods: A total of 970 subjects (215 in ATB group and 755 in non-ATB group) underwent both an interferon-γ release assay (IGRA) and a TB-DNA PCR assay. Using clinical diagnosis as the gold standard, both qualitative and quantitative test results for IL-2 and IFN-γ were analyzed. Subsequently, the diagnostic ability of IL-2 and IFN-γ to screen for ATB among the high-risk population was then evaluated.

Results: IL-2 exhibited higher specificity, while IFN-γ demonstrated higher sensitivity in distinguishing between ATB and non-ATB subjects. The sensitivity of the serial application of IL-2 and IFN-γ had no significant difference ($p = 1.000$) compared with IFN-γ; the specificity of the serial application of IL-2 and IFN-γ had no significant difference ($p = 0.708$) compared with IL-2. Quantitative analysis of the results revealed that the IL-2 and IFN-γ values were significantly higher in the ATB group compared with the non-ATB group. Additionally, the combined predictors of IL-2 and IFN-γ did not show a significant difference compared with IL-2 alone ($p = 0.324$) or IFN-γ alone ($p = 0.405$).

Conclusions: This study demonstrated that IL-2 and IFN-γ release assays induced by the fusion protein (ESAT-6/CFP-10/Rv1985c) were valuable for distinguishing ATB from non-ATB subjects, with IL-2 exhibiting higher specificity and IFN-γ demonstrating higher sensitivity.

1 | Introduction

The 2023 WHO Global Tuberculosis Report [1] revealed that 7.5 million individuals were diagnosed with tuberculosis in 2022. Approximately a quarter of the global population was infected by *Mycobacterium tuberculosis* (MTB), and 5%–10% of those infected would develop into active tuberculosis (ATB). There was some support for preventive medication in patients with latent tuberculosis infection (LTBI) [2]. However, the current literature presents unclear findings regarding the advantages and disadvantages of preventive treatment for LTBI [3, 4], highlighting the need for future research to develop effective and reliable experimental methods for tuberculosis prevention and treatment [5]. Interferon-γ (IFN-γ) release assays (IGRAs), which assess T-cell responses to MTB-specific antigens, are commonly used to detect MTB infection [6]. Current research focuses on screening serum markers for ATB to enhance sensitivity and specificity, as well as on optimizing MTB-specific antigens [7–11]. The

aim of this study was to evaluate the values of ESAT-6/CFP-10/Rv1985C-induced IFN-γ and IL-2 in the screening for ATB among 970 subjects at high risk of ATB [12].

2 | Materials and Methods

2.1 | Subjects

This study was performed at Beijing Tsinghua Changgung Hospital affiliated to Tsinghua University (Beijing, China) from November 2021 to October 2023. This study was conducted in accordance with the Declaration of Helsinki. It received ethical approval from the Ethics Committee of Beijing Tsinghua Changgung Hospital (approval number: 24337–0-01).

A total of 970 subjects at high risk of ATB, as judged by the physicians, were enrolled in this study. The subjects who met all the following criteria were included in this study [13]: (1) exhibiting symptoms associated with ATB or undergoing regular ATB monitoring; (2) having results from both IGRA and TB-DNA PCR testing. Exclusion criteria required subjects to meet one of the following: (1) subjects lacking regular monitoring results for ATB; (2) subjects with unqualified IGRA results according to the manufacturer's instructions. The 970 subjects were categorized into either an ATB group or a non-ATB group, separately. The diagnostic criteria of ATB were judged by the physicians, meanwhile fulfilling at least one of the following criteria [13]: (1) MTB DNA was positively detected by PCR; (2) MTB DNA was detected positively by metagenomics next-generation sequencing (mNGS); (3) a positive smear with acid-fast staining; (4) ATB typical findings from pathological examination; (5) ATB typical imaging findings; (6) effective response to empirical treatment; (7) confirmed TB infection at other hospitals.

Among the 970 subjects, 215 were assigned to the ATB group, while the remaining 755 were classified as the non-ATB group. The ATB group was further divided into pulmonary tuberculosis, extra-pulmonary tuberculosis, and multiple-site infection subgroups based on lesion location. Similarly, the non-ATB group was subdivided into pulmonary infections, extra-pulmonary infections, autoimmune diseases, tumors, old tuberculosis, non-tuberculous mycobacteria (NTM), and other diseases subgroups. The study flowchart is shown in Figure 1, and the clinical and epidemiological characteristics of the enrolled 970 subjects are summarized in Table 1.

2.2 | Laboratory Procedures

IL-2 and IFN-γ assays were conducted using the MTB-specific cytokine detection kit (Deaou Bio-technology Co. Ltd., Guangzhou, China) following the manufacturer's instructions. The fusion protein (ESAT-6/CFP-10/Rv1985c) from the kit was used to stimulate T lymphocytes to release IL-2 and IFN-γ, and then the cytokine concentrations of IL-2 and IFN-γ were measured. Peripheral blood mononuclear cells (PBMCs) were isolated from heparin-anticoagulated blood by density gradient centrifugation. The cell suspension was adjusted to a concentration of 2.5×10^6 cells/mL. Subsequently, 100 μL of this suspension was added in triplicate to each of the three cell culture wells: the test well (T well), the negative well (N well) and the positive well (P well). The T wells contained ESAT-6/CFP-10/Rv1985c fusion protein, The N wells contained serum-free medium as the negative control, and the P wells contained phytohemagglutinin as the positive control. The samples were then incubated in a carbon dioxide incubator at 37°C for 16 h. At the end of the incubation, the supernatant was collected, and the concentrations of IL-2 and IFN-γ were quantitatively measured using an enzyme-linked immunosorbent assay (ELISA). Meanwhile, the qualitative results were interpreted according to the manufacturer's instructions; a positive IL-2 reaction (IL-2 +) was defined as an IL-2 difference greater than 19 pg/mL between the T wells and the N wells. Similarly, a positive IFN-γ reaction (IFN-γ +) was defined as an IFN-γ difference greater than 7 pg/mL between the T wells and the N wells. The quantitative results were also analyzed using the receiver operating characteristic (ROC) curve.

2.3 | DNA Detection for *mycobacterium tuberculosis*

TB-PCR was utilized to detect MTB deoxyribonucleic acid (DNA) using the commercial kit (Capital Bio Technology Co Ltd., Beijing, China) following the manufacturer's instructions. The kit was capable of reporting results for MTB and non-tuberculous mycobacteria (NTM) separately.

2.4 | Statistical Analysis

Differences in the sensitivity and specificity of IL-2 and IFN-γ for ATB screening were evaluated using the chi-square test. The Youden index was calculated as 'sensitivity + specificity—1' and was applied to determine the screening experiment's effectiveness and validity. The concordance between IL-2 and IFN-γ and ATB diagnosis was determined using the *kappa* values, with *kappa* values between 0.41 and 0.60 indicating moderate agreement, between 0.61 and 0.80 indicating substantial agreement, between 0.81 and 1.00 indicating almost perfect agreement. The values of IL-2 and IFN-γ in ATB and non-ATB groups did not follow a normal distribution. A Mann–Whitney U test was used to compare the differences of IL-2 and IFN-γ between ATB and non-ATB groups. Receiver operating characteristic (ROC) curves were constructed for IL-2 and IFN-γ based on clinical diagnosis. The area under the ROC curve (AUC), optimal cut-off values, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Statistical analyses were performed using SPSS 26.0 (IBM Corporation, Armonk, New York, USA), SPSSAU, the R language, and GraphPad Prism 10 software (GraphPad Software, Boston, Massachusetts, USA). A p-value of less than 0.05 was considered statistically significant.

3 | Results

3.1 | Sensitivity and Specificity of IL-2 and IFN-γ in the Two Groups

The positive rate of IL-2 in the ATB group was 66.5%, significantly higher than that in the non-ATB group (22.4%) ($\chi^2 = 150.499$, $p = 0.000$). Similarly, the positive rate of IFN-γ in

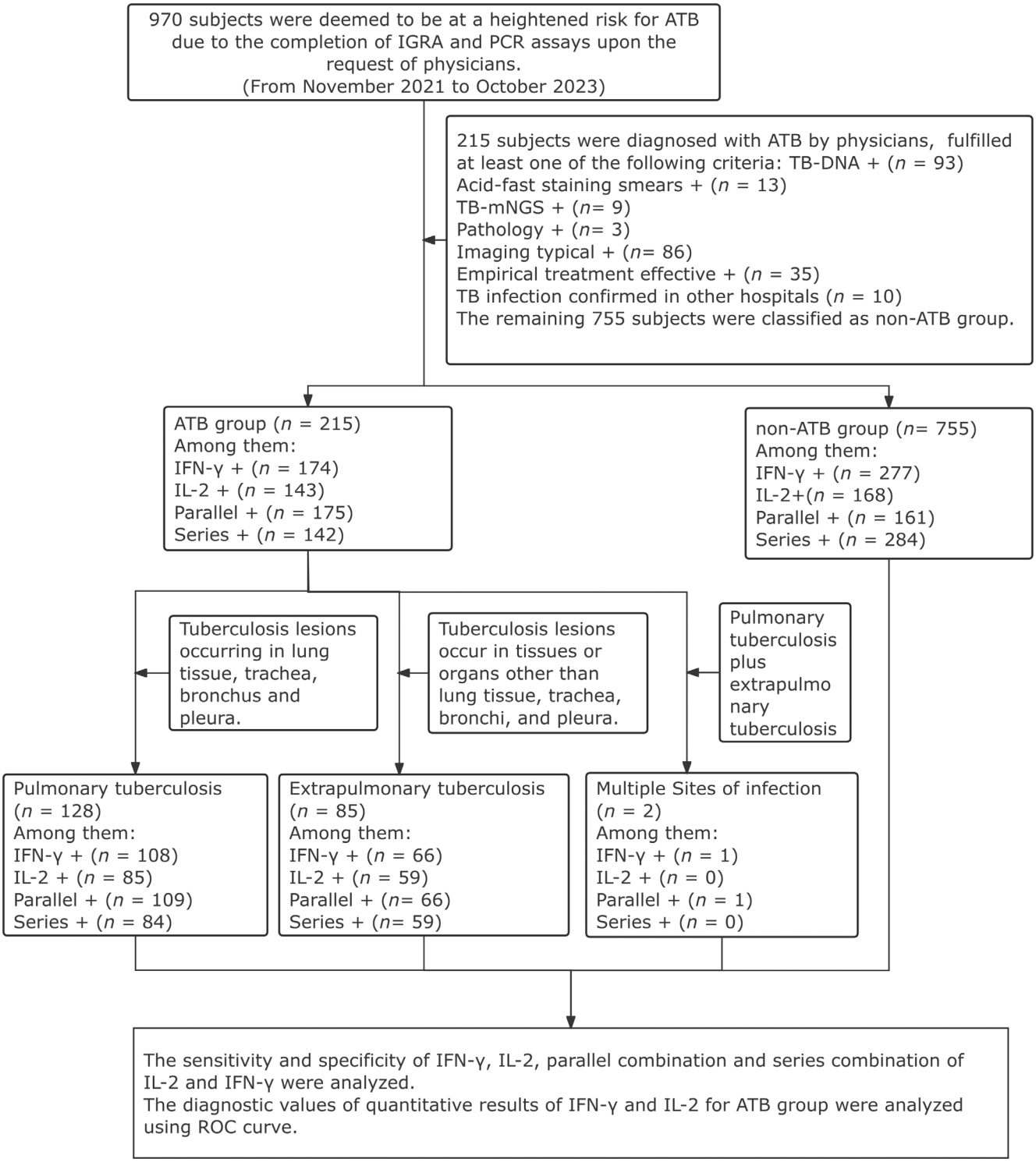

FIGURE 1 | The flow chart of this study.

the ATB group was 80.9%, which was significantly higher than that in the non-ATB group (36.7%) ($\chi^2 = 131.666$, $p = 0.000$). The sensitivities of IL-2 and IFN-γ for the diagnosis of ATB were 66.5% and 80.9%, respectively, and the specificities were 77.6% and 63.3%, respectively. IFN-γ demonstrated a higher sensitivity than IL-2 ($\chi^2 = 11.536$, $p = 0.001$), whereas IL-2 showed a higher specificity than IFN-γ ($\chi^2 = 37.115$, $p = 0.001$).

The sensitivities of IL-2 and IFN-γ for the diagnosis of pulmonary tuberculosis were 66.4% and 88.4%, respectively; IFN-γ demonstrated a higher sensitivity than IL-2 ($\chi^2 = 11.138$, $p = 0.001$). However, there was no significant difference in the sensitivity of IL-2 and IFN-γ for the diagnosis of extra-pulmonary tuberculosis ($\chi^2 = 1.481$, $p = 0.224$). The summarized results were shown in Table 2.

TABLE 1 | Clinical and epidemiological characteristics of subjects enrolled.

	ATB group ($n=215$)			Non-ATB group ($n=755$)							
Variables	**Pulmonary tuberculosis ($n=128$)[a]**	**Extra-pulmonary tuberculosis ($n=85$)[b]**	**Multiple Sites of infection ($n=2$)[c]**	**Pulmonary infection ($n=307$)[d]**	**Extra-pulmonary infections ($n=137$)[e]**	**Other Diseases ($n=146$)**	**Autoimmune diseases ($n=50$)**	**Tumors ($n=82$)**	**Old tuberculosis ($n=18$)[f]**	**NTM ($n=15$)[g]**	***p***
Male, *n* (%)	74 (57.8)	47 (55.3)	1 (50.0)	195 (63.5)	66 (48.2)	89 (61.0)	25 (50.0)	54 (65.9)	9 (50.0)	4 (26.7)	0.223[h]
Age, year	54.4 ± 19.4	47.5 ± 18.2	55.0 ± 25.0	60.7 ± 17.6	52.3 ± 19.4	56.9 ± 16.9	45.0 ± 21.7	65.3 ± 11.5	60.3 ± 15.5	64.3 ± 6.4	0.301[i]
Smear, *n* (%)	8 (6.3)	5 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	NA
mNGS, *n* (%)	4 (3.1)	5 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA
PCR, *n* (%)	64 (50.0)	29 (34.1)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA
pathology, *n* (%)	0 (0.0)	3 (3.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA
Medical imaging, *n* (%)	46 (35.9)	40 (47.1)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA
Empirical treatment is effective, *n* (%)	19 (14.8)	15 (17.7)	0 (0.0)	NA	NA	NA	NA	NA	NA	NA	NA
IFN-γ +, *n* (%)	108 (84.4)	66 (77.7)	1 (50.0)	107 (34.9)	49 (35.8)	61 (41.8)	13 (26.0)	32 (39.0)	11 (61.1)	4 (26.7)	NA
IL-2 +, *n* (%)	85 (66.4)	59 (69.4)	0 (0.0)	60 (19.5)	34 (24.8)	39 (26.7)	6 (12.0)	19 (23.2)	9 (50.0)	2 (13.3)	NA

[a]These include *Mycobacterium tuberculosis* infections occurring in the lung parenchyma, bronchi, bronchioles, and pleura.
[b]The tuberculosis infections excluded pulmonary tuberculosis.
[c]There are a subject with pulmonary tuberculosis plus esophageal tuberculosis and a subject with pulmonary tuberculosis plus cutaneous tuberculosis.
[d]Pulmonary infections were caused by bacteria, viruses, or fungi instead of *Mycobacterium tuberculosis*.
[e]Other infectious diseases exclude pulmonary infection, include bacterial, viral, and fungal infections, but exclude *Mycobacterium tuberculosis*.
[f]At the time of the initial visit during the study period, the tuberculosis infection had been cured or there was a clear previous history of cured tuberculosis.
[g]The subjects infected with non-tuberculous mycobacteria.
[h]Compare the sex of the ATB group with that of the non-ATB group.
[i]Compare the age of the ATB group with that of the non-ATB group.

The Youden indices for IL-2 and IFN-γ in the diagnosis of ATB were 0.441 and 0.442, respectively. Both IL-2 and IFN-γ showed substantial agreement in the diagnosis of ATB, and the *kappa* values were 0.614 for agreement in sensitivity and 0.618 for agreement in specificity.

3.2 | Sensitivity and Specificity of Combined Application of IL-2 and IFN-γ

The results can be interpreted in two ways: parallel combination (negative results for both IL-2 and IFN-γ lead to a negative diagnosis; all other combinations were positive) and serial combination (positive results for both IL-2 and IFN-γ lead to a positive diagnosis; all other combinations were negative). The sensitivity of the parallel combination was 81.4% (CI: 76.2–86.6), higher than that of the serial combination, which was 66.0% (CI: 59.7–72.4) ($\chi^2 = 13.072$, $p = 0.000$). The specificity of the serial combination was 78.7% (CI: 75.8–81.6), higher than that of the parallel combination, which was 62.4% (CI: 58.9–65.8) ($\chi^2 = 48.203$, $p = 0.000$). The Youden index of the serial combination of IL-2 and IFN-γ in the diagnosis of ATB was 0.447, which was higher than the Youden index of 0.438 of the parallel combination approach. There was no significant difference in sensitivity between the serial combination and the parallel combination in the diagnosis of extra-pulmonary tuberculosis ($\chi^2 = 1.481$, $p = 0.224$). The diagnostic accuracy of the combination of IL-2 and IFN-γ for the diagnosis of ATB was shown in Table 3.

Although the sensitivity of the parallel combination was high, it had no significant difference from that of IFN-γ alone ($\chi^2 = 0.015$, $p = 1.000$). Also, the specificity of the serial combination had no significant difference compared with that of IL-2 alone ($\chi^2 = 0.190$, $p = 0.780$).

3.3 | The Distribution of IL-2 and IFN-γ Values in the ATB Group and Non-ATB Group

The median IL-2 level in the ATB group was 45.25 pg/mL (IQR: 5.96–156.58 pg/mL), which was higher than the median IL-2 level of 23.3 pg/mL (IQR: 0.00–11.72 pg/mL) in the non-ATB group ($Z = 38{,}963$, $p = 0.000$). Similarly, the median IFN-γ level in the ATB group was 123.12 pg/mL (IQR: 18.93–474.52 pg/mL), which was significantly higher than the median IFN-γ level of 2.43 pg/mL (IQR: 0.00–28.26 pg/mL) in the non-ATB group ($Z = 37{,}145$, $p = 0.000$). The distribution of IL-2 and IFN-γ values in the ATB and non-ATB groups is illustrated in Figure 2.

3.4 | ROC Curve Analysis of IL-2 and IFN-γ

The ROC curves of IL-2 and IFN-γ for the diagnosis of ATB are shown in Figure 3. The area under the curve (AUC) was 0.762 for IL-2, 0.771 for IFN-γ, and 0.761 for the combination of IL-2 and IFN-γ.

The optimal cut-off value for the diagnosis of ATB was 10.890 pg/mL for IL-2 and 23.885 pg/mL for IFN-γ. Based on the cut-off values, sensitivity, specificity, positive predictive value, and negative predictive values of IL-2, IFN-γ, and the combined application of IL-2 and IFN-γ were summarized in Table 4.

4 | Discussion

In China, a country with a high burden of TB, the application of preventive treatment for LTBI is limited. Within clinical settings, the focus has primarily been on screening for ATB in patients presenting with TB-like symptoms. Since physicians implemented intensive screening programs for tuberculosis infection, it is reasonable to believe that these subjects are at high risk of ATB. From the perspective of clinical diagnosis and treatment, the real application scenario of IFN-γ and IL-2 was to effectively screen for ATB in subjects with similar symptoms, at least those with ATB screening indications. In the non-ATB group, although LTBI cannot be 100% ruled out, compared with the natural population, the proportion of LTBI in the non-ATB group in this study should be much lower than that in the natural population. Also, there is no gold standard for the diagnosis of LTBI. WHO recommends classifying IGRA-positive people without related symptoms as having LTBI. As a result, this

TABLE 2 | Diagnostic sensitivity and specificity of the IL-2 and IFN-γ for ATB.

Classification	IFN-γ		IL-2		χ^2	*p*
	n/N	Estimate% (95% CI)	*n*/N	Estimate% (95% CI)		
Sensitivity for active tuberculosis						
All active tuberculosis	174/215	80.9 (75.7–862.)	143/215	66.5 (60.2–72.8)	11.536	0.001[a]
Pulmonary tuberculosis	108/128	84.4 (78.1–90.7)	85/128	66.4 (58.2–74.6)	11.138	0.001[b]
Extra-pulmonary tuberculosis	66/85	77.6 (68.8–86.5)	59/85	69.4 (59.6–79.2)	1.481	0.224[c]
Multiple Sites of infection	1/2	NA	0/2	NA	NA	NA
Specificity for active tuberculosis	478/755	63.3 (59.9–66.8)	586/755	77.6 (74.6–80.6)	37.115	0.000[d]

[a]Sensitivity of IL-2 VS IFN-γ in all active tuberculosis.
[b]Sensitivity of IL-2 VS IFN-γ in pulmonary tuberculosis.
[c]Sensitivity of IL-2 VS IFN-γ in extra-pulmonary tuberculosis.
[d]Specificity of IL-2 VS IFN-γ in active tuberculosis.

TABLE 3 | Diagnostic sensitivity and specificity of the combined IL-2 and IFN-γ for ATB.

Classifications	Parallel combination of IFN-γ and IL-2[e]		Serial combination of IFN-γ and IL-2[f]		χ^2	*p*
	n/N	Estimate (95% CI)	*n*/N	Estimate (95% CI)		
Sensitivity for ATB						
All ATB	175/215	81.4 (76.2–86.6)	142/215	66.0 (59.7–72.4)	13.072	0.000[a]
Pulmonary tuberculosis	109/128	85.2 (79.0–91.3)	84/128	65.6 (57.4–73.9)	13.159	0.000[b]
Extra-pulmonary tuberculosis	66/85	77.6 (68.8–86.5)	59/85	69.4 (59.6–79.2)	1.481	0.224[c]
Multiple Sites of infection	1/2	NA	0/2	NA	NA	NA
Specificity for ATB	471/755	62.4 (58.9–65.8)	594/755	78.7 (75.8–81.6)	48.203	0.000[d]

[a]Sensitivity of parallel combination versus serial combination in all active tuberculosis.
[b]Sensitivity of parallel combination versus serial combination in pulmonary tuberculosis.
[c]Sensitivity of parallel combination versus serial combination in extra-pulmonary tuberculosis.
[d]Specificity of parallel combination versus serial combination in active tuberculosis.
[e]Positive results for both IL-2 and IFN-γ lead to a positive diagnosis, while all other combinations are negative.
[f]Negative results for both IL-2 and IFN-γ lead to a negative diagnosis, while all other combinations are positive.

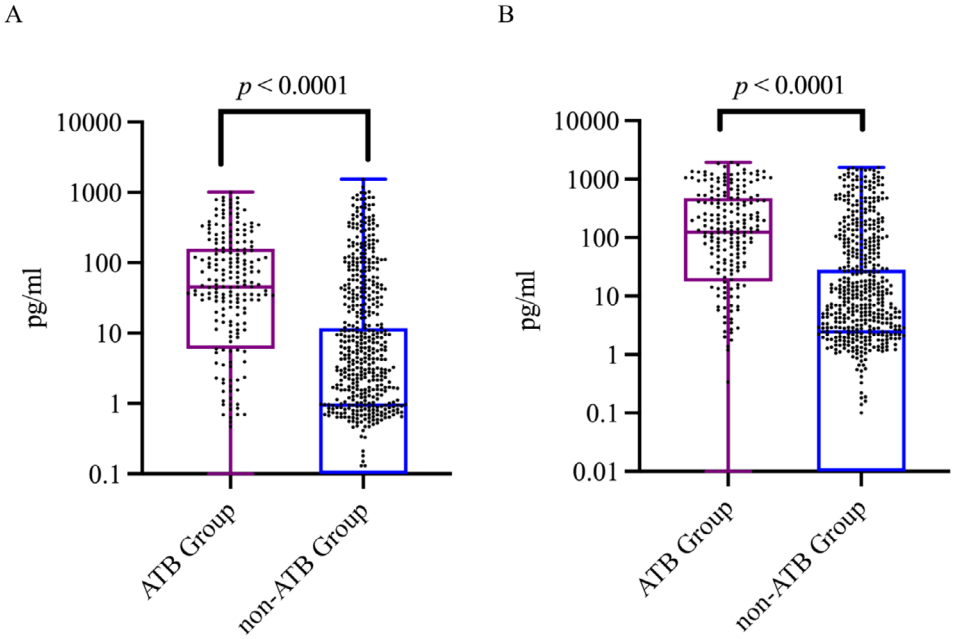

FIGURE 2 | Distribution of IL-2 (A) and IFN-γ (B) values in the ATB and non-ATB groups. The *Y*-axis is plotted on a log10 scale to improve data visualization. Each box represents the interquartile range (IQR), with the median indicated by the central line. The whiskers extend to 1.5 times the IQR. The log10 transformation is applied to compress the scale and highlight differences in IL-2 and IFN-γ levels between the two groups.

study did not deliberately distinguish LTBI subjects, but rather categorized all subjects into either the ATB group or the non-ATB group.

While IGRAs exhibit sensitivity and specificity in diagnosing tuberculosis infection [14, 15], they struggle to effectively distinguish between ATB and LTBI Scholars have made efforts to enhance the diagnostic performance of IGRAs for ATB by improving specific antigens and identifying new markers. For instance, the addition of new antigens like Rv1733c and SLP to traditional ESAT-6 and CFP-10 antigens has shown promise in enhancing the sensitivity and specificity of T-Spot for the diagnosis of ATB [16]. The MTB protein encoded by the Rv1986 gene is a target for memory T cells in patients with tuberculosis, and the Rv1985c protein positively upregulates the expression of Rv1986 [17]. In addition to ESAT-6 and CFP-10, the new generation of IGRAs includes the MTB-specific secreted protein Rv1985c as an antigen to enhance the host cellular immune response, thereby improving the sensitivity in detecting ATB. The Rv1985c protein has the promising potential to distinguish patients with active tuberculosis from *M. bovis* BCG-vaccinated individuals [18]. A study utilizing IGRAs showed that the addition of Rv-1985c increased the sensitivities of

ESAT-6, CFP-10, and the ESAT-6/CFP-10 combination in detecting TB from 82.1% to 89.2% ($p=0.125$), 67.9% to 87.5% ($p<0.001$), and 85.7% to 92.9% ($p=0.125$), respectively [19]. The fusion protein ESAT-6/CFP-10/Rv1985c exhibited higher potency in stimulating IFN-γ release compared with the conventional ESAT-6-CFP-10 fusion protein [8, 20]. Wang Jie et al. reported the potential utility of HP16118P protein in distinguishing between LTBI and ATB [3, 21]. A previous study conducted in a tertiary teaching hospital demonstrated that the antigen-stimulated IL-2 exhibited a sensitivity of 71.88% and a specificity of 77.49% in screening ATB [11]. In addition, IL-2 has shown potential in improving the treatment outcomes of Zoledronate for multidrug-resistant tuberculosis [22]. Research using the Luminex technology suggested that IL-2 and IFN-γ may serve as valuable biomarkers for TB diagnosis [23]. The production of IL-2 induced by Ala-DH, as measured by the LIOSpot TB kit, demonstrates high sensitivity and specificity for ATB [24]. Pavithra Sampath et al. [25] discovered that the value of IL-2 in the peripheral blood of patients with ATB was significantly elevated compared with that in the control and LTBI groups. Nadege Gourgouillon et al. [21] reported that the TNF-α/IL-2 ratio was higher in ATB patients compared with LTBI individuals. A FluoroSpot test using ESAT-6 and CFP-10 antigens showed that the IFN-γ (+) IL-2 (−) T cell count was more diagnostically valuable, with a higher AUC of the ROC curves, than the IFN-γ (+) T cell count for the diagnosis of ATB [26]. By combining ESAT-6-induced IP-10 with HBHA-induced IL-2 and GM-CSF, 90%–93% of ATB patients were accurately identified among infected individuals in the training and validation cohorts [2]. The inconsistent results of IL-2 and IFN-γ in the diagnosis of ATB reported in the literature can be attributed to variations in research protocols, including differences in detection methods, study populations, used antigens, and experimental groups.

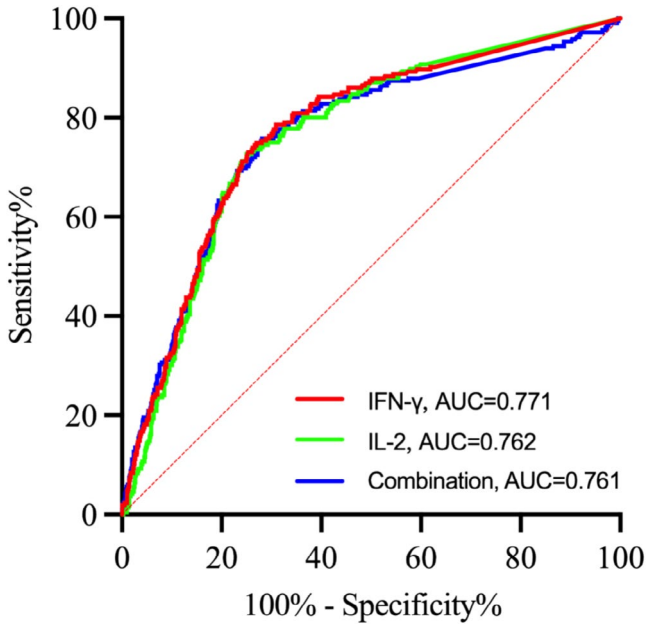

FIGURE 3 | ROC curve of IL-2 and IFN-γ for differentiating ATB from non-ATB.

This study focused on analyzing the diagnostic value of IFN-γ and IL-2 for ATB using the ESAT-6/CFP-10/Rv1985c fusion protein as the specific antigen for the diagnosis of ATB in a high-risk population. The qualitative analysis revealed that the positive rates of IL-2 and IFN-γ in the ATB group were significantly higher than those in the non-ATB group, indicating their potential diagnostic values for ATB. The sensitivity of IFN-γ was found to be higher than that of IL-2, while the specificity of IL-2 was observed to be higher than that of IFN-γ. Both IL-2 and IFN-γ demonstrated good concordance in distinguishing ATB from non-ATB. Notably, IFN-γ showed a higher sensitivity in the diagnosis of ATB than that of IL-2, but there was no significant difference in the diagnosis of extra-pulmonary tuberculosis.

This study showed that there was no significant difference in the sensitivity between IL-2 and IFN-γ in the diagnosis of extrapulmonary tuberculosis. Tuberculosis infection can occur in all tissues and organs except the nails, hair, and teeth. Different lesions may have different effects on the generation of memory lymphocytes in vivo. Hao Zhang et al. reported that the ratio of IFN-γ to IL-2 had shown good diagnostic efficacy in lymph node tuberculosis [27]. Thus, the ratio of IFN-γ to IL-2 may be one of the feasible evaluation indices for the diagnosis of extrapulmonary tuberculosis. The limited number of subjects and the lack of further subgrouping may also account for the bias in this study. The diagnostic ability of IL-2 and IFN-γ for extrapulmonary tuberculosis requires further investigation in multicenter studies.

The study found that the parallel combination of IL-2 and IFN-γ resulted in enhanced sensitivity and a higher Youden index,

TABLE 4 | Diagnostic accuracy of IL-2, IFN-γ, and the combined application of IL-2 and IFN-γ for the diagnosis of ATB.

Cytokine	AUC (95% CI)	Cut-of value (pg/mL)	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)
IL-2	0.761 (0.726–0.798)	10.89	0.726 (0.661–0.784)	0.747 (0.714–0.778)	0.450 (0.396–0.504)	0.905 (0.880–0.927)
IFN-γ	0.771 (0.735–0.807)	23.885	0.740 (0.675–0.797)	0.738 (0.705–0.769)	0.445 (0.393–0.499)	0.909 (0.883–0.930)
Combination	0.761 (0.723–0.799)	0.173	0.758 (0.695–0.814)	0.718 (0.684–0.750)	0.434 (0.383–0.485)	0.913 (0.887–0.934)

Note: Combination: Combined predictor, which were obtained after binary logistic regression analysis of the results of IL-2 and interferon assays. $Z_{\text{IL-2 vs IFN-}\gamma}=0.3536$, $p_{\text{IL-2 vs IFN-}\gamma}=0.7237$; $Z_{\text{il-2 vs Combination}}=0.0382$, $p_{\text{IL-2 vs Combination}}=0.9695$; $Z_{\text{IFN-}\gamma\text{ vs Combination}}=0.3821$, $p_{\text{IFN-}\gamma\text{ vs Combination}}=0.7024$.
Abbreviations: AUC: The area under the receiver operating characteristic curve, NPV: negative predictive value, PPV: positive predictive value.

whereas the serial combination of IL-2 and IFN-γ led to higher specificity. However, the sensitivity of the parallel combination wasn't significantly different compared with that of IFN-γ. Similarly, the specificity of serial combination had no significant differences compared with IL-2. The combination of IL-2 and IFN-γ didn't show a higher value.

The quantitative measurement values of IL-2 and IFN-γ in the ATB group were found to be significantly higher than those in the non-ATB group. Yu Zhou et al. [28] showed that the AUC of IFN-γ (AUC = 0.59) for distinguishing ATB and LTBI was lower than that of IL-2 (AUC = 0.76) based on the principle of the QFT-TB (QuantiFERON-TB Gold Test) experimental method. However, Yaoju Tan et al. [29] reported that the AUC of IFN-γ and IL-2 to distinguish ATB and non-ATB was similar (0.865, 0.859). The results of this study showed that the AUC of IL-2 and IFN-γ for distinguishing ATB and non-ATB had no difference (0.771, 0.762), which was consistent with the conclusion of Yaoju Tan et al. [29]. The AUC of the combined predictor of IL-2 and IFN-γ also had no difference compared with that of IL-2 and IFN-γ separately. No advantage was shown for the combined application of IL-2 and IFN-γ. The difference of AUC between IL-2 and IFN-γ was not statistically significant, which may be due to the serious skewness of the qualitative test data and poor data continuity. This study indicated that IL-2 exhibits higher specificity, and IFN-γ demonstrates higher sensitivity for the diagnosis of ATB, suggesting that patients with positive IL-2 results should be considered for the possibility of ATB, and those with negative IFN-γ results could be ruled out for ATB.

It should be noted that this study had several limitations. Firstly, this study consecutively collected 970 subjects; the number of subjects in the non-ATB group (755 subjects) was significantly higher than that in the ATB group (215 subjects), with an unmatched number of subjects in the two groups. Secondly, this study was conducted in a single center with geographical limitations, which warrant further studies with larger sample sizes to draw more general conclusions. Thirdly, the combined application of IL-2 and IFN-γ did not show improved diagnostic effectiveness compared with the individual use of IL-2 or IFN-γ. Other forms of combination, such as IFN-γ/IL-2 [2], need further investigation.

In conclusion, this study indicated that IL-2 and IFN-γ release assays induced by the fusion protein (ESAT-6/CFP-10/Rv1985c) were valuable for distinguishing ATB from non-ATB subjects, with IL-2 exhibiting higher specificity and IFN-γ demonstrating higher sensitivity.

Author Contributions

Zhipeng Zhao and Runqing Li conceptualized and wrote the manuscript. Other authors participated in the operation and data collection. All authors edited the manuscript and approved the final version.

Acknowledgments

The authors are grateful to the staff of the infectious disease department of Beijing Tsinghua Changgung Hospital for their efforts in this study. This study was funded by the Incubation Program Project of Beijing Municipal Administration of Hospitals of China (PG2018013), the Education Reform Project of Tsinghua University (ZY01_02), and the Beijing High-level Public Health Technical Personnel Project (2023-03-03).

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. World Health Organization, "World Health Statistics 2023: Monitoring Health for the SDGs, Sustainable Development Goals," (2023), https://www.who.int/publications/i/item/9789240074323.

2. S. Rajpal and V. K. Arora, "Latent TB (LTBI) Treatment: Challenges in India With an Eye on 2025: 'to Treat LTBI or Not to Treat, That Is the Question'," *Indian Journal of Tuberculosis* 67, no. 4S (2020): S43–S47, https://doi.org/10.1016/j.ijtb.2020.09.028.

3. D. G. Assefa, A. Bedru, E. D. Zeleke, et al., "Efficacy and Safety of Different Regimens in the Treatment of Patients With Latent Tuberculosis Infection: A Systematic Review and Network Meta-Analysis of Randomized Controlled Trials," *Archives of Public Health* 81, no. 1 (2023): 82, https://doi.org/10.1186/s13690-023-01098-z.

4. P. Yoopetch, T. Anothaisintawee, A. Gunasekara, et al., "Efficacy of Anti-Tuberculosis Drugs for the Treatment of Latent Tuberculosis Infection: A Systematic Review and Network Meta-Analysis," *Scientific Reports* 13, no. 1 (2023): 16240, https://doi.org/10.1038/s41598-023-43310-8.

5. A. Diatlova, N. Linkova, A. Lavrova, et al., "Molecular Markers of Early Immune Response in Tuberculosis: Prospects of Application in Predictive Medicine," *International Journal of Molecular Sciences* 24, no. 17 (2023): 13261, https://doi.org/10.3390/ijms241713261.

6. R. Diel, D. Goletti, G. Ferrara, et al., "Interferon-γ Release Assays for the Diagnosis of Latent *Mycobacterium tuberculosis* Infection: A Systematic Review and Meta-Analysis," *European Respiratory Journal* 37, no. 1 (2011): 88–99, https://doi.org/10.1183/09031936.00115110.

7. M. M. Vivekanandan, E. Adankwah, W. Aniagyei, et al., "Plasma Cytokine Levels Characterize Disease Pathogenesis and Treatment Response in Tuberculosis Patients," *Infection* 51, no. 1 (2023): 169–179, https://doi.org/10.1007/s15010-022-01870-3.

8. E. da Silva Graça Amoras, T. G. de Morais, R. do Nascimento Ferreira, et al., "Association of Cytokine Gene Polymorphisms and Their Impact on Active and Latent Tuberculosis in Brazil's Amazon Region," *Biomolecules* 13, no. 10 (2023): 1541, https://doi.org/10.3390/biom13101541.

9. A. Strzelak, A. Komorowska-Piotrowska, A. Borowa, M. Krasińska, W. Feleszko, and M. Kulus, "IP-10 for the Diagnosis and Treatment Monitoring of Tuberculosis in Children," *Diagnostics (Basel)* 14, no. 2 (2024): 177, https://doi.org/10.3390/diagnostics14020177.

10. K. Takeda, H. Nagai, M. Suzukawa, et al., "Comparison of QuantiFERON-TB Gold Plus, QuantiFERON-TB Gold in-Tube, and T-SPOT. TB Among Patients With Tuberculosis," *Journal of Infection and Chemotherapy* 26, no. 11 (2020): 1205–1212, https://doi.org/10.1016/j.jiac.2020.06.019.

11. N. S. Sheffee, P. Rubio-Reyes, M. Mirabal, et al., "Engineered *Mycobacterium tuberculosis* Antigen Assembly Into Core-Shell Nanobeads

for Diagnosis of Tuberculosis," *Nanomedicine* 34 (2021): 102374, https://doi.org/10.1016/j.nano.2021.102374.

12. C. Meng, T. Wan, Z. Xu, et al., "Screening Putative Antigens as Stimulators in the *Mycobacterium bovis* Interferon-Gamma Release Assay for Cattle," *Veterinary Immunology and Immunopathology* 168, no. 1–2 (2015): 111–117, https://doi.org/10.1016/j.vetimm.2015.09.001 PMID:26375813.

13. National Health and Family Planning Commission of the People's Republic of China, "Diagnostic Criteria for Pulmonary Tuberculosis (WS 288-2017)," *Electronic Journal of Emerging Infectious Diseases* 3, no. 1 (2018): 59–61.

14. Association CA, "Expert Consensus on Diagnosis and Prevention of Inactive Pulmonary Tuberculosis," *Journal of Tuberculosis and Lung Disease* 2, no. 3 (2021): 197–201, https://doi.org/10.3969/j.issn.2096-8493.20210085.

15. L. Yanming, L. Yuhong, Z. Hui, and C. Bin, "Clinical Practice Guidelines for Early Detection of Pulmonary Tuberculosis in General Medical Facilities," *Chinese Journal of Antituberculosis* 5, no. 1 (2024): 1–14, https://doi.org/10.19982/j.issn.1000-6621.20230428.

16. L. Zhang, H. Ma, S. Wan, Y. Zhang, M. Gao, and X. Liu, "*Mycobacterium Tuberculosis* Latency-Associated Antigen Rv1733c SLP Improves the Accuracy of Differential Diagnosis of Active Tuberculosis and Latent Tuberculosis Infection," *Chinese Medical Journal* 135, no. 1 (2022): 63–69, https://doi.org/10.1097/CM9.0000000000001858.

17. M. Schneefeld, T. Busche, R. Geffers, J. Kalinowski, and F. C. Bange, "The Transcriptional Regulator LysG (Rv1985c) of *Mycobacterium Tuberculosis* Activates lysE (Rv1986) in a Lysine-Dependent Manner," *PLoS One* 12, no. 10 (2017): e0186505, https://doi.org/10.1371/journal.pone.0186505.

18. S. Wang, J. Chen, Y. Zhang, et al., "*Mycobacterium tuberculosis* Region of Difference (RD) 2 Antigen Rv1985c and RD11 Antigen Rv3425 Have the Promising Potential to Distinguish Patients With Active Tuberculosis From *M. bovis* BCG-Vaccinated Individuals," *Clinical and Vaccine Immunology* 20, no. 1 (2013): 69–76, https://doi.org/10.1128/CVI.00481-12 PMID:23136116.

19. J. Chen, S. Wang, Y. Zhang, et al., "Rv1985c, a Promising Novel Antigen for Diagnosis of Tuberculosis Infection From BCG-Vaccinated Controls," *BMC Infectious Diseases* 10 (2010): 273, https://doi.org/10.1186/1471-2334-10-273.

20. Z. Zhipeng, X. LI, P. X, M. L, and X. Z, "A Comparison Study of the Diagnostic Value of Two Gamma- Interferon Release Assays for *Mycobacterium tuberculosis* Infection," *Labeled Immunoassays and Clinical Medicine* 29, no. 12 (2022): 2116–2120.

21. J. Wang, F. Jiang, P. Cheng, et al., "Construction of Novel Multi-Epitope-Based Diagnostic Biomarker HP16118P and Its Application in the Differential Diagnosis of *Mycobacterium tuberculosis* Latent Infection," *Molecular Biomedicine* 5, no. 1 (2024): 15, https://doi.org/10.1186/s43556-024-00177-z.

22. H. Shen, E. Yang, M. Guo, et al., "Adjunctive Zoledronate + IL-2 Administrations Enhance Anti-Tuberculosis Vγ2Vδ2 T-Effector Populations, and Improve Treatment Outcome of Multidrug-Resistant Tuberculosis(1)," *Emerging Microbes & Infections* 11, no. 1 (2022): 1790–1805, https://doi.org/10.1080/22221751.2022.2095930.

23. M. P. La Manna, V. Orlando, P. Li Donni, et al., "Identification of Plasma Biomarkers for Discrimination Between Tuberculosis Infection/Disease and Pulmonary Non Tuberculosis Disease," *PLoS One* 13, no. 3 (2018): e0192664, https://doi.org/10.1371/journal.pone.0192664.

24. C. Della Bella, M. Spinicci, A. Grassi, et al., "Novel *M. tuberculosis* Specific IL-2 ELISpot Assay Discriminates Adult Patients With Active or Latent Tuberculosis," *PLoS One* 13, no. 6 (2018): e0197825, https://doi.org/10.1371/journal.pone.0197825.

25. P. Sampath, A. Rajamanickam, K. Thiruvengadam, et al., "Cytokine Upsurge Among Drug-Resistant Tuberculosis Endorse the Signatures of Hyper Inflammation and Disease Severity," *Scientific Reports* 13, no. 1 (2023): 785, https://doi.org/10.1038/s41598-023-27895-8.

26. L. Zhang, S. Wan, S. Ye, et al., "Application of IFN-γ/IL-2 FluoroSpot Assay for Distinguishing Active Tuberculosis From Non-Active Tuberculosis: A Cohort Study," *Clinica Chimica Acta* 499 (2019): 64–69, https://doi.org/10.1016/j.cca.2019.08.022.

27. H. Zhang, Y. Chen, J. Huang, et al., "Exploration of the Diagnostic Value and Expression Levels of Blood IFN-γ and IL-2 in Patients With Lymph Node Tuberculosis," *Heliyon* 2024 (2024): e39008, https://doi.org/10.1016/j.heliyon.2024.e39008.

28. Y. Zhou, F. Zhang, H. Shi, P. Wu, and Y. Zhou, "Host Biomarkers Other Than Interferon Gamma in QFT-TB Supernatants for Identifying Active Tuberculosis," *Tuberculosis (Edinburgh, Scotland)* 136 (2022): 102256, https://doi.org/10.1016/j.tube.2022.102256.

29. Y. Tan, Y. Tan, J. Li, et al., "Combined IFN-γ and IL-2 Release Assay for Detect Active Pulmonary Tuberculosis: A Prospective Multicentre Diagnostic Study in China," *Journal of Translational Medicine* 19, no. 1 (2021): 289, https://doi.org/10.1186/s12967-021-02970-8.