JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Exploration of the diagnostic value and expression levels of IFN-γ and IL-2 in patients with lymph node tuberculosis

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Abstract

Introduction: Lymph node tuberculosis (LNTB) is the most common form of extrapulmonary tuberculosis (TB). The symptoms of LNTB lack specificity, making diagnosis difficult and increasing the likelihood of missed or incorrect diagnoses. Along with IFN-γ Release Assay (IGRA), the detection method using enzyme-linked immunosorbent assay (ELISA) can simultaneously detect IFN-γ and interleukin-2 (IL-2). However, the diagnostic value and expression levels of IFN-γ and IL-2 in patients with LNTB remain unclear.

Methodology: A total of 95 patients with enlarged lymph nodes were enrolled. Baseline data were collected, including age, gender, history of TB, histopathology, microbiology test results, and blood levels of IFN-γ and IL-2. Receiver operating characteristic (ROC) curve was used to investigate the diagnostic effect of the IFN-γ and IL-2. A double antibody sandwich ELISA was used to detect the concentrations of IFN-γ and IL-2 in culture supernatants.

Results: Based on ROC curves, the cut-off values for IFN-γ/IL-2 for differentiating LNTB from non-TB-lymphadenopathy (LAP) were determined to be 58.267 and 12.207 pg/mL, respectively. In patients with LNTB and non-TB-LAP, the sensitivity of IFN-γ/IL-2 in parallel was 83.33% (95% CI: 71.71–90.99), significantly higher ($p < 0.05$) than that for IFN-γ alone. The highest specificity (93.10%) (95% CI: 75.78–98.8) was observed when IFN-γ and IL-2 were applied in tandem.

Conclusions: The combined detection of IFN-γ and IL-2 showed good diagnostic efficacy in LNTB. In addition, in patients diagnosed with LNTB, the expression level of IL-2 may be related to the burden of MTB and the severity of TB infection.

Key words: lymph node tuberculosis; IFN-γ; IL-2; diagnosis; biomarker.

J Infect Dev Ctries 2026; 20(7):1049-1060. doi:10.3855/jidc.22313

(Received 31 August 2025 – Accepted 13 November 2025)

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (MTB) and ranks among the 10 leading causes of death globally [1,2]. Extrapulmonary TB accounts for 10% to 25% of all TB cases, with superficial lymph node tuberculosis (LNTB) being the most common form [3,4]. LNTB occurs when MTB invades the lymph nodes, leading to hyperplasia or granulomatous inflammation of the lymph nodes and the surrounding soft tissues [5]. It can present with symptoms such as fever and the presence of masses, commonly referred to as tuberculous lymphadenitis [6]. This type of TB is most frequently detected in the neck, but is also observed in the axilla and groin [7,8].

The symptoms and signs of LNTB are non-specific, which complicates its differentiation from non-tuberculous lymphadenitis, lymphoma, and metastatic carcinoma of lymph nodes, among other lymph node enlargement diseases, potentially leading to missed or incorrect diagnosis [3,9]. LNTB diagnosis can be confirmed via lymph node aspiration biopsy, a method that provides pathological or pathogenetic evidence of MTB. However, this procedure is invasive, and may thus be poorly accepted by patients. Additionally, it cannot be used for the routine screening of latent infections in high-risk, high-priority, or healthy populations [10,11]. This can lead to delayed diagnosis of LNTB, ultimately resulting in missed opportunities for early and optimal treatment. *Mycobacterium tuberculosis* culture is the gold standard for the diagnosis of superficial LNTB. However, this procedure requires a long culture period (2–6 weeks), which greatly delays diagnosis and treatment initiation [5]. Moreover, its positivity rate is low, similar to that of smear antacid bacillus staining [12]. While molecular

biology methods for detecting MTB in lymph node aspirates or extracted pus samples have a high degree of sensitivity and specificity, technical complexity, combined with an inability to distinguish between dead and live bacteria, limits their application [13]. Consequently, most of these techniques must still be combined with the evaluation of clinical symptoms, treatment history, imaging data, and immunological examination to enable early and accurate diagnosis [14,15]. Therefore, there remains a requirement to discover additional innovative, precise, and non-intrusive biomarkers for the diagnosis of LNTB.

The currently recommended immunological detection methods for MTB infection mainly include the traditional tuberculin skin test (TST) and the blood interferon-γ (IFN-γ) release assay (IGRA) [16,17]. In the TST, a pure protein derivative (PPD) of tuberculin is injected into the subject, and MTB infection is determined through the body's immune response [18]. Although this method is widely used in clinical practice, it presents challenges in China, where the population is generally vaccinated with the Bacillus Calmette–Guérin (BCG) vaccine [19]. Notably, there is cross-reactivity between PPD and BCG, as well as between PPD and most non-tuberculous *Mycobacteria* (NTM), leading to low detection specificity and a high false-positive rate [20]. The IGRA is used to determine the presence of MTB infection by detecting MTB-specific antigens that stimulate the production of IFN-γ by T cells. The indications for IGRA primarily include the diagnosis of latent tuberculosis infection (LTBI) and active TB [21,22]. The IGRA offers obvious advantages over the TST, namely, high specificity, and no interference from BCG vaccination or most NTM [23,24]. The World Health Organization (WHO) recommends the IGRA as an in vitro immunological method for the diagnosis of MTB infection; however, LTBI and MTB cannot be completely excluded when the IGRA is employed, even in pathologically positive TB patients, nor can the IGRA distinguish between LTBI and active TB [25].

IL-2 is a T helper-1 (Th1)-like cytokine primarily produced by dendritic cells and CD4+ and CD8+ T cells, and is involved in promoting the growth, proliferation, and differentiation of lymphocytes [26]. Additionally, IL-2 activates macrophages and enhances their ability to kill MTB, which has an important role in the immune response to MTB infection [27]. Qiu *et al.* demonstrated that IL-2 can serve as a novel and effective biomarker for distinguishing between active TB, LTBI, and non-TB populations [28]. Building on the IGRA, a new immunological detection method, based on enzyme-linked immunosorbent assay (ELISA), was developed for the simultaneous detection of IFN-γ and IL-2. This method is different from the IGRA and incorporates the TB-specific antigen, Rv1985c, found in the RD2 region of MTB, allowing its differentiation from other NTM [29, 30]. Tan *et al.* showed that this assay, combining IFN-γ and IL-2, could differentiate between active TB and other lung diseases [31]. However, the utility of this assay in improving the diagnosis of LNTB, a localized infection with a distinct immunopathology, is unknown. It remains unclear whether this combined strategy can effectively address the key diagnostic challenge of differentiating LNTB from non-tuberculous lymphadenopathy (non-TB-LAP).

To address these challenges, this study aimed at evaluating the diagnostic performance of IFN-γ and IL-2—individually and combined—in differentiating LNTB from non-TB LAP within a defined cohort. Further, the combined strategy's accuracy was compared against single-marker assays to establish the optimal diagnostic cutoffs for both cytokines. It was hypothesized that the IFN-γ/IL-2 combination would yield greater diagnostic value than any single marker, providing a new effective tool for the precise and non-invasive diagnosis of LNTB.

Methodology

Participants

In this retrospective study, data were collected for patients diagnosed with extrapulmonary TB in The Second Hospital of Nanjing and Guangzhou Chest Hospital from June 2022 to May 2023. The participants were selected according to predefined inclusion and exclusion criteria (see below). The clinical data collected included age, gender, history of TB, histopathology, microbiology test results, and blood levels of IFN-γ and IL-2. Pathogenetic examinations were performed on lymph node puncture specimens and encompassed Xpert MTB/RIF targeted next-generation sequencing (t-NGS), whole-genome sequencing (WGS), *Mycobacterium* culture, and smears. Pathological analysis included antacid staining and molecular polymerase chain reaction (PCR) analysis of biopsy specimens. In the case of patients with non-lymph node TB, the final diagnosis was made by the hospital clinician based on medical records. However, it should be noted that no pre-study sample size calculation was conducted for this retrospective investigation.

The inclusion criteria (all criteria had to be met) were: (1) patients hospitalized for lymph node

enlargement; (2) patients receiving primary treatment; (3) lymph node puncture biopsy tissue subjected to pathogenic and pathological testing; (4) synchronized blood IFN-γ and IL-2 testing; (5) complete clinical data; (6) informed consent obtained.

The exclusion criteria (failure to meet any one of the criteria meant exclusion) were no lymph node enlargement, failure to synchronize blood IFN-γ and IL-2 testing, no lymph node aspiration biopsy, human immunodeficiency virus (HIV)-positive, and diabetic with poor glycemic control.

LNTB diagnostic criteria

LNTB was diagnosed as follows:

(1) Confirmed LNTB: Subjects admitted with enlarged lymph nodes were adjudged to have a confirmed diagnosis of LNTB when at least one of the laboratory test results was positive for MTB pathogenicity and/or pathology.

(2) Clinically diagnosed LNTB: A clinical diagnosis of LNTB required meeting all of the following criteria: typical clinical and imaging features of LNTB; negative results for MTB pathogenicity and pathological tests, but confirmed MTB infection by immunological testing or positive MTB antibody, with exclusion of other diseases causing LAP; no response to empirical antibiotic therapy; and initiation of standard anti-tuberculosis treatment followed by at least 3 months (or longer) of follow-up, demonstrating significant clinical and/or imaging improvement [32].

(3) Non-TB-LAP: In the case of patients admitted with enlarged lymph nodes who had negative pathogenetic, pathology, and immunologic marker test results, clinicians combined their epidemiologic history and imaging manifestations to clinically exclude LNTB [33].

Lymph node biopsy

Ultrasound-guided superficial site mass puncture biopsy was performed. With the patient in the supine position, the target area was identified and marked with color ultrasound. After routine disinfection and towel placement, local anesthesia was performed with 2% lidocaine hydrochloride. A puncture needle was inserted into the lymph node mass under ultrasound guidance, and the biopsy gun was activated to obtain a tissue sample. Following the withdrawal of the biopsy needle, the tissues were fixed and sent for pathological examination.

IL-2/IFN-γ assay

Instruments and reagents

The following reagents and instruments were used in this study: Duplex assay kit for human IL-2 and IFN-γ (Guangzhou Diao Medical Technology Co., Ltd., Guangzhou, China); trypan blue stain solution (Shanghai Bioengineering Co., Ltd., Shanghai, China); cell culture media and reagents: RPMI-1640 basal medium, ready-to-use serum-free complete medium, and lymphocyte separation solution (Gibco, Grand Island, NY, USA); biological safety cabinet (Model HR40-IIA2, Haier, Qingdao, China); refrigerated centrifuge and microplate reader (Shenzhen Shengxinkang Biotechnology Co., Ltd., Shenzhen, China); and cell culture incubator (Changsha Changjin Science and Technology Co., Ltd., Changsha, China).

Methods of operation

A double antibody sandwich ELISA was used to detect the concentrations of IFN-γ and IL-2 in culture supernatants. For this, 4 mL of peripheral blood was drawn into a blood collection tube containing heparin anticoagulant. Subsequently, peripheral blood mononuclear cells (PBMCs) were isolated, quantified, and separated using a lymphocyte separation solution. The negative control, protein stimulation, and positive control wells were set up for cell culture. Then, 100 μL of a prepared lymphocyte suspension (2.5×10^6/mL) was added to each well, followed by incubation with 5% CO_2 at 37 ℃ for 16–20 hours. After culture, the supernatant was pipetted from each well of the cell culture plate, avoiding disturbing the cell precipitates. At least 110 μL was collected per well. The samples and calibrators were collected from each well (negative control, TB-specific recombinant protein stimulation, positive control) of both the IL-2 and the IFN-γ antibody-precoated plates. Then, 50 μL of dilution buffer was added to each well, followed by 50 μL of sample supernatant (negative control, TB-specific recombinant protein stimulation, positive control), ensuring that no precipitate was aspirated. The plate was incubated at room temperature for 2 hours after adding samples and calibrators and sealing with a plate cover. The plate was washed 3 times and then patted dry. After incubation with the antibodies, the plates were washed again and then incubated with the enzyme conjugate, separately for IL-2 and IFN-γ. Another round of washing was followed by color development. Finally, the results for IL-2 and IFN-γ were read and determined based on predefined judgment criteria.

Statistical analysis

SPSS 26.0 and GraphPad Prism 9 software were used for data analysis [34]. All variables were assessed for missing data. Only cases with complete data for all key variables were included in the final analysis. When there were missing core diagnostic indicators (IFN-γ/IL-2), no imputation was performed, and analyses were conducted using available data. The small amount of missing data in baseline characteristics was handled by listwise deletion/multiple imputation. Non-normally distributed measurements were presented as medians with interquartile ranges [M (Q1, Q3)]. The Mann-Whitney U test was used to compare differences between groups. Categorical data were described using frequencies. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were used for the assessment of diagnostic performance. The area under the curve and the cut-off value were determined from the receiver operating characteristic (ROC) curve of the subjects. The optimal cut-point was selected by maximizing the Youden index, which identifies the point on the ROC curve that yields the highest sum of sensitivity and specificity minus one, with the corresponding concentration value adopted as the final threshold. A statistically significant difference was considered at $p < 0.05$.

Results

Demographic and clinical features of the participants

A total of 225 patients with extrapulmonary TB who sought treatment at the tuberculosis departments in The Second Hospital of Nanjing and Guangzhou Chest Hospital from June 2022 to May 2023 were selected for the study. Of these, 130 were excluded for a variety of reasons. Ultimately, 95 patients with enlarged lymph nodes were enrolled, of whom 38 had confirmed LNTB through pathogenicity testing with puncture tissue analysis (Figure 1). The methods employed for diagnosis were the Xpert MTB/RIF assay (X-PERT) (17 cases), next-generation sequencing (NGS, 17 cases), antacid staining of fresh tissues or pathology slides (16 cases), and molecular PCR for MTB (7 cases) (Figure 2). Several lymph node puncture tissue samples tested positive using more than one method, reflective of diagnostic overlap between different detection methods.

Of the 66 patients diagnosed with LNTB, 71.21% (47/66) were female. Ten had negative culture results from puncture specimens; among these, two patients were tested via X-PERT, with one showing a positive result. In four cases, NGS was employed for MTB detection, yielding two positive results. Of the 38 patients with confirmed TB, 78.95% (30/38) were also diagnosed with pulmonary TB.

Figure 1. Technology roadmap.

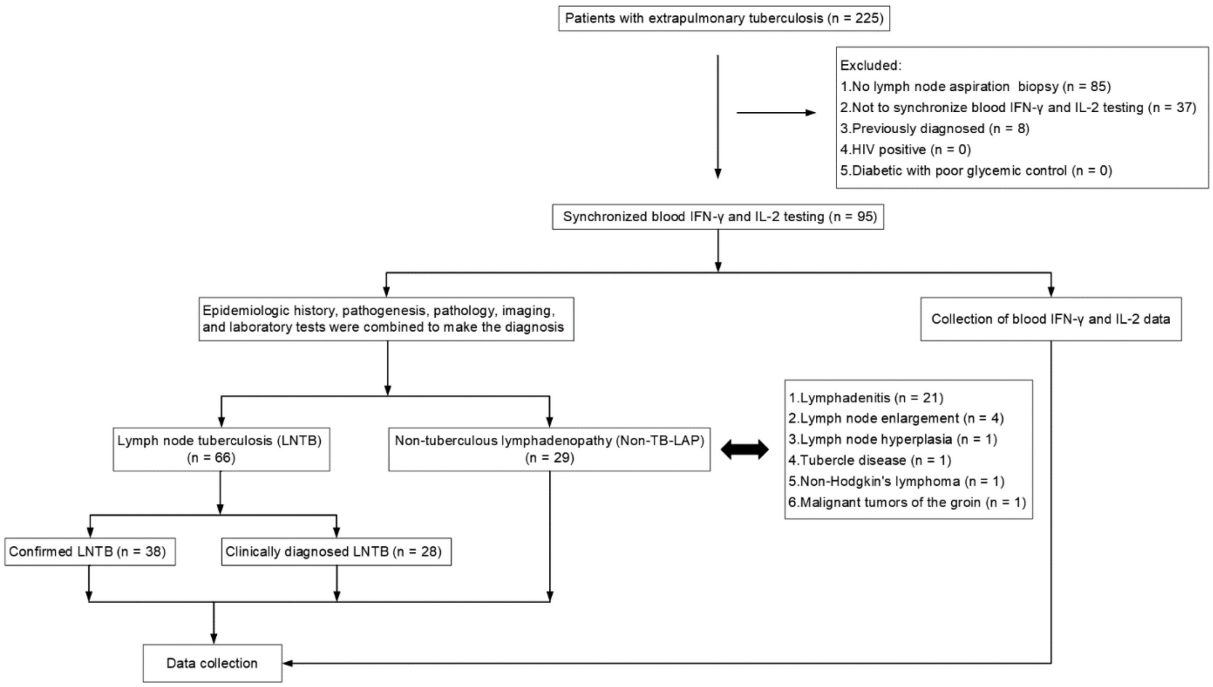


Table 1. Demographic and clinical characteristics of the participants enrolled in this study.

Characteristics of participants		Confirmation (N = 38)	Clinical diagnosis (N = 28)	Non-lymph node tuberculosis (LNTB) (N = 29)
Median age (years)		38 (28–51.25)	45.5 (30.25–51.75)	32 (21–48)
Gender (n)				
Male		12 (31.58)	7 (25.00)	19 (65.52)
Female		26 (68.42)	21 (75.00)	10 (34.48)
Complicated by tuberculosis (n)		30 (78.95)	14 (50.00)	7 (24.14)
Lymph node puncture tissue pathogenetic testing (n)				
X-PERT	*Positive*	17 (44.74)	0 (0.00)	0 (0.00)
	Negatives	7 (18.42)	14 (50.00)	9 (31.03)
	NA	14 (36.84)	14 (50.00)	20 (69.97)
NGS	*Positive*	17 (44.73)	0 (0.00)	0 (0.00)
	Negatives	3 (7.89)	6 (21.43)	8 (27.59)
	NA	18 (47.38)	22 (78.57)	21 (72.41)
Culture	*Positive*	0 (0.00)	0 (0.00)	0 (0.00)
	Negatives	5 (13.16)	6 (21.43)	4 (13.79)
	NA	33 (86.84)	22 (78.57)	25 (86.21)
Smear	*Positive*	0 (0.00)	0 (0.00)	0 (0.00)
	Negatives	4 (10.53)	2 (7.14)	0 (0.00)
	NA	34 (89.47)	26 (92.86)	29 (100.00)
Lymph node puncture for histopathologic testing (n)				
Pathological results	Granulomatous inflammation	34 (89.48)	18 (64.29)	14 (48.28)
	Lymphadenitis	0 (0.00)	7 (25.00)	0 (0.00)
	Lymph node hyperplasia	2 (5.26)	3 (10.71)	7 (24.13)
	Other	2 (5.26)	0 (0.00)	8 (27.59)
Antacid staining of fresh tissue or sections	*Positive*	16 (42.11)	0 (0.00)	0 (0.00)
	Negatives	22 (57.89)	11 (39.29)	19 (65.52)
	NA	0 (0.00)	17 (60.71)	10 (34.48)
Molecular PCR	*Positive*	7 (18.42)	0 (0.00)	0 (0.00)
	Negatives	0 (0.00)	5 (17.86)	15 (51.72)
	NA	31 (81.58)	23 (82.14)	14 (48.28)

Figure 2. Distribution of confirmed lymph node tuberculosis (LNTB) cases using four different diagnostic methods: X-PERT, next-generation sequencing (NGS), antacid staining, and molecular polymerase chain reaction (PCR).

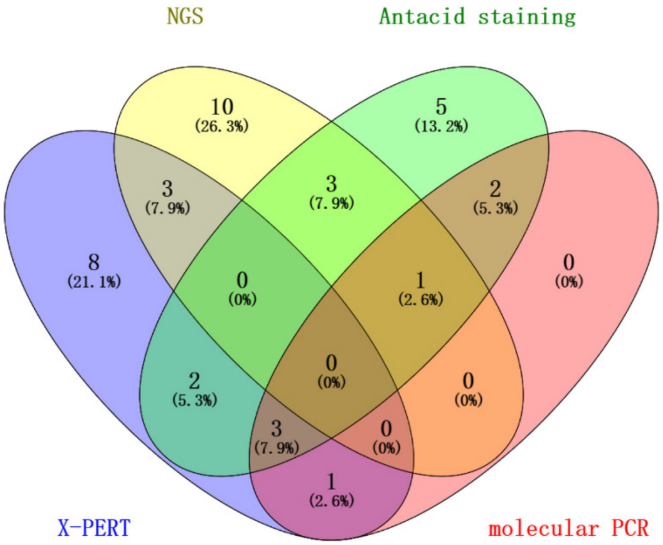


Figure 3. Receiver operating characteristic (ROC) curve for IFN-γ/IL-2 for differentiating lymph node tuberculosis (LNTB) from non-tuberculous lymphadenopathy (non-TB-LAP).

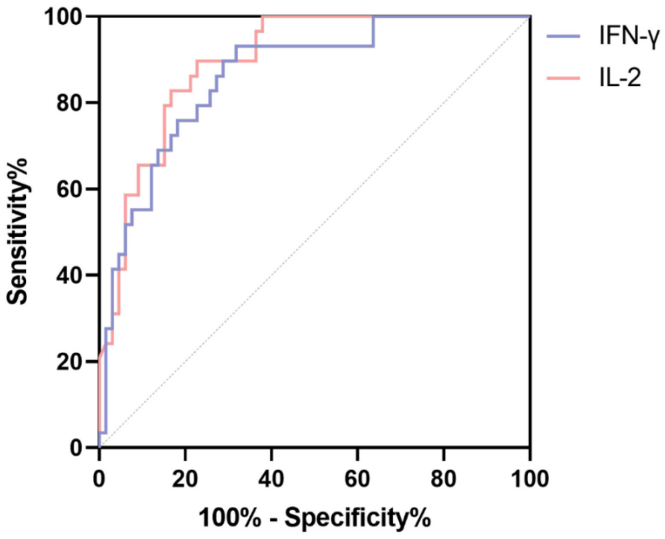


Table 2. ROC curve analysis of IFN-γ, IL-2 for differentiating Lymph node tuberculosis (LNTB) from non-tuberculous lymphadenopathy (non-TB-LAP).

Classifications	Cut-off (pg/mL)	AUC	95% CI lower limit	95% CI upper limit
IFN-γ	58.267	0.864	0.786	0.941
IL-2	12.207	0.896	0.835	0.958

The percentage of combined pulmonary TB in patients with LNTB was significantly higher than that in patients with non-TB-LAP ($p < 0.001$) (Table 1).

Effectiveness of single IL-2 and IFN-γ in the diagnosis of LNTB and non-TB-LAP

The efficacy of IFN-γ and IL-2 in differentiating LNTB from non-TB-LAP was evaluated by ROC curves (Figure 3). The results showed that the area under the ROC curve was 0.864 (95% CI: 0.786–0.941) for IFN-γ and 0.896 (95% CI: 0.835–0.958) for IL-2. The cut-off values were 58.267 and 12.207 pg/mL, respectively (Table 2). The sensitivity and specificity of IFN-γ were 68.18% (95% CI: 55.43–78.8) and 89.66% (95% CI: 71.51–97.29), respectively. Meanwhile, compared to IFN-γ, IL-2 showed higher sensitivity (77.27%; 95% CI: 65.01–86.32) but lower specificity (86.21%; 95% CI: 67.43–95.49) (Table 3).

Diagnostic performance of the combined IL-2 and IFN-γ assay in LNTB and non-TB-LAP

Seeking to further improve the sensitivity and specificity of diagnosis, a combined test was undertaken using both IL-2 and IFN-γ (Table 3). In the concurrent test, a positive result for either IL-2 or IFN-γ was considered indicative of LNTB, achieving a sensitivity of 83.33% (95% CI: 71.71–90.99). In the serial test, LNTB was indicated only when results were positive for both IL-2 and IFN-γ. Here, the specificity was determined to be 93.1% (95% CI: 75.78–98.8). The sensitivity of the concurrent diagnosis approach for LNTB was significantly higher than that of IFN-γ alone (Figure 4).

Expression levels of IFN-γ/IL-2 among the different subgroups

As shown in Figure 5, the expression levels of FN-γ and IL-2 differed significantly between LNTB and non-TB-LAP patients ($p < 0.001$).

Figure 4. Comparison of the sensitivity of IFN-γ, IL-2, parallel, and tandem detection methods for lymph node tuberculosis (LNTB). * p < 0.05, ** p < 0.01.

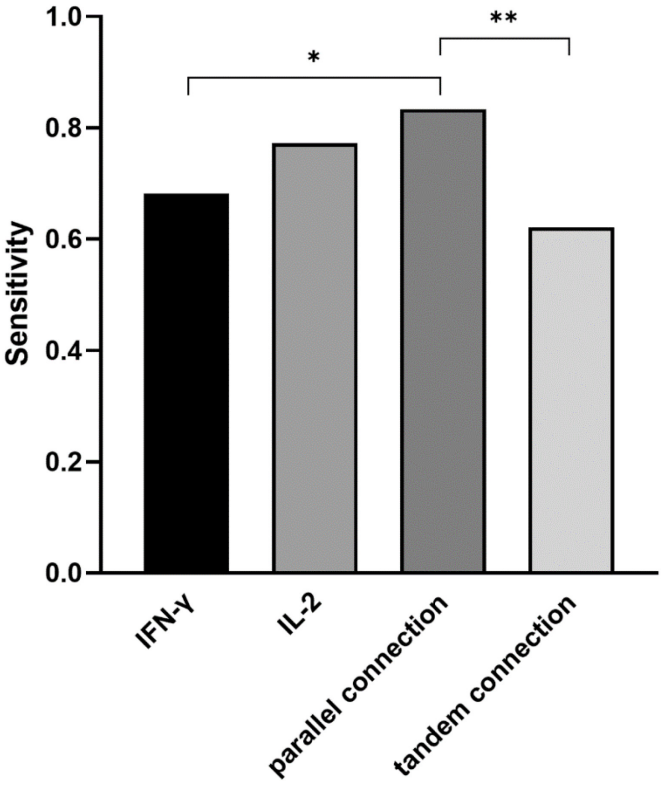


Table 3. Diagnosis of lymph node tuberculosis (LNTB) by IFN-γ/IL-2 combined detection.

Classification	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)
IFN-γ	68.18 (55.43–78.80)	89.66 (71.51–97.29)	93.75 (81.79–98.37)	55.32 (40.24–69.54)
IL-2	77.27 (65.01–86.32)	86.21 (67.43–95.49)	92.73 (81.58–97.65)	62.50 (45.81–76.83)
Parallel test	83.33 (71.71–90.99)	86.21 (67.43–95.49)	93.22 (82.73–97.81)	69.44 (51.73–83.08)
Tandem test	62.12 (49.30–73.52)	93.10 (75.78–98.80)	95.35 (82.95–99.19)	51.92 (37.78–65.78)

The difference in the levels of IFN-γ produced in response to TB antigen stimulation between patients with confirmed LNTB and those with clinically diagnosed LNTB was not statistically significant ($p > 0.05$) (Figure 5A). Furthermore, there was a statistically significant difference in the levels of IL-2 produced in response to TB antigen stimulation between patients with confirmed LNTB and those with clinically diagnosed LNTB ($p < 0.05$), with the median level of IL-2 being higher in the former (Figure 5B).

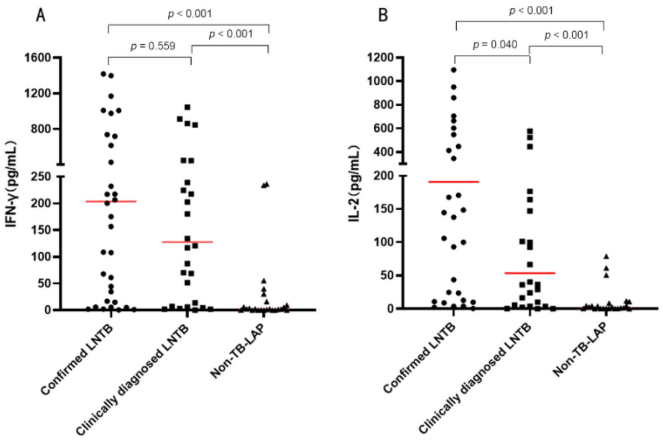
Comparison of various tests for diagnosing LNTB

In order to compare the positivity rates of various methods for diagnosing LNTB, a positive result was defined as IFN-γ/IL-2 exceeding the established cut-off. As shown in Table 4, in lymph node biopsy tissue, the positivity rate was 44.74% for X-PERT-based detection, 65.38% for NGS, 58.33% for molecular PCR detection, and 24.24% for acid-fast staining of lymph node biopsy tissue or pathologic sections. Meanwhile, the positivity rate for IFN-γ was 68.18%, that for IL-2 was 77.27%, that for blood IFN-γ or IL-2 (in parallel) was 83.33%, and that for blood IFN-γ and IL-2 (in tandem) was 62.12%. These results highlight the superior diagnostic efficacy of the parallel method.

Discussion

LNTB is primarily characterized by the absence of typical systemic symptoms or a clear history of contact with TB patients. Despite advancements in diagnostic techniques, the diagnosis of LNTB remains highly challenging [35]. Many immunological methods exist for the diagnosis of this condition. However, given that MTB expresses an abundance of protein and polysaccharide antigens, the specificity of these methods is poor, and the sensitivity of routine positive culture and other TB pathogen identification methods remains low [36]. In this study, 10 patients with LNTB were assessed for the presence of MTB by fresh tissue culture, which yielded negative results. Lymph node aspirates were sent for pathology testing, resulting in a positivity rate of only 16.84% for antacid staining, and

Figure 5. The levels of (**A**) IFN-γ and (**B**) IL-2 among patients with confirmed lymph node tuberculosis (LNTB), clinically diagnosed lymph node tuberculosis (LNTB), and non-tuberculosis lymphadenopathy (non-TB-LAP) following tuberculosis antigen stimulation. The red line indicates the median value. p values were calculated using the Mann-Whitney U test.



a slightly higher positivity rate of 58.33% for molecular PCR testing for MTB, likely due to the low number of cases undergoing this type of testing in the clinic, as well as potential selection bias by clinicians. Meanwhile, the diagnosis of patients with suspected LNTB was more challenging due to difficulties in sample collection, the small number of cells obtained from the samples, and the insufficient bacterial content [37]. In this study, among the 95 patients with enlarged lymph nodes, 66 were diagnosed with LNTB, of whom 47 were female, accounting for 71.21%. This gender bias may be related to differences in the immune response, potentially linked to genetic susceptibility and endocrine factors. Among the remaining 29 patients presenting with non-TB-LAP, 21 were finally diagnosed with lymphadenitis, accounting for 72.41% of the total, underscoring the need to distinguish clinically diagnosed LNTB from lymphadenitis. Efforts should be made to improve the identification of the underlying cause of enlarged lymph nodes; if necessary, lymph node puncture biopsy and tissue culture should be performed to clarify the diagnosis. Patients with

Table 4. Comparison of positive rates of different tests for the diagnosis of lymph node tuberculosis (LNTB).

Detection methods	Positive cases	Number of cases detected	Positivity rate
Lymph node puncture tissue X-PERT	17	38	44.74%
Lymph node puncture tissue NGS	17	26	65.38%
Lymph node aspiration tissue culture	0	6	0.00%
Lymph node puncture tissue smear	0	6	0.00%
Special antacid staining of lymph node puncture tissue or pathology sections	16	66	24.24%
Molecular PCR for lymph node puncture histopathology of tuberculosis	7	12	58.33%
Blood IFN-γ	45	66	68.18%
Blood IL-2	51	66	77.27%
Blood IFN-γ or IL-2 (parallel)	55	66	83.33%
Blood IFN-γ and IL-2 (tandem)	41	66	62.12%

LNTB have a higher proportion of co-occurrence with pulmonary TB than those without LNTB, implying that when the patients with enlarged lymph nodes present with concurrent pulmonary TB, LNTB should be strongly suspected.

In this study, assays for IFN-γ and IL-2 were used, which are widely abundant in the blood. Obtaining cytokines from blood for the relevant assays is not as physically or psychologically taxing on patients as compared to the collection of lymph node aspirates for pathological or etiological diagnosis. Additionally, cytokine assays are more cost-effective and have greater global applicability, particularly in regions with a high prevalence of TB or limited facilities for complex diagnostics. The main immunological tests for MTB infection are the TST and IGRA, with the latter being the most commonly used and the one recommended by the WHO [38]. In this study, novel detection markers were added to the IGRA and a comparative analysis of the performance of the combined IFN-γ/IL-2 test and the blood IGRA was performed. The positivity rate for detecting LNTB varied among the different methods. The detection sensitivity for blood IFN-γ and IL-2 in parallel was 83.33%, which was higher than that for IFN-γ (68.18%), suggesting that the combined detection of IFN-γ and IL-2 allows for earlier and more accurate diagnosis. In patients with suspected lymph node enlargement, the combined use of IFN-γ and IL-2 can identify a greater number of patients with LNTB, thereby facilitating timely treatment with anti-TB drugs. This is conducive to recovery from the disease, as well as to the reduction of transmission and the improvement of public health outcomes. In this study, the cut-off values for IFN-γ and IL-2 differed from those reported in a domestic large-sample study (7.38 and 20.19 pg/mL, respectively). Several reasons may explain this discrepancy. First, the large-sample study was conducted to compare TB with non-tuberculous lung diseases or a healthy condition rather than for differentiating LNTB from non-TB-LAP. Secondly, the sample size in this study was relatively small, potentially leading to greater fluctuations in the cut-off values. However, compared with IGRA, the method used in the present study directly and quantitatively measures the concentrations of two specific cytokines (IFN-γ and IL-2) in plasma. More importantly, we expanded from a single marker to multiple markers, thereby capturing richer immune response information. The inclusion of IL-2 may reflect the activity of T-cell subsets with different functional states (such as central memory T cells), which could help identify cases that might be missed by single IFN-γ testing, thereby potentially improving detection sensitivity [39]. Furthermore, the double antibody sandwich ELISA enables the simultaneous quantification of both markers in a single test using the same sample. This is not merely the sum of two separate ELISAs but offers greater diagnostic efficiency. In summary, the integrated dual-marker assay provides a more accurate tool than conventional IGRA and a more efficient alternative to sequential single-marker ELISAs, thereby offering clinicians stronger decision-making support in the complex differential diagnosis of LNTB.

The increased sensitivity of the combined assay is mainly attributable to the introduction of a new target, IL-2. The area under the ROC curve for IL-2 was slightly higher than that for IFN-γ, suggesting that the cytokine IL-2 was superior as a diagnostic marker for distinguishing LNTB from non-TB-LAP. This phenomenon can be reasonably explained by the immunopathological characteristics of tuberculosis. As a localized infection, the immune response in LNTB is primarily concentrated within granulomas and draining lymphoid tissues [40]. IFN-γ, being a core cytokine in the effector phase, may more readily reach a plateau or become exhausted in chronic, localized infections, leading to greater variability among patients. In contrast, IL-2—a key growth factor for T cell proliferation and survival (particularly of naïve and central memory T cells)—may more accurately reflect the size and activity of the antigen-specific T cell pool [39,41]. In the context of LNTB, where persistent antigen stimulation occurs in a relatively confined environment, maintaining a functional T cell pool may rely more heavily on IL-2 signaling, making IL-2 levels a more stable immunological marker [42]. Furthermore, studies suggest that the association between antigen-specific IL-2 responses and bacterial load or disease activity may differ from that of IFN-γ. In LNTB, a moderate yet sustained IL-2 response may be crucial for maintaining T cell survival and function within granulomas, allowing IL-2 levels to more precisely reflect the local immune status and thereby provide better resolution in differential diagnosis. In summary, the superior performance of IL-2 over IFN-γ is not coincidental but likely reflects the unique immune microenvironment of LNTB as a chronic focal infection. While IFN-γ more directly indicates immediate effector output, IL-2 better represents the potential and sustainability of the immune response. In the specific setting of LNTB, IL-2 may serve as a more discriminative biological marker.

It was further observed that the difference in IL-2 levels between patients with confirmed LNTB and

those with clinically diagnosed LNTB was statistically different and the median IL-2 level was higher in the former group. Qiu *et al.* [28] also showed that IL-2 levels were significantly higher in patients with TB and LTBI than in healthy individuals, and progressively decreased during treatment. These observations suggest that, although the primary focus of this study was diagnostic performance, a potential trend was noted between IL-2 levels and disease severity. This finding raises the intriguing hypothesis that IL-2 may not only serve as a diagnostic marker but could also possess prognostic potential for reflecting bacterial load or immune response intensity. However, it must be emphasized that as this study did not include longitudinal follow-up to assess treatment response, no conclusions can be drawn regarding the prognostic value of IL-2. The observations on the relationship between IL-2 and disease severity remain preliminary and speculative, requiring validation in future studies.

The IFN-γ/IL-2 combined assay developed in this study offers value not only through its superior diagnostic performance but also through its potential for clinical translation. For patients with suspected LNTB, particularly in high tuberculosis burden regions, diagnosis often relies on invasive lymph node biopsies and time-consuming pathogen-based tests [43]. The combined assay, as a relatively rapid blood test, serves as an efficient auxiliary diagnostic or screening tool. Furthermore, prioritizing the IFN-γ/IL-2 test for suspected cases can significantly enhance diagnostic confidence: a positive result supports earlier empirical treatment or more targeted biopsy site selection, while a negative result provides strong evidence to explore non-tuberculous causes such as malignancy or other infections. Finally, compared to "gold-standard" methods requiring sophisticated instrumentation and specialized expertise, this assay can be standardized in regional central laboratories. Although reagent costs require evaluation, the potential to improve diagnostic efficiency, avoid unnecessary invasive procedures, and reduce misdiagnosis may lead to overall cost savings, especially in LNTB-prevalent areas with limited pathological resources [44]. Certainly, translating these potential advantages into real-world benefits will require further cost-effectiveness analyses and prospective clinical validation studies. Nevertheless, this study represents a solid step toward developing an accurate and practical novel diagnostic tool for LNTB.

This study has several noteworthy limitations. Firstly, its retrospective, two-center design may inevitably introduce selection bias and limit the generalizability of the findings to broader populations. The results should be interpreted with caution when applied to other regions, hospital levels, or ethnic groups, and require further external validation. Secondly, although the overall sample size is acceptable, the subgroup analyses (comparison between confirmed and clinically diagnosed cases) involved relatively small numbers, resulting in limited statistical power. This means that even though differences in some indicators were reported, the possibility of undetected but clinically meaningful differences cannot be excluded. Therefore, these comparisons should be interpreted with great caution and are primarily hypothesis-generating rather than conclusive. Thirdly, and importantly, the cut-off values for IFN-γ and IL-2 in this study were based on the results of a specific cohort, and subsequent validation in a larger and more diverse population is needed. Fourthly, immunosuppression, diabetes mellitus, concurrent infections, and genetic susceptibility in TB patients may affect their immune status, and, consequently, the reliability of the results. However, given that a large proportion of TB patients also presented with diabetes mellitus, only HIV-positive patients and diabetes mellitus patients with poor glycemic control were excluded from the present study, which may have influenced the results. Furthermore, factors such as concurrent infections and genetic susceptibility, which may affect the levels of cytokines, were not controlled for. Finally, IFN-γ and IL-2 were not used as biomarkers to monitor long-term efficacy in terms of treatment response or disease progression.

Conclusions

This study demonstrates that the combined detection of IFN-γ and IL-2 shows good diagnostic performance in distinguishing LNTB from non-TB-LAP, offering a potential supplementary tool for the current complex diagnostic process. Using these markers in tandem has the potential for improving the specificity of diagnosing LNTB and reducing the rate of misdiagnosis. When assessed in parallel, the IFN-γ/IL-2 combination can improve the sensitivity of diagnosing LNTB and reduce the missed detection rate. However, its clinical applicability still requires further validation. Future studies should focus on validating this model in broader populations, including other forms of extrapulmonary TB. In addition, the preliminary observation of an association between IL-2 levels and disease severity provides a clear rationale for future prospective longitudinal studies to validate the role of IL-2 in monitoring treatment response and predicting disease outcomes.

Acknowledgements
The authors thank the participants in this study.

Ethical approval and consent
The study protocol was approved by the Ethics Committee of The Second Hospital of Nanjing (SN:2023-LS-021). All participants provided informed consent to utilize and disclose clinical data for research objectives, adhering to the principles outlined in the Declaration of Helsinki.

Availability of data and materials
The datasets used and analyzed during the current research are available from the corresponding author on reasonable request.

Funding
This study was supported by the Social Development Project of the Key Research and Development Plan of Jiangsu Province (BE2023660) Innovation Center for Infectious Disease of Jiangsu Province (NO. CXZX202232) and the "333 Talent Project" of Jiangsu province.

Authors' contributions
CH designed the project and obtained financial support. CZ and YC coordinated the project and wrote the manuscript. JH and YL conducted data analysis. HZ and TH revised the manuscript. LH and JQ prepared the figures and citations. CZ and YC collected the specimens and clinical information. All authors read and approved the final manuscript.

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Conflict of interest
No conflict of interest is declared.

References
1. Furin J, Cox H, Pai M (2019) Tuberculosis. Lancet 393: 1642–1656. doi: 10.1016/S0140-6736(19)30308-3.
2. Harding E (2020) WHO global progress report on tuberculosis elimination. Lancet Respir Med 8: 19. doi: 10.1016/S2213-2600(19)30418-7.
3. Rodriguez-Takeuchi SY, Renjifo ME, Medina FJ (2019) Extrapulmonary tuberculosis: pathophysiology and imaging findings. Radiographics 39: 2023–2037. doi: 10.1148/rg.2019190109.
4. Ganchua SKC, White AG, Klein EC, Flynn JL (2020) Lymph nodes-the neglected battlefield in tuberculosis. PLoS Pathog 16: e1008632. doi: 10.1371/journal.ppat.1008632.
5. Sarfaraz S, Iftikhar S, Salahuddin N (2020) Frequency, clinical characteristics, risks, and outcomes of paradoxical upgrading reactions during anti-tuberculosis treatment in tuberculous lymphadenitis. Pakistan Journal of Medical Sciences 36: S27–S32. doi: 10.12669/pjms.36.ICON-Suppl.1711.
6. Suárez I, Fünger SM, Kröger S, Rademacher J, Fätkenheuer G, Rybniker J (2019) The diagnosis and treatment of tuberculosis. Deutsches Arzteblatt International 116: 729–735. doi: 10.3238/arztebl.2019.0729.
7. Wang Y, Meng X, Chen P, Luo J, Tong J, Yan X, Lin T, Yu Y, Zhang Y, Yang G (2025) To predict cervical lymph node tuberculosis based on clinical-multimodal ultrasound radiomics model. Clin Hemorheol Microcirc 89: 324–334. doi: 10.1177/13860291241304060.
8. Cao J, Gan H, Wu J-H, Yuan Y, Ye F (2025) Case of nasopharyngeal tuberculosis complicated with cervical lymph node and pulmonary tuberculosis. Open Life Sciences 20: 20221041. doi: 10.1515/biol-2022-1041.
9. Li J, Li D, Zhang W (2025) Value of contrast-enhanced ultrasound assisted core needle biopsy in the diagnosis of cervical lymph node tuberculosis. Front Oncol 15: 1570133. doi: 10.3389/fonc.2025.1570133.
10. Meng X, Fu H, Jia W, Wang Y, Yang G (2022) A comparative study of ultrasound-guided puncture biopsy combined with histopathology and Xpert MTB/RIF in the diagnosis of lymph node tuberculosis. Front Public Health 10: 1022470. doi: 10.3389/fpubh.2022.1022470.
11. Viswanathan S, Shroff CP (2025) Cytomorphology in tuberculous lymphadenitis and correlation with acid fast bacilli detection by Papanicolaou, auramine-rhodamine fluorescence and Ziehl-Neelsen techniques. Diagn Cytopathol, 3: 125–132. doi: 10.1002/dc.25486.
12. Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, Borroni E, Mondo A, Piana F, Scarparo C, Coltella L, Lombardi C, M Cirillo D (2012) Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary

tuberculosis. Eur Respir J 40: 442–447. doi: 10.1183/09031936.00176311.
13. Fantahun M, Kebede A, Yenew B, Gemechu T, Mamuye Y, Tadesse M, Brhane B, Jibriel A, Solomon D, Yaregal Z (2019) Diagnostic accuracy of Xpert MTB/RIF assay and non-molecular methods for the diagnosis of tuberculosis lymphadenitis. PloS One 14: e0222402. doi: 10.1371/journal.pone.0222402.
14. Ghosh P, Anwar S, Siegel M, Okuni JB, Weidmann M, Mithila NT, Cassar K, Müller SE, Luba FR, Kobialka RM, Dey BP, Ceruti A, Nakiyingi L, Kiracho EE, Morshed SN, Arpa C, Chowdhury MAA, Bhowmik B, Saleh AA, Wahed AAE, Mondal D, Schneitler S (2025) Assessing the performance of a point-of-need diagnostic algorithm in rapid detection of peripheral lymph node tuberculosis (Mobile-TB-Lab): a diagnostic evaluation study protocol. BMJ Open 15: e097001. doi: 10.1136/bmjopen-2024-097001.
15. Zhu X-L, Liu X-Y, Wen L, Li R, Lv S-X, Wang G-X (2025) Clinical and CT characteristics of abdominal tuberculous lymphadenopathy: a comparative analysis of hematogenous and non-hematogenous dissemination. BMC Infect Dis 25: 710. doi: 10.1186/s12879-025-11101-3.
16. Thillai M, Pollock K, Pareek M, Lalvani A (2014) Interferon-gamma release assays for tuberculosis: current and future applications. Expert Rev Respir Med 8: 67–78. doi: 10.1586/17476348.2014.852471.
17. Abubakar I, Drobniewski F, Southern J, Sitch AJ, Jackson C, Lipman M, Deeks JJ, Griffiths C, Bothamley G, Lynn W, Burgess H, Mann B, Imran A, Sridhar S, Tsou CY, Nikolayevskyy V, Rees-Roberts M, Whitworth H, Kon OM, Haldar P, Kunst H, Anderson S, Hayward A, Watson JM, Milburn H, Lalvani A; Team PS (2018) Prognostic value of interferon-γ release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study. Lancet Infect Dis 18: 1077–1087. doi: 10.1016/S1473-3099(18)30355-4.
18. Zhou G, Luo Q, Luo S, He J, Chen N, Zhang Y, Yang R, Qiu Y, Li S, Ping Q, Cai S, Guo X, Song C (2022) Positive rates of interferon-γ release assay and tuberculin skin test in detection of latent tuberculosis infection: a systematic review and meta-analysis of 200,000 head-to-head comparative tests. Clin Immunol 245: 109132. doi: 10.1016/j.clim.2022.109132.
19. Fritschi N, Gureva T, Eliseev P, Jackson C, Milanzi E, Crichton S, Collins IJ, Turkova A, Mariandyshev A, Ritz N (2024) Diagnosis of tuberculosis infection in children with a novel skin test and the traditional tuberculin skin test: an observational study. PloS One 19: e0293272. doi: 10.1371/journal.pone.0293272.
20. Larson EM, O'Donnell M, Chamblee S, Horsburgh CR, Marsh BJ, Moreland JD, Johnson LS, von Reyn CF (2011) Dual skin tests with *Mycobacterium avium* sensitin and PPD to detect misdiagnosis of latent tuberculosis infection. Int J Tuberc Lung Dis 15: 1504–1509. doi: 10.5588/ijtld.11.0015.
21. Tang J, Huang Y, Cai Z, Ma Y (2021) Mycobacterial heparin-binding hemagglutinin (HBHA)-induced interferon-γ release assay (IGRA) for discrimination of latent and active tuberculosis: a systematic review and meta-analysis. PloS One 16: e0254571. doi: 10.1371/journal.pone.0254571.
22. Peng Z, Li J, Rong Z, Zhou Y, Wang Y, Wang Y, Zhang G, Yang Y, Tian X, Zeng X (2024) Incidence, risk factors for active tuberculosis infection and changes of IGRA in patients with Takayasu arteritis: a prospective cohort study. Emerg Microbes Infect 13: 2302099. doi: 10.1080/22221751.2024.2302099.
23. Redelman-Sidi G, Sepkowitz KA (2013) IFN-γ release assays in the diagnosis of latent tuberculosis infection among immunocompromised adults. Am J Respir Crit Care Med 188: 422–431. doi: 10.1164/rccm.201209-1621CI.
24. Matteo MJ, Latini MC, Martinovic DN, Bottiglieri M (2025) Update of diagnostic methods in tuberculosis (TB). Revista Argentina de Microbiologia 57: 49–53. doi: 10.1016/j.ram.2024.12.008.
25. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, Metcalfe JZ, Cattamanchi A, Dowdy DW, Dheda K, Banaei N (2014) Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. Clin Microbiol Rev 27: 3–20. doi: 10.1128/CMR.00034-13.
26. Spolski R, Li P, Leonard WJ (2018) Biology and regulation of IL-2: from molecular mechanisms to human therapy. Nat Rev Immunol 18: 648–659. doi: 10.1038/s41577-018-0046-y.
27. Liu X, Li F, Niu H, Ma L, Chen J, Zhang Y, Peng L, Gan C, Ma X, Zhu B (2019) IL-2 restores T-cell dysfunction induced by persistent *Mycobacterium tuberculosis* antigen stimulation. Front Immunol 10: 2350. doi: 10.3389/fimmu.2019.02350.
28. Qiu X, Wang H, Tang Y, Su X, Ge L, Qu Y, Mu D (2020) Is interleukin-2 an optimal marker for diagnosing tuberculosis infection? A systematic review and meta-analysis. Ann Med 52: 376–385. doi: 10.1080/07853890.2020.1800073.
29. Chen J, Wang S, Zhang Y, Su X, Wu J, Shao L, Wang F, Zhang S, Weng X, Wang H, Zhang W (2010) Rv1985c, a promising novel antigen for diagnosis of tuberculosis infection from BCG-vaccinated controls. BMC Infect Dis 10: 273. doi: 10.1186/1471-2334-10-273.
30. Zhao Z, Li R, Zhao X, Wang Y, Lin M, Wei Q, Li X, Xiong P (2025) The diagnostic value of interleukin-2 and interferon-γ induced by fusion protein (ESAT-6/CFP-10/Rv1985c) for active *Mycobacterium tuberculosis* infection. J Clin Lab Anal 39: e70010. doi: 10.1002/jcla.70010.
31. Tan Y, Tan Y, Li J, Hu P, Guan P, Kuang H, Liang Q, Yu Y, Chen Z, Wang Q, Yang Z, AiKeReMu D, Pang Y, Liu JX (2021) Combined IFN-γ and IL-2 release assay for detect active pulmonary tuberculosis: a prospective multicentre diagnostic study in China. J Transl Med 19: 289. doi: 10.1186/s12967-021-02970-8.
32. Carabalí-Isajar ML, Rodríguez-Bejarano OH, Amado T, Patarroyo MA, Izquierdo MA, Lutz JR, Ocampo M (2023) Clinical manifestations and immune response to tuberculosis. World J Microbiol Biotechnol 39: 206. doi: 10.1007/s11274-023-03636-x.
33. Yang J-S, Du Z-X: (2019) Comparison of clinical and pathological features of lymph node tuberculosis and histiocytic necrotizing lymphadenitis. J Infect Dev Ctries 13: 706–713. doi: 10.3855/jidc.10860.
34. Fan S, Abulizi A, You Y, Huang C, Yimit Y, Li Q, Zou X, Nijiati M (2024) Predicting hospitalization costs for pulmonary tuberculosis patients based on machine learning. BMC Infect Dis 1: 875. doi: 10.1186/s12879-024-09771-6.
35. Mathiasen VD, Lillebaek T, Wejse C (2022) Tuberculous lymphadenitis: a forgotten and delayed diagnosis in low-incidence countries. Infection 50: 277–280. doi: 10.1007/s15010-021-01632-7.
36. Park W-J, Yoon Y-K, Kim Y, Park J-S, Pansuriya R, Cho S-N, Seok Y-J, Ganapathy R (2021) Development of a bivalent conjugate vaccine candidate against rotaviral diarrhea and tuberculosis using polysaccharide from *Mycobacterium*

tuberculosis conjugated to ΔVP8* protein from rotavirus. Vaccine 39: 6644–6652. doi: 10.1016/j.vaccine.2021.09.067.
37. Bennani K, Khattabi A, Akrim M, Mahtar M, Benmansour N, Essakalli Hossyni L, Karkouri M, Cherradi N, El Messaoudi MD, Lahlou O, Cherkaoui I, Khader Y, Maaroufi A, Ottmani SE (2019) Evaluation of the yield of histopathology in the diagnosis of lymph node tuberculosis in Morocco, 2017: cross-sectional study. JMIR Public Health Surveill 5: e14252. doi: 10.2196/14252.
38. Zhou G, Luo Q, Luo S, Teng Z, Ji Z, Yang J, Wang F, Wen S, Ding Z, Li L, Chen T, Abi ME, Jian M, Luo L, Liu A, Bao F (2020) Interferon-γ release assays or tuberculin skin test for detection and management of latent tuberculosis infection: a systematic review and meta-analysis. Lancet Infect Dis: 20: 1457–1469. doi: 10.1016/S1473-3099(20)30276-0.
39. Shouse AN, LaPorte KM, Malek TR (2024) Interleukin-2 signaling in the regulation of T cell biology in autoimmunity and cancer. Immunity 57: 414–428. doi: 10.1016/j.immuni.2024.02.001.
40. Sahmoudi K, Abbassi H, Bouklata N, El Alami MN, Sadak A, Burant C, Henry Boom W, El Aouad R, Canaday DH, Seghrouchni F (2018) Immune activation and regulatory T cells in *Mycobacterium tuberculosis* infected lymph nodes. BMC Immunol 19: 33. doi: 10.1186/s12865-018-0266-8.
41. Zhang R, Zhao Y, Chen X, Zhuang Z, Li X, Shen E (2023) Low-dose IL-2 therapy in autoimmune diseases: an update review. Int Rev Immunol 43: 113–137. doi: 10.1080/08830185.2023.2274574.
42. Raeber ME, Zurbuchen Y, Impellizzieri D, Boyman O (2018) The role of cytokines in T-cell memory in health and disease. Immunol Rev 283: 176–193. doi: 10.1111/imr.12644.
43. Meng X, Fu H, Jia W, Wang Y, Yang G (2023) A comparative study of ultrasound-guided puncture biopsy combined with histopathology and Xpert MTB/RIF in the diagnosis of lymph node tuberculosis. Front Public Health 10: 1022470. doi: 10.3389/fpubh.2022.1022470.
44. Fontanilla J-M, Barnes A, von Reyn CF (2011) Current diagnosis and management of peripheral tuberculous lymphadenitis. Clin Infect Dis 53: 555–562. doi: 10.1093/cid/cir454.