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Serum lymphocytes and cytokines: diagnostic value and influence on the immune status in patients with pulmonary tuberculosis

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ABSTRACT

Objective: To determine the absolute number of serum T lymphocytes and cytokine levels and the characteristics of patients with active pulmonary tuberculosis and to assess their effect on the immune status of these patients and their diagnostic and predictive value for tuberculosis. **Methods:** We included 1,069 patients with active tuberculosis, 51 patients with latent tuberculosis infection, and 600 health individuals. Absolute serum T-lymphocyte counts and cytokine levels were quantified. **Results:** T lymphocytes were significantly reduced in patients with active tuberculosis when compared with healthy individuals. The immune function of patients gradually decreased with age and was stronger in female patients than in males. Th1 cells expressed higher levels of cytokines than did Th2 cells. Logistic regression analysis showed that reduced $CD3^+$ T, $CD8^+$ T, and NK cell counts, as well as reduced IL-4 and IFN-γ expression, were independent influencing factors for active tuberculosis. ROC analysis showed that the sensitivity and specificity of absolute $CD3^+$ T and $CD8^+$ T lymphocyte counts and combined factors were significantly higher than were those of IL-4 and IFN-γ for diagnosing active tuberculosis. **Conclusions:** Serum T-lymphocyte counts and cytokine levels can assess the immune status of tuberculosis patients; they are also useful biomarkers for predicting and diagnosing tuberculosis.

Keywords: Lymphocytes; Cytokines; Tuberculosis; Immunity.

INTRODUCTION

Tuberculosis is an inflammatory disease caused by *Mycobacterium tuberculosis* (Mtb) infection, and pulmonary tuberculosis is the most common form of clinical presentation.[1] The WHO Global Tuberculosis Report 2022[2] reported 10.6 million new cases of tuberculosis globally in 2021, with an incidence rate of 134 per 100,000 population. Although antituberculosis drugs are available and the number of related deaths has decreased globally in recent years, tuberculosis is still the leading cause of death from a single infectious disease.[3]

Efficacious $CD4^+$ T lymphocytes can be divided into at least three distinct subpopulations: Th1, Th2, and Th17 cells. These subpopulations are functionally controlled by regulatory $CD4^+$ T cells and have a high degree of specialization. Moreover, IFN-γ produced by Th1 cells plays an essential role in host resistance to primary infection with Mtb. In addition, IFN-γ enhances the antibacterial activity of macrophages and promotes the production of reactive nitrogen intermediates, which can eliminate bacteria intracellularly.[4,5] Both animal models and human trials have shown that IFN-γ expression levels do not provide relevant and reliable protection against Mtb infection.[3]

Analysis of the number of T lymphocytes and levels of cytokines, such as IL-2, IL-4, TNF-α, IFN-α, and IFN-γ, can provide a precious research base for disease through noninvasive methods to study the state of the immune system in patients.[6,7] In this study, we analyzed the absolute number of T lymphocytes and cytokine levels in the serum of patients with active tuberculosis (ATB) and patients with latent tuberculosis infection (LTBI), as well as those of healthy individuals (HI), to provide a reference for diagnosing tuberculosis and assessing the immune status of the participants.

METHODS

In this study, we included 1,069 patients with ATB, 600 HI, and 51 patients with LTBI. According to the WHO report,[2] the diagnosis of ATB is based on clinical evaluation, microbiology, molecular testing (such as GeneXpert Ultra), and imaging findings. LTBI is defined as a positive IFN- γ release assay result with no other clinical manifestations or with negative microbiological, molecular, and imaging results (Table 1).

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Exclusion criteria were as follows: autoimmune diseases or concomitant immune disorders; AIDS; glucocorticoid therapy; concomitant heart, liver, kidney, or other important organ diseases; severe hypertension; poor mental condition; concomitant diabetes mellitus; pregnancy or lactation; and drug-resistant tuberculosis. The study was approved by the Research Ethics Committee of the Third People's Hospital of Kunming (protocol no. KSLL20230320007).

Peripheral blood specimens from HI, patients with ATB, and those with LTBI were collected in 3 mL blood collection tubes. For cytokine assays, the blood samples were centrifuged at 1,000 × g for 10 min, and the serum was tested using cytokine assay kits (Risskell Bio, Qingdao, China) in order to determine the levels of cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, TNF-α, IFN-α, and IFN-γ) in accordance with the manufacturer instructions, We used the MR Flow Analysis software (AZE Ltd., Tokyo, Japan) for the analysis of results. For flow cytometry assays, the blood specimens were mixed and a flow cytometry kit (DIALAB GmbH, Wiener Neudorf, Austria) was used for determining $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, $CD4^+$/$CD8^+$, $CD19^+$B, NK, and NKT lymphocyte counts in accordance with the manufacturer instructions. We used the LEGENDplex software (BioLegend, San Diego, CA, USA) for result analysis. Both cytokine and flow cytometer assays were performed using a BriCyte E6 flow cytometer (Mindray, Shenzhen, China).

Statistical analyses were performed and plotted using the IBM SPSS Statistics software package, version 24.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism, version 8.0 (GraphPad Software Inc., San Diego, CA, USA), respectively. Quantitative data were described as mean ± SD when they conformed to a normal distribution using a t-test, whereas those with non-normal distribution were described as median [IQR] using the nonparametric Mann-Whitney test. Comparisons between multiple groups were made using the Kruskal-Wallis test, and differences between multivariate models were determined using the nested t-test. The analysis of the relevant influencers was performed by logistic regression analysis. The diagnostic value of these variables was analyzed using ROC curves. Statistical significance was set at $p < 0.05$.

RESULTS

The number of $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, and NK cells was lower in patients with ATB when compared with HI, and this difference reached statistical significance. In addition, the absolute number of $CD3^+$ T cells in the LTBI group was lower and significantly different than in the HI group, whereas the absolute numbers of $CD4^+$ T, $CD8^+$ T, and NK cells were higher, but not statistically significant. In the ATB group, the levels of IL-1β, IL-2, IL-8, and IL-17 were higher, and those of IL-4, IL-6, TNF-α, and IFN-γ were lower than those in the HI group. As for the LTBI group, the levels of IL-1β, IL-5, and IFN-γ were lower, and those of IL-6, IL-8, IL-10, IL-12p70, IL-17, and TNF-α were higher than those in the HI group. All of these differences reached statistical significance, which suggested that inflammatory factors are differentially expressed in ATB and LTBI (Figure 1).

We analyzed the expression of indicators associated with statistically significant differences between the ATB and HI groups by sex and age. In the ATB group, the number of $CD3^+$ T, $CD4^+$ T, and $CD8^+$ T cells was lower in men than in women; the difference was statistically significant, indicating that female patients with ATB are more immunocompetent than are males (Figure 2A). The expression of $CD3^+$ T, $CD4^+$ T, and $CD8^+$ T cell numbers gradually decreased with age, this difference reaching statistical significance. This suggests that older ATB patients have poorer immune function than do adolescent patients. Cytokine IFN-γ expression was significantly lower in men than in women, while the remaining expression levels did not differ significantly across age and sex (Figure 2B).

To determine the effect of ATB on the frequency of Th1/Th2 cells, we analyzed the expression levels of IL-1β, IL-2, TNF-α, IFN-γ, IL-4, IL-5, IL-6, and IL-10 in that group. As shown in Figure 3, those with ATB exhibited significant Th1 (IFN-γ and IL-1β) expression, while only IL-6 levels were increased (Th2 cells). In contrast, Th1 cells expressed higher levels of cytokines than did Th2 cells, and the difference was statistically significant, suggesting that the levels of cytokines expressed by Th1 cells in ATB may be specific for Mtb.

To investigate the factors associated with the influence of T lymphocytes and cytokines on ATB and LTBI, we screened for statistically different factors between the ATB, LTBI, and HI groups to be included in the impact factor analysis (Figure 1). Logistic regression results showed that reduced absolute numbers of $CD3^+$ T, $CD8^+$ T, and NK cells, as well as reduced expression levels of IL-4 and IFN-γ, were independent influencing factors for ATB (Figure 4A). Reduced expression levels of IL-1, IL-5, and IFN-γ

Table 1. Characteristics of the study participants.[a]

Characteristic	ATB	LTBI	HI
Number of participants	1,069	51	600
Male	587 (54.9)	27 (52.9)	331 (55.2)
Age, years	45.4 ± 17.4	36.3 ± 17.6	41.7 ± 17.2
Positive IGRA	1,069 (100)	51 (100)	0 (0)
Positive imaging	1,032 (96.5)	0 (0)	0 (0)

ATB: active tuberculosis; LTBI: latent tuberculosis infection; HI: healthy subjects; and IGRA: IFN-γ release assay. [a]Values expressed as n, n (%), or mean ± SD.

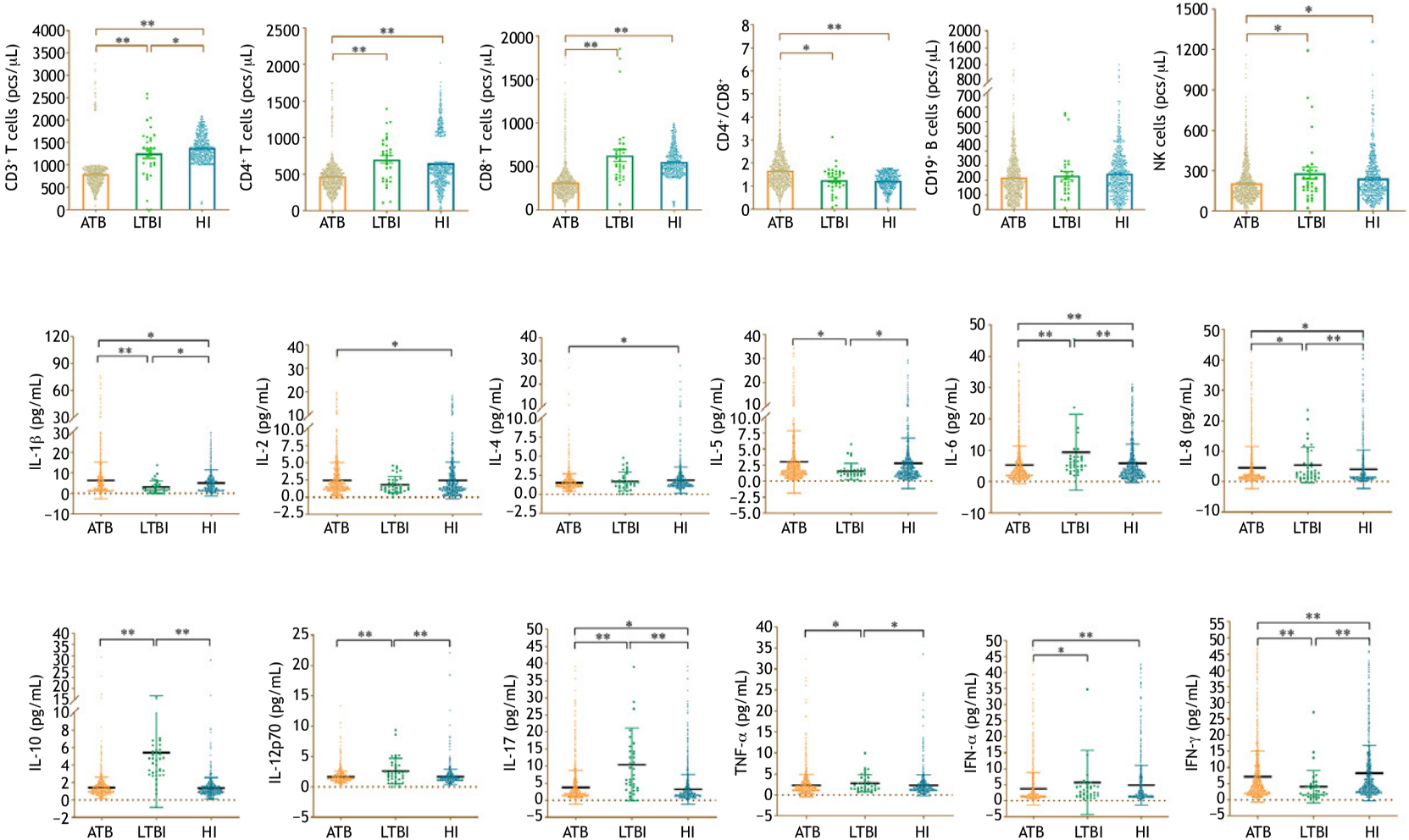

Figure 1. Expression of lymphocytes and cytokines by study group showing that the absolute numbers of $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, and NK cells in the active tuberculosis (ATB) group were all lower than normal. The latent tuberculosis infection (LTBI) group had a higher-than-normal number of lymphocytes, except for the absolute number of $CD3^+$ T cells. The ATB group had higher levels of IL-1β, IL-2, IL-8 and IL-17, but lower levels of IL-4, IL-6, TNF-α, and IFN-γ, than did the healthy individual (HI) group. pcs: pieces. *$p < 0.05$. **$p < 0.01$.

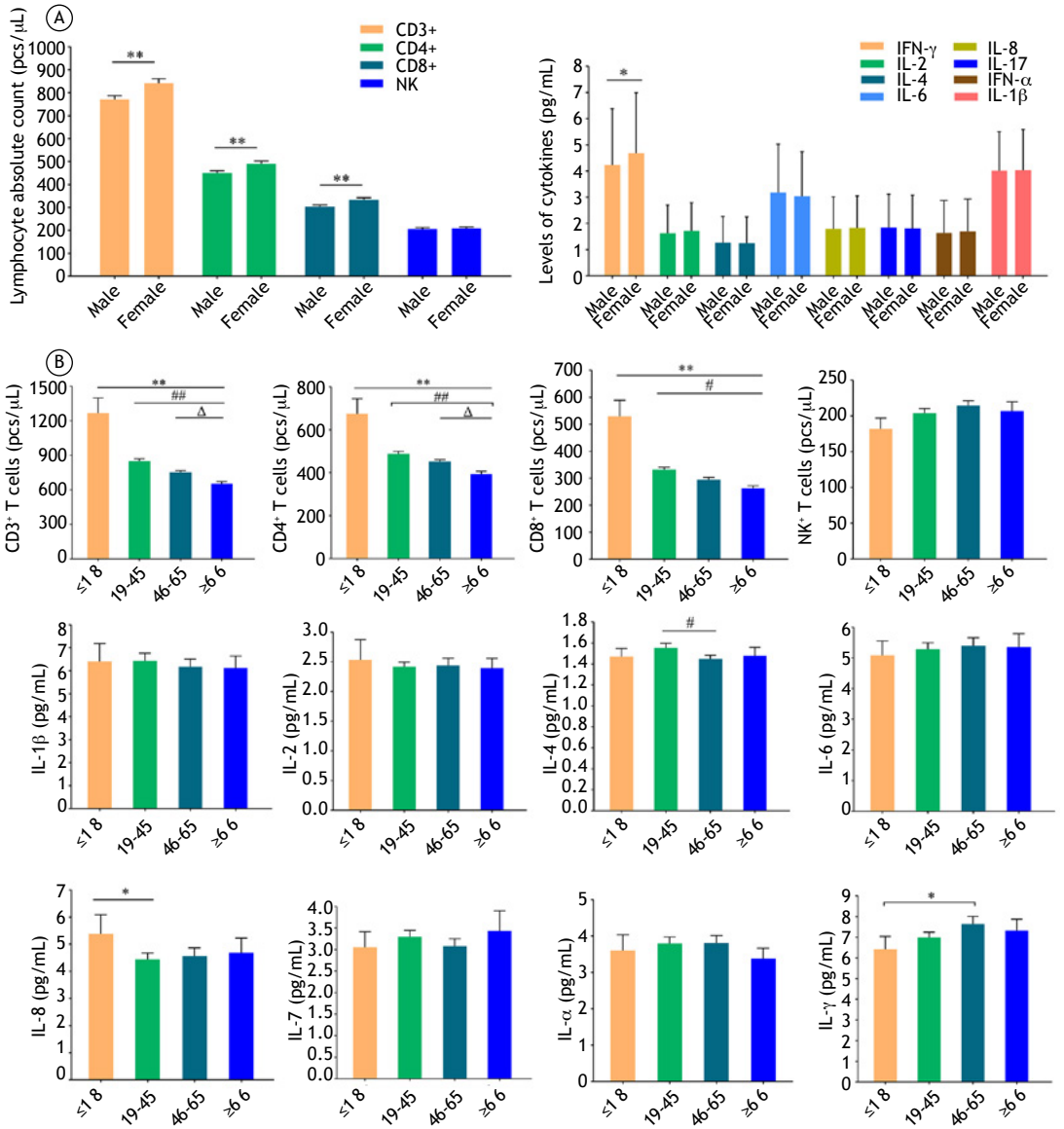

Figure 2. In A, absolute numbers of $CD3^+$ T, $CD4^+$ T, and $CD8^+$ T lymphocytes in active tuberculosis patients showing significant differences between men and women, except for NK cells. There were no significant differences in cytokines levels between male and female patients, except for IFN-γ levels. In B, the absolute numbers of $CD3^+$ T, $CD4^+$ T, and $CD8^+$ T lymphocytes showing significant differences among the age groups (in years) of patients with active tuberculosis. The overall cytokine levels did not significantly differ among the age groups. pcs: pieces. *$p < 0.05$. **$p < 0.01$.

and increased expression levels of IL-12p70 were independent influencing factors for LTBI (Figure 4B). These influencing factors are a guide to the diagnosis and differentiation of ATB and LTBI.

To assess the diagnostic and predictive value of the associated factors that have an impact on ATB and LTBI, we performed ROC analyses of the associated factors. The results showed that the absolute $CD3^+$ T and $CD8^+$ T lymphocyte counts and combined factors (sensitivity and specificity were, respectively, 0.955 and 0.993; 0.774 and 0.955; and 0.951 and 0.977) were significantly more valuable for the diagnosis of ATB than were the absolute number of NK cells and the levels of IL-4 and IFN-γ (Figure 5A). As for the ROC analysis for LTBI, IL-12p70 and IFN-γ expression levels, as well as combined factors, showed diagnostic value (sensitivity and specificity were, respectively, 0.568 and 0.862; 0.493 and 0.908; and 0.649 and 0.725), and combined factors had the highest diagnostic value (Figure 5B).

DISCUSSION

Tuberculosis is an inflammatory disease whose progression or cure is largely determined by the relative strength of Mtb against the host immune system and is therefore considered to be caused by an unbalanced

immune response to Mtb infection.[8] Immunity in tuberculosis patients is dominated by cellular immunity, in which T cells are believed to play a vital role in the containment of Mtb infection for controlling the infection directly or indirectly. The cytokines of the organism are a vital part of the immune system as messengers, and their role in the fight against the invasion of Mtb is complex and multifaceted and is influenced by different host states.[9] Therefore, a comprehensive and systematic exploration of the relationship of lymphocytes and cytokines with Mtb infection is needed to provide a theoretical basis for a better understanding of the immune status of tuberculosis patients by immunology-based detection and diagnosis.

The present study showed that the absolute number of T lymphocytes was lower in patients with ATB than in HI. The difference was statistically significant, ATB patients having a poorer immune status than the healthy population. The absolute numbers of $CD4^+$ T, $CD8^+$ T, and NK cells were higher in LTBI patients when compared with HI, although this difference was not significant; however, the number of T cells available from peripheral blood can help assess the progression of the infection.[10] This study also showed that female patients with ATB are more immunocompetent than males. This is also consistent with findings of a previous study on sex differences and immune function.[11] In addition, the immune function of the patient gradually decreases with age. In contrast, as for cytokine-mediated inflammatory responses, the differences in expression in regard to age and sex were not significant. Our results showed that the levels of some cytokines, such as IL-1β, IL-5, IL-6, IL-10, and TNF-α, differed in ATB and LTBI. The reason for this phenomenon is that the cytokines produced in response to Mtb infection may reduce their immune response to limit tissue damage, and overproduction of cytokines may lead to failure to control the infection.[12] This can provide us with ideas for identifying ATB and LTBI.

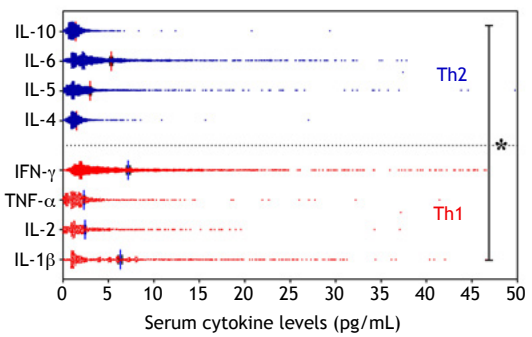

Figure 3. In the active tuberculosis group, Th1-type cytokine levels were higher than were Th2-type cytokine levels. The dot plot shows the mean and standard error of each cytokine level. Differences between overall Th1/Th2 cytokine levels were analyzed by nested t-test. *p < 0.05.

An essential factor in the control of tuberculosis is the characteristics of $CD4^+$ T cells that react after infection.[13] Usually, $CD4^+$ T cells produce more than one cytokine, especially Th1-type cytokines (IFN-γ and TNF-α), which are thought to be associated with protective immune responses and play an essential role in antituberculosis immunity.[14] Our data showed that ATB has significantly elevated Th1-type cytokine (IFN-γ) levels. These are cytokines specific for Mtb antigens.[15] Thus, our study confirms the vital role of Th1 cells in the pathogenesis of tuberculosis and suggests that Th1 cell occurrence may be specific for Mtb. However, higher amounts of Th1 cytokines may lead to a more severe disease that could reflect an increased bacterial load.[16] In conclusion, our study suggests that cytokines expressed by Th1 play a vital role in the pathogenesis of ATB.

We can speculate on the correlates of risk of ATB by the absolute number of T lymphocyte subpopulations and the levels of some cytokines. Logistic regression analysis showed that significantly lower absolute $CD3^+$ T, $CD8^+$ T, and NK cell counts, as well as significant lower IL-4 and IFN-γ expression levels, had an independent influence on ATB and they could be used as evidence of potential biomarkers

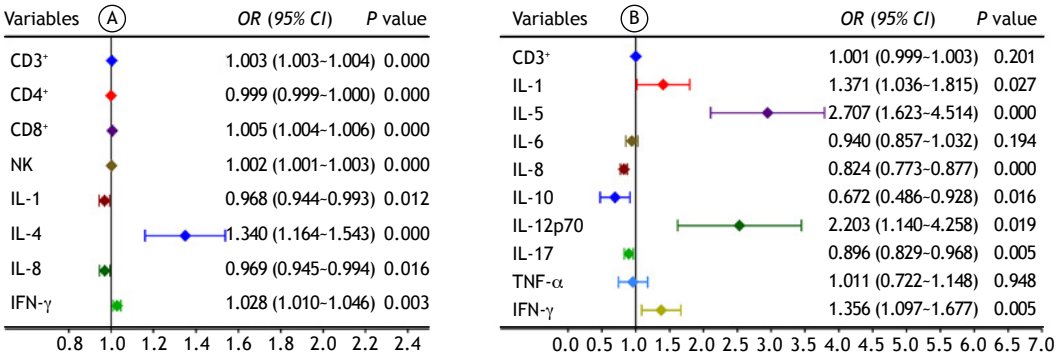

Figure 4. In A, logistic regression analysis shows that reduced absolute numbers of $CD3^+$ T, $CD8^+$ T, and NK cells, and reduced expression levels of IL-4 and IFN-γ were independent influencing factors for active tuberculosis ($p < 0.05$). In B, the analysis shows that reduced expression levels of IL-1, IL-5, and IFN-γ and increased expression levels of IL-12p70 were independent influencing factors for latent tuberculosis infection ($p < 0.05$).

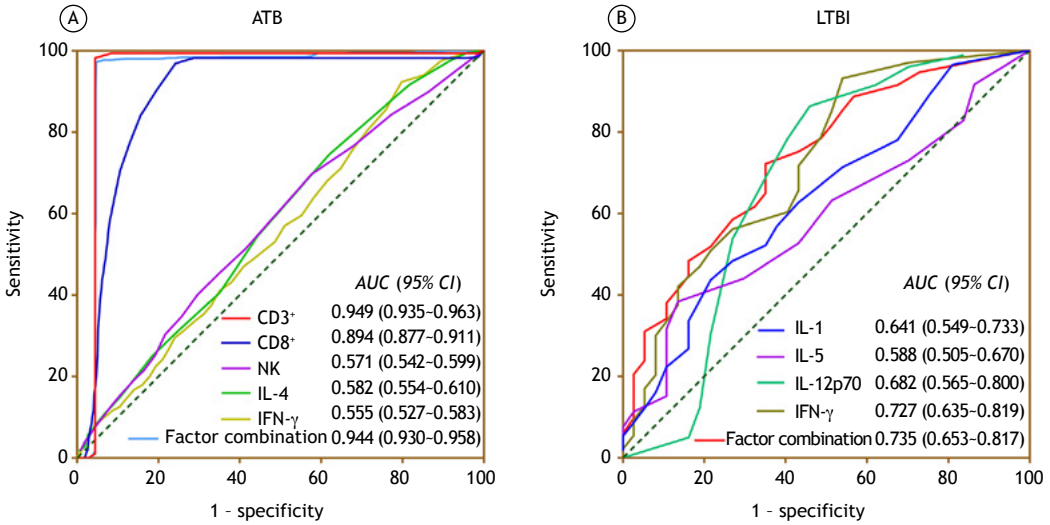

Figure 5. In A, ROC curves for active tuberculosis (ATB). Absolute CD3+ T and CD8+ T lymphocyte counts, as well as those of combined factors, showed a high diagnostic value for ATB ($p < 0.01$). In B, ROC curves for latent tuberculosis infection (LTBI). Expression levels of IL-12p70, IFN-γ, and combined factors showed diagnostic value for LTBI assessment. Combined factors had the highest diagnostic value ($p < 0.01$).

for tuberculosis surveillance. To identify LTBI, we also analyzed its influencing factors, and the results suggest that influencing factors of related diseases are mostly different; this may be a reference for the differential diagnosis of ATB and LTBI. To assess the diagnostic and predictive value of these influencers, we performed a ROC analysis. The results showed that the absolute $CD3^+$ T and $CD8^+$ T lymphocyte counts and combined factors were significantly more valuable, sensitive, and specific for the diagnosis of ATB than were IL-4, IL-12p70, and IFN-γ expression levels, and that combined factors are valuable in the diagnostic evaluation of LTBI. The diagnostic value of these biomarkers can also be assessed from the sensitivity and specificity of the ROC curves. When these factors are considered together, they have a better value in the diagnosis of Mtb infection or ATB.

In conclusion, the results of this study suggest that serum T lymphocytes and cytokines are essential reference indicators for assessing the immune status of patients with ATB. The absolute numbers of $CD3^+$ T, $CD8^+$ T, and NK cells, as well as the expression levels of IL-4 and IFN-γ, can be useful biomarkers for predicting and diagnosing tuberculosis. These markers, especially the combined factors, play a reference role in diagnosing and treating tuberculosis.

AUTHOR CONTRIBUTIONS

ZM: study concept; drafting, editing, and reviewing of the manuscript; and software analysis. SL: data analysis; draft preparation; methodology; and statistical analysis. YL, CL, and XW: data collection and experimental observation. XT, RD, and SZ: test operation. LW: funding acquisition; and editing and reviewing of the manuscript. All of the authors approved the final version of the manuscript.

CONFLICTS OF INTEREST

None declared.

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