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ORIGINAL RESEARCH

Analysis of Lymphocyte Immunologic Indexes in the Early Diagnosis of Active Pulmonary Tuberculosis of Adolescents in China

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Introduction: The aim of this study was to detect lymphocyte subpopulations to discover potential immunologic indicators to differentiate active tuberculosis (ATB) from latent tuberculosis infection (LTBI) and healthy controls (HC) and to predict the risk of progression of LTBI to ATB.

Methodology: Flow cytometry was used to detect lymphocyte subsets in ATB, LTBI and HC to compare the differences in lymphocyte subpopulation levels between groups, and Logistic regression was used to screen ATB-related immune indices, development of a novel nomogram model to predict the risk of progression to ATB in individuals with LTBI.

Results: Compared to the LTBI group, the ATB group had significantly higher $CD3^+CD4^+$T cell percentage, whereas $CD3^-CD16^+CD56^+$NK cell percentage, lymphatic cell, $CD3^+$T cell number, $CD3^+CD8^+$T cell number, and $CD3^-CD16^+CD56^+$NK cell number were significantly lower ($P<0.05$). Compared with the HC group, the ATB group had significantly higher $CD3^+$T cell percentage and $CD3^+CD4^+$T cell percentage, whereas $CD3^-CD16^+CD56^+$NK cell percentage, lymphatic cell, $CD3^+$T cell number, and $CD3^-CD16^+CD56^+$NK cell number were significantly lower ($P<0.05$); logistic regression analysis showed that $CD3^+CD4^+$T cell percentage, $CD3^+$T cell number, and $CD3^+CD8^+$T cell number were all independent indicators for the diagnosis of ATB ($P<0.05$), and based on these three immune indicators, we constructed diagnostic feature to distinguish ATB and LTBI, ATB from HC, and successfully developed a novel nomogram model to predict the risk of progression to ATB in individuals with LTBI.

Conclusion: A combined assay of lymphocyte-associated immune markers serves as a biomarker for early ATB diagnosis in adolescents, and established a predictive model to evaluate the risk of progression of LTBI to ATB.

Keywords: active tuberculosis, latent tuberculosis infection, lymphocyte subsets, mycobacterium tuberculosis, Tuberculosis

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis (MTB) and remains a major global public health problem.[1,2] According to the Global Tuberculosis Report 2024 published by the World Health Organization (WHO), 10.8 million new TB cases will occur globally in 2023, and 12% will be in children and adolescents, of which 1.25 million deaths.[3] TB is ranking above HIV/AIDS and PTB/EPTB diagnosis exhibits serious challenges owing to paucibacillary nature of specimens and localization of disease at sites that are difficult to access.[4] Until the COVID-19 pandemic, TB was the foremost cause of death from a single infectious agent and is now the second leading infectious killer after COVID-19.[5] Individuals with latent tuberculosis infection (LTBI) are potential "reservoirs" of active tuberculosis (ATB), and 5–10% of individuals with LTBI may develop ATB during their lifetime.[6] Adolescents are in the critical period of growth and development, and their immune system has not yet fully matured. Adolescent ATB has become the "invisible short board" in the prevention and control of TB worldwide due to "atypical symptoms, diagnostic difficulties, and neglected policies".[7] Patient deaths are partly due to misdiagnosis and underdiagnosis due to

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the lack of simple and effective diagnostic methods. Confirmation of the diagnosis of patients with ATB relies mainly on insensitive antacid bacillus smears or time-consuming mycobacterial culture methods, and the clinical use of these methods often leads to delayed treatment.[8,9] There are also tuberculin skin tests (TST) and Interferon gamma release assay (IGRA) that can be used to detect MTB infection, but they do not differentiate between ATB and LTBI and are not recommended for ATB diagnosis.[10,11] Additionally, molecular detection tools such as PCR, LAMP, and GeneXpert also have limitations. PCR cannot distinguish between live and dead bacteria, potentially leading to false positives, and relies on specialized laboratories and high costs.[12,13] LAMP cannot detect drug resistance, exhibits low sensitivity in smear-negative samples, and is susceptible to contamination interference.[14,15] GeneXpert demands high standards for equipment maintenance and power supply, making it difficult to implement in resource-poor areas. Furthermore, it only detects rifampicin resistance and cannot cover other drugs.[16,17] Therefore, there is an urgent need for effective biomarkers to differentiate ATB, LTBI, and healthy controls (HC) for early diagnosis and treatment of ATB. Studies indicate that in pediatric tuberculosis patients, the percentage and absolute counts of CD3+T cells and CD4+T cells, as well as the absolute count of NK cells, are significantly reduced, while the percentage of B lymphocytes is elevated.[18] In adult tuberculosis patients, previous studies indicate that CD3+T cells, CD4+T cells, B cells, and NK cell counts are lower than in healthy individuals, while CD8+T lymphocyte counts may be mildly elevated or show no significant difference to healthy individuals;[19–22] regarding trends in the percentage changes of lymphocyte subsets such as CD3+T cells, CD4+T cells, and CD8+T cells, previous studies have not reached consistent conclusions.[23–25] Dramatic fluctuations in sex hormone concentrations can influence immune responses and the pathogenesis of immune-related diseases, with age also serving as a significant factor affecting immune responses.[18,26] Furthermore, an analysis of MTB-specific antibody responses in an adolescent cohort from South Africa revealed that adolescents who progressed to disease exhibited a distinct immune phenotype.[27]

Most domestic and international studies categorize populations as either “children” or “adults”, with only a limited number of adolescents included in adult studies and no further age-stratified analysis. There has been no rigorous comparison of lymphocyte immune markers between adolescents with ATB, LTBI and HC. Existing adolescent tuberculosis research has primarily focused on epidemiology, clinical characteristics, and diagnostic method evaluations, while studies delving into cellular immunity remain relatively scarce. The aim of this study is to focus on this special group of adolescents, to understand the immune characteristics of ATB in adolescents by detecting their peripheral blood T-lymphocytes, B-lymphocytes, and NK-cells by flow cytometry, to explore the clinical value of related immune indexes in the diagnosis of early ATB, as well as to predict the risk of progression of LTBI to ATB, and to provide a clinical decision for the diagnosis of early ATB.

Methods

Study Design and Patients

This study enrolled 43 ATB patients diagnosed at the Fifth Hospital of Shijiazhuang between December 2024 and February 2025 (22 males, 21 females; average age 16.67±2.6 years), 45 LTBI cases (23 males, 22 females; mean age 16.13±1.0 years) were selected and diagnosed by tuberculosis specialists from the same hospital, and 20 HC participants (10 males, 10 females; mean age 17 years) were recruited. There was no difference between the three groups in terms of gender and age. Inclusion criteria: (1) ATB patients: with obvious clinical symptoms such as low-grade fever, night sweats, cough and coughing up blood; positive X-ray and bacteriologic examination to exclude other non-tuberculous lung diseases. (2) LTBI: strong positive results of tuberculin pure protein derivative (PPD) or positive results of recombinant Mycobacterium tuberculosis fusion protein (EC); no history of TB and negative X-rays and bacteriologic examination; no clinical symptoms. (3) HC persons: negative PPD results or negative EC results; no evidence of any suspected ATB or other disease. Exclusion criteria: (1) With primary or secondary immune system diseases, immune-related diseases, hematologic diseases. (2) Serious infection or transfusion of blood products within the last 3 months. The exclusion criteria apply equally to ATB, LTBI, and HC. This study was approved by the Ethical Review Committee of Shijiazhuang No. 5 Hospital (No. 202307–1). Informed consent was obtained from the parents or legal guardians of participants under the age of 18.

Research Method

All subjects underwent peripheral blood sampling in a fasting state. Two milliliters of blood were collected from each participant into EDTA anticoagulant tubes. Lymphocyte subset analysis was performed within 6 hours. If timely testing was not feasible, samples were stored at 4°C and analyzed within 48 hours. Samples from ATB patients, LTBI patients, and HC controls were collected by medical staff from the Department of Tuberculosis at Shijiazhuang Fifth Hospital. Flow cytometry detection of peripheral blood lymphocyte subpopulations: 10μL of lymphocyte subpopulation detection reagent (labeled antibodies CD3FITC, CD16PE+CD56PE, CD45PerCP-Cy5.5, CD4PE-Cy7, CD19APC, CD8APC-Cy7) was aspirated in a BD Trucount tube, and 50μL of the blood was added. Peripheral blood specimens were shaken and mixed, and then incubated at room temperature for 15 minutes, protected from light. 450 μL of BD FACS hemolysin was added, mixed, and incubated at room temperature for 15 minutes, protected from light, and the percentages of lymphocyte subpopulations and absolute counts were obtained using a flow cytometer (BD FACSCanto, USA).

Statistical Analysis

Statistical analyses were performed using SPSS 27.0, Graphpad Prism 10.1, and R 4.5.0; quantitative data were expressed as mean ± standard deviation or M (*P*25, *P*75); consistent with normal distribution and homogeneity of variance, comparisons between multiple groups were analyzed by one-way ANOVA, and nonconformity was tested by the nonparametric Kruskal Wallis test; Variables with $P<0.05$ in the univariate analysis were included in the multivariate logistic regression (stepwise method) to identify independent risk factors. The diagnostic value of the immune indicators was evaluated using the subject's work characteristics (ROC) curve; nomogram model were developed to predict the risk of progression to ATB in individuals with LTBI; ROC curves, calibration curves, and clinical decision curves were used to evaluate the discriminability, calibration performance, and clinical efficacy of the nomogram model, respectively. A P-value < 0.05 was considered statistically significant.

Results

Percentage of Lymphocyte Subsets in ATB, LTBI and HC

The $CD3^+CD4^+$T cell percentage was significantly higher in the ATB group compared with the LTBI group (P<0.05), whereas the $CD3^-CD16^+CD56^+$NK cell percentage was significantly lower than that in the LTBI group ($P<0.05$); $CD3^+$T cell percentage and $CD3^+CD4^+$T cell percentage were significantly higher in the ATB group compared with the HC group ($P<0.05$), while $CD3^-CD16^+CD56^+$NK cell percentage was significantly lower than that of the HC group ($P<0.05$) (Table 1 and Figure 1); there was no significant difference between the LTBI and HC groups in any of the lymphocyte subpopulation percentages (Figure 1).

Absolute Counts of Lymphocyte Subpopulations in ATB, LTBI, and HC

The lymphatic cell, $CD3^+$T cell number, $CD3^+CD8^+$T cell number, and $CD3^-CD16^+CD56^+$NK cell number in ATB compared with LTBI group were all significantly lower than those in the LTBI group ($P < 0.05$); lymphatic cell, $CD3^+$T cell number, and $CD3^-CD16^+CD56^+$NK cell number were all significantly lower in the ATB group compared with the HC group ($P < 0.05$) (Table 2 and Figure 1); there was no significant difference in the absolute number of lymphocyte subsets between the LTBI and HC groups (Figure 1).

Table 1 Percentage of Lymphocyte Subpopulations by Group

Variables (%)	ATB (n=43)	LTBI (n=45)	HC (n=20)	*F/H*	*P* value
$CD3^+$Tcell	73.00(69.45, 78.30)	70.70(65.80, 77.40)	67.60(63.05, 73.57)	9.06	0.01
$CD3^+CD4^+$Tcell	41.27±6.59	35.48±6.75	31.91±6.10	16.27	<0.01
$CD3^+CD8^+$Tcell	26.62±4.55	27.42±4.36	25.99±6.38	0.67	0.51
$CD3^-CD16^+CD56^+$NK cell	7.50(5.75, 10.65)	13.10(9.20, 18.00)	15.90(9.30, 18.18)	18.85	<0.01
$CD3^-CD19^+$B cell	14.00(9.60, 16.60)	11.60(9.60, 14.60)	11.25(8.35, 12.83)	3.99	0.14

Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection; HC, healthy controls.

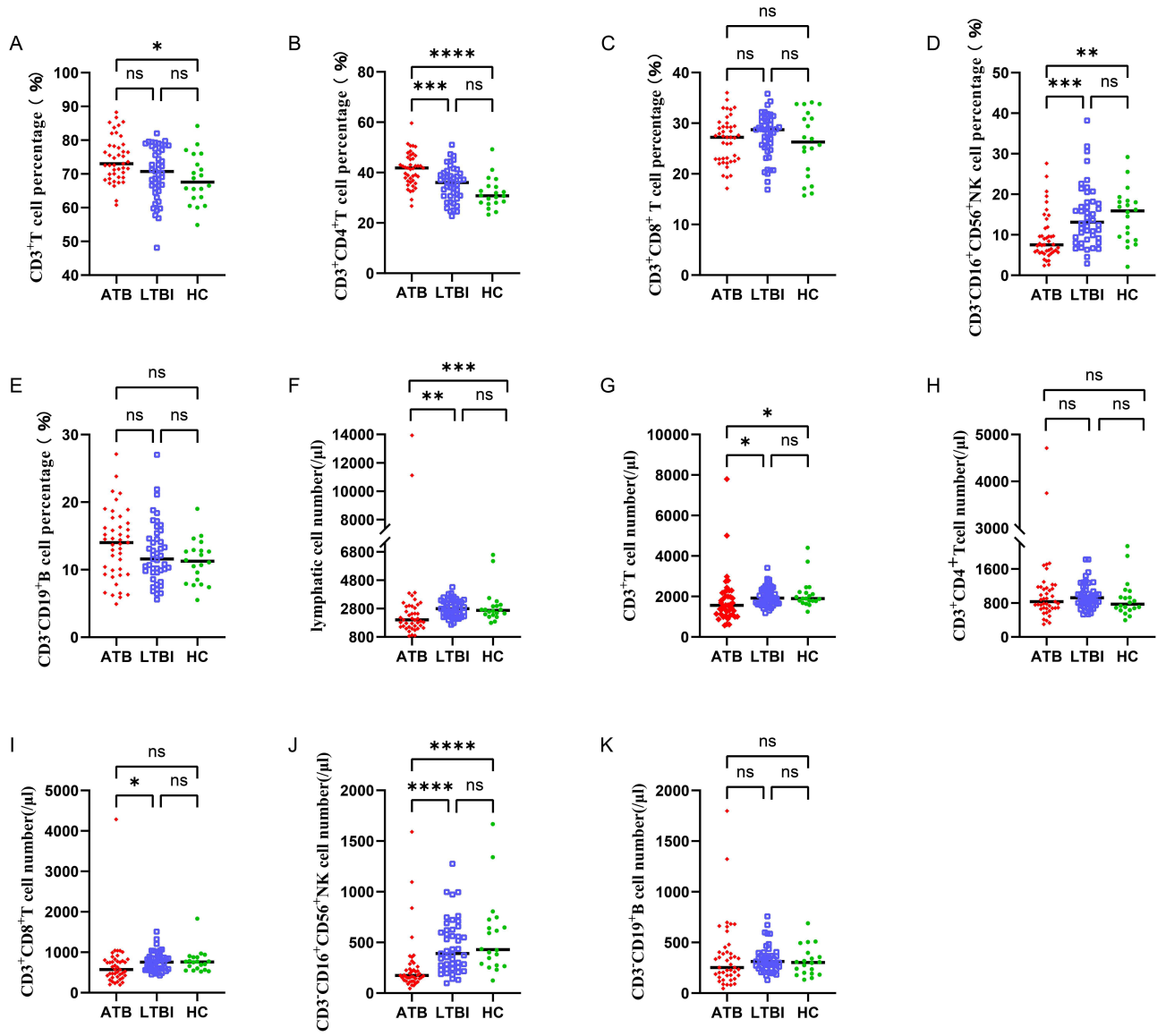

Figure 1 Lymphocyte subpopulation results for ATB, LTBI, and HC. ATB (n=43), LTBI (n=45), and HC (n=20), horizontal lines indicate median (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; **** $P < 0.0001$; ns, not significant). Red diamonds represent the ATB patients, blue squares represent the LTBI patients, and green dots represent the HC individuals. (**A**) Scatter plot of $CD3^+$T cell percentage among ATB, LTBI, and HC groups; (**B**) Scatter plot of $CD3^+CD4^+$T cell percentage among ATB, LTBI, and HC groups; (**C**) Scatter plot of $CD3^+CD8^+$T cell percentage among ATB, LTBI, and HC groups; (**D**) Scatter plot of $CD3^-CD16^+CD56^+$NK cell percentage among ATB, LTBI, and HC groups; (**E**) Scatter plot of $CD3^-CD19^+$B cell percentage among ATB, LTBI, and HC groups; (**F**) Scatter plot of lymphatic cell number among ATB, LTBI, and HC groups; (**G**) Scatter plot of $CD3^+$T cell number among ATB, LTBI, and HC groups; (**H**) Scatter plot of $CD3^+CD4^+$T cell number among ATB, LTBI, and HC groups; (**I**) Scatter plot of $CD3^+CD8^+$T cell number among ATB, LTBI, and HC groups; (**J**) Scatter plot of $CD3^-CD16^+CD56^+$NK cell number among ATB, LTBI, and HC groups; (**K**) Scatter plot of $CD3^-CD19^+$B cell number among ATB, LTBI, and HC groups.

Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection; HC, healthy controls.

Multiple Logistic Regression Analysis of ATB Related Immune Indicators

Multiple logistic regression analysis with statistically significant lymphocyte subpopulations as independent variables was performed with ATB as the reference category, and ATB was compared with LTBI and HC, respectively, and the results showed that $CD3^+CD4^+$T cell percentage, $CD3^+$T cell number and $CD3^+CD8^+$T cell number were independent risk factors for the diagnosis of ATB ($P < 0.05$) (Table 3).

Diagnostic Value of Subject Work Characteristics (ROC) Curve Evaluation Indicators

The diagnostic value of the three immune indicators screened based on multivariate logistic regression analysis was evaluated by ROC curves, and the results showed that in distinguishing ATB from LTBI, the AUCs for $CD3^+CD4^+$T cell

Table 2 Absolute Number of Lymphocyte Subpopulations in Each Group

Variables (/μL)	ATB (n=43)	LTBI (n=45)	HC (n=20)	*H*	*P* value
Lymphatic cell	2007.00(1541.50, 2961.50)	2789.00(2351.00, 3311.00)	2675.50(2392.00, 3003.75)	13.82	<0.01
$CD3^+$Tcell	1562.00(1118.50, 2093.50)	1921.00(1646.00, 2336.00)	1899.50(1780.75, 2189.50)	9.97	<0.01
$CD3^+CD4^+$Tcell	831.00(677.50, 1163.50)	921.00(772.00, 1095.00)	772.50(664.50, 969.25)	2.10	0.35
$CD3^+CD8^+$Tcell	545.00(360.50, 757.00)	755.00(584.00, 867.00)	760.00(594.00, 878.25)	10.78	<0.01
$CD3^+CD16^+CD56^+$NK cell	174.00(135.50, 249.50)	391.00(256.00, 621.00)	429.00(285.25, 666.50)	31.41	<0.01
$CD3^-CD19^+$B cell	253.00(175.00, 405.00)	313.00(244.00, 373.00)	302.00(198.25, 366.50)	1.53	0.47

Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection; HC, healthy controls.

Table 3 Multiple Logistic Regression Analysis of ATB-Related Immune Indicators

Variables	*β* value	Standard Error	*Wald* χ^2 value	*P* value	*OR (95% CI)*
Comparison of ATB and LTBI					
$CD3^+$Tcell percentage (%)	0.143	0.075	3.594	0.058	1.154(0.995~1.337)
$CD3^+CD4^+$Tcell percentage (%)	−0.270	0.083	10.537	0.001	0.763(0.649~0.899)
$CD3^-CD16^+CD56^+$NK cell percentage (%)	0.191	0.102	3.482	0.062	1.210(0.990~1.479)
Lymphatic cell number (/ul)	−0.001	0.001	1.355	0.244	0.999(0.997~1.001)
$CD3^+$Tcell number (/ul)	0.005	0.002	10.136	0.001	1.005(1.002~1.008)
$CD3^+CD8^+$Tcell number (/ul)	−0.006	0.002	5.333	0.021	0.994(0.990~0.999)
$CD3^-CD16^+CD56^+$NK cell number (/ul)	0.000	0.003	0.005	0.943	1.000(0.995~1.005)
Comparison of ATB and HC					
$CD3^+$Tcell percentage (%)	0.169	0.097	3.041	0.081	1.185(0.979~1.433)
$CD3^+CD4^+$Tcell percentage (%)	−0.411	0.101	16.511	0.000	0.663(0.544~0.808)
$CD3^-CD16^+CD56^+$NK cell percentage (%)	0.140	0.117	1.426	0.232	1.150(0.914~1.446)
Lymphatic cell number (/ul)	−0.002	0.001	1.402	0.236	0.998(0.996~1.001)
$CD3^+$Tcell number (/ul)	0.007	0.002	12.593	0.000	1.007(1.003~1.011)
$CD3^+CD8^+$Tcell number (/ul)	−0.008	0.003	6.484	0.011	0.992(0.986~0.998)
$CD3^-CD16^+CD56^+$NK cell number (/ul)	0.001	0.003	0.179	0.673	1.001(0.995~1.008)

Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection; HC, healthy controls.

percentage, $CD3^+$T cell number and $CD3^+CD8^+$T cell number were 0.728 (*95% CI*: 0.623–0.833), 0.676 (*95% CI*: 0.559–0.793), 0.686 (*95% CI*: 0.570–0.801), and the combined assay AUC was 0.814 (*95% CI*: 0.726–0.902); and in distinguishing between ATB and HC, the AUCs for $CD3^+CD4^+$T cell percentage, $CD3^+$T cell number and $CD3^+CD8^+$Tcell number were 0.867 (*95% CI*: 0763–0.972), 0.685 (*95% CI*: 0.557–0.814), 0.691 (*95% CI*: 0.564–0.818), and the AUC of the combined assay was 0.897 (*95% CI*: 0.801–0.992) (Figure 2).

Development of a Nomogram Model to Predict the Risk of Progression to ATB in Individuals with LTBI

To predict the risk of progression to ATB in individuals with LTBI, weighted scores for $CD3^+CD4^+$T cell percentage, $CD3^+$T cell number, and $CD3^+CD8^+$T cell number, and a novel visual column-line graph model was developed, which can determine whether an individual with LTBI has a higher risk of progressing to ATB based on the results of risk coefficient calculations (Figure 3). The ROC curve analysis showed that the AUC of the three indicators for combined detection was 0.814 (*95% CI*: 0.726~0.902), indicating that the model had a good discriminatory degree, and the calibration curve was generated by plotting the actual probability of progression (y-axis) versus the predicted probability (x-axis), and the results of the Hosmer-Lemeshow test (P=0.480) indicated that there was a good agreement between the actual probability and the predicted probabilities were in good agreement with each other (Figure 4), and the clinical utility of the model was assessed by clinical decision curves, which showed that significant net clinical benefit was demonstrated in most of the high-risk threshold ranges (Figure 5). Finally, in order to verify the stability and reliability of the model, Bootstrap method of internal cross-

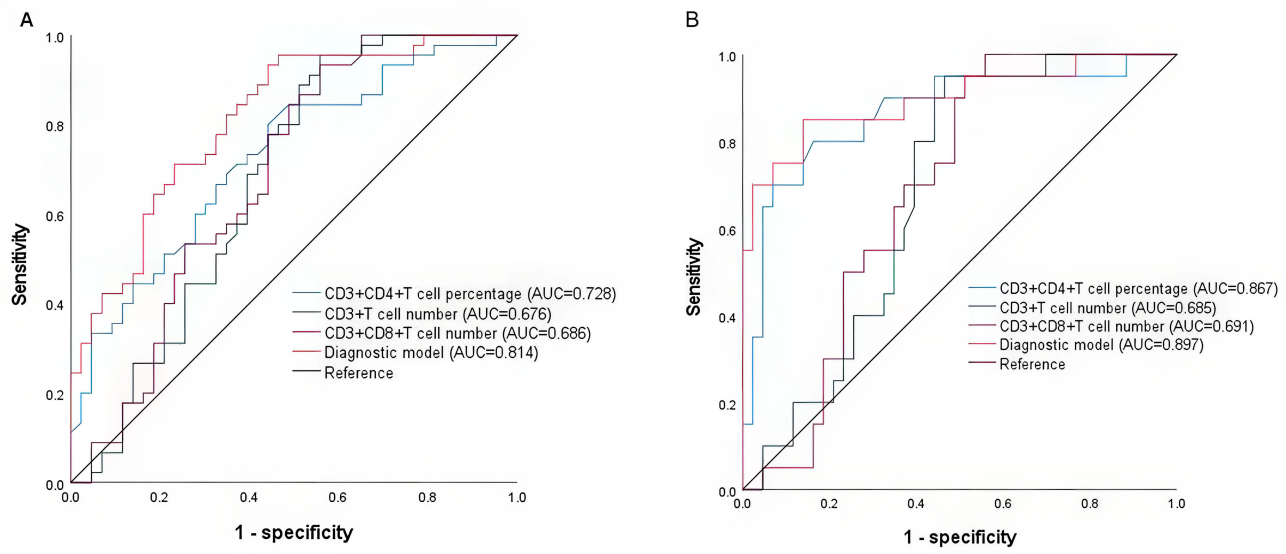

Figure 2 ROC curve analysis: (**A**) the ROC curves for diagnosis of ATB versus LTBI; (**B**) the ROC curves for diagnosis of ATB versus HC.
Abbreviations: ROC, receiver operating characteristic; ATB, active tuberculosis; LTBI, latent tuberculosis infection; HC, healthy controls.

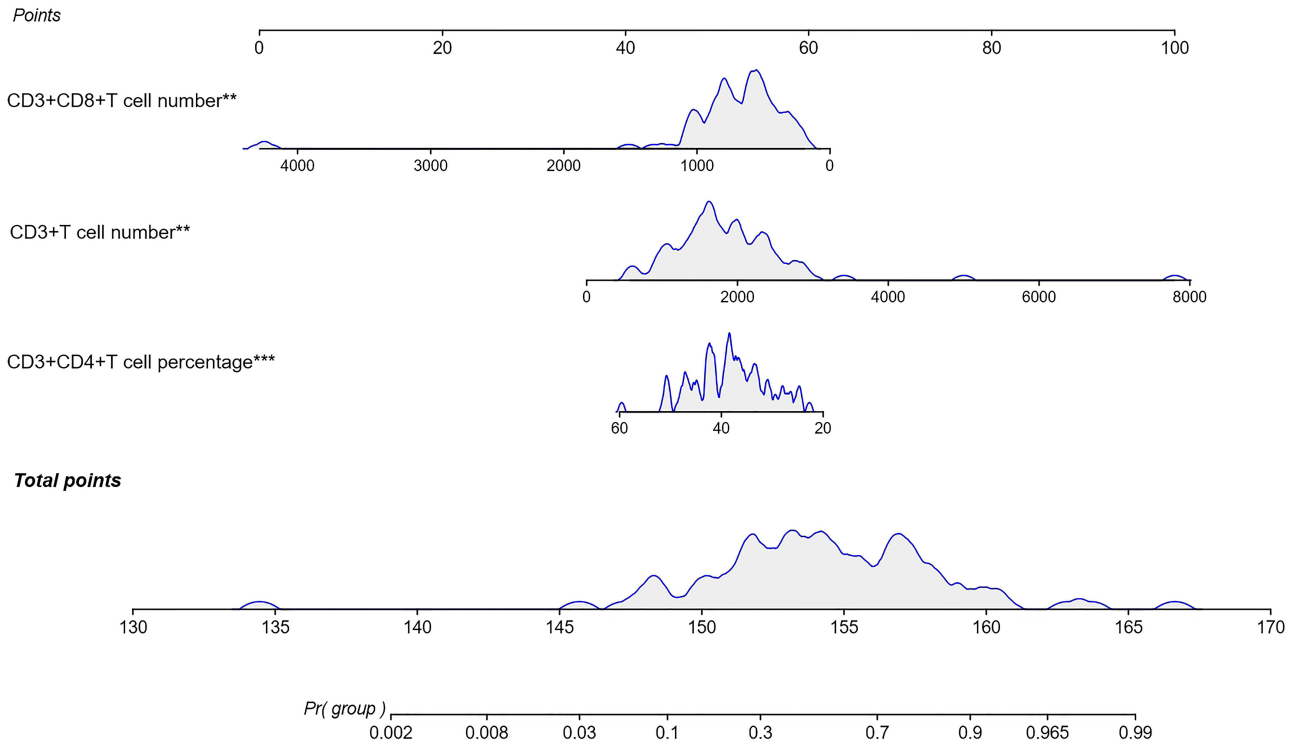

Figure 3 Construction of a nomogram for predicting the risk of progressing into ATB from LTBI. (**$P < 0.01$; ***$P < 0.001$).
Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection.

validation 1000 times and ten-fold cross-validation are used to validate the model, and the AUCs are 0.780 and 0.781, respectively, which confirms that the model performs stably under different data partitioning strategies.

Discussion

In this study, we systematically analyzed the characteristics of lymphocyte subpopulations in three different MTB infection states of ATB, LTBI and HC in a special group of adolescents by flow cytometry, and the core findings

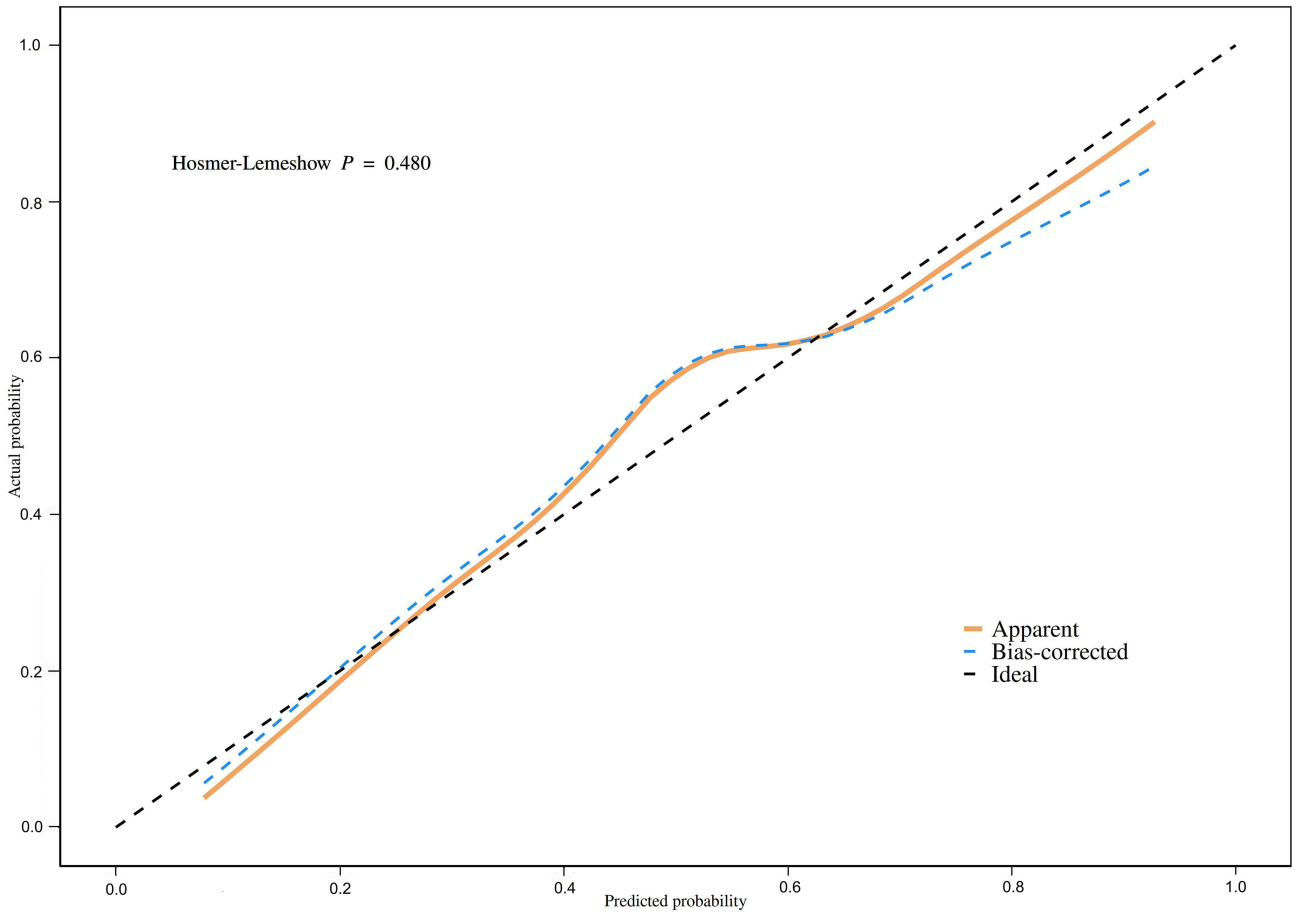

Figure 4 Calibration curve of the nomogram.

included the presence of a significant immune imbalance in patients with ATB and an immune homeostasis maintenance state in individuals with LTBI. These results reflect a pattern of immune response that is both similar to and different from that of adult patients in the adolescent population.

The results of this study indicate a significant reduction in $CD3^+$T cell counts, consistent with previous findings. $CD3^+$T lymphocytes participate in the body's cellular immune response. Persistent MTB infection continuously stimulates T cell activation, leading to decreased absolute $CD3^+$T cell counts in peripheral blood. This suggests the presence of immunodeficiency.[28]

The point of difference is the age-specificity of the immune response in adolescents versus adults. Our study found that the percentage of $CD3^+CD4^+$T cells in adolescent ATB patients was significantly higher than in the LTBI and HC groups, E. Venturini's findings indicate a reduction in the $CD3^+CD4^+$T cells percentage.[18] $CD4^+$T cells play a primary role in anti-tuberculosis immunity during the course of tuberculosis, primarily by aiding other cells in regulating the immune response.[29] A review suggests that the steep decline in thymic Tregs during adolescence may create a window of increased immune susceptibility during puberty.[30] Furthermore, the reconstitution of immune cell populations during infection may lead to alterations in the proportion of $CD4^+$T cells. However, concrete experimental data to substantiate this hypothesis are currently lacking. Future studies should validate the underlying mechanisms of this phenomenon through further immune function analysis and cell subset investigations. The $CD3^+CD8^+$T cell number in the adolescent ATB group was significantly lower than that in the LTBI group, and in D. Lewinsohn's study it was shown that the $CD3^+CD8^+$T cell number was in a maintained or mildly elevated.[22] $CD8^+$T cells primarily execute cellular immune functions, exhibiting strong and sustained responses. However, the protective role of $CD8^+$T cells is limited. Research indicates that the body mobilizes $CD3^+CD8^+$T cells to directly kill intracellular and extracellular MTB, leading to

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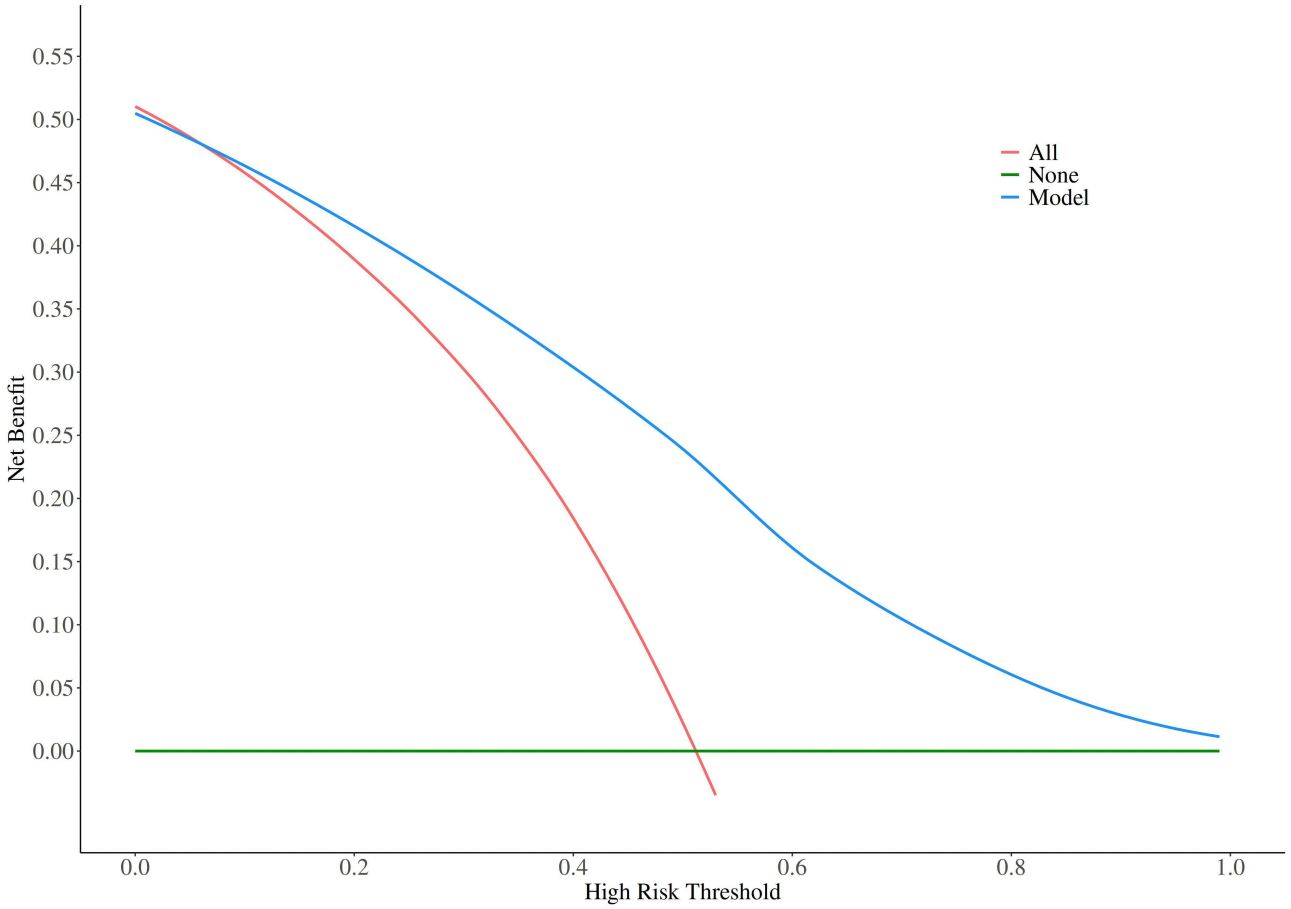

Figure 5 Clinical decision curve of the nomogram.

significant depletion of these cells.[28,31] There were no significant differences between the adolescent LTBI group and the HC group in any lymphocyte subset, which is different from the low level of Th1 response common in adult LTBI.[32,33] On one hand, these data suggest that the host immune systems of LTBI individuals may have temporarily succeeded in resisting MTB, particularly considering that most of the adolescent LTBI patients included in our study were primarily exposed for the first time and for a relatively short duration. Although the thymus has begun to degenerate at puberty, it still possesses a certain T-lymphocyte output capacity, and $CD4^{+}CD25^{+}FoxP3^{+}$regulatory T cells have a stronger immune-suppressor function, which, by secreting TGF-β and IL-10 inhibits effector T lymphocyte activation and avoids excessive immune responses.[34,35] Consequently, they did not exhibit overall characteristics indicative of an immune barrier or immune deficiency. On the other hand, the immunological markers employed in our study may have been insufficiently specific or comprehensive to fully capture the subtle immunological differences between LTBI patients and healthy controls. Therefore, we recommend that future research expand to include a broader range of immunological markers and potential risk factors to provide a more comprehensive analysis of the characteristics of the immune response. These differences suggest that the diagnosis of ATB in adolescents needs to take into account age-specific immune thresholds and avoid the direct application of adult criteria.[36]

We utilized multivariate logistic regression to further screen $CD3^{+}CD4^{+}$T cell percentage, $CD3^{+}$T cell number and $CD3^{+}CD8^{+}$T cell number three immune indicators were constructed to distinguish the diagnostic features of ATB from LTBI and ATB from HC. Based on this we developed a novel visual nomogram model to predict the risk of progression to ATB in individuals with LTBI, the nomogram model exhibits good discriminatory ability, calibration performance, and clinical utility, compared with the traditional model, the novel model not only effectively predicts the risk of an individual with LTBI evolving into an ATB patient, but also has the unique advantage of visualizing the distribution

of the number of patients with different values of the same predictor, thus presenting a more comprehensive picture of the distribution of patients in the dataset. The ROC curve shows that the model has good discriminatory power, and the calibration curve indicates good agreement between the model's predicted probabilities and the actual observations. The clinical decision curve shows that the model avoids over-treatment of low-risk individuals by screening for individuals with LTBI at high risk of progression (eg, $CD3^+$T cell number < 1500/μL and $CD3^+CD4^+$T cell percentage > 40%), which is consistent with the WHO guideline that "LTBI prophylaxis should prioritize coverage of high-risk populations",[37] the model significantly outperforms "all-intervention" or "no-intervention" strategies over a wide range of thresholds and has good clinical utility, which provides physicians with richer information for in-depth analyses and studies of the distribution of patients within a given range.[38] Based on the nomogram model proposed in this paper, the risk of progression from LTBI to ATB can be effectively calculated for each adolescent case. For example, an LTBI patient with a $CD3^+CD8^+$T cell count of 500/μL (score: 55 points), a $CD3^+$T cell count of 1700/μL (score: 50 points), and a $CD3^+CD4^+$T cell percentage of 40% (score: 50 points) would have a total score of 55 + 50 + 50 = 155 points. Based on this risk factor calculation, the LTBI patient is determined to have a high risk of progressing to ATB and is thus included in the close follow-up and preventive treatment cohort.

In summary, we focused on the diagnosis of ATB in adolescents, explored the immune differences that exist among ATB, LTBI, and HC in adolescents, compared the similarities and differences with the findings in adults or children, screened three immune indicators to construct two sets of diagnostic features for ATB versus LTBI, and ATB versus HC, and developed a novel visual nomogram model for predicting progression of an individual with LTBI to ATB, which has good differentiation, calibration performance and clinical utility.

This study has several limitations. First, The sample size was limited and the recruitment period was short, with only internal validation conducted, which may affect the stability of the predictive model. Second, the analysis did not incorporate activation or exhaustion markers (such as HLA-DR, CD38, etc), cytokines (such as IL-2, etc), or functional immune responses, limiting the biological depth of the research. Third, unmeasured confounding factors (eg, BCG vaccination status) introduce potential selection bias, limiting mechanistic exploration. Future research should prioritize external validation in larger, independent adolescent cohorts. Integrating additional immune biomarkers and potential risk factors is essential to expand the study's depth and breadth. Furthermore, leveraging advanced machine learning techniques to refine risk prediction models will enable more precise, personalized, and accurate testing.

Conclusions

In this study, we aimed to better understand this special group of adolescents from an immunological perspective, screened three immunological indicators to construct a diagnostic profile, and developed a novel visual nomogram model to predict the risk of progression to ATB in individuals with LTBI, which requires further exploration and validation in the future. Our findings provide the clinical value of early ATB diagnosis for this special group of adolescents.

Abbreviations

ATB, Active Tuberculosis; HC, Healthy Controls; IGRA, Interferon-gamma release assays; LTBI, Latent Tuberculosis Infection; MTB, Mycobacterium tuberculosis; TB, Tuberculosis; TST, Tuberculin skin tests; WHO, World Health Organization.

Data Sharing Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval Statement

This study followed the Declaration of Helsinki, the study was approved by the Medical Ethics Management Committee of Shijiazhuang No. 5 Hospital (202307-1), and informed consent was obtained from all study subjects before testing.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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