Original Article

Retrospective analysis of the clinical utility of multi-cytokine profiles in smear-negative pulmonary tuberculosis

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ABSTRACT

الأهداف: تقييم بروفيلات السيتوكين واختبار إنترفيرون–جاما (IGRA) لقدراتها التشخيصية في التمييز بين السل (TB) وغير السل، وكذلك السل الرئوي سلبي المسحة (SNPT) عن السل الرئوي إيجابي المسحة (SPPT).

المنهجية: اشتملت الدراسة على 125 مشاركًا، 77 منهم كانوا مصابين بالسل و 48 لم يكونوا كذلك، جمعنا البيانات الديموغرافية والسريرية والمعملية، بما في ذلك مستويات السيتوكين ونتائج IGRA. تم تقسيم مرضى السل إلى مجموعتين فرعيتين: SNPT (العدد = 42) و SPPT (العدد = 35).

النتائج: مقارنة بغير السل، كان لدى مجموعة السل مؤشر كتلة جسم أقل، وعدد كريات الدم البيضاء والخلايا متعادلة القطب والخلايا وحيدة النواة والتسرب الوريدي السريع والبروتين السي–التفاعلي أعلى ($p<0.05$). أظهر مرضى السل مستويات أعلى من IL–2 و IL–6 و IFN–γ و IL–8 القيمة الإحصائية ($p<0.001$) وإيجابية IGRA أعلى (88.3% مقابل 29.2%، $p<0.001$). بين SNPT و SPPT، لوحظت حجوم تأثير معتدلة ل IFN–α و IL–2 و IL–10 و IL–8 (كوهين د 0.59–0.76)، مع إيجابية IGRA أقل في SNPT مقابل 97.1%، $p=0.015$. أشارت تحليلات ROC إلى أن IFN–α و IL–2 و IL–10 و IL–8 لها دقة متوسطة لتشخيص SNPT (مساحات تحت المنحنى= 0.668–0.734)، وأن الجمع بينها حسّن الدقة (مساحة تحت المنحنى=0.759، حساسية 80%، نوعية 64.2%).

الخلاصة: إن النهج متعدد الحيومات الذي يجمع بين هذه السيتوكينات يظهر دقة تشخيصية محسنة للسل.

Objectives: To evaluate cytokine profiles and interferon-gamma release assay (IGRA) for their diagnostic capabilities in the differentiation of tuberculosis (TB) from non-TB conditions, as well as smear-negative pulmonary tuberculosis (SNPT) from smear-positive pulmonary tuberculosis (SPPT).

Methods: A total of 125 participants were included, 77 of whom had TB and 48 who didn't, and demographic, clinical, and laboratory data were collected, including cytokine levels and IGRA results. The TB patients were further divided into 2 subgroups: SNPT (n=42) and SPPT (n=35).

Results: Compared to non-TB, the TB group had lower BMI, higher WBC, neutrophils, monocytes, ESR and CRP ($p<0.05$). TB patients showed higher IL-2, IL-6, IFN-γ, IL-8 ($p<0.001$) and higher IGRA positivity (88.3% versus [vs.] 29.2%, $p<0.001$). Between SNPT and SPPT, moderate effect sizes were observed for IFN-α, IL-2, IL-10, IL-8 (Cohen's d 0.59-0.76), with lower IGRA positivity in SNPT (81.0% vs. 97.1%, $p=0.015$). ROC analysis indicated IFN-α, IL-2, IL-10, IL-8 had moderate accuracy for SNPT diagnosis (AUCs 0.668-0.734), and combining these improved accuracy (AUC 0.759, 80% sensitivity, 64.2% specificity).

Conclusion: A multi-biomarker approach combining these cytokines demonstrates enhanced diagnostic accuracy for tuberculosis.

Keywords: *Mycobacterium tuberculosis*, smear-negative pulmonary tuberculosis, cytokine profiles, retrospective analysis

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Tuberculosis (TB) continues to pose a considerable global health burden, and smear-negative pulmonary tuberculosis (SNPT) represents a significant proportion of TB cases.[1,2] Smear-negative pulmonary tuberculosis is defined by the absence of detectable bacteria in sputum smears, despite clinical and radiological indications of pulmonary TB.[3] The limited bacterial presence in SNPT complicates diagnosis using conventional techniques, resulting in delays in treatment initiation and heightened risk of disease transmission.[4] Given these challenges, the investigation of alternative diagnostic biomarkers is crucial in addressing the diagnostic limitations associated with SNPT.[5,6]

The academic community has been actively engaged in efforts to enhance the diagnosis of SNPT, with a focus on investigating new biomarkers and detection methods.[7-9] Recent research initiatives have explored a wide range of potential SNPT biomarkers through various investigative approaches. Adopting the Xpert MTB/RIF assay, a type of nucleic acid amplification test (NAAT), has greatly advanced the accuracy of TB diagnostics and the identification of SNPT.[10,11] Luo et al. have conducted research on the assessment of novel antigens, including Rv0310c and Rv1255c, which have demonstrated potential in differentiating between SNPT and smear-positive pulmonary TB patients (SPPT) patients.[12] Furthermore, proteomic analyses have garnered interest for their ability to reveal the unique protein profiles associated with SNPT. Through the examination of protein expression patterns in clinical samples, researchers have identified potential biomarkers that could aid in distinguishing SNPT from other pulmonary conditions.[13] An innovative methodology has integrated machine learning algorithms with metabolomic and clinical data, utilizing advanced data analytics to develop predictive models. These models demonstrate a high level of accuracy in distinguishing SNPT from SPPT, providing a promising advancement beyond conventional diagnostic approaches. The combination of multimodal biomarkers and computational methods is expected to significantly enhance the diagnosis of SNPT in clinical settings.[14] Interferon-gamma release assays (IGRAs) are commonly utilized in the diagnosis of tuberculosis; however, their limitations in differentiating between active infection, past TB infection, and vaccine-induced responses underscore the necessity for more advanced diagnostic methods, particularly for SNPT.[15,16]

As part of the immune system's response to *Mycobacterium tuberculosis* (Mtb), cytokines play a crucial role and have been investigated as possible systemic indicators for TB diagnosis.[17] The interaction between various cytokines, including IFN-γ, interlukin (IL)-12, IL-6, and IL-1, is important in the development and control of TB.[18,19] This study aims to assess the clinical utility of multiple cytokine detection for the diagnosis of SNPT, with the objective of enhancing diagnostic precision and facilitating prompt initiation of treatment.

Methods. This retrospective study analyzed a cohort of 77 tuberculosis patients treated at the First Affiliated Hospital of Dali University, Yunnan, China from September 2022 to January 2024. The cohort comprised 42 patients with sputum smear-negative results and 35 patients with sputum smear-positive results. In accordance with the Chinese Pulmonary Tuberculosis Diagnostic Criteria (WS288-2017), the study included participants aged 18 years and older. Smear-negative pulmonary tuberculosis patients were identified as those with 3 consecutive negative acid-fast staining results from morning sputum samples, whereas SPPT exhibited at least one positive acid-fast staining result from 3 consecutive morning sputum samples. The exclusion criteria for patients with TB included: i) prior anti-tuberculosis therapy; ii) concurrent infections with HIV, HBV, HCV, autoimmune disorders, malignancies, or other comorbidities; and iii) pregnancy or breastfeeding status. Additionally, a control group of 48 individuals matched for age and gender, diagnosed with non-TB pneumonia during the same time frame, were included in the study. These individuals tested negative for TB-DNA. Exclusion criteria for the control group encompassed co-infection with HIV, HBV, HCV, autoimmune diseases, malignant tumors, or other medical conditions, as well as pregnancy or lactation. Approval for the study was obtained from the Ethics Committee at the First Affiliated Hospital of Dali University. The research adhered to the principles of the Declaration of Helsinki, under reference number #DFY20220605001.

A thorough literature search was carried out utilizing Cochrane Library databases, Embase, and PubMed, incorporating relevant keywords and Medical Subject Headings (MeSH) pertaining to tuberculosis, cytokines, interferon-gamma release assays (IGRA), and diagnostic accuracy. To ensure the latest and highest quality evidence, we also included systematic

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reviews and meta-analyses. The search was limited to English articles from the past 10 years to focus on recent advancements. We reviewed titles, abstracts, and full texts to identify studies relevant to our objectives, including those on the use of cytokines and IGRA in tuberculosis diagnosis.

Sample collection. Each participant provided 5 milliliters of peripheral venous blood following an overnight fast. To prepare for subsequent analysis, the plasma was spun at 3000 rpm for 10 minutes and then kept at -80°C. Whole blood samples for the IGRA were collected in lithium heparin anticoagulant tubes.

Cytokine measurement. The concentrations of various cytokines including IL-2, IFN-a, IL-4, TNF-α, IL-8, IFN-γ, IL-17, IL-12p70, IL-10, IL-6, IL-5, and IL-1β were quantified using the BriCyte E6 flow cytometer (Mindray, Shenzhen, China) in conjunction with the RAISECARE reagent kit from Qingdao, China. Based on manufacturer's guidelines, the measured cytokine levels ranged from as low as 2.44 pg/mL to as high as 10,000 pg/mL.

IGRA. The assay known as QuantiFERON-TB Gold In-Tube (QFT-GIT) was carried out following the manufacturer's instructions (Qiagen, Carnegie, Australia). The outcomes were classified as positive, negative, or indeterminate following the criteria established by the manufacturer.

TB-DNA testing. Using a nucleic acid detection kit by DaAn Gene (Guangzhou, China), the TB-DNA was identified via PCR-fluorescent probe technique. Each assay iteration adhered to the manufacturer's guidelines and incorporated both positive and negative controls. The minimum detectable limit was determined to be 500 copies/mL.

Statistical analysis. The data analysis was carried out with the Statistical Package for the Social Sciences, version 27.0 Windows (IBM Corp., Armonk, NY, USA). A *p*-value of <0.05 was considered statistically significant. To evaluate the significance of differences in proportions between the SNPT and SPPT groups, as well as between the TB and non-TB groups, we applied the chi-square test to evaluate categorical variables. Continuous variables were first assessed for normality. Data with a normal distribution were reported as mean with standard deviation (SD), and group differences were tested using the Student's t-test. For data not following a normal distribution, results were shown as median with interquartile range (IQR), and the Wilcoxon rank-sum test was used for group comparisons. Given the study's limited sample size, we utilized Cohen's d to quantify the effect sizes, allowing for quantification of differences in various cytokines between groups. We categorized effect sizes into 3 groups: small (ranging from 0.20 to 0.49), medium (0.50 to 0.79), and large (0.80 or greater). For further investigation, cytokines with the highest effect sizes were selected. To evaluate the diagnostic potential of these cytokines, both individually and collectively, receiver operating characteristics (ROC) curve analysis was employed. The analysis included calculating the area under the curve (AUC) and assessing sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The Youden index was used to determine the best cutoff points.

Results. Table 1 provides an overview of the demographic and clinical characteristics of the 125 participants, consisting of 77 individuals in the TB group and 48 in the non-TB group. The median age in the TB group was 53 years (IQR: 40-64), while in the non-TB group, it was 59 years (IQR: 46-67), with no significant difference (p=0.266). Both groups exhibited similar gender distributions (p=0.175). The TB group, however, had a significantly lower BMI and higher counts of white blood ceslls (WBC), neutrophils, and monocytes compared to the non-TB group (all p<0.05). Inflammatory markers such as ESR and CRP were dramatically elevated in the TB patients. Specifically, the median (IQR) ESR was 59 (21.5-96.8) mm/h in the TB group versus 27.5 (12.0-57.0) mm/h in the non-TB group (p=0.002), and the median (IQR) CRP was 29.21 (6.53-59.46) ng/mL in the TB group compared to 3.26 (1.27-21.00) ng/mL in the non-TB group (p<0.001).

This study further examined cytokine profiles and IGRA test results in individuals with and without tuberculosis, revealing significant immunological differences as presented in Table 1. The TB group exhibited notably higher levels of IL-6, IFN-γ, IL-2, and IL-8, highlighting significant variations in key immunological markers. Specifically, IFN-γ and IL-6 levels were significantly elevated in TB individuals (all p<0.001). While IL-2 and IL-8 also showed significant differences, other cytokines such as TNF-α, IL-5, IFN-α, IL-1β, IL-4, IL-17, IL-10, and IL-12p70 did not display notable differences between the 2 groups. The IGRA test results revealed a substantial disparity in positivity rates, with 88.3% of individuals in the TB group testing positive, compared to only 29.2% in the non-TB group. This highlights the diagnostic utility of the test in distinguishing between tuberculosis and non-tuberculosis conditions.

Next, this study involved the categorization of 77 tuberculosis patients into SNPT (n=42) and SPPT (n=35) groups, with their demographic characteristics

Table 1 - Demographic and clinical characteristics of TB and non-TB groups.

Characteristics	TB (n=77)	Non-TB (n=48)	*P*-value
Age, median (IQR)	53 (40, 64)	59 (46, 67)	0.266
Gender, n (%)			0.175
Male	48 (62.3%)	24 (50.0%)	
Female	29 (37.7%)	24 (50.0%)	
BMI, mean ± SD	20.11 ± 3.53	23.46 ± 4.50	<0.001
WBC (×10^9/L), median (IQR)	7.11 (5.42, 8.67)	5.89 (4.64, 7.79)	0.027
Neutrophil (%), median (IQR)	72.2 (66.0, 77.4)	59.6 (50.1, 70.2)	<0.001
Monocyte (%), median (IQR)	8.7 (6.6, 11.0)	8.0 (6.3, 9.1)	0.042
ESR (mm/h), median (IQR)	59 (21.5, 96.8)	27.5 (12.0, 57.0)	0.002
CRP (ng/mL), median (IQR)	29.21 (6.53, 59.46)	3.26 (1.27, 21.00)	<0.001
IL-5, median (IQR)	2.36 (1.41, 3.32)	1.94 (1.58, 3.165)	0.392
IFN-a, median (IQR)	1.40 (0.88, 2.06)	1.22 (0.89, 1.63)	0.161
IL-2, median (IQR)	1.41 (1.04, 1.85)	1.18 (0.86, 1.52)	0.027
IL-6, median (IQR)	12 (6.4, 25.46)	3.01 (1.50, 4.94)	<0.001
IL-1b, median (IQR)	5.17 (1.95, 10.26)	3.25 (1.39, 8.10)	0.087
IL-10, median (IQR)	1.68 (1.25, 4.09)	1.39 (1.08, 2.31)	0.079
IFN-g, median (IQR)	15.02 (5.47, 32.03)	4.46 (1.99, 6.3)	<0.001
IL-8, median (IQR)	6.44 (1.8, 26.19)	4.05 (0.85, 9.34)	0.023
IL-17, median (IQR)	2.68 (1.00, 7.20)	2.72 (1.63, 13.02)	0.195
IL-4, median (IQR)	1.36 (0.69, 1.90)	1.19 (1.00, 1.62)	0.471
IL-12p70, median (IQR)	1.36 (0.80, 1.85)	1.48 (1.05, 1.87)	0.575
TNF-α, median (IQR)	1.99 (1.09, 5.29)	1.41 (0.82, 3.16)	0.110
IGRA positive, n (%)	68 (88.3%)	14 (29.2%)	<0.001

Chi-square for gender, t test for body mass index (BMI), Wilcoxon test for others. SD: standard deviation, IQR: interquartile, TB: tuberculosis, IL: interlukin, TNF-α: tumor necrosis factor-alpha, IGRA: interferon gamma release assay test, WBC: white blood cells, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein

Table 2 - Demographic and clinical characteristics of smear-negative pulmonary tuberculosis (SNPT) and smear-positive pulmonary tuberculosis (SPPT) groups.

Characteristics	SNPT (n=42)	SPPT (n=35)	*P*-value
Age, mean±standard deviation	53.6 ± 14.7	50.7 ± 17. 9	0.432
Gender, n (%)			0.699
Male	27 (35.1%)	21 (27.3%)	
Female	15 (19.5%)	14 (18.2%)	
BMI, median (IQR)	20.4 (17.3, 22.1)	18.9 (17.8, 22.5)	0.943
ESR (mm/h), median (IQR)	42.5 (12.5, 95)	70.5 (47.75, 99.75)	0.091
CRP (ng/mL), median (IQR)	27.36 (5.87, 55.83)	34.29 (9.95, 65.39)	0.563
WBC (×10^9/L), median (IQR)	6.64 (5.09, 7.77)	7.67 (6.57, 9.35)	0.056
Neutrophil (%), median (IQR)	72.55 (65.2, 77.7)	71.9 (66, 77.4)	0.747
Monocyte (%), mean ± SD	8.7 ± 3.8	9.5 ± 3.2	0.319
IL-5, median (IQR)	2.83 (1.51, 4.22)	2.09 (1.35, 2.83)	0.104
IFN-a, median (IQR)	1.9 (1.09, 3.03)	1.2 (0.74, 1.59)	<0.001
IL-2, median (IQR)	1.66 (1.17, 2.44)	1.22 (0.9, 1.48)	0.012
IL-6, median (IQR)	11.44(6.44, 25.49)	12.44 (6.06, 22.96)	0.963
IL-1b, median (IQR)	7.86 (2.38, 13.9)	3.32 (1.88, 5.71)	0.032
IL-10, median (IQR)	2.17 (1.47, 4.6)	1.53 (1.06, 1.88)	0.005
IFN-g, median (IQR)	14.87 (7.44, 39.89)	15.02 (3.84, 28.50)	0.385
IL-8, median (IQR)	15.82 (1.99, 51.78)	3 (1.00, 8.40)	0.003
IL-17, median (IQR)	4.28 (1.44, 8.46)	1.89 (0.89, 3.29)	0.074
IL-4, median (IQR)	1.43 (0.64, 1.89)	1.34 (0.87, 1.89)	0.771
IL-12P70, median (IQR)	1.61 (0.92, 1.95)	1.12 (0.74, 1.75)	0.060
TNF-a, median (IQR)	2.81 (1.09, 5.66)	1.7 (1.06, 3.51)	0.231
IGRA positive, n (%)	34 (81.0%)	34 (97.1%)	0.015

Chi-square test for gender and IGRA positive proportion, t test for age and monocyte, Wilcoxon test for others. BMI: body mass index, IQR: interquartile, IL: interlukin, TNF-α: tumor necrosis factor-alpha, IGRA: interferon gamma release assay test, WBC: white blood cells, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein

outlined in Table 2. The mean age for the SNPT group was 53.6 ±14.7 years, while it was 50.7±17.9 years for the SPPT group, indicating no statistically significant age discrepancy (p=0.432). Similarly, gender distribution and BMI values were comparable between the groups, with no significant variations noted (p=0.699 for gender; p=0.943 for BMI). In this study, an analysis was conducted on inflammatory and hematological markers, such as ESR and CRP levels, as well as percentages of WBC, neutrophils, and monocytes. While the p-value (0.091) indicated no statistical significance, the SPPT group tended to have higher ESR levels. Conversely, no notable changes were observed in CRP levels or the percentages of WBC, neutrophils, and monocytes between the groups, suggesting a similarity in inflammatory profiles (p=0.563 for CRP; all other p>0.05).

Cytokine levels were analyzed and compared between the SNPT and SPPT groups (Table 3). Because the sample size was restricted, we did not carry out a regression analysis; instead, the magnitude of differences in cytokine levels between the 2 groups was assessed using Cohen's d. The results revealed a statistically significant increase in IFN-α levels in the SNPT group compared to the SPPT group, with a Cohen's d value of 0.59, indicating a medium effect size and moderate differences in cytokine concentrations between the groups. Similarly, levels of IL-2, IL-10, and IL-8 were significantly higher in the SNPT group, with Cohen's d values of 0.61, 0.62, and 0.76, all reflecting medium effect sizes. Comparatively, the levels of TNF-α, IFN-γ, IL-6, and IL-5 did not differ significantly between SNPT and SPPT patients, with Cohen's d values between 0.01 and 0.35 indicating minimal effect sizes. A notable disparity was identified in the IGRA test outcomes, wherein 81% (34/42) of the SNPT cohort tested positive compared to 97.1% (34/35) in the SPPT group (p=0.015). Nevertheless, the ROC analysis revealed inadequate diagnostic efficacy of the IGRA results for SNPT (p>0.05, data not presented). In summary, these findings suggest that the concentrations of IFN–α, IL-2, IL-10, and IL-8 hold promise for distinguishing SNPT from SPPT, exhibiting moderate effect sizes.

In this research, we assessed the diagnostic efficacy of different cytokines in differentiating between SNPT and SPPT populations through the utilization of ROC curve analysis. The AUC serves as a metric for the comprehensive assessment of the cytokines' effectiveness as diagnostic biomarkers for the SNPT population. IFN-α showed significant diagnostic potential with an AUC of 0.734, indicating moderate accuracy. The optimal cut-off value identified was 1.91 pg/mL, yielding a sensitivity of 91.4% and a specificity of 50%. Interferon alfa -α demonstrated significant potential as a rule-out test for non-SNPT, with a p<0.001. For IL-2, the diagnostic accuracy was found to be 0.668, with a Youden index of 0.367 at a cut-off value of 1.55 pg/mL. This resulted in a sensitivity of 77.1%, specificity of 59.5%, a PPV of 75.8%, and an NPV of 36.7%, with a p=0.009. Interlukin-10 exhibited an AUC of 0.686, a cut-off value of 2.06 pg/mL, a sensitivity of 85.7%, and a specificity of 54.8%, indicating substantial diagnostic utility similar to IFN-α. IL-8 showed comparable diagnostic accuracy to IL-10, with a cut-off value of 9.46 pg/mL, sensitivity of 80%, specificity of 59.5%, PPV of 90.5%, and NPV of 58.9%, and a significant

Table 3 - Effect size of cytokine levels in smear-negative pulmonary tuberculosis (SNPT) and smear-positive pulmonary tuberculosis (SPPT) population.

Characteristics	SNPT (n=42)	SPPT (n=35)	Effect size	*P*-value
IL-5, median (IQR)	2.83 (1.51, 4.22)	2.09 (1.35, 2.83)	0.35 (-0.11-0.80)	0.104
IFN-a, median (IQR)	1.90 (1.09, 3.03)	1.20 (0.74, 1.59)	0.59 (0.13-1.05)	<0.001
IL-2, median (IQR)	1.66 (1.17, 2.44)	1.22 (0.90, 1.48)	0.61 (0.15-1.07)	0.012
IL-6, median (IQR)	11.44(6.44, 25.49)	12.44 (6.06, 22.96)	0.12 (-0.33-0.57)	0.963
IL-1b, median (IQR)	7.86 (2.38, 13.9)	3.32 (1.88, 5.71)	0.37 (-0.08-0.83)	0.032
IL-10, median (IQR)	2.17 (1.47, 4.6)	1.53 (1.06, 1.88)	0.62 (0.17-1.08)	0.005
IFN-g, median (IQR)	14.87 (7.44, 39.89)	15.02 (3.84, 28.50)	0.19 (-0.26-0.64)	0.385
IL-8, median (IQR)	15.82 (1.99, 51.78)	3.00 (1.00, 8.40)	0.76 (0.29-1.22)	0.003
IL-17, median (IQR)	4.28 (1.44, 8.46)	1.89 (0.89, 3.29)	0.03 (-0.42-0.48)	0.074
IL-4, median (IQR)	1.43 (0.64, 1.89)	1.34 (0.87, 1.89)	0.01 (-0.45-0.45)	0.771
IL-12P70, median (IQR)	1.61 (0.92, 1.95)	1.12 (0.74, 1.75)	0.36 (-0.09-0.82)	0.060
TNF-a, median (IQR)	2.81 (1.09, 5.66)	1.70 (1.06, 3.51)	-0.03 (-0.48-0.42)	0.231
IGRA positive, n (%)	34 (81.0%)	34 (97.1%)	NA	0.015

NA: not analyzed. Effect sizes were calculated using the Cohen d. Absolute values of 0.20–0.49 represent a small change; values of 0.50–0.79 a medium change; and values of ≥0.80 a large change. IQR: interquartile, IL: interlukin, TNF-α: tumor necrosis factor-alpha, IGRA: interferon gamma release assay test, WBC: white blood cells

p<0.001 (Table 4 & Figure 1A). Combining IL-2, IL-10, IL-8, and IFN-α enhanced diagnostic accuracy, resulting in an AUC of 0.759 (95% CI: 0.652-0.865). The sensitivity was 80%, specificity was 64.2%, and the Youden index was calculated to be 0.443. The PPV was 79.4% and the NPV was 65.1%, suggesting that a multi-biomarker approach could provide more precise diagnostics (p<0.001, Table 4 & Figure 1B). Collectively, the results indicate the substantial diagnostic value of cytokine profiling in our research. The joint evaluation

Table 4 - Receiver operating characteristic analysis of cytokine levels in (SNPT) and smear-positive pulmonary tuberculosis (SPPT) population.

Cytokines	AUC (95%CI)	Cut-off	Youden value	*P*-value	Sensitivity	Specificity	PPV	NPV
IFN-a (pg/mL)	0.734 (0.623-0.844)	1.91	0.414	<0.001	91.4%	50.0%	87.5%	60.4%
IL-2 (pg/mL)	0.668 (0.546-0.790)	1.55	0.367	0.009	77.1%	59.5%	75.8%	36.7%
IL-10 (pg/mL)	0.686 (0.564-0.809)	2.06	0.405	<0.001	85.7%	54.8%	82.1%	61.2%
IL-8 (pg/mL)	0.698 (0.580-0.816)	9.46	0.395	<0.001	80.0%	59.5%	90.5%	58.9%
IFN-a+ IL-2+IL-10+IL-8	0.759 (0.652-0.865)	-	0.443	<0.001	80.0%	64.2%	79.4%	65.1%

CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value, AUC: area under curve

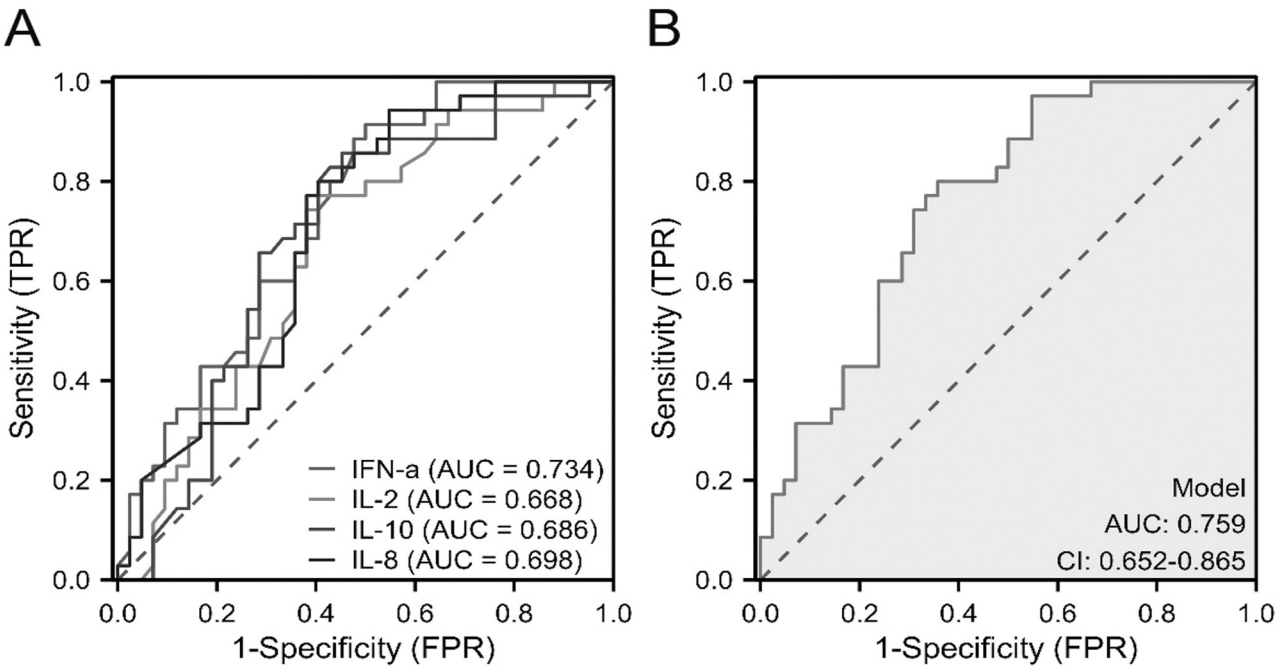

Figure 1 - Diagnostic value of four cytokines alone or in combination for the smear-negative pulmonary tuberculosis. **A**) The receiver operating characteristic (ROC) curve of Interferons (IFN)-α, interleukin (IL)-2, IL-10, and IL-8. **B**) represents the ROC curve of the combination of IFN-α, IL-2, IL-10, and IL-8 for diagnosing smear-negative pulmonary tuberculosis. AUC: area under curve

of IFN-α, IL-2, IL-10, and IL-8 shows improved diagnostic accuracy compared to analyzing each cytokine separately, implying a synergistic usefulness in clinical diagnostics.

Discussion. The importance of cytokine profiling in TB diagnosis has gained substantial attention. This study aimed to evaluate the diagnostic utility of cytokine detection in distinguishing TB patients from non-TB individuals and in differentiating between SPPT and SNPT cases. In this section, we interpret our results and their implications for clinical application and future research directions.

Our initial findings indicated no significant age or gender differences between TB and non-TB groups, suggesting that these demographic factors do not predispose individuals to TB. However, TB patients exhibited significantly lower BMI and higher levels of inflammatory markers (WBC count, neutrophils percentage, ESR, and CRP), aligning with the recognized

pathophysiological impact of TB on nutritional status and systemic inflammation.[20] The analysis revealed markedly elevated levels of IL-6, IL-2, IL-8, and IFN-γ in TB patients, suggesting a robust Th1-mediated immune response alongside potential roles for these cytokines in TB pathogenesis.[21] Notably, the IGRA results demonstrated high sensitivity in distinguishing TB from non-TB cases, affirming its utility in clinical practice.[22,23]

There have been previous studies exploring the role of various cytokines in TB pathogenesis and diagnosis.[24,25] Importantly, this study further delved into the comparative cytokine profiles between SNPT and SPPT patients. Interferon-α, a member of the type I interferon family, has been demonstrated to be pivotal in the host immune defense against Mtb infection.[26] Elevated levels of IFN-α have been observed in TB patients compared to healthy controls, suggesting its potential as a biomarker for TB diagnosis.[27,8] Similarly, our study found significantly higher levels of IFN-α in SNPT patients compared to SPPT patients, with an AUC of 0.734, indicating its discriminatory power in distinguishing between these 2 groups. Interlukin-2 is involved in activating and proliferating T cells, which are essential for controlling Mtb infection.[29] Our study found significant differences in the levels of these cytokines between SNPT and SPPT patients, with an AUC of 0.668, indicating its potential as diagnostic marker. Interkukin-10, known for its anti-inflammatory properties, contributes to regulating the immune response during Mtb infection.[30] Studies have reported increased IL-10 levels in TB patients, suggesting its role in the suppression of the protective immune response.[31,32] Our findings are consistent with these reports, demonstrating significantly higher IL-10 levels in SNPT patients compared to SPPT patients, with an AUC of 0.686. The chemokine CXCL8/IL-8 is linked to neutrophil recruitment and granuloma formation in TB.[33] Previous studies have reported elevated IL-8 levels in TB patients, indicating its involvement in the inflammatory response.[34] We observed a notable elevation in IL-8 levels among SNPT patients as opposed to SPPT patients, with an AUC of 0.698. Our findings point to the possibility that IL-8 can be used as a diagnostic marker for SNPT.

The joint evaluation of IL-10, IL-8, IL-2, and IFN-α showed an improved AUC value of 0.759 (95% CI: 0.652-0.865), indicating improved overall diagnostic precision. With a Youden index of 0.443, this multi-cytokine panel showed sensitivity of 80% and specificity of 64.2%. The PPV was 79.4%, and the NPV was 65.1%, highlighting the efficacy of a multi-biomarker strategy for more accurate diagnosis. Collectively, these findings demonstrate the significant diagnostic potential of cytokine profiling in our study. The combined assessment of IL-8, IL-10, IFN-α, and IL-2 offers an enhanced diagnostic performance compared to the individual cytokines, suggesting a synergistic utility in clinical diagnostics. This discovery has significant implications for clinical practice, suggesting that in cases where patients exhibit a strong suspicion of pulmonary tuberculosis despite negative sputum smear results, elevated levels of the 4 cytokines identified may warrant consideration of antituberculosis treatment due to the inability to definitively exclude the possibility of tuberculosis.

The key strengths of this study are: i) the comprehensive evaluation of cytokine profile differences between SNPT and SPPT patients, providing a basis for further identification of TB subtypes; ii) the discovery that the combined detection of IFN-α, IL-2, IL-10, and IL-8 can significantly improve the diagnostic accuracy of SNPT, offering a potential biomarker panel for clinical application.

Study limitation. The study has some limitations: i) the relatively small sample size, which warrants further validation in larger cohorts; ii) the lack of in-depth exploration of the potential associations between cytokine level changes and the pathophysiology of TB, which could enhance the understanding of their diagnostic utility; iii) the lack of a validation cohort to verify the clinical utility of the combined cytokine analysis.

In conclusion, this study proposes a novel diagnostic strategy based on the combined cytokine measurement, which holds significant implications for improving the diagnosis of SNPT. This multi-biomarker approach has the potential to become a valuable complement to clinical decision-making, providing a more accurate tool for TB diagnosis.

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