Research Square

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Circulating immune biomarkers indicate patients' immune imbalance and predict their severity and poor prognosis in pulmonary tuberculosis

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Research Article

Keywords: soluble immune checkpoints, tuberculosis, Immune Imbalance Score (IIS), CD8 + T cell, prognosis

Posted Date: June 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10012150/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Infection on October 29th, 2026. See the published version at https://doi.org/10.1007/s15010-026-03025-0.

Abstract

Background.

A comprehensive understanding of the host immune response and identifying valuble markers to predict disease severity and patient's prognosis is a crucial issue.

Methods.

Plasma levels of 12 soluble immune checkpoints (sICPs), 12 cytokines/chemokines and blood immune cell subsets were analyzed through flow cytometry.

Results.

Seven sICPs, such as sCD25 (P < 0.001), sCD27 (P < .0.001), sPD-L1 (P < 0.001), sPD-L2 (P = 0.031), sTim-3 (P < 0.001), sLAG-3 (P = 0.003), sGal-9 (P < 0.001) were positively correlated with disease severity in PTB patients (n = 78). Four cytokines/chemokines, such as IL-6 (P < 0.001), IL-8 (P < 0.01), IP-10 (P < 0.001) and IFN-γ (P < 0.01), and some routine laboratory examinations, such as absolute neutrophil count (Neutrophil, P < 0.001), were also significantly elevated in severe PTB (SE). However, absolute lymphocyte count (LY, P < 0.001) was significantly lower in SE patients. Through Lasso regression analysis, six factors, including sCD25,sTim-3,IP-10, LY, Neutrophil, LDH were screened and weighted to form the Immune Imbalance Score (IIS). Patients with higher IIS had higher possibility to experience severe illness (P < 0.001) and shorter survival time (P < 0.001). Furthermore, the result of blood immune cell revealed that the frequencies of exhausted CD8 + T (Tim-3 + CD8+T, n = 48, P = 0.0374) were significantly increased in SE patients than those in non-SE patients.

Conclusion.

Patients with severe PTB exhibited a high non-specific inflammatory status, but their specific adaptive immunity was suppressed. Patients' IIS could potentially serve as biomarkers to predict PTB patients' severity and progress.

Introduction

Tuberculosis (TB) is an infectious disease triggered by bacteria belonging to the *Mycobacterium tuberculosis* (Mtb) complex. In humans, the disease is primarily transmitted via inhaling aerosol droplets containing Mtb bacilli. Prior to the COVID-19 pandemic, TB was the leading cause of death from a single infectious agent, claiming approximately 1.5 million deaths annually [1–2]. While the majority of patients infected with Mtb may exhibit asymptomatic, mild or moderate TB, some may progress to advanced illness. For patients with advanced TB, fibrosis, cavitation, and necrosis inflict long-lasting and

irreversible harm on lung tissue and function, potentially resulting in respiratory failure and ultimately death [1–3]. The in-depth research on host-pathogen interactions and post-mortem lung lesions reveals that the pathological manifestations of TB primarily result from the damage to bystander tissues due to excessive inflammatory response of host immune system [4–6]. Therefore, a comprehensive understanding of the host immune response is crucial for identifying diagnostic markers, developing more effective treatment interventions, and optimizing prognoses. These efforts could substantially enhance clinical outcomes [7–10].

Immune exhaustion and immune system dysfunction is an characteristic of chronic infections [11, 12]. Our previous study found that in severe PTB (SE) patients, there is a reduction in peripheral lymphocytes, pronounced activation of cell apoptosis pathways, widespread immune exhaustion, and cytokine storms [13]. Immune checkpoints (ICPs) play a pivotal role in maintaining the balance of the immune system, essential for its optimal function [14–16]. Soluble isoforms of checkpoints arise from the cleavage of membrane-bound proteins or alternative splicing of mRNA and they competitively modulate the functions of their membrane-bound counterparts [14, 17, 18]. Numerous studies revealed membrane-bound and soluble isoforms of checkpoints are linked to T cell exhaustion across various chronic infections, tumors etc [19–22]. However, it remains unclear whether soluble checkpoint molecules are involved in the immune dysfunction of severe PTB and whether they could indicate PTB patients' disease severity and prognosis.

In this study, we analyzed the hematological and immunological characteristics of PTB patients and healthy individuals, focusing on soluble checkpoints, inflammatory cytokine/chemokines, and T lymphocyte subsets. The findings reveal that both stimulatory and inhibitory immune checkpoints, along with certain cytokines were significantly elevated in severe patients. Furthermore, CD8 + T cells in blood of patients with severe PTB demonstrate significant exhausted and dysfunction. Among all these, six factors were screened and constitute a better predictor of PTB patients' disease severity and prognosis.

Materials and Methods

Patients

This study enrolled active PTB patients and they were confirmed with bacteriological evidence by culture or Xpert MTB/RIF and underwent treatment in Beijing Chest Hospital between May 2022 and February 2023.The patients were divided into two cohorts: one is retrospective cohort (n = 78) who were enrolled for plasma checkpoints and cytokines/chemokines detection; the other cohort (n = 48) was for blood immune cell subpopulation analysis. Criteria of inclusion were: age > 18 years; confirmed with active PTB; adequate cardiac, pulmonary, renal, liver, and bone marrow function. Criteria of exclusion were: autoimmune disease; history of organ allograft; severe pneumonia; incomplete clinical data. In every cohort, patients are divided into severe PTB (SE) and non-severe PTB (non-SE) groups according to the guidelines on drug-susceptible tuberculosis treatment (Module 4) by WHO and other references. Briefly, SE patients: (1) had dense lesions occupying greater than 1/3 of one lung, or bilateral lesions greater

than 1/2 whole lung, or cavitation up to 4 cm, or with a large amount of pleural effusion; (2) serum CRP≥75mg/L, and PO2 < 80mmHg or oxygen index < 300 mmHg. Non-SE patients: lesion density did not reach the criteria for severe. All patients were followed up until February 2024. This research was approved by the ethics committee of Beijing Chest Hospital (JYS-2023-10) and all patients provided written informed consent.

Sample collection and preparation

Peripheral blood was collected in EDTA anticoagulant tubes upon admission. For soluble factor detection, plasma was aliquoted after centrifugation (3,000rpm, 10min, 4°C) and stored at − 80°C within 4h after collection. All samples were dissolved uniformly during testing to avoid repeated freeze-thaw cycles. For flow cytometric analysis, anticoagulated blood samples are analyzed within 4 hours of collection.

Plasma multiplexed beads immunoassay

44 SE, 34 non-SE patients and 10 HCs' plasma multiple soluble proteins were tested, these proteins consisted of two parts: part I, the LEGENDPlex Human Immune Checkpoint Panel 1 (BioLegend, Calsbad, CA, USA), including sCD25, s4-1BB, sCD27, sCD86, free active TGF-β1, sCTLA-4, sPD-1, sPD-L1, sPD-L2, sTim-3, sLAG-3, and sGalectin-9; part II, the LEGENDplex™ Human Essential Immune Response Panel, including IL-1β, IL-2, IL-4, IL-6, CXCL8(IL-8), IL-10, IL-12p70, IL-17A, CXCL10 (IP-10), CCL2(MCP-1), TNF-α, and IFN-γ (BioLegend). Data were acquired according to manufacturer's protocol using LSRFortessa instrument (BD Biosciences).

Blood immune cell subsets flow cytometric analysis

For blood immune cell subpopulation analysis, EDTA-anticoagulated blood samples were incubated with two panel of antibodies, panel 1: FITC-anti-CD3 (HIT3a, BD Biosciences), APC-anti-CTLA-4(L3D10, Biolegend), BV421-anti-CD25 (M-A251, BD Biosciences), percp-Cy5.5-anti-BTLA (MIH26, Biolegend); panel 2: FITC-anti-CD3, PE-Cy7-anti-CD27 (O323, BD Biosciences), PE-anti-CD137(4B4-1, BD Biosciences), APC-anti-PD-1(NAT105, Biolegend), BV421-anti-Tim-3 (F38-2E2, Biolegend), BUV-395-anti-CD8(RPA-T8, BD Biosciences), APC-H7-anti-CD4 (RPA-T4, BD Biosciences), for 25 min at room temperature, then erythrocytes (RBCs) were lysed for 15 min, and the stained cells were washed twice for analysis with LSRFortessa instrument (BD Biosciences).

Statistical analysis

SPSS 24.0 statistical software was used for statistical analysis. One-way ANOVA and inter group testing were used to analyze the difference between HCs and different groups of PTB patients; if the variance is homogeneous, LSD-t test is used for multiple comparisons between groups; If the variance is uneven, the

Dunnett's test will be used for inter group comparison. Mean ± standard deviation was used to analyze the difference between 2 groups of normally distributed variables, Media, (interquartile range, IQR) and Mann-Whitney U test was used for nonnormally distributed variables. The Systemic Immune-Inflammation Index (IIS) was obtained from a LASSO regression model. A receiver operating characteristic (ROC) curve was used to evaluate the diagnostic sensitivity and specificity of one checkpoint, cytokine/ chemokines. The area under the ROC curve (AUC) was used as a measure of discriminatory ability for the signature scores. Survival analysis was performed using the Kaplan–Meier survival plot, and the log-rank test. A Cluster Heatmap analysis was conducted using R and R Studio. Multivariable Cox regression mode was used to examine the death risk of different factors.

Results

Clinical characteristics and laboratory data

Totally, 126 PTB patients, including 70 non-SE and 56 SE cases, and 19 healthy controls were enrolled. There was no significant difference in median age between the SE and the non-SE group. The study schema was illustrated in Fig. 1. Patients' blood routine indicators showed that the absolute white blood cell count (WBC,10^9/L), absolute neutrophils count (NEUT,10^9/L), D-dimer (mg/L), hs-CRP (mg/L), lactic dehydrogenase (LDH, U/L) were higher in patients with SE compared to those in non-SE patients, whereas absolute lymphocytes count (LY,10^9/L), Hemoglobin (g/L), total protein (TP, g/L), Albumin (g/L) were lower in SE patients compared to non-SE patients. Table 1 provides a detailed summary of these characteristics.

Table 1
Association of Patients' Laboratory data and the severity of PTB

Characteristics	**HD patients (n = 19)**	**Non-SE patients (n = 70)**	**SE patients (n = 56)**	***P* values**
Age	19(30,80)	69(19, 89)	56(22,85)	*P* = 0.180
Gender				
Male n(%)	10(52.6%)	39(56.5%)	45(80.4%)	
Female n(%)	9(47.4%)	30(43.5%)	11(19.6%)	
Laboratory data				
WBC(10^9/L)	6.20(5.35 ~ 9.46)	6.19(4.92 ~ 8.60)	8.37(6.66 ~ 11.70)	*P* < 0.001
Lymphocyte(10^9/L)	1.71 ± 0.58	1.30 ± 0.57	0.74 ± 0.40	*P* < 0.001
Neulrophil(10^9/L)	3.93(3.21 ~ 6.68)	3.41(1.90 ~ 6.18)	7.65(5.41 ~ 10.18)	P < 0.001
$CD3^{\pm}T$ (%)	/	73.66 ± 7.33	70.78 ± 7.62	*P* = 0.253
$CD4^{\pm}T$ (%)	/	44.79 ± 10.6	39.81 ± 10.56	*P* = 0.167
$CD8^{\pm}T$ (%)	/	24.6 ± 9.25	28.14 ± 10.56	*P* = 0.282
Hemoglobin(g/L)	128.28 ± 19.72	116.81 ± 21.62	99.91 ± 19.45	*P* < 0.001
Platelets (10^9/L)	222.5(188.5 ~ 258.5)	268(177.5 ~ 311)	289.66 ± 132.15	*P* = 0.390
D-dimer(mg/L)	0.85(0.36 ~ 0.95)	0.97(0.43 ~ 3.03)	3.49(1.51 ~ 4.60)	*P* < 0.001
CRP(mg/L)	7.90(1.00 ~ 47.53)	23.19(4.53 ~ 60.92)	69.86 ± 39.59	*P* < 0.001
LDH(U/L)	167(143 ~ 216.75)	166(147.5 ~ 205.5)	208(148 ~ 291)	*P* = 0.042
TP(g/L)	70.59 ± 5.96	65.91 ± 7.82	58.94 ± 6.99	*P* < 0.001
Albumin(g/L)	40.01 ± 7.38	36.92 ± 5.82	28.93 ± 5.51	*P* < 0.001

Elevations in seven plasma soluble checkpoints are associated with an increased risk of severe illness in PTB patients

We analyzed the plasma soluble checkpoints by immunofluorescence microsphere technology, constructing a heatmap to illustrate the relative plasma levels in HCs (n = 10), non-SE (n = 34), and SE (n = 44) patients. Each column of the heatmap represented a patient, while the rows represented different checkpoint molecules (Fig. 2A). The Plasma levels of sCD25 ($P < 0.001$), sCD27 ($P < 0.001$), sPD-L1($P < 0.001$), sPD-L2 (0.031), sTim-3 ($P < 0.001$), sLAG-3 ($P = 0.003$), sGal-9 ($P < 0.001$) levels were 6.087 (4.48–10.87) ng/ml, 114.5 ± 81.12 ng/ml, 0.07(0.03–0.12) ng/ml, 7.36 ± 1.91 ng/ml, 69.67 ± 44.77 ng/ml, 2.12 (1.63–5.01)ng/ml, 68.82 (47.31–105.4) ng/ml in SE patients; were 1.72 (1.12–2.93) ng/ml, 46.93 (31.77–72.76) ng/ml, 0.03 (0.01–0.05) ng/ml, 6.20 ± 1.70 ng/ml, 13.79 (6.71–23.68) ng/ml, 1.41 (0.81–2.43)ng/ml, 37.50 (21.91–56.40) ng/ml in non-SE, and were 0.79 (0.62–0.99) ng/ml, 55.80 ± 17.27ng/ml, 0.02 ± 0.01 ng/ml, 7.39 (6.40–8.39) ng/ml, 9.43 ± 4.18ng/ml, 1.91 (0.81–1.50) ng/ml, 26.40 ± 5.41 ng/ml in HCs, respectively. These checkpoints were significantly higher in SE patients than in non-SE and HCs, but there was no significantly difference between non-SE and HCs (Fig. 2B). Data were shown as mean ± standard deviation (SD) or median (interquartile range, IQR). The other five checkpoints showed no significant difference between SE and non-SE patients (data not shown).

Furthermore, we assessed the predictive value of these seven molecules in severity of PTB, using receiver operating characteristic curve (ROC) to estimate the sensitivity and specificity, the area under the curve (AUC) to estimate the capacity to distinguish non-SE from SE patients. sCD25 and sTim-3 showed superior predictive value (AUCs > 0.8). The ROC curves and AUCs for the seven checkpoint molecules were displayed in Fig. 2C.

The plasma concentrations of four inflammatory cytokines/chemokines are significantly increased in SE patients

To thoroughly assess the systemic inflammatory response in patients, we detected 12 inflammatory cytokines/chemokines in plasma testing cohort. Cytokine/ chemokines heatmap depicted their relative plasma levels in HCs and PTB patients (Fig. 3A). Plasma IP-10 ($P < 0.001$), IL-6 ($P < 0.001$), IFN-γ ($P < 0.01$), IL-8 ($P < 0.01$) were 786.60 ± 532.70 pg/ml, 71.42 (26.30-193.50) pg/ml, 19.54 (9.17–28.60) pg/ml, 5.67 (2.96–21.79) pg/ml in SE patient, were 242.00 (150.40–411.0) pg/ml, 11.19 (6.20-25.36) pg/ml, 7.43 (3.31–14.52) pg/ml, 2.41 (1.38–4.85) pg/ml in non-SE, and were 164.90 (76.00-314,70) pg/ml, 22.11 (8.93–35.20) pg/ml, 7.96 ± 7.12 pg/ml, 2.81 ± 1.86 pg/ml in HCs, respectively. The levels of these cytokines/chemokines were significantly increased in SE patients compared to non-SE and HCs. However, there was no significantly difference between non-SE and HCs (Fig. 3B). The ROC curves and AUCs for these four cytokines were detailed in Fig. 3C. Importantly, no significant differences were observed in other eight cytokines between SE and non-SE patient (data not shown).

Immune Imbalance Score (IIS) could better predict PTB patients' severity and prognosis

Soluble checkpoint molecules, such as sCD25, sCD27, sPD-L1, sPD-L2, sTim-3, sLAG-3, sGal-9, as well as cytokine/chemokines such as IP-10, IL-6, IFN-γ, IL-8 were both significantly elevated in SE Patients. Interrelationships analysis showed that there were positive correlations between these molecules (Fig. 4A). We next conducted an in-depth analysis of the correlations between these molecules with blood routine index. The concentrations of sCD25, sCD27, sTim-3, sGal-9, IP-10, sPD-L2 in plasma demonstrated a moderate negative correlation with absolute lymphocyte count, but a weak to moderate positive correlated with Neutrophil counnt (Fig. 4B).

Among 78 TB patients, the median follow-up time was 365 days (range: 0-365 days), and 14 deaths occurred (all SE) as of February 27, 2023. Through Kaplan–Meier survival plot and log-rank test, we found that patients with higher levels of sCD25, sCD27, sPD-L1, sTim-3, sGal-9 or higher levels of IP-10, IL-6, IL-8, experienced shorter survival time (Fig. 5A,B and supplementary Table 1). Furthermore, 19 variables, including sCD25, sCD27, sPD-L1, sPD-L2, sTim-3, sLAG-3, sGal-9, IP-10, IL-6, IFN-γ, IL-8, WBC, NEUT, LY, D-dimer, hs-CRP, LDH, Hemoglobin, Albumin were analyzed to evaluate their predictive value for SE. Lasso regression model was employed to identify potential parameters. The result showed that NEUT, sCD25, sTim-3, IP-10, LDH were the positive and LY was the negative indicators of SE (Supplementary Table 2). These variables weighted to form the Immune Imbalance Score (IIS), and the formula to calculate the IIS for patients was as follow:

$$IIS = \frac{1}{1+e^{-(0.3935+0.2857\cdot sCD25+0.4633\cdot sTim\text{-}3+0.2709\cdot IP\text{-}10-0.4704\cdot Lymphocyte+0.6947\cdot Neulrophil+0.0003\cdot LDH)}}$$

SE patients had significantly higher IIS than non-SE patients ($P < 0.001$), and patients with higher IIS have a shorter survival time ($P < 0.001$). The AUCs of IIS to predict severity and patients' prognosis were 0.9098 and 0.9063, and IIS showed to improve the predict probability for SE and the poor prognosis of patients compared any individual variable, (Fig. 6B,C). Sankey diagram showing that IIS integrated the six factors and is convenient to analyze patients' severity and prognosis (Fig. 6D)

Higher frequency of exhausted CD8 T cells in SE patients

Given the pivotal role of immune checkpoint molecules in T cell-mediated precision immunity and the central role of T cells in the eradication of Mtb, we analyzed the membrane checkpoint expressed on peripheral blood T cells to evaluate T cell status in PTB patients. We employed flow cytometry to analyze peripheral blood T cell subsets in 48 PTB patients, with the detailed gating strategy presented in supplement Fig. 1, the result revealed that there were no differences in the frequencies of BTLA+, CTLA-4 + CD137+, CD27+, CD25+, PD-1 + CD3+T cell, only a significant difference in the frequency of Tim-3 + CD3+T cell between SE and non-SE patients (supplementary Fig. 2). Subsequently, we conducted a detailed analysis of Tim-3 expressed on different T subsets The result indicated that there were no differences in the frequencies of CD3+, CD4 + T and CD8 + T cells between HCs (n = 9), non-SE (n = 36) and SE group (n = 12) (Fig. 7A), the frequencies of Tim-3 + CD3+T, Tim-3 + CD4+T, and Tim-3 + CD8+T

cells were significantly increased in PTB patients compared with HCs. However, only the frequency of Tim-3 + CD8+T cells demonstrated a significantly increased between SE and non-SE patients (Fig. 7B).

Discussion

People's health and homeostasis depend critically on a well-balanced immune system: too little inflammation leds to an inability to eliminate viruses, bacteria and malignant cells etc. Conversely, excessive inflammation results in autoimmunity or unwanted immunopathology. T cells are fundamental to achieve immune system balance, as they differentiate between "self" and "non-self", react with strongly and specifically to an almost unlimited range of "non-self". Immune checkpoint molecules play an essential role in modulating the T cell immune response[14, 23]. In tuberculosis, most individuals maintain the immune balance and clear bacterial infection or establish a homeostatic balance between host immune system and pathogen, accompanied by inflammation resolution and minimal tissue damage, thereby preventing disease progression in the initial phase of TB. In the minority, TB may escalate to a severe stage characterized by permanent lung tissue damage and extensive pulmonary dysfunction due to persistent inflammation[3, 24]. However, it remains unclear whether the cause of this immune dysregulation lies in the overproduction of immune checkpoint molecules and pro-inflammatory cytokines and chemokines. In this study, we found that the plasma levels of sCD25, sCD27, sPD-L1, sPD-L2, sTim-3, sLAG-3 and sGal-9 levels in SE patients were significantly enhanced than those in non-SE and HCs. Both CD25 and CD27 play critical roles in T lymphocyte activation, proliferation, survival, and differentiation. Soluble CD25 and sCD27 may be derived from membrane-expressed CD25 and CD27 and increased these molecules could interfere with the interaction between their ligands and membrane-bound receptors, thereby inhibiting the activity of T cells through these receptor/ligand systems [25, 26]. PD-L2, Tim-3, LAG-3 and Gal-9 are inhibitory receptors and ligands. In chronic antigen stimulation, co-inhibitory receptors are highly and constitutively expressed on T cells to limit excessive T cell over activation and host tissue damage [22]. Qinfang Ou et al found that soluble stimulatory and inhibitory checkpoint are upregulated in PTB than in HCs, however their research did not involve the correlations between the plasma marker levels and disease severity and patients prognosis [27]. In Systemic lupus erythematosus (SLE), elevated stimulatory and inhibitory checkpoint molecules are predictive biomarker for severe manifestationss and long-term outcome [28]. Premeaux TA et al'study found that elevated soluble checkpoint (stimulatory and inhibitory) are associated with poor immune reconstitution and adverse outcomes in HIV patients [21]. Both stimulatory and inhibitory soluble checkpoint molecules are significantly elevated, indicating immune dysregulation [29].

Soluble checkpoints are identified to not only associated with disease severity, but also possess significant predictive values for disease progression [25, 29, 30]. In cancer patients, sCD27, sTim-3 serve as markers of T-cell dysfunction, with elevated levels indicating poorer overall survival [25, 30]. In our study, we discovered that the plasma levels of sCD25, sCD27, sPD-L1, sTim-3 and sGal-9 all negatively correlate with the prognosis of PTB patients, especially for sTim-3. Over the past decade, researchers have increasingly identified an expanding number of soluble immune checkpoints. These checkpoints precisely indicate the exhaustion of T cells and NK cells. The highly specific and fine-tuned regulation of

the immune response offers significant potential for therapeutic intervention in immune system imbalances, such as tuberculosis.

T cells play an irreplaceable role in TB clearance, particularly through secretion of cytotoxicity molecules such as perforin, granzyme and IFN-γ, which eliminate TB-infected cells. Boosting the numbers and functionality of T cells is crucial for successful recovery in PTB patients [31]. However, the exhausted CD8 + T cells in patients with severe PTB failed to secrete cytotoxic molecules than patients with mild PTB patients, and expressed higher levels of exhausted markers PD-1, Tim-3, CTLA-4 and TIGIT. These co-inhibitory receptors are essential for maintaining homeostasis and preventing tissue damage, but they can also impede pathogen clearance [22, 31, 32]. Through flow cytometry analysis we observed an increase in the percentage Tim3 + T cell in PTB patients. This combined with our previous single-cell sequencing results, provide a demonstration of abnormal immune responses and immune dysfunction, especially the abnormal activation, dysregulation, and exhausted characteristics of CD8 + T cells in SE patients [13]. Tim-3 is demonstrated to be the most functionally dysfunctional subset of CD8 + T cells in chronic viral infections and cancer in both preclinical models and in humans. Blocking Tim-3 improves IFN-γ production, and facilitates the eradication of pathogen and tumors. [22, 33]. In chronically TB infected mice, Tim-3-mediated T cell exhaustion impairs host resistance to Mtb, and anti-Tim-3 neutralized antibodies emerge as an effective therapeutic strategy [34]. These observations point to a new therapeutic direction: the potential to more effectively control tuberculosis, particularly in SE patients, through the use of immunomodulators such as anti-Tim-3 agents to reverse the exhaustion process of T cells [35].

Although immune checkpoint molecules such as sCD27 and sTim3 offer significant predictive value, they inadequately consider the levels of nonspecific inflammation, including centrilobular inflammation. To evaluate the difference or severity of inflammatory response in SE and non-SE patients, this study systematically evaluated a panel of inflammatory cytokines. We found that plasma concentrations of Il-6, IL-8, IP-10 and IFN-γ were significantly increased in SE patients compared to non-SE patients. Cytokines/chemokines play an important role in the immune response during early infection. For instance, IL-6 had an role in potentiating immunity during early Mtb infection through potentiate IFN-γ expression at the site of infection; IP-10 is important for activated CD4 + and CD8 + T cell migrated from lymph node to infection site, IFN–γ can activate macrophage, and they are crucial for granuloma formation[36, 37]. But they can also drive inflammation that increases lung tissue injury and promote a pathogen-permissive host environment. Such as elevated cytokines (IL-8 and IL-6) recruit more neutrophils to the lesion site, these neutrophils not only create a favorable environment for the proliferation of Mtb, resulting in a significant increase in bacterial counts, but also produce specific matrix metalloproteinases (MMPs), which can exacerbate the nonspecific inflammatory response, leading to tissue damage, cavity formation, lung structure destruction, and reduced lung function [36–40]. These enzymes are also suspected of being involved in the transition from membrane to soluble checkpoints, resulting in increased soluble checkpoint levels in blood, which contributes to the depletion of antigen specific T cells [36, 41–43]. These findings explained that elevated checkpoint molecules and cytokine were positively correlated with each other, and negatively with absolute lymphocyte count, but

positively correlated with absolute neutrophil count. Tuberculosis, as a disease model rich in information, offers exceptional resources for examining the intricate immune network among checkpoint molecules, cytokines, and immune cells.

In summary, our research revealed the dysregulated, unresolved inflammation in SE patients and identified IIS which integrated specific and nonspecific immune factors, could better evaluate the immune imbalance of PTB patients, and be a biomarker that could classify individuals with severe PTB. The predictive severity model holds promise for the management of individuals with SE, aiming to guide host-targeted therapy and reduce lung tissue damage, function impairment, and mortality rate.

This study has several limitations. First, due to the soluble checkpoints levels and cellular immune checkpoint molecules were got from different cohort of patients, it is difficult to determine the association between them. Second, we conducted a singlecenter patients; more studies based on larger cohort are necessary to verify our findings. Third, deeper studies were needed to investigate whether some of these checkpoints might serve as potential therapeutic targets, with an aim to reverse immune imbalance of sever PTB.

Declarations

Declaration of interests

All authors declare no competing interests.

Clinical trial number

Not applicable.

Ethics statement

The present study involved the collection of blood from healthy humans, patients, all related protocols were approved by the ethics committee of Beijing Chest Hospital (JYS-2023-10) and patients were informed and signs an informed consent form.

Funding declaration

This work was supported by grants from National natural science foundation of China (82572642), Prevention and Control of Emerging and Major Infectious Diseases-National Science and Technology Major Project (2025ZD01907900), Beijing High-level Innovative and Entrepreneurial Young Talent (G202522153), Beijing Tongzhou Municipal Science & Technology Commission (WS2024045).

Author Contribution

Guirong Wang affirms that the manuscript is an honest, accurate, and transparent study. Guirong Wang, Ling Yi and Shuye Lin conceived and designed the analysis; Sibo Long, Bin Yang, Yu Xue, Yiheng Shi,Huimin Li, Weibing Lin, Yun Zhang, Xinting Yang collected and analyzed the data; Guirong Wang, Shuye Lin, Ling Yi, Sibo Long, Bin Yang contributed in data analysis, interpretation of the data, and drafting the manuscript. All authors reviewed and approved the manuscript for submission.

Data Availability

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

References

1. Global Tuberculosis Report. 2021. World Health Organization 2021.
2. Bagcchi S, WHO's Global Tuberculosis Report 2022. Lancet Microbe. 2023;4(1):e20. 10.1016/S2666-5247(22)00359-7.
3. Ravimohan S, Kornfeld H, Weissman D, Bisson GP. Tuberculosis and lung damage: from epidemiology to pathophysiology. Eur Respir Rev. 2018;27(147):170077.
4. McCaffrey EF, Donato M, Keren L, Chen Z, Delmastro A, Fitzpatrick MB, et al. The immunoregulatory landscape of human tuberculosis granulomas. Nat Immunol. 2022;23(2):318–29.
5. Brown RE, Hunter RL. Early Lesion of Post-Primary Tuberculosis: Subclinical Driver of Disease and Target for Vaccines and Host-Directed Therapies. Pathogens. 2021;10(12):1572.
6. Hunter RL. The Pathogenesis of Tuberculosis-The Koch Phenomenon Reinstated. Pathogens. 2020;9(10):813.
7. Allwood BW, Byrne A, Meghji J, Rachow A, van der Zalm MM, Schoch OD. Post-Tuberculosis Lung Disease: Clinical Review of an Under-Recognised Global Challenge. Respiration. 2021;100(8):751–63.
8. Krug S, Parveen S, Bishai WR. Host-Directed Therapies: Modulating Inflammation to Treat Tuberculosis. Front Immunol. 2021;12:660916.
9. Kilinç G, Saris A, Ottenhoff THM, Haks MC. Host-directed therapy to combat mycobacterial infections. Immunol Rev. 2021;301(1):62–83.
10. Tsenova L, Singhal A. Effects of host-directed therapies on the pathology of tuberculosis. J Pathol. 2020;250(5):636–46.
11. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15(8):486–99.

12. Belk JA, Daniel B, Satpathy AT. Epigenetic regulation of T cell exhaustion. Nat Immunol. 2022;23(6):848–60.
13. Wang Y, Sun Q, Zhang Y, Li X, Liang Q, Guo R, et al. Systemic immune dysregulation in severe tuberculosis patients revealed by a single-cell transcriptome atlas. J Infect. 2023;86(5):421–38.
14. oseph J, Rahmani B, Cole Y, Puttagunta N, Lin E, Khan ZK, Jain P. Can Soluble Immune Checkpoint Molecules on Exosomes Mediate Inflammation? J Neuroimmune Pharmacol. 2022;17(3–4):381–97.
15. Zahavi DJ, Weiner LM. Targeting Multiple Receptors to Increase Checkpoint Blockade Efficacy. Int J Mol Sci. 2019;20(1):158.
16. Ward-Kavanagh LK, Lin WW, Šedý JR, Ware CF. The TNF Receptor Superfamily in Co-stimulating and Co-inhibitory Responses. Immunity. 2016;44(5):1005–19.
17. Hayashi H, Chamoto K, Hatae R, Kurosaki T, Togashi Y, Fukuoka K, et al. Soluble immune checkpoint factors reflect exhaustion of antitumor immunity and response to PD-1 blockade. J Clin Invest. 2024;134(7):e168318.
18. Gu D, Ao X, Yang Y, Chen Z, Xu X. Soluble immune checkpoints in cancer: production, function and biological significance. J Immunother Cancer. 2018;6(1):132.
19. Clayton KL, Douglas-Vail MB, Nur-ur Rahman AK, Medcalf KE, Xie IY, Chew GM, et al. Soluble T cell immunoglobulin mucin domain 3 is shed from CD8 + T cells by the sheddase ADAM10, is increased in plasma during untreated HIV infection, and correlates with HIV disease progression. J Virol. 2015;89(7):3723–36.
20. Osuch S, Kumorek A, Kozłowski P, Berak H, Kochanowicz AM, Cortés-Fendorf K. Plasma levels of soluble PD-1, TIM-3, LAG-3 and galectin-3 and the degree of liver fibrosis in CHC and the impact of successful antiviral treatment on their levels. Sci Rep. 2025;15(1):15436.
21. Moar P, Bowler S, Landay AL, Gianella S, Ndhlovu LC, Premeaux TA. Alterations in circulating immunoregulatory proteins discriminate poor CD4 T lymphocyte trajectories in people with HIV on suppressive antiretroviral therapy. mBio. 2024;15(10):e0226524.
22. King HAD, Lewin SR. Immune checkpoint inhibitors in infectious disease. Immunol Rev. 2024;328(1):350–71.
23. Wang J, Zhao X, Wan YY. Intricacies of TGF-β signaling in Treg and Th17 cell biology. Cell Mol Immunol. 2023;20(9):1002–22.
24. Torrelles JB, Schlesinger LS. Integrating Lung Physiology, Immunology, and Tuberculosis. Trends Microbiol. 2017;25(8):688–97.
25. Benhamouda N, Sam I, Epaillard N, Gey A, Phan L, Pham HP, et al. Plasma CD27, a Surrogate of the Intratumoral CD27-CD70 Interaction, Correlates with Immunotherapy Resistance in Renal Cell Carcinoma. Clin Cancer Res. 2022;28(22):4983–94.
26. Joller N, Anderson AC, Kuchroo VK. LAG-3, TIM-3, and TIGIT: Distinct functions in immune regulation. Immunity. 2024;57(2):206–22.

27. Chen H, Zhou J, Zhao X, Liu Q, Shao L, Zhu Y, Ou Q. Characterization of multiple soluble immune checkpoints in individuals with different Mycobacterium tuberculosis infection status and dynamic changes during anti-tuberculosis treatment. BMC Infect Dis. 2022;22(1):543.
28. Dreveton LS, Spencker JJ, Rincon-Arevalo H, Aue A, Osmanodja B, Wiedemann A, et al. Soluble checkpoint molecules as predictive biomarker for disease activity and long-term outcome in SLE. Front Immunol. 2025;16:1685275.
29. Kong Y, Wang Y, Wu X, Han J, Li G, Hua M, Han K, Zhang H, Li A, Zeng H. Storm of soluble immune checkpoints associated with disease severity of COVID-19. Signal Transduct Target Ther. 2020;5(1):192.
30. Wang Q, Zhang J, Tu H, Liang D, Chang DW, Ye Y, Wu X. Soluble immune checkpoint-related proteins as predictors of tumor recurrence, survival, and T cell phenotypes in clear cell renal cell carcinoma patients. J Immunother Cancer. 2019;7(1):334.
31. Wang X, Cao Z, Jiang J, Li Y, Dong M, Ostrowski M, Cheng X. Elevated expression of Tim-3 on CD8 T cells correlates with disease severity of pulmonary tuberculosis. J Infect. 2011;62(4):292–300.
32. Wang J, Dai Y, Liu J, Yin Y, Pei H. MTB-specific lymphocyte responses are impaired in tuberculosis patients with pulmonary cavities. Eur J Med Res. 2017;22(1):4.
33. Marin-Acevedo JA, Dholaria B, Soyano AE, Knutson KL, Chumsri S, Lou Y. Next generation of immune checkpoint therapy in cancer: new developments and challenges. J Hematol Oncol. 2018;11(1):39.
34. Jayaraman P, Jacques MK, Zhu C, Steblenko KM, Stowell BL, Madi A, et al. TIM3 Mediates T Cell Exhaustion during Mycobacterium tuberculosis Infection. PLoS Pathog. 2016;12(3):e1005490.
35. Qin Y, Wang Q, Shi J. Immune checkpoint modulating T cells and NK cells response to Mycobacterium tuberculosis infection. Microbiol Res. 2023;273:127393.
36. Monin L, Khader SA. Chemokines in tuberculosis: the good, the bad and the ugly. Semin Immunol. 2014;26(6):552–8.
37. Domingo-Gonzalez R, Prince O, Cooper A, Khader SA. Cytokines and Chemokines in Mycobacterium tuberculosis Infection. Microbiol Spectr. 2016;4(5):10.
38. Lowe DM, Bandara AK, Packe GE, Barker RD, Wilkinson RJ, Griffiths CJ, Martineau AR. Neutrophilia independently predicts death in tuberculosis. Eur Respir J. 2013;42(6):1752–7.
39. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature. 2010;466(7309):973–7.
40. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol. 2011;11(8):519–31.
41. ernández Do Porto DA, Jurado JO, Pasquinelli V, Alvarez IB, Aspera RH, Musella RM, García VE. CD137 differentially regulates innate and adaptive immunity against Mycobacterium tuberculosis. Immunol Cell Biol. 2012;90(4):449–56.
42. Bailly C, Thuru X, Goossens L, Goossens JF. Soluble TIM-3 as a biomarker of progression and therapeutic response in cancers and other of human diseases. Biochem Pharmacol.

2023;209:115445.

43. Yi L, Yan J, Wei P, Long S, Wang X, Gu M et al. The levels of soluble CD137 are increased in tuberculosis patients and associated with disease severity and prognosis. Eur J Immunol 2024 Jun 25:e2350796.

Figures

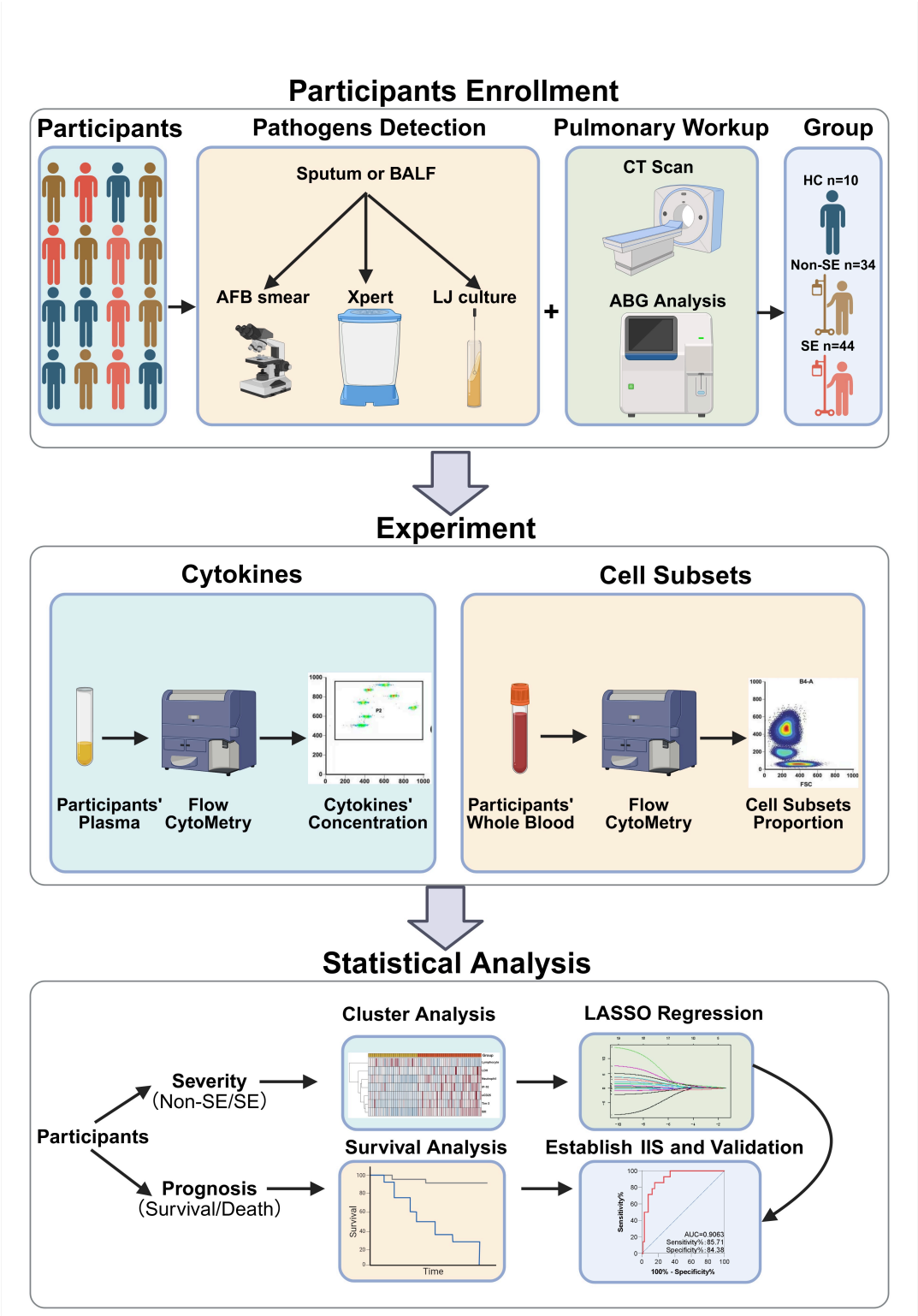


Figure 1

Schematic diagram of study design.

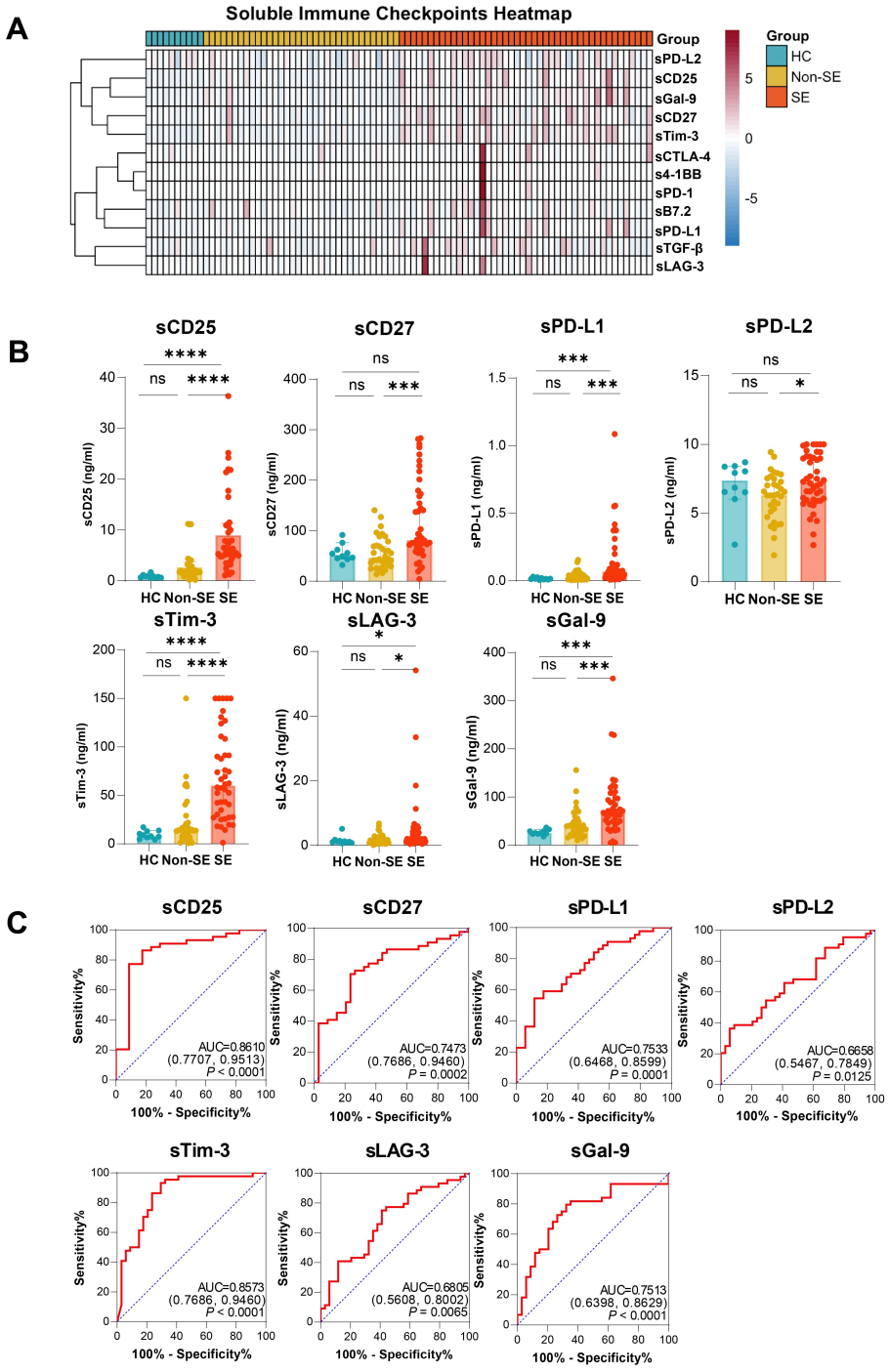


Figure 2

Plasma checkpoints were elevated and correlated with severe illness in PTB patients.

A. Cluster heatmap depicting the relative plasma levels of soluble checkpoint molecules in HCs (n=10, green), mild/moderate (n=34, yellow), and severe/critical (n=44, red) patients with PTB. Each column of the heatmap shows a patient, while the rows represent different checkpoint molecules. Significantly different soluble checkpoints between HCs, mild and severe PTB patients. Differences are indicated as *P* values. One-way ANOVA followed by post-hoc test was used to calculate the P-value. **P*0.05,**P0.01, ***P*0.005, ****P*0.001. C. Receiver operating characteristic (ROC) curves and the area under the curve (AUC)s of significantly different soluble checkpoints. ROC curves were used to estimate the sensitivity, specificity, and AUCs were performed to estimate the capacity of sCD25,sCD27,PD-L1,PD-L2,Tim-3, LAG-3, Gal-9 to distinguish non-SE from SE patients.

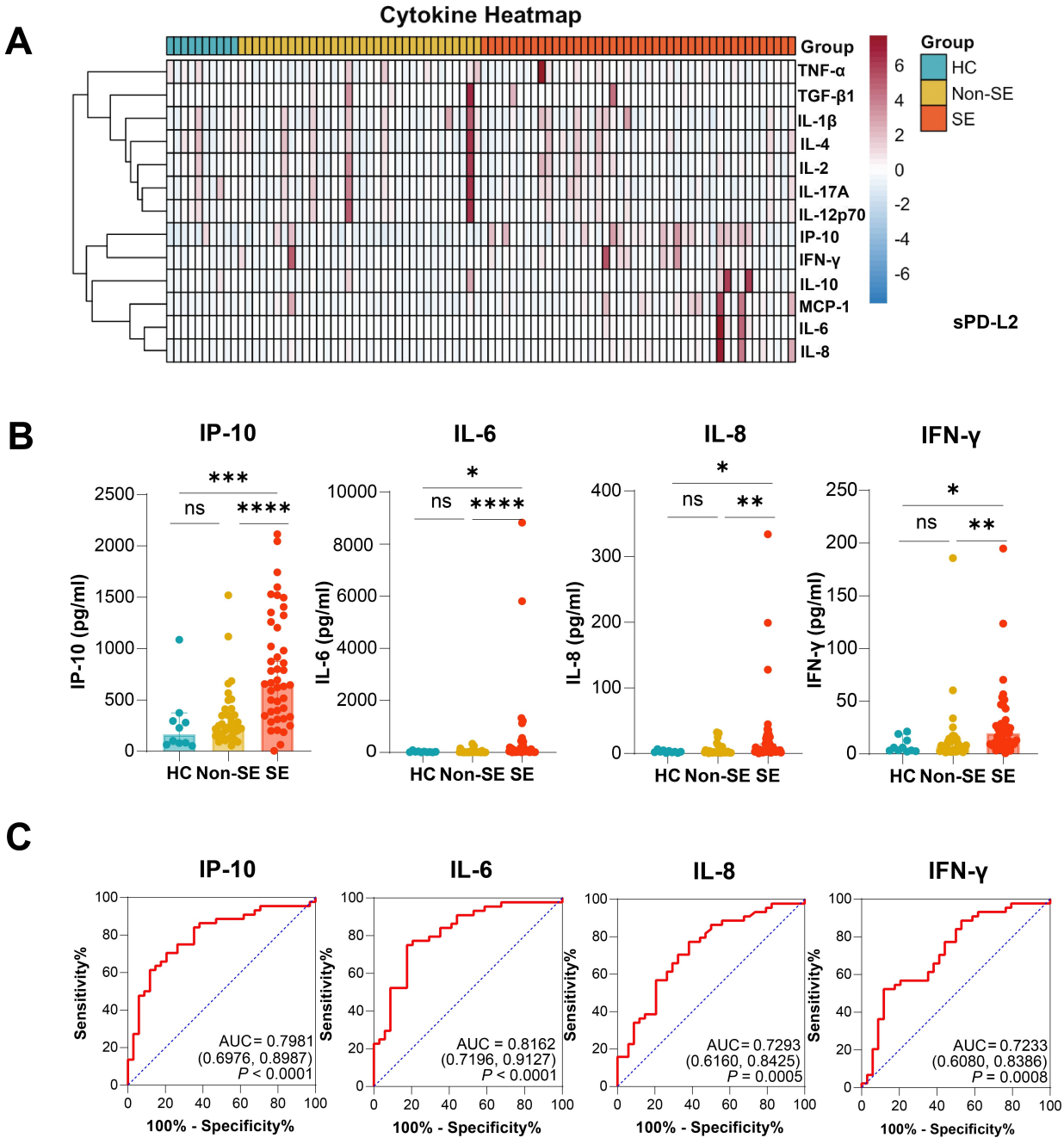


Figure 3

Comparison of plasma cytokine/chemokines concentrations between groups.

A. Cytokine heatmap of HCs (n=10, green), mild/moderate (n=34, yellow), and severe/critical (n=44, red) patients with PTB.

B. Significantly different cytokines between HCs, mild and severe patients. Differences are indicated as P values. One-way ANOVA followed by post-hoc test was used to calculate the *P*-value.*$P \leq 0.05$, ***$P \leq 0.005$.

C.ROC curves of IP-10, IL-6, IFN-γ, IL-8.

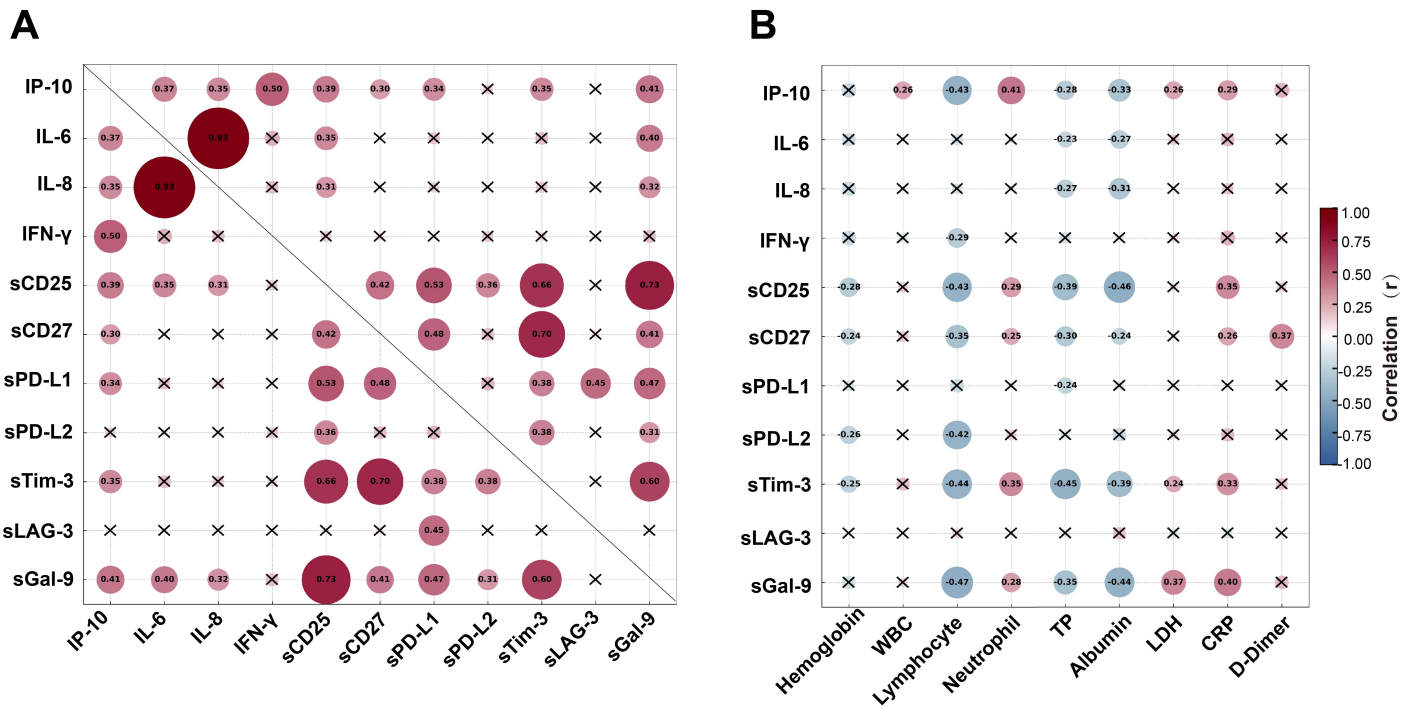


Figure 4

Correlations between elevated soluble checkpoint molecules, cytokines/chemokines and Laboratory Examination index in PTB patients

A, Correlations between elevated checkpoint molecules and cytokines.

Checkpoint molecules,such as sCD25,sCD27, PD-L1, PD-L2, Tim-3, LAG-3, Gal-9, and cytokine/chemokines such as IP-10, IL-6, IFN-γ, IL-8, were both significantly different between non-SE and SE Patients, we analyzed the correlations between each other and found that there were all positive correlations between them. The spot size is proportional to the correlation coefficient. P values with no significant correlation are marked with a black cross.

B, Correlations between Laboratory Examination index and elevated checkpoint molecules, cytokines/chemokines Spearman correlation method was used to calculated the P value. The spot size

is proportional to the correlation coefficient. Red spot: positive correlation; Blue spot: negative correlation; P values with no significant correlation are marked with a black cross.

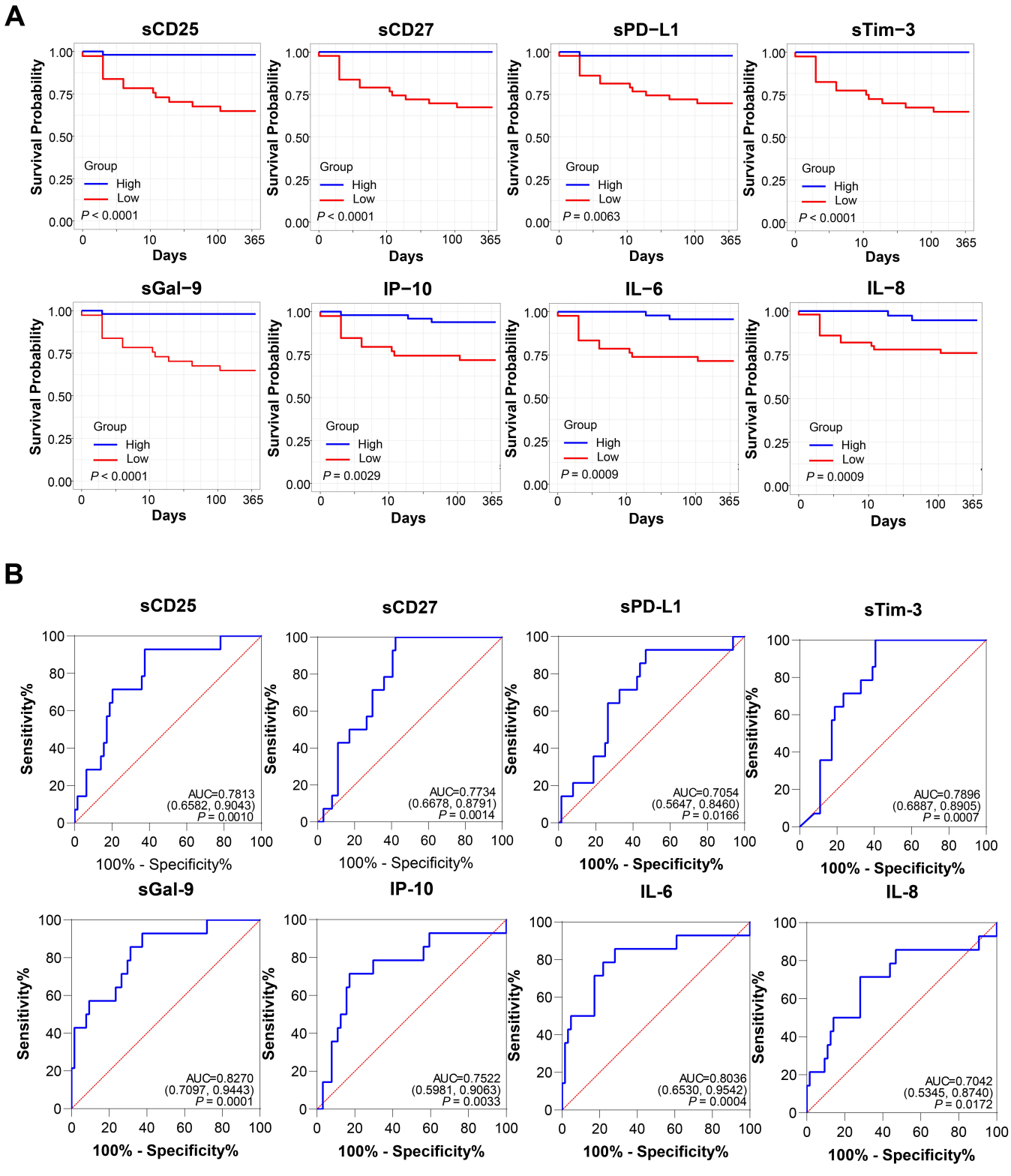


Figure 5

Plasma checkpoints and cytokines/chemokines correlate with PTB patients' prognosis

A. Plasma checkpoints and cytokines/chemokines correlate with PTB patients' prognosis. PTB patients (n=78) were subdivided into two groups, dichotomization was based on the cut-off values of patients' blood checkpoint or cytokines/chemokines levels. Patients with high levels of sCD25, sCD27, PD-L1, Tim-3, Gal-9, or IP-10, IL-6, IL-8, had shorter survival time. Survival analysis was performed using a Kaplan–Meier survival plot, and the log-rank test P value was calculated.

B. The AUC curves of sCD25, sCD27, PD-L1, Tim-3, Gal-9,IP-10, IL-6, and IL-8.

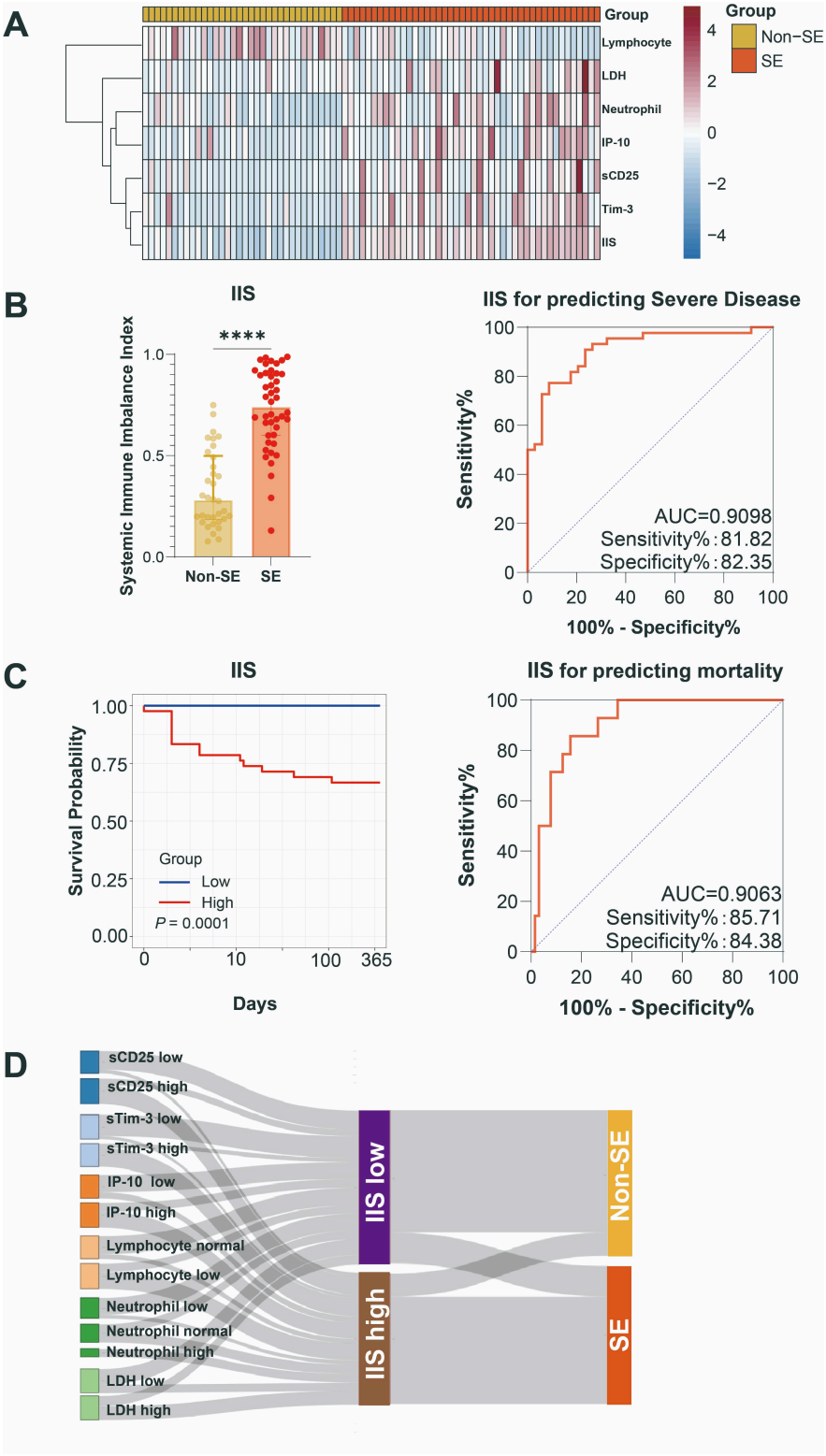


Figure 6

IIS could better predict PTB patients' severity and prognosis.

A. Cluster heatmap depicting the relative levels, counts or scores of six variables (Lymphocyte, LDH, Neutrophil, IP-10, sCD25, Tim-3) and Immune Imbalance Index (IIS) in mild/moderate (n=34, yellow), and severe/critical (n=44, red) patients with PTB. Each column of the heatmap shows a patient, while the rows represent different variables. B. Left: Comparison of IIS between Non-SE and SE patients. The difference was indicated by P value.****,P < 0.0001. Right: The AUC of IIS for predicting the severity of PTB disease. C. Left panel: Correlation of PTB patients' IIS and their prognosis. Dchotomization was based on the cut-off of IIS. Patients with high levels of IIS had shorter survival time. Kaplan-Meier was used. Right panel: The AUC of IIS for predicting mortality. D, Sankey diagram showing the distribution of patients based on levels of biomarkers (sCD25, Tim-3, IP-10, Lymphocyte, Neutrophil, LDH), and their relation to IIS categories (low or high) and disease severity groups (Non-SE or SE).

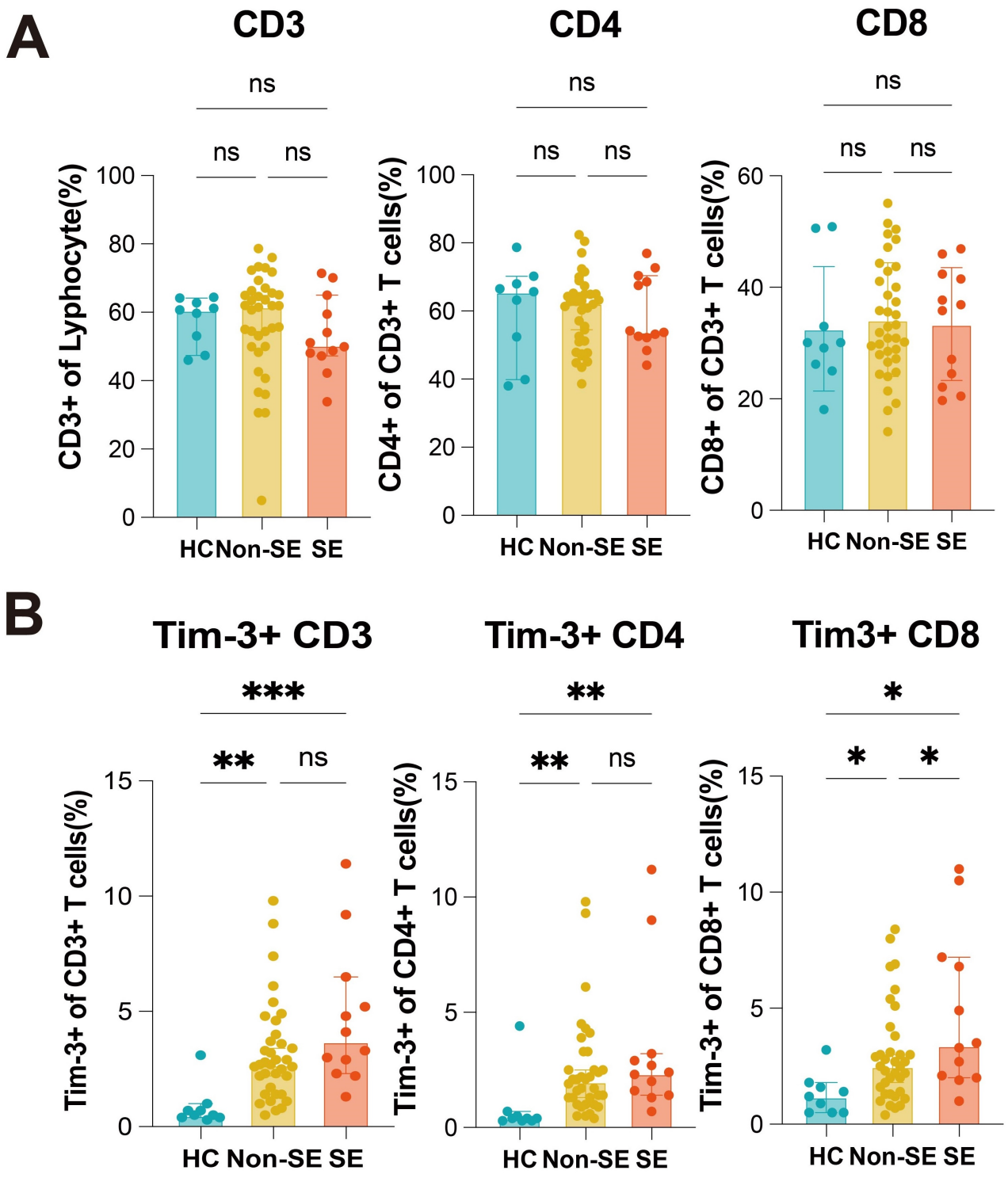


Figure 7

T cell subsets were dysfunctional and exhausted in PTB patients

A, The frequencies of CD3+T, CD4+T, CD8+T cell in HCs ,non-SE and SE patients. There was no difference in cell frequency of CD3+T cell, CD4+T cell, CD8+T cell between HCs, non-SE patients and SE patients. B,The frequencies of Tim-3+CD3+T, Tim-3+CD4+T, and Tim-3+CD8+T cell in HCs ,non-SE and SE patients.

There was significant difference in the frequencies of these subsets between HCs and non-SE patients, as well as between HC and SE patients. However, between non-SE and SE patients, only the frequencies of Tim-3+CD8+T cells show significant difference. Kruskal-Wallis test was used to assess overall differences across groups, and post-hoc comparisons were performed using Dunn's multiple comparison test to identify specific differences between pairs of groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryData610.docx