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ORIGINAL RESEARCH

Elderly Patients with Tuberculosis Combined with Diabetes Mellitus: A Comprehensive Analysis of Lymphocyte Subpopulation Dynamics, Clinical Features, Drug Resistance and Disease Regression

Xin Wang[1,*], Yu Song[2,*], Na Li[3], JingYi Huo[3], BingBing Wang[3], Xue Jiang[3], YuLiang Zhang[3]

[1]Department of General Internal Medicine, Infectious Disease Hospital of Heilongjiang Province, Harbin City, Heilongjiang Province, 150500, People's Republic of China; [2]Heilongjiang Agricultural Engineering Vocational College, Harbin City, Heilongjiang Province, 150025, People's Republic of China; [3]Department of Clinical Laboratory, Infectious Disease Hospital of Heilongjiang Province, Harbin City, Heilongjiang Province, 150500, People's Republic of China

*These authors contributed equally to this work

Correspondence: YuLiang Zhang, Department of Clinical Laboratory, Infectious Disease Hospital of Heilongjiang Province, No. 1, Jianshe Street, Hulan District, Harbin City, Heilongjiang Province, 150500, People's Republic of China, Email YLZ_zhangyuliang02@outlook.com

Objective: The aim of this study was to evaluate lymphocyte subsets and clinical outcomes in patients with pulmonary tuberculosis (PTB) combined with type 2 diabetes mellitus (T2DM).

Methods: Between January 2022 and March 2024, 320 patients aged > 60 years were included, 95 of whom had comorbid DM. Lymphocyte subsets (T-lymphocytes, B-lymphocytes, and natural killer cells, NK) were assessed in both groups of patients using fluorescence-associated cell sorting. Clinical characteristics, drug resistance, regression and lymphocyte subsets were compared in PTB patients with DM versus PTB patients alone. The correlation between lymphocyte subsets-related indices and the above characteristics in PTB patients with DM was analyzed.

Results: Compared with PTB patients alone, PTB patients with comorbid DM had a higher positive rate (81.05% vs 62.67%, $P = 0.001$), prolonged course of antituberculosis treatment (298 [244, 339] days vs 223 [179, 254], $P < 0.001$) and a lower cured rate (64.21% vs 75.56%, $P = 0.039$). Compared with PTB patients only, PTB patients with comorbid DM had higher T-lymphocytes and $CD4^+$ T-lymphocytes ($P < 0.001$, $P = 0.006$) and lower NK cells ($P < 0.001$). In PTB patients with comorbid DM, both treatment-prolonged patients and drug-resistant patients showed reduced NK cells. NK cells were slightly elevated in cured patients, although there was no significant difference ($P = 0.082$).

Conclusion: Lymphocyte homeostasis is altered in PTB patients with comorbid DM, making them more susceptible to severe clinical manifestations and therapeutic outcomes. Notably, NK cells have been identified as key regulatory cells with a significant impact on prolonged treatment course and drug resistance. This study provides new ideas and methods for individualized treatment of PTB combined with DM in the elderly, which can help to optimize the treatment plan and improve the outcome and quality of life of patients.

Keywords: tuberculosis, diabetes mellitus, lymphocyte subsets, clinical regression

Introduction

It has long been documented that diabetes mellitus (DM) is associated with the prevalence of pulmonary tuberculosis (PTB).[1] PTB remains the leading cause of mortality and morbidity worldwide, with an estimated 1.7 million deaths per year.[2] The latest data show that the prevalence of tuberculosis (TB) in China is still high.[3] At present, the number of Mycobacterium tuberculosis (MTB) infections in China is about 500 million, with about 1.5 million new cases each year, and the incidence of TB in China is characterized by a high rate of infection, high prevalence, and high rate of drug resistance.[4] At the same time, the prevalence of DM is soaring due to globalization, urbanization, population growth and

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rapid aging.[5] The management of TB in elderly patients is a special problem.[6] First, older people are less tolerant of anti-TB treatment with more frequent side effects. Second, older adults have more underlying disease and age-related immunosuppression, which can increase disease recurrence and reduce treatment success. Thirdly, older people with PTB often have non-specific symptoms, making early diagnosis difficult. Therefore, there is a need to improve the understanding and management of PTB in the elderly. The prevalence of DM in China is rapidly increasing.[7]

DM is closely associated with PTB, and the combination of the two can make the patient's condition more severe. A meta-analysis has shown that the risk of treatment failure and death in PTB patients with DM is higher than that in PTB patients without DM.[8] Therapeutically, DM requires strict dietary control, whereas PTB is a wasting disease and requires increased nutrition. In addition, PTB-DM is more prone to complications such as lung cavitation and pulmonary aspergillosis.[9] These increases bring more complexity and risk to the clinical treatment of PTB.

T cells play an important role in the immune response to PTB, and the measurement of peripheral blood T cells can reflect the immune status of the organism. MTB-induced anti-TB immunity is mainly based on T cell-mediated cellular immune response, and CD4 and CD8 T cells can recognize presented antigenic epitopes and also directly respond to antigens in an ultimate response. Previous investigators have found that the percentage of peripheral blood $CD3^+$ and $CD4^+$ T-cells are significantly lower and B-lymphocytes are increased in PTB patients than in healthy controls, with no significant difference in NK cells.[10] In another study, the percentage of peripheral blood $CD4^+$ is decreased and the percentage of NK cells is increased in PTB patients with DM compared to healthy controls.[11] Thus, lymphocyte subsets are complex in PTB and may be associated with various clinical features or disease processes. In terms of the pathomechanisms of DM, patients with DM may suffer from altered immune system function due to their hyperglycemic state, which may increase the susceptibility to PTB and the severity of the disease. Studies have shown that the immune system of DM patients exhibits dysfunction in response to MTB infection, particularly in the function of monocytes and macrophages.[12] In addition, hyperglycemia in diabetic patients may lead to abnormal cytokine secretion, which affects the immune system's response to MTB.[13]

Compared to younger patients, older patients usually have lower immune function, which is related to the aging of the immune system. The elderly often have some chronic inflammatory state in their bodies, which itself reduces the body's resistance to TB bacteria.[14] In turn, TB infection can exacerbate this chronic inflammatory state, creating a vicious cycle. In addition, PTB may also cause immunosuppression, making elderly patients also less resistant to other pathogens, increasing the risk of secondary infections.[15] Based on the difficulty of managing and treating PTB in the elderly and the complex immunopathologic mechanisms between DM and PTB, there is a need to investigate the characterization of lymphocyte subsets in the peripheral blood of elderly PTB patients with DM. However, there appear to be no studies comparing the characteristics of lymphocyte subsets between PTB patients with DM and PTB patients without DM alone.

The aim of this study was primarily to assess the correlation of lymphocyte subsets with clinical features, drug resistance and regression in PTB patients with DM. We hypothesized that lymphocyte subsets in PTB patients with DM would be associated with clinical characteristics, drug resistance, and regression.

Materials and Methods

Study Population

This was a prospective cross-sectional study in accordance with STROBE guidelines. Elderly PTB patients in Infectious Disease Hospital of Heilongjiang Province from January 2022 to March 2024 were included in this study.

Inclusion criteria: (1) Age greater than 60 years. (2) Clinical diagnostic criteria were based on clinical features, imaging results and laboratory tests, and met the clinical diagnosis of PTB patients in accordance with the WS 288–2017 Diagnosis for pulmonary tuberculosis standards. (3) Patients had not received standardized anti-TB drug therapy. Patients who met the above criteria and were clinically diagnosed with DM were included in the PTB with DM group, and the diagnostic of type 2 DM was in accordance with the criteria of the World Health Organization.[16]

Exclusion criteria: (1) patients with concomitant HIV infection, viral hepatitis; (2) patients with concomitant rheumatic diseases: systemic lupus erythematosus; (3) patients with tumors and those taking immunosuppressive drugs; (4) patients with other concomitant extrapulmonary infections, chronic obstructive pulmonary emphysema,

asthma, and bronchodilatation; (5) pregnant women or breastfeeding women; and (6) patients with irrational or irregular use of anti-TB drugs due to PTB for more than 1 month.

Finally, 95 PTB patients with DM and 225 PTB patients without DM were included. All subjects were asked to participate voluntarily and sign an informed consent form.

General Information and Clinical Characteristics

A pre-designed questionnaire was used to collect information on demographic characteristics such as age, gender, and history of alcohol and tobacco. Clinical characteristics (lung cavities, sputum smear and sputum culture results, drug resistance, course of treatment) and outcomes were collected through chart review. Glycosylated protein (HbAc1) values of PTB patients with DM were obtained during the last laboratory examination before treatment. Treatment status was categorized as cured, completed treatment, and adverse outcomes.[17] Adverse outcomes were defined as death, default, treatment failure, transfer to another jurisdiction, or other outcomes (eg, interruption of PTB treatment on medical advice). Overall treatment success was defined as cured + completed treatment. Course of treatment was defined as the time from the start to the end of anti-TB chemotherapy.

Treatment

Newly diagnosed PTB patients were treated with a standard regimen consisting of 2 months of isoniazid, rifampicin, pyrazinamide, and ethambutol (or streptomycin) followed by 4 months of isoniazid and rifampicin (2 h/4 h). The course of anti-TB therapy may be extended based on available radiologic and bacteriologic data and the clinical judgment of the physician. All pretreatment-positive cultures were sent to the laboratory for drug sensitivity testing and strain identification. Multidrug resistant-TB (MDR-TB) is defined as: MTB infected by TB patients confirmed in vitro to be resistant to at least both isoniazid and rifampicin. Rifampicin-resistant TB (RR-TB) is defined as MTB infected by TB patients confirmed to be resistant to rifampicin by in vitro DST.[18] Follow-up visits were conducted every 2 weeks after the start of the initial phase of treatment (within the first 2 months of treatment) and at least once a month during the middle and late phases of treatment. Follow-ups included regular outpatient visits and telephone calls.

Specimen Collection and Preparation

Fasting venous blood samples (2 mL) were taken before breakfast and collected into tubes containing anticoagulant (dipotassium ethylenediaminetetraacetate). All samples were collected before treatment.

Flow Cytometry Data Acquisition and Analysis

Four-color flow cytometry was performed on a flow cytometer using FACS Diva (version 4.0) software (Becton Dickinson). Cells were stained with antibodies conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE) with the following color schemes: (1) T lymphocytes, CD3-PE; (2) $CD4^+$ T lymphocytes, CD3-PE/CD4-FITC; (3) $CD8^+$ T lymphocytes, CD3-PE/CD8-FITC; (4) B lymphocytes, CD19-PE; (5) NK cells, CD3-PE/CD (16+56)-FITC. Cells were incubated with the dye for 30 min and then assayed. Lymphocyte gating was obtained at event rates of 30,000 to 100,000 by forward and sideways light scatterings. For data analysis, a sequential gating was used, with gating (forward vs sideways scattering) set transiently at visible lymphocytes, CD3 lymphocytes were then gated in CD3 vs side scatter histograms. CD3-CD4, CD3-CD8, CD14-CD16 and CD16-CD56 cells were further gated at diagnosis and at different follow-up intervals. Background staining obtained from isotype-matched control antibodies was removed, and the positivity threshold was determined.

Data Analysis

Statistical analyses were performed using SPSS 20.0 software. The Shapiro–Wilk test was used to determine the normality of the data. For normal distribution, measurement data were shown as mean ± standard deviation, and Student's *t*-test was used for between-group comparisons; data for continuous variables with skewed distributions were shown as median (M1, M3), and Mann–Whitney *U*-test was used for between-group comparisons. Count data were expressed as frequencies (n) and ratios (%) and were tested by chi- square test or Fisher's exact test. Spearman's

test was used to analyze the correlation between HbAc1 and lymphocyte subsets with Bonferroni correction. Factors associated with cure in PTB patients with DM were analyzed using univariate and multivariate logistic analyses, and those with *P* less than 0.05 in the univariate analysis were included in the multivariate logistic regression. Images were plotted using Hiplot (ORG) (https://hiplot.cn/).

Results

Demographic and Clinical Characteristics of Patients

A total of 320 patients were included in this study, of which 95 had combined DM. Among the PTB patients, 204 (63.75%) were males and 116 (36.25%) were females, with the median age of 63 years. Table 1 shows the demographic and clinical characteristics of patients. In terms of demographic characteristics, there was a higher proportion of males in the PTB with DM group (76.84% vs 58.22%, $P = 0.002$) and a higher number of patients with a history of smoking (50.53% vs 32.00%, $P = 0.002$); and in terms of clinical characteristics, there was a higher rate of sputum smear positivity in the PTB with DM group (73.68% vs 54.22%, $P = 0.001$), higher sputum culture positivity (81.05% vs 62.67%, $P = 0.001$) and longer treatment course ($P < 0.001$). In addition, on MDR/RR-TB resistance, the percentage was slightly higher in the PTB with DM group than in the PTB without DM group (7.37% vs 3.11%, $P = 0.089$). Consistent with expectations, patients in the PTB with DM group had higher FBG and HbA1c than those in the PTB without DM group (both $P < 0.001$); in terms of lipids, LDL-C was only observed to be slightly higher in the PTB with DM group than in the PTB without DM group ($P = 0.042$). No significant difference was observed between the two groups in terms of age and alcohol abuse ($P > 0.05$).

Table 1 Comparison of Demographic and Clinical Characteristics of Patients

Characteristics	PTB with DM (n = 95)	PTB Without DM (n = 225)	P value
Gender			0.002
Female	22 (23.16)	94 (41.78)	
Male	73 (76.84)	131 (58.22)	
Age	67 [63, 71]	68 [63, 72]	0.336
Alcoholism	29 (30.53)	52 (23.11)	0.163
Smoking history	48 (50.53)	72 (32.00)	0.002
Cavitary lesions	30 (31.58)	34 (15.11)	0.001
Sputum smear			0.001
Negative	25 (26.23)	103 (45.78)	
Positive	70 (73.68)	122 (54.22)	
Sputum culture			0.001
Negative	18 (18.95)	84 (37.33)	
Positive	77 (81.05)	141 (62.67)	
Drug resistance			0.089
MDR/RR-TB	7 (7.37)	7 (3.11)	
Absent	88 (92.63)	218 (96.89)	
Course of treatment (days)	298 [244, 339]	223 [179, 254]	< 0.001
FBG (mmol/L)	4.37±0.37	7.36±0.35	< 0.001
HbA1c (%)	6.3±0.46	7.37±1.60	< 0.001
TC (mmol/L)	4.19±1.38	4.52±1.50	0.374
TG (mmol/L)	1.60±0.44	1.72±0.50	0.325
HDL-C (mmol/L)	1.14±0.33	1.15±0.40	0.958
LDL-C (mmol/L)	2.23±0.30	2.62±0.68	0.042

Notes: Categorical data were expressed as n (%) and chi-square test was used. Continuous variable data were expressed as median (M1, M3) or $\bar{x} \pm S$, and the Mann–Whitney *U*-test or student's *t* test was used for between-group comparisons. $p < 0.05$ was significantly different.
Abbreviations: MDR/RR-TB, multidrug-resistant tuberculosis or rifampicin-resistant tuberculosis; FBG, fasting blood glucose; HbA1c, glycated protein; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Clinical Regression in Patients

All patients received standardized anti-TB treatment and Table 2 shows the outcome at the end of treatment. The results showed that 61 cases (64.21%) in the PTB with DM group were cured, 30 completed treatment (31.58%), and 4 adverse outcomes (of which 2 died from PTB, 1 failed, and 1 transferred out); 170 cases (75.56%) in the PTB without DM group were cured, 50 (22.22%) completed treatment, and 5 (2.22%) poor outcomes (2 died of PTB, 1 died of other, 1 failed, and 1 transferred out). In terms of overall success rate, there was no significant difference between the two groups (95.79% vs 97.78%, $P = 0.326$). However, for the cured rate, the PTB with DM group was lower than that of the PTB without DM group ($P = 0.039$).

Lymphocyte Subsets

Prior to treatment, these patients were analyzed for lymphocyte subsets (Table 3). The two groups had different characteristics in different subtypes of lymphocytes. Total lymphocytes included T lymphocytes, B lymphocytes, and NK cells. T lymphocytes were mainly categorized into T lymphocytes expressing CD4 surface antigens and T lymphocytes expressing CD8 surface antigens. Changes in the $CD4^+/CD8^+$ ratio reveal the dynamic changes in the immune system. Compared with the PTB without DM group, the PTB with DM group had a higher ratio of T lymphocytes and $CD4^+$ T lymphocytes ($P < 0.001$, $P = 0.006$) and a lower ratio of NK cells ($P < 0.001$). In addition, the proportion of $CD8^+$ T lymphocytes was slightly higher in the PTB with DM group, but there was no statistical difference ($P = 0.082$). No differences in the ratios of total lymphocytes, B-lymphocytes, and $CD4^+/CD8^+$ T-lymphocytes were observed ($P > 0.05$).

Lymphocyte Subsets in PTB with DM Patients with Different Clinical Characteristics, Drug Resistance, and Regression Profiles

Next, we assessed the correlation between HbAc1 levels and lymphocyte subsets in PTB with DM patients, as shown in Figure 1. Weak correlations were observed between HbAc1 and $CD8^+$ T cells (%) and $CD4^+/CD8^+$ (both $P < 0.05$), with a negative correlation with $CD8^+$ T cells (%) and a positive correlation with $CD4^+/CD8^+$. The characterization of

Table 2 Comparison of Patients' Treatment Regression

Group	Cured	Completed Treatment	Adverse Outcomes
PTB with DM (n = 95)	61 (64.21)	30 (31.58)	4 (4.21)
PTB without DM (n = 225)	170 (75.56)	50 (22.22)	5 (2.22)
P^a value	0.039	0.077	0.326
P^b value	< 0.001		

Notes: Data are expressed as n (%) and were tested using the chi-square test or Fisher's exact test. a, 2×2 columns compared with each other, b, 3×2 columns compared with each other. $p < 0.05$ is significantly different.

Table 3 Characterization of Lymphocyte Subsets in Patients with PTB with or Without DM with Different Clinical Features, Drug Resistance, and Regression

Types	PTB with DM (n = 95)	PTB Without DM (n = 225)	P value
Total Lymphocytes (%)	21.06 [14.31, 25.97]	18.10 [11.84, 26.4]	0.338
T lymphocytes (%)	70.4 [62.61, 76.29]	64.28 [57.41, 73.46]	< 0.001
$CD4^+$ T lymphocytes (%)	42.31 [35.60, 48.05]	39.07 [29.69, 45.55]	0.006
$CD8^+$ T lymphocytes (%)	21.73 [17.93, 28.17]	21.10 [15.42, 27.01]	0.082
B lymphocytes (%)	10.35 [7.30, 14.08]	10.18 [6.32, 15.66]	0.752
NK cells (%)	15.01 [9.52, 21.10]	18.45 [12.31, 25.82]	< 0.001
Ratio of $CD4^+/CD8^+$	2.01 [1.29, 2.72]	1.69 [1.26, 2.67]	0.514

Notes: Data for continuous variables were expressed as median (M1, M3), and the Mann–Whitney *U*-test was used for between-group comparisons. $P < 0.05$ was significantly different.

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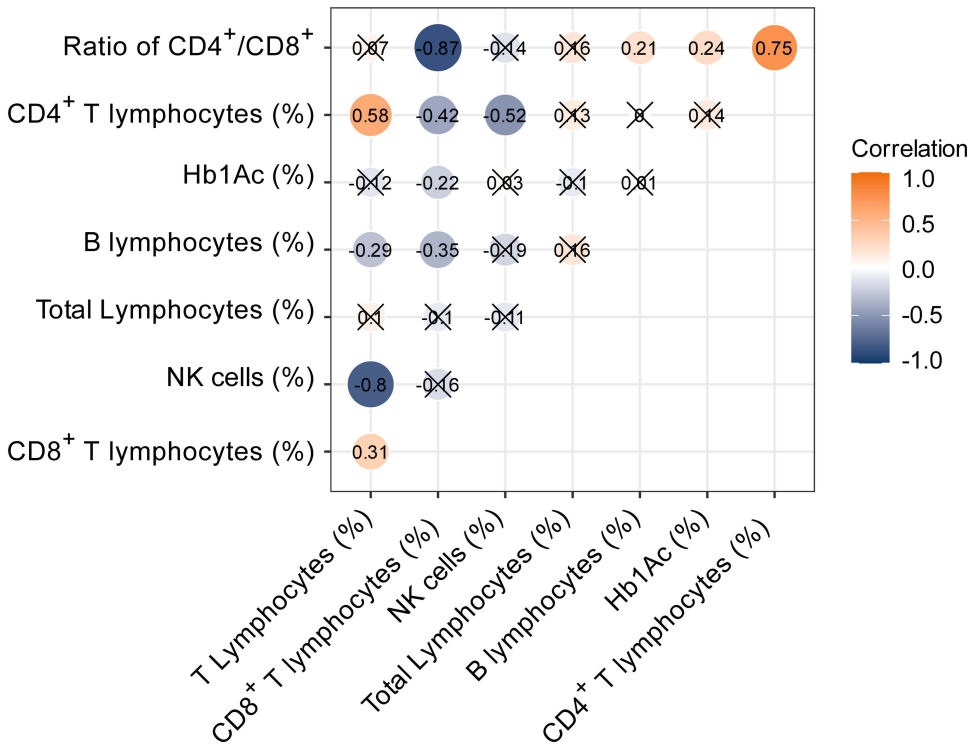

Figure 1 Correlation of HbAc1 with T lymphocyte subsets. Spearman's test was used to analyze the correlation between HbAc1 and lymphocyte subsets with Bonferroni correction.

lymphocyte subsets in PTB patients with DM with different clinical features, drug resistance, and regression is shown in Figure 2. Figure 2A shows that in PTB patients with DM, the levels of T lymphocytes (%) and $CD4^+$ T lymphocytes (%) were elevated (both $P < 0.001$), whereas the levels of B lymphocytes (%) and NK cells (%) were decreased in patients with pulmonary cavitary compared with those without pulmonary vacuoles ($P = 0.041$, $P < 0.001$). Figure 2B shows the levels of total lymphocytes (%), T lymphocytes (%), $CD4^+$ T lymphocytes (%), and the ratio of $CD4^+/CD8^+$ T lymphocytes were decreased in sputum culture-positive patients compared to sputum culture-negative patients ($P = 0.023$, $P < 0.001$, $P < 0.001$, $P < 0.001$), whereas the NK cell (%) levels increased ($P < 0.001$). Figure 2C shows that the lymphocyte subset profile of patients with extended treatment courses was similar to that of patients with pulmonary alveoli, with increased levels of T lymphocytes (%) ($P < 0.001$) and decreased levels of NK cells (%) ($P < 0.001$). The increased levels of T lymphocytes (%) appeared to be attributable to the increased levels of $CD8^+$ T lymphocytes (%) ($P = 0.007$). Figure 2D shows that both B lymphocytes (%) and NK cells (%) levels were reduced in resistant patients compared to non-resistant patients ($P = 0.039$, $P < 0.001$). Figure 2E shows that cured patients had a slight reduction in Total Lymphocytes (%) ($P = 0.047$), and no significant differences were observed for other subtypes of lymphocytes (all $P > 0.05$). Finally, univariate and multivariate logistic regression analyses were conducted to identify the factors influencing the cure of PTB in patients with DM following treatment. The results indicated that a positive sputum culture was the sole independent protective factor for the cure of PTB in patients with DM after anti-TB therapy (OR 0.026 [0.06~0.98], $P = 0.05$), as detailed in Table 4. Conversely, total lymphocyte (%) and NK cell levels were not found to be significant factors affecting the cure of PTB in this patient cohort.

Discussion

Among 320 elderly PTB patients, 29.69% had DM. In terms of clinical features and clinical regression, PTB patients with DM had higher rates of positive pathology, prolonged course of anti-TB treatment, and lower cure rates. Importantly, peripheral blood lymphocyte subsets in PTB patients with DM correlated with clinical features. Our findings

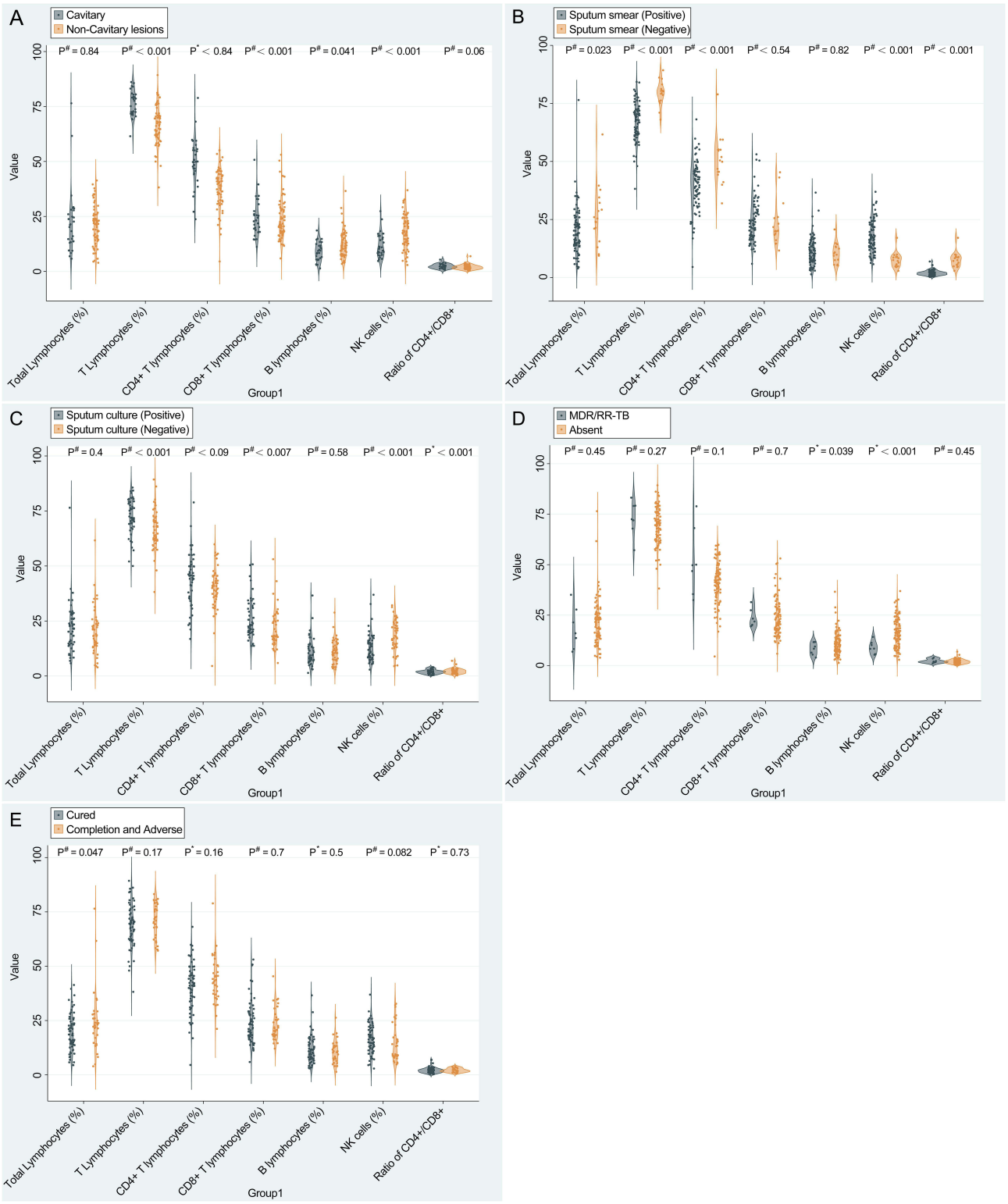

Figure 2 Characterization of lymphocyte subsets in PTB patients with DM with different clinical features, drug resistance, and regression. (**A**) Pulmonary vacuoles versus no pulmonary vacuoles; (**B**) Sputum culture positive and sputum culture negative; (**C**) Extension of anti-TB treatment course; (**D**) Drug resistance; and (**E**) Clinical outcome. Data are shown as violin plots. Student's *t*-test (*) or Mann–Whitney *U*-test (#) was used for between-group comparisons. $p < 0.05$ was significantly different.

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Table 4 Univariate and Multivariate Logistic Regression Analysis of Factors Associated with Healing in Patients With PTB With DM

Variables	Univariate			Multivariate
	P	OR (95% CI)	*P*	OR (95% CI)
Gender				
Female		1.00 (Reference)		
Male	0.264	0.30 (0.04–2.48)		
Age	0.123	1.12 (0.97–1.31)		
Alcoholism				
No		1.00 (Reference)		
Yes	0.744	1.26 (0.31–5.14)		
Smoking				
No		1.00 (Reference)		
Yes	0.36	0.54 (0.15–2.00)		
Sputum Smear				
Negative		1.00 (Reference)		
Positive	0.939	1.06 (0.26–4.34)		
Cavitary lesions				
Negative		1.00 (Reference)		1.00 (Reference)
Positive	0.009	0.18 (0.04–0.77)	0.05	0.026 (0.06–0.98)
Drug resistance				
MDR/RR-TB		1.00 (Reference)		
Absent	0.817	0.77 (0.08–7.06)		
Course of treatment	0.295	0.99 (0.99–1.00)		
Hb1Ac (%)	0.098	0.71 (0.50–1.02)	0.157	0.80 (0.54–1.18)
Total Lymphocytes (%)	0.149	0.97 (0.92–1.01)		
NK cells (%)	0.148	1.08 (0.97–1.19)		

Notes: $P < 0.05$ was significantly different.
Abbreviations: OR, Odds Ratio; CI, Confidence Interval.

have important implications for the development of an early two-way screening and personalized clinical treatment regimen for PTB patients with comorbid DM to improve the prognosis of PTB patients.

DM is now known to be one of the risk factors for PTB, and the proportion of DM in PTB increases with the prevalence of DM.[19] This study focused on elderly PTB patients, and the prevalence of DM in PTB patients was found to be 29.69%, which is higher than the prevalence in the general population. Our findings are consistent with those of other countries with a high burden of PTB.[20] In addition, the highest prevalence of PTB with comorbid DM has been reported in a study in the Asian population, which could be as high as 33.1%.[21] However, in the elderly population of Shanghai, the prevalence of DM among PTB patients is 19.9%. This discrepancy is associated with different degrees of population aging[22] and the prevalence of DM in different cities. Consistent with a previous study,[23] the present study also observed that the prevalence of DM was higher in male patients than in females among PTB patients.

The immune response induced by MTB relies primarily on cellular immunity, specifically T lymphocytes. When MTB infects the body, the immune system activates T lymphocytes to fight the pathogen. In this process, T lymphocytes (including $CD4^+$ T cells and $CD8^+$ T cells) proliferate and differentiate into effector cells that participate in immune responses, such as activating other immune cells (macrophages) and promoting inflammatory responses.[24,25] Furthermore, activated $CD3^+CD4^+$ T cells in the peripheral blood of patients with DM is significantly higher compared to healthy individuals, which attributes to the persistent increase in blood glucose leading to a chronic inflammatory response in the body, which activates the immune system.[26] Our results showed an increase in T-lymphocytes (%) and $CD4^+$ T-lymphocytes (%) and a decrease in NK cells (%) in PTB patients with DM compared to PTB patients alone (PTB without DM). Therefore, we hypothesized that when PTB patients with comorbid DM, the organism is subjected to dual stimulation by both MTB and DM. This dual stimulation would further exacerbate the proliferation and

differentiation of T lymphocytes and $CD4^+$ T lymphocytes. However, inconsistent with a previous report, PTB patients with DM have lower $CD4^+$ T-cell counts compared to patients with TB alone or DM alone.[27] In this regard, we believe that the immune status of patients with PTB with DM is complex and closely related to the body's immune stress response, immune dysfunction, increased risk of infection, and autoimmune response. These changes not only reflect the abnormal immune status of the patient, but may also have an important impact on the progression of the disease and the occurrence of complications. Therefore, changes in the immune status of diabetic patients should be closely monitored in clinical practice and appropriate therapeutic measures should be taken to maintain the stability of their immune function. For NK cells, unlike T lymphocytes, their ability to kill target cells does not depend on antibodies and complement, and is the first immune defense barrier in the organism. When the organism is infected with MTB, NK cells are able to respond rapidly to inhibit the replication and spread of MTB through their killing effect. It is known that an increase in the number of NK cells and their activity has a better therapeutic effect on the patients.[28,29] Consistent with these results, the present study showed that PTB patients with DM had significantly lower levels of NK cells (%) and lower cure rates after standardized treatment compared to PTB patients alone. It is known that due to metabolic disorders in the body, immune function is impaired, especially both intrinsic and acquired immunity are reduced, resulting in increased susceptibility to MTB. Therefore, blood glucose stabilization in DM patients is important for treatment. We correlated HbAC1, an indicator that responds to the glycemic stability of DM patients in the past 2–3 months, with lymphocyte subsets. The results showed that HbAc1 had a negative correlation with $CD8^+$ T cells (%) and a positive correlation with $CD4^+/CD8^+$. Normally, elevated blood glucose is associated with elevated $CD8^+$ T cells (%).[30] However, this is closely related to the immune state the patient is in, and hyperglycemia may reduce the number of $CD8^+$ T cells only by inducing apoptosis.[30] Based on the complexity of immunity, we intensively investigated the characterization of lymphocyte subsets in the peripheral blood of PTB patients with DM with different clinical characteristics, drug resistance, and regression. Compared with patients without cavitary, patients with cavitary had increased levels of $CD4^+$ T lymphocytes (%) and decreased levels of B lymphocytes (%) and NK cells (%); for pathologically positive patients, the levels of T lymphocytes (%), $CD4^+$ T lymphocytes (%), and $CD4^+/CD8^+$ T lymphocytes were decreased, whereas NK cell (%) levels increased. Drug resistance in PTB patients is the main reason for prolonged course of therapy.[31] In this study, patients with prolonged treatment had similar characteristics to those who were resistant, both showing reduced levels of NK cells (%). In addition, NK cells (%) were slightly elevated in cured patients, although there was no significant difference. These results show that the expression of lymphocyte subsets in patients with different clinical features of the same disease is complex and inconsistent, and that reduced levels of NK cells (%) are strongly associated with prolonged treatment and drug resistance. At the time of disease, NK cells provide effective immune protection for the organism through mechanisms such as rapid response, extensive killing and immunomodulation, and can, to some extent, most directly respond to the current immune status of the organism.[32] In addition, in drug-resistant patients, the level of B lymphocytes (%) was reduced. B lymphocytes are mainly involved in the humoral immune response of the organism, and under normal conditions, the immune system balances the relationship between the immune response and immune tolerance through a variety of mechanisms. However, when humoral immunity is reduced, this balance may be disturbed, resulting in an excessive or insufficient immune response. This may promote drug resistance.[33] In addition, when humoral immunity is reduced, the body's immune pressure against pathogens is weakened, making it easier for pathogens to survive and multiply in the body. This increases the opportunity and duration of exposure of the pathogen to the drug, thereby promoting drug resistance.[34] A previous study reports that in patients with chronically infected diabetic foot ulcers, B-lymphocytes are significantly lower in patients infected with drug-resistant pathogens compared to the group infected with sensitive bacteria.[35] However, this study only looked at the analysis of drug resistance in PTB patients with DM and actually encountered the limitation of insufficient sample size for the importance of subgroup (drug resistance) analysis in TB patients without DM. Specifically, there were only 7 patients with MDR/RR-TB (multidrug/rifampicin-resistant TB) in the cohort studied, compared to a high of 218 drug-free patients. This sample size imbalance leads to the possibility that analyses of MDR/RR-TB subgroups may lack sufficient statistical power to affect the reliability and accuracy of the results. We believe that for subgroup analyses, the results of the current analysis need to be further validated in a larger cohort to have high confidence. Based on the above characteristics, we evaluated the factors associated with healing after treatment in PTB patients with DM and observed only positive sputum culture as

a protective correlate of healing after anti-TB treatment. For this, glycemic control has also been reported as a factor affecting cure. Hyperglycemia may affect the metabolism and efficacy of antiTB drugs. The metabolism and excretion of some anti-TB drugs in the body may be affected by blood glucose levels, which may lead to treatment failure.[36] In addition, hyperglycemia may increase the risk of diabetic complications in patients, such as diabetic nephropathy and diabetic retinopathy. These complications may further exacerbate the patient's condition and increase the difficulty of treatment and the incidence of adverse effects. However, glycemic control of patients was not adequately considered in this study. In future studies, we should record more comprehensive information about patients' glycemic management, including frequency of glucose monitoring, use of hypoglycemic agents, dietary and exercise interventions. This will help us to more accurately assess the impact of differences in glycemic management on patient outcomes.

The strength of this study is that it assessed the characterization of lymphocyte subsets in PTB patients with DM across different clinical features, drug resistance and regression scenarios. This study has some limitations; the immune status of TB patients, especially those with comorbid DM, is complex when it comes to their immune status, and changes in lymphocyte subsets during treatment are more useful to explore the correlation between the immune status of the organism and drug resistance and regression. Second, we underestimated the complexity of immunomodulation in the clinic, and based on the available data, the mechanism of these lymphocyte subset changes in patients, which involves further multiclassification of lymphocyte subsets and cytokine secretion, cannot be well explained. Finally, since anti-TB treatment is a standardized cyclic treatment for PTB patients with comorbid DM, the control of blood glucose during treatment greatly affects the immune status and regression of patients. In addition, our study sample did not cover the entire population of patients with PTB, especially those who had received anti-TB treatment. This means that the results of our study may not be directly applicable to the PTB community as a whole, especially those patient groups who have received treatment. To compensate for this limitation, we make recommendations for future studies that could expand the sample size. Specifically, we suggest that future studies could include more patients with PTB from different backgrounds, with different conditions, and at different stages of treatment in order to more comprehensively assess treatment outcomes in different patient groups. This will help improve the generalizability and applicability of the study results and provide a more accurate and reliable basis for the treatment of TB. However, our findings may suggest a unique role for certain immunomodulatory mechanisms, especially NK cell reduction, in PTB-DM, which deserves further exploration in future studies. Therefore, it is necessary to design a prospective study to investigate the effect of glycemic control on final regression and the correlation between glycemic changes and fluctuations in lymphocyte subtypes in these patients.

Conclusion

PTB patients with comorbid DM are more likely to have severe clinical manifestations and suboptimal treatment outcomes. The immune status of PTB patients, especially those with comorbid DM, is complex. In this context, NK cells have been identified as key regulatory cells that significantly influence patient treatment course and drug resistance. This suggests that in the course of treatment, in addition to focusing on the efficacy of anti-TB drugs, we should also pay attention to the patient's immune status, so as to improve drug sensitivity and reduce the occurrence of drug resistance by regulating the immune function. In the future, we can try to combine immunomodulation with anti-TB treatment to develop a more comprehensive treatment program to improve the cure rate and quality of life of patients. This study provides new ideas and methods for the treatment of elderly patients with TB combined with DM, with a view to bringing better therapeutic effects and quality of life to patients.

Data Sharing Statement

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics Approval

The present study was approved by the Ethics Committee of Infectious Disease Hospital of Heilongjiang Province and written informed consent was provided by all patients prior to the study start. All procedures were performed in accordance with the ethical standards of the Institutional Review Board and The Declaration of Helsinki, and its later amendments or comparable ethical standards.

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Disclosure

The authors have no conflicts of interest to declare for this work.

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