Immune Dysfunction of T Lymphocytes may be a Risk Factor for Pleurisy Tuberculoma

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**Immune dysfunction of T lymphocytes may be a risk factor for pleurisy
tuberculoma**

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Running head: Immune dysfunction of pleurisy tuberculoma

ABSTRACT

Background: Pleurisy tuberculoma (PTM) is a benign proliferative disease that occurs most commonly after effective treatment of tuberculous pleurisy. In this study, we investigated the risk factors for PTM. **Methods:** The data of 56 consecutive tuberculoma patients treated at the Nanjing Thoracic Hospital and the Second Hospital between March 2013, and April 2020, were extracted from the hospital database and reviewed retrospectively. Sixty patients treated effectively for tuberculous pleurisy without PTM were included as the control group as most cases of PTM have a history of tuberculous pleurisy. We analyzed the clinical characteristics, laboratory examination results, and imaging features to identify potential risk factors for PTM. **Results:** The mean age of the PTM patients was 29.04 ± 6.95 years, with no difference between males and females. The onset of PTM was 4.29 ± 2.34 months from the diagnosis of tuberculous pleurisy. The PTM lesions were more commonly located in the lower lobes with no difference in the left and right lung distribution. There were no significant differences in the presenting symptoms and underlying disease of the patients in the PTM and control groups. Pleural thickening was more common in the PTM group (44.64% vs. 23.33% respectively, $P = 0.015$) and ADA activity was higher (48.32 ± 19.19 vs. 44.79 ± 24.57) compared with the control group. There were no significant differences between the groups in terms of the absolute numbers of CD4⁺ and CD8⁺ T lymphocyte cells or the CD4⁺/CD8⁺ T cell ratio ($P > 0.05$). Expression of the activation marker Ki-67 on CD4⁺ and CD8⁺ T cells was significantly increased in the PTM group compared with the control group ($P < 0.05$).

Conclusions: Our results indicate that dysregulation of T lymphocytes may be a potential risk factor for PTM.

Keywords: pleural tuberculosis, tuberculous pleural effusion (TPE), pleural tuberculoma (PTM), adenosine deaminase (ADA), T lymphocyte subsets, T lymphocyte activation.

Introduction

Tuberculosis (TB) is the one of the world's most common infectious diseases. In 2019, around 1.2 million people died from TB and approximately 208,000 of these people were HIV-positive[1]. Pleural tuberculosis usually presents in the form of pleural effusions, and is accompanied on rare occasions by pleural-based nodules, which account for the 10% of the pleural tuberculosis-pleural tuberculoma (PTM) cases that were first reported by Suzuki et al., [2] in 1994. If left untreated, the size and numbers of these benign proliferative lesions increase and can severely damage lung function as shown in Figure 1. Some cases of PTM even exhibit necrosis and purulence. The empyema flows into the pleural space leading to tuberculous empyema, which requires surgical removal of the lesion. This causes great trauma to the patient and significantly reduces the quality of life in patients in addition to increasing health care expenses. Single or multiple round or quasi-round ('D-shaped') mass shadows, were observed in computed tomography (CT) imaging with central necrosis and a distinct border noted in contrast-enhanced CT imaging (Figure 2). Almost all PTMs occur during the course of treatment for tuberculous pleural effusion (TPE); therefore, we hypothesized that the incidence of PTM is closely related to TPE, although the pathogenesis is unknown. Based on currently available information[3], the mechanism underlying PTM may be related to several factors. PTM arising during the course of anti-tuberculous therapy and follow-up of a caseous pneumonia might be attributable to paradoxical or hypersensitivity reactions to anti-tuberculous therapy. The immune system is also known to play a critical role in

controlling *Mycobacterium tuberculosis* (Mtb) replication and may play a role in PTM. However, PTM may result from a localized fibrous tumor of the pleura, a localized mesothelioma, or metastasis, rather than chronic inflammation. Thus, the pathogenesis of PTM remains to be fully elucidated with only a few case reports of solitary or multiple PTM and a lack of extensive clinical observational and experimental studies. Recent advances in techniques for the diagnosis of pulmonary tuberculosis and an emphasis on PTM have improved the diagnosis of PTM, although the characteristics and pathogenesis of this condition have rarely been investigated. To provide a greater understanding of the disease and determine the clinical risk factors for PTM, we conducted a retrospective analysis of the clinical data of 56 PTM patients and cured TPE patients without PTM.

Patients and methods:

Patients:

The data of 56 consecutive tuberculoma patients treated at the Nanjing Thoracic Hospital and the Second Hospital between March 2013, and April 2020 were extracted from the hospital database and reviewed retrospectively; this group was defined as the PTM group. In addition, the data for 60 patients effectively treated for tuberculous pleurisy were included as the control group because most PTM patients have a history of tuberculous pleurisy. Diagnosis of PTM and TPE was based on accepted criteria[4]. The following exclusion criteria were applied: i) Patients with any medical illness including (auto)immune disorders such as psoriasis, inflammatory

bowel disease, chronic kidney disease, rheumatoid arthritis, HIV infection, asthma, and neuroinflammatory disorders including Parkinson's disease and multiple sclerosis but not tuberculosis; ii) Patients with a history of tuberculosis in the past; iii) Patients on current and lifetime treatment with immunomodulatory drugs including glucocorticoids; iv) Pregnant or lactating women. Both the PTM and control groups were treated with whole-body standard or extended chemotherapy regimen 3–6HRZE(S)/6–12HR (isoniazid, rifampin, ethambutol, and pyrazinamide add or don't add streptomycin for 3–6 months followed by isoniazid and rifampin for 6–12 months) according to World Health Organization (WHO) guidelines [5]. These patients were followed up for 6 months and the data from clinical, radiological, pleural fluid, and histological examinations were collected. The imaging of the lesions was evaluated independently by two highly experienced two radiologists.

This study was approved by the Ethics Committee of Nanjing Chest Hospital and the Second Hospital (no:2013-LY-kt022). All participants provided written informed consent for the collection of samples and subsequent analyses.

Flow cytometry analysis

To interrogate the phenotype of Mtb-specific CD4⁺ T cells and identify Mtb-specific CD4⁺ and CD8⁺ T cells from bulk T cells, we used the classic dual flow cytometric analysis of IFN γ ⁺CD4⁺ and IFN γ ⁺CD8⁺ T cells stimulated in vitro with the Mtb-derived reagents ESAT-6 and CFP-10. Heparinized blood samples were collected and total peripheral blood lymphocytes were counted using standard hematological techniques. Lymphocytes were obtained by density gradient centrifugation, Peripheral

blood mononuclear cells 1×10^6 / l (PBMC) were stimulated for 16 h at 37°C with a mixture of recombinant ESAT-6 and CFP-10 proteins (Lionex, Braunschweig, Germany). Cells were then stained using several combinations of the following monoclonal antibodies: phycoerythrin cychrome (PECy5) conjugated anti-CD3, allophycocyanin (APC) conjugated anti-CD4, fluorescein isothiocyanate (FITC) conjugated anti-CD8, fluorescein isothiocyanate, and FITC conjugated anti-HLA-DR, BV421 and Ki-67. Data were analyzed with CellQuest software (BD Biosciences). All samples obtained for the longitudinal study of each patient were processed in the same experiment by the same blinded researcher to avoid inter-assay variability.

Statistical analysis

All data were analyzed using SPSS 26.0 (SPSS Inc. Chicago, IL, USA). Continuous variables (age, ADA, LDH, total protein and the absolute count of CD3 and CD8) were expressed as means $\pm$ standard deviation (SD). Data from two groups were compared using Student's *t*-test (for mean values). Categorical data (sex, side of effusion, comorbid diseases, imaging findings, complications, and HLA-DR⁺ CD4⁺/CD4⁺, HLA-DR⁺ CD8⁺/CD8⁺, Ki-67⁺ CD4⁺/CD4⁺, Ki-67⁺ CD8⁺/CD8⁺ values were summarized as percentages. Chi-squared or Fisher's combined probability tests were used to evaluate differences between categorical variables. *P*-values < 0.05 were considered to indicate statistical significance.

Results

Clinical characteristics analysis of PTM patients and controls

A total of 56 PTM patients (mean age, 29.04 ± 6.95 years; 48.21% males) were enrolled in the study. All PTM patients had a history of tuberculous pleurisy and the onset of PTM was 4.7 ± 6.2 months after diagnosis of TPE. Thirty-five (62.50%) patients had a single lesion, and the PTM lesions were most common in the lower lobes with no significant difference between the left and right lung distribution ($P = 0.128$) (Table 1). The control group consisted of 60 patients treated effectively for tuberculous pleurisy and without PTM. There were no significant differences in the proportion of males and females and the mean age of patients in the PTM and control groups ($P > 0.05$). In terms of TPE, there were no significant differences in the proportions of patients with right-sided and left-sided effusion ($P > 0.05$). The most common presenting symptoms in the PTM and control groups were cough with/without expectoration (65.2% vs. 48.33%), fever (42.86% vs. 46.67%) and shortness of breath (35.71% versus 38.33%). The most common comorbidity was diabetes mellitus (8.93% vs. 10.00%), followed by chronic kidney disease (3.57% vs. 5.00%). No significant difference were observed in the pleurisy locations, presenting symptoms, and comorbidities between the experimental and control groups ($P > 0.05$). The most common imaging findings in the lungs of patients in the PTM and control groups included fibrous stripes (25.00% vs. 31.67%), patchy consolidation glass opacities (21.43% vs. 25.00%), and nodules or masses (19.64% vs. 16.07%). Compared with the control group, a significantly larger proportion of patients in the PTM group had pleural thickening (44.64% vs. 23.33%, $P = 0.02$). In addition, ADA

levels were higher in the PTM group than those in the control group (48.32 ± 19.19 vs 44.79 ± 24.57 ; $P = 0.03$) (Table 2 and Figure 4).

T lymphocyte subsets and their activation indexes

T cell subsets, such as CD4⁺ and CD8⁺ cells have roles in the acquired immune response to Mtb and controlling disease. Loss of CD4⁺T cells induced by HIV infection is recognized to increase susceptibility to TB[6]. The immune status of patients was determined not only by analysis of the number of immune cells, but also evaluation of cell activation. In this study, we analyzed the expression of Ki-67 and human leukocyte antigen (HLA)-DR on T cell subsets involved in TB infection as biomarkers of activation. Flow cytometric analyses showed that there were no significant differences between the PTM and control groups in terms of the absolute numbers of CD4⁺ and CD8⁺ T cells and the ratio of CD4⁺/CD8⁺ T cells ($P > 0.05$). In the PTM group, both CD4⁺ T and CD8⁺ T cells expressed higher levels of Ki-67 than those in the control group ($P < 0.05$), whereas there was no significant difference in the expression of HLA-DR by either of the T cells subsets in the two groups ($P > 0.05$) (Table 2 and Figures 3 and 5).

Table 1. Clinical and demographic characteristics of 56 PTM patients

Characteristic	n (%)	Chi-square/ <i>t</i> -value	<i>P</i> -value
Onset of PTM (mean $\pm$ SD), months	4.29 $\pm$ 2.34	-	-
Single	35 (62.50)	-	-
PTM location (n = 56)			
Right upper lobe	9 (16.07)	-	-

Right middle lobe	7 (12.50)	-	-
Right lower lobe	19 (33.93)	-	-
Left upper lobe	2 (3.57)	-	-
Left lower lobe	23 (41.07)	-	-
Right lobe	29 (51.79)	2.312	0.128
Left lobe	21 (62.50)		
Bilateral	6 (10.71)	-	-
Upper lobe	18 (32.14)	20.68	0.000
Lower lobe	42 (75.00)		

Table 2. Clinical and demographic characteristics of 56 patients with tuberculoma compared with the 60 pleurisy cured patients

Contents	PTM (n = 56)	Pleurisy cured group (n = 60)	Chi-square /t-value	P-value
Male, n (%)	27 (48.21)	32(53.33)	0.30	0.58
Age, (mean ±SD), years	29.04 ± 6.95	31.78± 13.11	-1.46	0.16
Location of the pleural fluid				
Left-sided	26 (48.43)	28 (46.67)	0.53	0.47
Right-sided	21 (37.50)	21 (35.00)	0.78	0.08
Bilateral	9 (16.07)	11 (18.33)	0.10	0.75
Symptoms (%)				
Asymptomatic	12 (21.43)	14 (23.33)	0.06	0.81
Cough with/without expectoration	35 (62.50)	29 (48.33)	2.35	0.13
Fever	24 (42.86)	28 (46.67)	0.17	0.68
Shortness of breath	20 (35.71)	23 (38.33)	0.09	0.77
Chest pain	15 (44.64)	12 (20.00)	0.75	0.39
Dyspnea	9 (16.07)	11 (18.33)	0.10	0.75
Night sweats	4 (7.14)	9 (15.00)	-	0.24
Combined imaging features				
Fibrous stripes	14 (25.00)	19 (31.67)	0.63	0.43
Patchy consolidation glass opacities	12 (21.43)	15 (25.00)	0.21	0.65
Nodule or mass	11 (19.64)	9 (16.07)	0.44	0.51
Bronchial disseminated lesion	6(10.71)	7 (11.67)	0.03	0.87
Caseous pneumonia	5 (8.93)	9 (15.00)	1.01	0.32
Cavity	6 (10.71)	8 (13.33)	0.19	0.67
Miliary opacities	3 (5.36)	5 (8.33)	-	0.72
Pleural thickening	25 (44.64)	14 (23.33)	5.89	0.02
Normal pulmonary imaging	12 (21.43)	9 (15.00)	0.81	0.37
Laboratory findings of pleural effusion				
ADA, (mean ± SD), (U/L)	48.32± 19.19	44.79± 24.57	t=2.17	0.03
LDH, (mean ± SD), IU/L	332.37±195.63	290.20±175.52	t= 1.05	0.30
Total protein, (mean ± SD), g/L	43.90±14.86	40.79±15.42	t=1.34	0.51

T cell subsets and activation				
marker expression				
Total CD3 ⁺ T	1104.87±387.06	999.48±332.76	t=1.72	0.09
Total CD4 ⁺ T	609.13±227.28	561.45±193.56	t=1.53	0.13
Total CD8 ⁺ T	442.69±184.10	389.15±155.47	t=0.66	0.51
CD4 ⁺ T/CD8 ⁺ T	1.45±0.44	1.49±0.37	t=0.28	0.78
HLA-DR ⁺ CD4 ⁺ T/CD4 ⁺ T	12.71±7.85	15.08±7.81	t=1.59	0.11
HLA-DR ⁺ CD8 ⁺ T/CD8 ⁺ T	22.84±11.22	20.05±9.76	t=0.52	0.65
Ki-67 ⁺ CD4 ⁺ T/CD4 ⁺ T	36.64±16.50	7.19±5.23	t=12.82	0.00
Ki-67 ⁺ CD8 ⁺ T/CD8 ⁺ T	33.31±15.94	5.02±7.13	t=11.74	0.00

Pleural thickening [7]: Chest CT image shows pleural thickness >2 mm.

Discussion

It is well known that adaptive immunity involving T-cell-mediated pathogenic immune response plays an important role in the inflammatory process during TB. PTM as a particular type of TPE, however, little is known about the mechanism underlying PTM. In this study, we retrospectively analyzed the clinical features, laboratory examination and imaging findings of 56 PTM patients compared with a control group of 60 patients following effective treatment for TPE and without PTM to investigate the risk factors for developing the disease. We found that most of the patients with PTM were young, with a mean age of 29.04 ± 6.95 years and there was no difference in the numbers of male and female patients in the group. The onset of PTM was 4.7 ± 6.2 months from diagnosis of TPE, which is consistent with a previous study showing the onset of PTM occurred within 6 months after the diagnosis [8]. Single nodular lesions were more common, although multiple nodular lesions were not rare. There were no differences in the distribution of lesions in the left and right lung, although PTM was more commonly located in the lower lobes, possibly due to the effects of gravity on the distribution of pleural fluid in tuberculous pleurisy.

Almost all PTM patients have a history of tuberculous pleurisy; therefore, we included 60 patients cured of tuberculous pleurisy as the control group to analyze the differences in clinical features and laboratory tests of patients with and without PTM. There were no significant differences between the two groups in terms of clinical and demographic characteristics. Interestingly, we found that a significantly higher proportion of the PTM patients also had pleural thickening compared with the

proportion of patients in the control group (44.64% vs. 23.33%; $P = 0.02$), which is consistent with other reports that PTM is associated with pleural thickening[9]. The appearance of pleural tuberculosis is commonly associated with the treatment of pleurisy, among which patients without timely treatment for PE[10]. This supports the hypothesis that the occurrence of tuberculoma is related to the delayed treatment of tuberculous pleurisy, thus highlighting clinicians of the importance of early treatment of TPE.

The pathology of PTM is caused by epithelioid granulomatous inflammation[11] and CD4⁺T lymphocytes are the main subset of immune cells involved in this microenvironment. As an enzyme that is widely distributed in human tissues and body fluids,

ADA released by activated lymphocytes, macrophages and neutrophils, is a nonspecific marker of inflammation, it is detected in lymphocyte-based exudates and has been identified as a marker of cell-mediated immune responses. A high ADA index is considered to be a component of the diagnostic criteria for TB pleurisy [12, 13]. In this study, we found the levels of ADA were significantly higher in PTM patients compared with those in the control group (48.32 ± 19.19 vs. 44.79 ± 24.57 ; $P = 0.03$). It can be speculated that this increase in ADA activity indicates functional hyperactivity of T lymphocytes in PTM patients. In contrast to the levels of ADA, there were no significant differences in the levels of the total protein and LDH activity between the two groups. LDH, which is the main enzyme involved in sugar metabolism, is widely distributed in the cytoplasm of tissue cells, and is of great

clinical significance in the diagnosis of leaky or exudative pleural effusion. LDH is an also an important indicator of an inflammatory response, but lacks specificity for etiological diagnosis; therefore, it has been recommended that the LDH/ADA ratio is used for the diagnosis of TB effusions rather than LDH alone[14] [15].

Pathogen-specific T cell immunity is a key mechanism by which the host controls Mtb infection. Studies in humans and animal models have demonstrated that acquired immunity to Mtb requires the contribution of multiple T cell subsets, with a dominant role for CD4⁺ T cells and CD8⁺ T cells. However, we are not aware of any studies of Mtb-specific T cell subsets in PTM patients. In this study, we compared the Mtb-specific T cell subsets and activation markers in cells from patients with PTM and the control group. The immune status of a patient is determined not only by enumeration of immune cells, but also by the detection of cell activation. Although there were no significant differences between the groups in terms of the absolute number of Mtb-specific CD4⁺, CD8⁺ and the ratio of CD4⁺/CD8⁺ T cells, we detected significantly increased expression of the activation marker Ki-67 in both in the Mtb-specific CD4⁺ and CD8⁺ T cell subsets of PTM patients compared with the corresponding cells in the control group. As the cornerstone of anti-mycobacterial immunity, CD4⁺ T cells act primarily by secreting a variety of cytokines that attract other immune cells to the site of infection and initiate the differentiation of different CD4⁺ T cell subsets capable of performing a variety of effector functions[16]. The importance of CD4⁺ T cells in the defense against Mtb is also supported by the fact that patients with HIV infection (which leads to reduced CD4⁺ T cell counts) are more

susceptible to primary Mtb infection, reinfection, and reactivation[17]. The cytolytic function of CD8⁺ T cells mediates the destruction of Mtb-infected cells through the release of cytotoxic granules or the induction of apoptosis. CD8⁺ T cells were initially thought be less important than CD4⁺ T cells in the immune response to Mtb. However, depletion of CD4⁺ T cells demonstrated that while CD8⁺ T cells and other immune cells play a protective role against Mtb, both the CD4⁺ and CD8⁺ T cells share several anti-mycobacterial effector functions in TB[17-19]. The CD4⁺/CD8⁺ T cell ratio is independently associated with an increased risk of TB[20]. Lack of function of T lymphocyte is a high factor for developing TB. Although there were no significant differences between the PTM and control groups in terms of the absolute number of Mtb-specific CD4⁺ and CD8⁺ T cells or the ratio of CD4⁺/CD8⁺ T cells in this study, we detected increased of the Ki-67 activation in both in the Mtb-specific CD4⁺ and CD8⁺ T lymphocyte subsets from PTM patients compared with the control group. Ki-67 is an early marker of intracellular proliferation, while HLA-DR expression appears later in this process[21, 22]. Some studies have indicated that HLA-DR and Ki-67 can be used as biomarkers of infection in TB patients[23]. Moreover, some studies have shown that HLA-DR and Ki-67 expression on Mtb-specific CD4 T⁺ cells correlate positively with mycobacterial load and expression is reduced after treatment[24-26]. The enhanced expression of Ki-67 by the CD4⁺ and CD8⁺ T cell subsets from PTM patients in response to in vitro re-stimulation with Mtb antigens is a reflection of higher mycobacteria antigenic burden in slow responders, which also suggests that the incidence of PTM may be a chronic infectious disease

with congenital and acquired immune participation in the response to tuberculous antigens. This imbalance in the activation of CD4⁺ and CD8⁺ T cell subsets suggests that immune dysregulation may be a feature of PTM. Therefore, further studies of cellular activation may provide important breakthroughs in our understanding of the pathogenesis of PTM.

Several limitations of this study should be noted. First, only 56 cases of PTM were included in this study; therefore, the findings may not be generalizable to all PTM patients. Second, although we found that a higher levels of Mtb-specific CD4⁺ T and CD8⁺ T lymphocyte activation in PTM, additional studies are required to clarify the underlying mechanism. Further investigation are also required to identify interventions that prevent the progression of TPE to PTM.

Conclusion

One of the most important revelations of this study is that patients with PTM have higher levels of Mtb-specific CD4⁺ T and CD8⁺ T lymphocyte activation, indicating that immune dysregulation is a feature of individuals at risk for this condition.

Furthermore, pleural thickening and high levels of ADA activity were related to long pleural fluid course and incomplete treatment of TPE. Thus, pleural thickening and high level of ADA activity were identified as potential risk factors in the differential diagnosis in PTM. Further studies of the immune function of T lymphocytes in PTM patients may give insights into the pathogenesis of PTM.

Abbreviations

TB: tuberculosis (TB); Mtb: *M. tuberculosis*; ATT: anti-tuberculous treatment; E: ethambutol; H: isoniazid; R: rifampicin; S: streptomycin; Z: pyrazinamide; ADA: adenosine deaminase; LDH: lactate dehydrogenase; CT: computed tomography; PTM: pleural tuberculoma; TPE: tuberculous pleural effusion; WHO: World Health Organization; FNAC: fine-needle aspiration cytology; HLA-DR: human leukocyte antigen-DR

Declarations

Ethics approval and consent to participate

All methods were performed in accordance with the relevant guidelines and regulations by the Ethics Committee of Nanjing Chest Hospital and the Second Hospital (no:2013-LY-kt022). All participants and parent/legal guardian of minor participants provided written informed consent for the collection of samples and subsequent analyses. This study was approved by the Ethics Committee of Nanjing Chest Hospital and the Second Hospital (no:2013-LY-kt022).

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Weiwei Gao and Nan Yang designed the study. Guangchuan Dai recruited the patients. Saiguang Ji and Weiyi Hu performed the practical work. Weiwei Gao and Yi Zeng prepared the manuscript. All authors reviewed the manuscript.

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Figure legends

Figure 1. A 26-year-old man was confirmed with PTM by CT-guided percutaneous lung biopsy after diagnosis with pulmonary tuberculosis and right tuberculous pleurisy following anti-tuberculosis treatment for 4 months. Chest CT image showing multiple semicircular ('D-shaped') masses with a clear border, situated close to the pleura, the width of the lesion in the right lung(as see in A、B、C by the white arrow). This patient not only has a large tumor body, but also has a large number of tumors, which caused great surgical trauma and post-operative lung function damage.

Figure 2. A 28-year-old man was diagnosed with pulmonary tuberculosis and tuberculous pleurisy. He was treated with 2HEZR/4HR. Chest CT performed 6 months later shows a pleural nodule situated close to the pleura(as see in A、B by the black and white arrow). This nodule appeared as a semicircular mass with a well-defined border. The width of the lesion, the non-strengthening of the lesion and the

reinforcement of some of the lesions. Histological examination revealed granulation, tissue hyperplasia and chronic inflammatory reaction. Staining for acid-fast bacilli was negative.

Figure 3. Flow cytometry analysis of peripheral blood T lymphocyte subsets

(CD4⁺,CD8⁺),HLA-DR⁺ and Ki-67⁺ expression on Mtb-specific CD4⁺ T and CD8⁺ T

cells in a TPE patient(A) and a PTM patient(B). The T lymphocyte activation marker-Ki-67 was obviously high expression in PTM than pleurisy cured patient, whereas the HLA-DR show no significant difference.

Figure 4. The index of pleural effusion in the two groups. A:The ADA was higher in tuberculoma patients group than the control group (48.32 ± 19.19 vs 44.79 ± 24.57 , * $p < 0.05$). B and C: There were no statistical difference about the level of LDH and the protein ($p > 0.05$).

Figure 5. The absolute count of Mtb-specific CD4⁺ T and CD8⁺ T cells, the ratio of CD4⁺/CD8⁺ T cells and distributions of activation markers (Ki67⁺ and HLA-DR⁺) on Mtb-specific CD4⁺ T and CD4⁺ T cells in PTM patients compared to subjects (* p<0.05). A+B+C: There were no statistical difference about the number of absolute CD4⁺, CD8⁺ T lymphocyte cells and the ratio of CD4⁺/CD8⁺ T cells (p>0.05). E+F: There were no statistical difference about the expression of activation markers HLA-DR⁺ on CD4⁺ T-cells and CD8⁺ T-cells in PTM group compared with the control group (p >0.05). G+H: The expression of activation markers ki-67⁺ on CD4⁺ T-cells

and CD8⁺ T-cells was significantly increased in PTM group compared with the control group ($p < 0.05$).

Supplementary Files

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

- [theoriginaldataofPTMANDPF.xlsx](#)