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## ORIGINAL ARTICLE

# Th1/Th2 imbalance and Elevated PD-L1 in Pleural Effusion Predict the Risk of Multi-Drug Resistant Tuberculous Pleuritis

Hongyan Xu[1], Yueqing Yang[2], Qianhong Wu[1], Yan Zhang[1*]

[1]Tuberculosis Prevention and Control Hospital of Shaanxi Province, [2]Traditional Chinese Medicine Hospital of Shaanxi Province, Xi'an City, Shaanxi

## ABSTRACT

**Background:** Patient immune status might be indicative of the variance in bacterial genetics in drug-resistant tuberculous pleuritis and could be used for predicting the risk of multi-drug resistant tuberculous pleuritis (MDR-TB). **Objective:** To determine the significance of Th2/Th1 ratio and concentration of PD-L1 in the pleural effusions for prediction of MDR-TB. **Methods:** We measured the ratio of Th2 to Th1 T cells from pleural effusions in 373 tuberculous pleuritis patients. We also measured the concentration of programmed death ligand-1 (PD-L1) in the pleural effusions of these patients. Afterwards, we determined the optimal cut-off value for predicting the occurrence of multi-drug resistant tuberculous based on the Youden index, diagnostic evaluation test, and receiver operation curve. Multiple logistic analysis was employed to identify the independent risk factors for MDR-TB occurrence. **Results:** The area under the curve (AUC) of the Th2 to Th1 ratio was 0.66 and the concentration of PD-L1 was 0.71. Based on the combined detection of PD-L1 concentration in pleural effusion and the Th2 to Th1 ratio, our AUC was 0.81 and had a specificity of 0.92. Only a combined detection was able to identify patients developing multidrug-resistant tuberculosis. Multiple logistic analysis showed that a high concentration of PD-L1 and a high Th2 to Th1 T ratio in pleural effusions were indicative of an immunocompromised status. Therefore, these measurements might be independent risk factors for the occurrence of multidrug-resistant tuberculous. **Conclusion:** Evaluation of immune status based on PD-L1 pleural concentration and Th2 to Th1 ratio might predict the risk of MDR-TB occurrence.

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**Keywords: Drug Resistant, PD-L1, Th1, Th2, Tuberculosis**

*Corresponding author: Dr. Yan Zhang, Tuberculosis Prevention and Control Hospital of Shaanxi Province, Xi'an City, Shaanxi, e-mail: zxasqw012@163.com

## INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. The disease attacks not only the lung but also other parts of the body (1). Tuberculous pleural effusion (TPE) is a major extra-pulmonary manifestation caused by *Mycobacterium* infection whose occurrence rate can vary greatly depending on the geographic location. TPE has a morbidity of 4% in the USA, 10% in Spain, and 20–25% in South Africa (2). This disease is characterized by an uncontrolled inflammatory response in the pleural cavity to mycobacterial antigens. Such response occurs due to the rupture of subpleural caseous focus secondary to the tuberculous infection of pulmonary parenchyma, leading to the entrance of *Mycobacterium* into the pleural cavity (1-3). For more than half a century, administration of anti-TB drugs has resulted in a treatment success rate of more than 80%, achieved in drug-sensitive TB. However, TB is still one of the leading causes of death worldwide and the most common infectious cause of global mortality (4). One of the main reasons for these figures is the appearance of mycobacteria with acquired drug-resistance to various anti-TB drugs. These drug-resistant mycobacteria have been associated with treatment failure rates of up to 70% (5). Evidence suggests that a lower dose and a shorter course of treatment is sufficient to treat TPE compared with standard intensive regimens recommended in the current WHO guidelines. However, data regarding patients with multi-drug resistance are still scarce. For an early diagnosis and management of patients with drug-resistant TB, WHO strongly recommends drug susceptibility testing (DST) prior to the initiation of standard anti-TB regimen (consisting of isoniazid, rifampicin, pyrazinamide, and ethambutol in intensive phase for two months, followed by the continuation phase for four months with just isoniazid and rifampicin) in patients with pulmonary TB (4,5). The host's cellular immune response induced by MTB-specific $CD4^+$ T cells is known to be involved in the progression and resolution of lung tuberculous inflammation (6,7). Different subsets of T cells play distinct roles in the pathogenesis of TB. $CD4^+$ Th1 cells secret a large amount of pro-inflammatory cytokines, including interleukin (IL-2), interferon (IFN)-gamma, and tumor necrosis factor (TNF)-alpha. $CD4^+$ Th1 cells have been observed to have an anti-infectious role in TB. $CD4^+$ Th2 cells are characterized by the production of cytokines involved in humoral immunity and having anti-inflammatory effect, such as IL-4, IL-5, IL-10, and TGF-beta (8). IFN-gamma, mainly generated by Th1 cells, is essential in the process of granuloma formation and for the enhancement of pro-inflammatory and anti-infectious effects (8,9). Production of type II cytokines by Th2 cells, such as IL-4, IL-10, and transforming growth factor (TGF)-β, in response to MTB, dull the immune response and restrict tissue injury. However, uncontrolled production of these anti-inflammatory cytokines may lead to a failure to eliminate TB infection (10). A previous study reported that the PD-1/PD-L1 pathway inhibited both MTB-specific $CD4^+$ T-cell-mediated immunity and innate immunity. Therefore, higher concentrations of PD-L1 in the pleural effusions of patients infected by TB might be associated with immune escape and occurrence of bacterial drug resistance (11). Because the immune response is associated with the pathogenesis of TB, it is important to explore immunological parameters in order to specify factors that might be conducive to assessing treatment response and predicting the occurrence of multi-drug resistance. Accordingly, it is of interest to investigate the immunological status of patients with the occurrence of multidrug resistant TB. The objective of the present study was to determine the frequency value of $CD4^+$ Th1/Th2 cells and the

concentration of PD-L1 in pleural effusions so as to predict the occurrence of multi-drug resistance TB.

## MATERIALS AND METHODS

**Study Design.** This is a cross-sectional study of patients under TB treatment at the Tuberculosis Prevention and Control Hospital in Shaanxi Province from October 2009 to March 2015. This study was approved by the Ethics Committee of our institution. All patients had positive results for the MTB (DST) culture. Patients who were resistant to both isoniazid and rifampicin, possibly associated with other anti-TB drugs, were defined as MDR-TB cases and were then selected and categorized into the drug-resistant group. Patients infected with non-resistant tubercle bacilli were classified as the drug-sensitive group. Cases of tuberculosis pleural effusions were defined according to the WHO guidelines based on etiology, detection, and levels of pleural adenosine deaminase activity (ADA) and interferon-gamma. Etiological examinations for the identification of tuberculous infection included T-SPOT, TB, or rapid detection of cell-free *Mycobacterium tuberculosis* DNA in pleural effusions. Patients were considered as having tuberculous pleurisy if they had an ADA value of >40 IU/L combined with an IFN-γ concentration above 140 pg/mL in a lymphocyte-predominant pleural exudate. Those with a history of lung tuberculous infection and pleural effusions were also considered to have tuberculous pleurisy, regardless of their ADA and IFN-γ levels. Anti-TB drug treatment was administered through a directly observed therapy 5−7 days/week, with the recommended treatment regimens. Exclusion criteria were: i) exclusively hematogenous disseminated TB; ii) age <18 years; iii) lack of information (no registries found); iv) serologic HIV-positive patients, and v) concurrence of extra-thoracic tuberculosis. To characterize our study population, demographic, laboratory, and clinical data were obtained from the medical records. An immunocompromised status, manifested by a high ratio of Th2 to Th1 cells and a high concentration of PD-L1 in pleural effusions, were evaluated as possible predictors of MDR-TB occurrence. We further assessed other possible predictors of anti-TB drug resistance such as patient's age (years), sex, concurrence of lung active tuberculous infection, education, occupation (farmers or non-farmers), previous anti-TB therapy, and hemoglobin levels.

**Flow cytometry**. Pleural periphery mononuclear cells were collected from patients by Ficoll-Hypaque density centrifugation and seeded into six-well plates at a density of $3\times10^5$ cells/well. To measure the production of intracellular cytokines, cells from PE or PBMCs were stimulated by PMA (0.08 μM), ionomycin (1.3 μM), and brefeldin A (10 μM) at 37°C, and 5% $CO_2$ atmosphere for 5h. Cells were then harvested and stained with primary antibody conjugated with fluorescein, including IFN-γ-FITC, IL4-PE, and CD4-APC. All primary antibodies utilized for flow cytometry detection were purchased from BD Biosciences (New Jersey, USA). Frequency of IL4-producing Th2 and IFN-γ-producing Th1 CD4+ T cells were evaluated by flow cytometry. The ratio of Th2 to Th1 cells was directly calculated based on the frequency of Th2 and Th1 CD4+ cells.

**ELISA Assay**. Serum samples pertaining to peripheral blood and pleural effusion (50 ml) samples were centrifuged at 543× g for 10 min and the supernatants were stored at −80°C for subsequent ELISA assays. Expression levels of soluble PD-L1 (sPD-L1) in pleural effusion samples were determined in duplicate wells using a commercial human ELISA kit (Abcam, UK) according to manufacturer's instructions.

**Definition of Anti-TB Therapy Failure and MDR-TB Occurrence.** WHO guidelines were used as a reference when defining the occurrence of MDR-TB and treatment outcomes. Treatment failure was defined as a termination of treatment or a need for a permanent regimen change of at least two anti-TB drugs due to a lack of culture conversion by the end of the intensive phase, or the recurrence of sputum smear positive after the intensive phase, or evidence of additional acquired resistance to fluoroquinolones or second-line injectable drugs. MDR-TB was defined as a resistance to at least both isoniazid and rifampicin according to drug susceptibility testing based on phenotypic method, that is, by evaluating the growth ability of *Mycobacterium* in culture media containing the critical concentration of a certain drug.

**Statistical Analysis.** Data are presented as mean ± SD or frequency (%). Differences between cases and controls were expressed as odds ratios (ORs). Multiple logistic regression models were used to evaluate the associations between patients with immunocompromised status and each outcome of MDR-TB occurrence and treatment failure following adjustment for other covariables (demographic and baseline clinical data). Univariable analysis was performed using the chi-square test. The predictive value of single or combined detection of Th2 to Th1 ratio was determined according to diagnostic test evaluation based on AUC method and Youden index. The statistical level of significance was set to 0.05. R software, version 3.2.2, was used for all statistical analyses.

## RESULTS

**Patients' characteristics.**

A total of 449 patients newly diagnosed with tuberculous pleuritis in our institution between 2009 and 2015 were identified. Among these, 221 patients with pleural effusions were culture or sputum smear positive at the beginning of treatment, and all patients were diagnosed according to T-SPOT TB (a unique, single-visit blood test, also known as an interferon-gamma release assay for TB infection) or rapid detection of cell-free *Mycobacterium tuberculosis* DNA in pleural effusions. A total of 142 (31.6%) patients had a history of previous lung tuberculosis or anti-TB treatment. The following patients were excluded from the study: 10 patients suffering from extra-thoracic tuberculosis, 34 HIV positive patients, 22 patients who were forced to stop the full course of anti-TB treatment following the initiation of anti-TB therapy, and 10 patients with unknown clinical outcomes. A total of 373 patients received the WHO recommended standard anti-TB chemotherapy as the first line of regimen, including rifampin, isoniazid, ethambutol, and pyrazinamide. Out of 161 patients with treatment failure or death, 145 experienced bacteriologically confirmed MDR-TB during the three-year follow-up period. Among these, 101 patients (70%) were male, and 112 (77%) were farmers. Median age was 49.54 years (range, 25-79 years). Figure 1 is a flow diagram related to the process of patient enrollment. Baseline demographic and clinical characteristics of patients with or without multi-drug resistant tuberculosis are presented in Table 1. Based on the results, the drug-resistant group had more farmers and more patients with a previous history of tuberculous infection compared to the drug-sensitive group. Previous anti-TB treatment and anemia were also associated with MDR-TB occurrence.

**Table 1. The basic demographic and clinical features of the study population grouped by occurrence of MDR-TB.**

| Variables | Drug-sensitivity group (n=196) | Drug-resistance group (n=145) | p-value |
|---|---|---|---|
| **Age (yrs)** | 47.39 ± 11.21 | 49.54 ± 14.78 | 0.313 |
| **Male (n,%)** | 129 (65.8) | 101 (69.7) | 0.371 |
| **No employment (n,%)** | 34 (17.3) | 73 (50.3) | |
| **Farmer (n,%)** | 78 (39.8) | 112 (77.2) | 0.012* |
| **Alcoholism (n,%)** | 42 (21.4) | 22 (15.2) | 0.129 |
| **Smoking (n,%)** | 87 (43.1) | 76 (52.4) | 0.165 |
| **Education** | | | 0.267 |
| **Primary school (n,%)** | 123 (62.7) | 110 (75.8) | |
| **Junior high school (n, %)** | 34 (17.3) | 21 (14.5) | |
| **Senior high school (n, %)** | 37 (18.8) | 10 (6.9) | |
| **College or university (n, %)** | 2 (1) | 4 (2.8) | |
| **History of previous lung tuberculosis (n,%)** | 57 (29.0) | 75 (51.7) | 0.006* |
| **Previous anti-TB therapy (n,%)** | 99 (50.5) | 124 (85.5) | 0.013* |
| **Anemia (n, %) (Hemoglobin<12g/L)** | 67 (34.18) | 104 (71.7) | 0.015* |
| **Number of lung cavity** | | | 0.163 |
| **No cavity (n,%)** | 141 (71.9) | 87 (60.0) | |
| **≥1 (n,%)** | 55 (28.1) | 58 (40.0) | |
| **PD-L1 levels in serum (ng/ml)** | 1.82 ± 1.09 | 1.89 ± 1.23 | 0.232 |
| **PD-L1 levels in the PE (ng/ml)** | 2.47 ± 1.23 | 3.76 ± 1.18 | 0.046 |
| **Ratio of Th2 to Th1 CD4+ T cells in the PE** | 0.44 ± 0.34 | 0.54 ± 0.21 | 0.041 |
| **Ratio of Th2 to Th1 CD4+ T cells in the blood** | 0.14 ± 0.05 | 0.16 ± 0.08 | 0.170 |

Data were presented as Mean ± SD or number (frequency). $p<0.05$ indicated significant difference. *$p<0.05$. MDR-TB; multidrug resistant- tuberculosis. PD-1, programmed death ligand-1.

**The diagnostic value of Th2 to Th1 cells ratio or PD-L1 concentration in the pleural cavity.**

A flow cytometry assay was employed to measure the frequency of $CD4^+$ Th1 and Th2 cells. In all enrolled patients, the average frequency of Th1 cells from the total $CD4^+$ T cells was 47.84 ± 12.35% while the average frequency of Th2 $CD4^+$ cells was 12.54 ± 9.89%. The average and standard deviation frequency ratio of Th2 to Th1 cells was 0.47 ± 0.14. In the 145 MDR-TB patients with treatment failure, the frequency ratio of Th2 to Th1 cells was 0.54 ± 0.21. There was a significant difference regarding the ratio of Th2 to Th1 cells between patients with and without MDR-TB. Based on the ratio of Th2 to Th1, the AUC was 0.66 (Table 2) and the specificity was 0.59 (Table 2). Based on the AUC value and Youden index, the optimal cut-off value was 0.48. Based on the optimal cut-off values, the patients were grouped into two subsets. The chi-square test revealed that patients with a higher Th2 to Th1 ratio, based on the optimal cut-off value, had a slightly increased tendency towards a higher incidence of treatment failure and MDR-TB occurrence (Table 3).

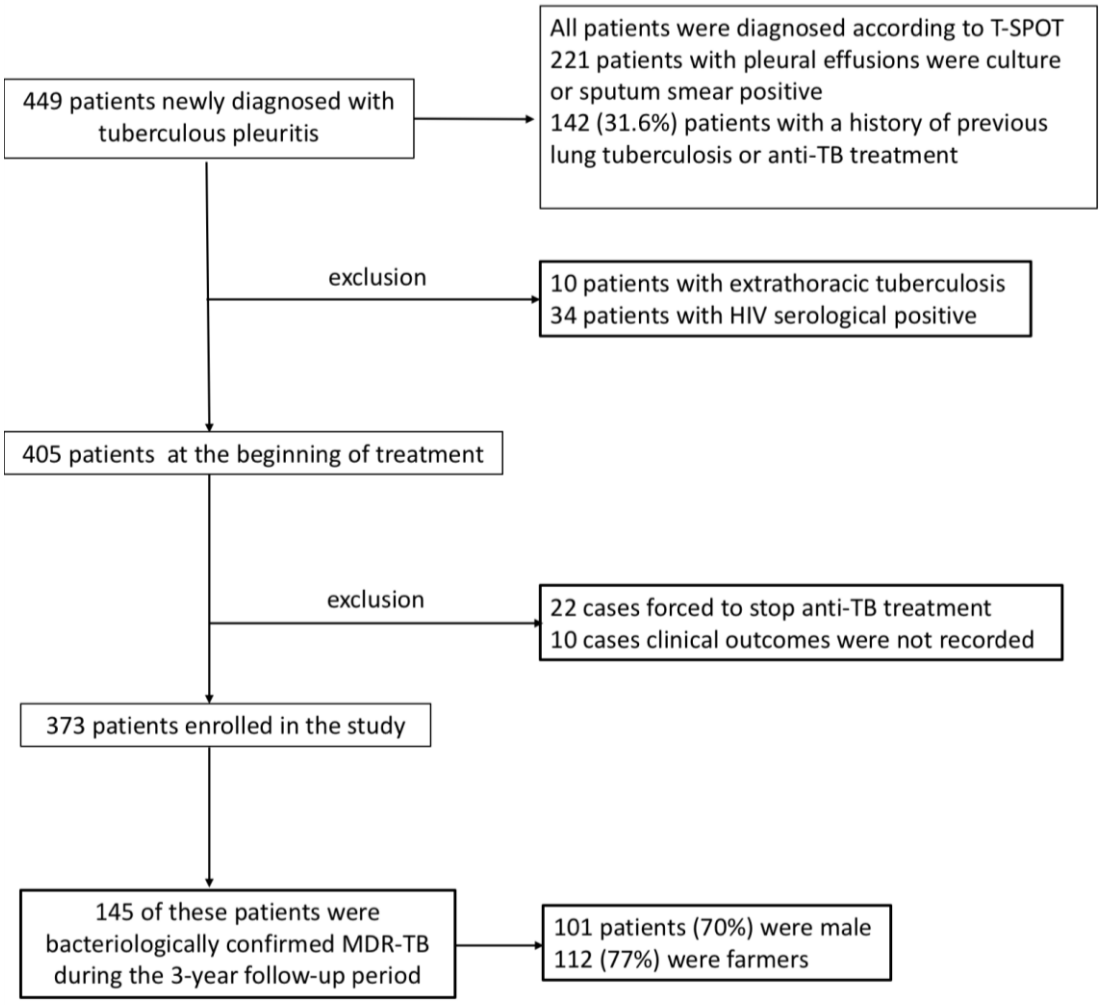


**Figure 1.** Flow diagram related to the process of patient enrollment.

In all enrolled patients, the average concentration of PD-L1 in pleural effusions was 3.56 ± 1.34 ng/ml. In MDR-TB patients, the average concentration of PD-L1 in pleural effusions was 3.76 ± 0.91 ng/ml while in drug-sensitive TB patients, the average was 2.47 ± 0.84.

**Table 2. The results of diagnostic evaluation test according to single and combined detection of ratio of Th2 to Th1 cell and concentration of PD-L1 in the pleural effusions.**

| | Sp | Se | AUC | Youden index | The optimal Cut-off value |
|---|---|---|---|---|---|
| **Concentration of PD-L1** | 0.53 | 0.91 | 0.71 | 0.44 | 3.24 |
| **Ratio of Th2 to Th1 CD4+ T cells** | 0.59 | 0.89 | 0.66 | 0.48 | 0.48 |
| **Combined detection** | 0.94 | 0.86 | 0.80 | 0.83 | / |

Sp, specificity. Se, Sensitivity. AUC, area under the receiver operation curve.

There was a significant difference between the two groups concerning the concentration of PD-L1 in pleural effusions. In the diagnostic test evaluation, regarding the predictive value of PD-L1 concentration for the occurrence of MDR-TB, the AUC was 0.71 and the optimal cut-off value was 3.24 ng/ml for PD-L1 concentration according to the AUC value and the Youden index. The specificity was 0.53 (Table 2). Patients with a higher concentration of PD-L1 in pleural effusions had a significantly increased incidence of treatment failure and a slight but non-significant increased risk of MDR-TB occurrence (Table 3). These data showed that due to poor specificity, the prediction of MDR-TB occurrence based on a single biomarker was relatively inefficient.

**Table 3. The results chi-square test for the correlation of grouping according to single or combined biomarker and outcomes.**

| outcomes | Grouping | $X^2$ | p- value |
|---|---|---|---|
| **Failure of treatment** | Single biomarker (Ratio of Th2 to Th1 CD4+ T cells) | 2.56 | 0.078 |
| | Single biomarker (Concentration of PD-L1) | 4.52 | 0.023* |
| | Double biomarker | 7.81 | 0.013* |
| **Occurrence of MDR-TB** | Single biomarker (Ratio of Th2 to Th1 CD4+ T cells) | 2.43 | 0.067 |
| | Single biomarker (Concentration of PD-L1) | 1.21 | 0.012* |
| | Double biomarker | 11.31 | 0.006* |

*P<0.05 indicated significant difference

**The diagnostic value of combined detection of Th2 to Th1 cells ratio or PD-L1 concentration in the pleural cavity**.

Given the low specificity of a single biomarker, we applied a combined detection of $CD4^+$ Th2 to Th1 cells ratio and PD-L1 concentration in pleural effusions to predict MDR-TB occurrence. The patients were regrouped according to the optimal cut-off value of the Th2 to Th1 ratio and PD-L1 concentration in pleural effusions. We divided the patients into two groups: one group included patients with a higher ratio of Th2 to Th1 cells (over the optimal cut-off value of 0.48) accompanied by higher levels of PD-L1 in pleural effusions (pleural concentration of PD-L1 over 3.24 ng/ml). The other group included patients higher Th2/Th1 ratio and higher levels of PD-L1 simultaneously. The specificity and sensitivity for the prediction of MDR-TB occurrence with the combined detection method was 0.94 and 0.86, respectively (Table 2). The chi-square test revealed that patients with a higher Th2 to Th1 cells ratio had a significantly higher incidence rate of MDR-TB and higher levels of PD-L1 in pleural effusions compared with the other group. A higher incidence rate of treatment failure was further detected in these patients (Table 3). The AUC was 0.90. Our data showed that a combined detection of $CD4^+$ Th2 to Th1 cells ratio and PD-L1 concentration in tuberculous pleural effusions could better predict the risk of treatment failure and MDR-TB occurrence compared with single detection.

**Multivariable logistics regression analysis revealed that immune status might be a risk factor for developing multi-drug resistant tuberculosis.**

A univariable regression analysis was performed to identify the independent risk factors for MDR-TB occurrence. Results showed that following adjustment for other variables, neither a higher ratio of Th2 to Th1 nor a higher concentration of PD-L1 were independent risk factors. The odds ratio for Th2 to Th1 cells ratio was 0.92 (95% confidence interval [CI] 0.81-1.34) while that of PD-L1 concentration was 0.89 (0.78-1.05) (Table 4).

**Table 4. The results of multivariable logistics regression analysis for single or combined detection of ratio of Th2 to Th1 cells and concentration of PD-L1 in the pleural effusions.**

| | Odds ratio | 95% Confidence internal (CI) | P value |
|---|---|---|---|
| **Single biomarker (Ratio of Th2 to Th1 CD4+ T cells)** | 0.92 | 0.81, 1.34 | 0.158 |
| **Single biomarker (Concentration of PD-L1)** | 0.89 | 0.78, 1.05 | 0.096 |
| **Double biomarker** | 0.45 | 0.31, 0.78 | 0.007* |

*P<0.05 indicated significant difference

However, the results showed that patients with an immunocompromised status, based on a higher Th2 to Th1 ratio (a Th2 to Th1 ratio of over 0.48) accompanied by a high PD-L1 concentration in pleural effusions (pleural concentration of PD-L1 over 3.24 ng/ml), had a higher incidence of MDR-TB occurrence based on the multiple adjustment model (OR=2.24, 95% CI 1.48-3.02) (Table 4). In addition to the immunocompromised status, being a farmer, previous treatment, and unemployment might also be independent risk factors for MDR-TB occurrence (Table 5).

Furthermore, multiple logistic regression analysis revealed that low education, unemployment, hemoglobin <12 g/L, being a farmer, and immunocompromised status might be independent risk factors for first-line treatment failure (Table 6).

## DISCUSSION

Drug resistance is a serious issue that has raised the need for the prevention and treatment of drug-resistant TB, which is known to have distinct and complicated regional trends (12). It is also necessary to conduct further research into the risk factors associated with the occurrence of MDR-TB in tuberculous patients, particularly for the development of a national policy for public health interventions in regard to tuberculous pleuritis and pleural effusions. Unfortunately, poor treatment compliance is not always explained by TB resistance.

**Table 5. The results of independent risk factors in the multivariable logistics regression analysis for the prediction of occurrence of MDR-TB.**

| Variable | OR | 95% CI | P value |
|---|---|---|---|
| **Age** | 1.21 | 0.97, 1.34 | 0.091 |
| **≥60 yrs, =1** | | | |
| **<60 yrs, =0** | | | |
| **Gender** | 1.05 | 0.89, 1.20 | 0.241 |
| **Male=1** | | | |
| **Female=0** | | | |
| **Concurrence of active lung TB** | 1.56 | 0.96, 2.12 | 0.067 |
| **Yes=1** | | | |
| **No=0** | | | |
| **Unemployment** | 1.63 | 1.21, 1.94 | 0.031* |
| **Yes=1** | | | |
| **No=0** | | | |
| **Hemoglobin levels** | 0.96 | 0.91, 1.18 | 0.164 |
| **≥12g/L=0** | | | 0.164 |
| **<12g/L=1** | | | |
| **Previous anti-TB therapy** | 1.54 | 1.19, 1.86 | 0.029* |
| **Yes=1** | | | |
| **No=0** | | | |
| **Occupation** | 1.46 | 1.12, 1.78 | 0.031* |
| **Farmer =1** | | | |
| **Non-farmer=0** | | | |
| **Education** | 0.87 | 0.79, 1.02 | 0.058 |
| **Primary school=0** | | | |
| **Junior high school=1** | | | |
| **Senior high school=2** | | | |
| **University or college=3** | | | |
| **Immunocompromised status** | 2.24 | 1.48, 3.02 | **0.013*** |
| **Yes=1** | | | |
| **No=0** | | | |

*P<0.05 indicated significant significance

Therefore, finding other serum biomarkers and genetic markers could be of great value. Nowadays, immune-related gene polymorphisms such as SLC11A1, certain HLA genes, TLR7, TLR8, and INFG have been associated with increased susceptibility to pulmonary TB. Plasma levels of specific chemokines and inflammatory markers, including MCP-1/CCL2, IP-10, sIL-2Rα, SAA, CRP, and AFB measured prior to MDR-TB treatment are candidate predictive markers of delayed sputum culture conversion (13).

**Table 6. The results of independent risk factors in the multivariable logistics regression analysis for the prediction of failure of treatment.**

| Variable | OR | 95% CI | P value |
|---|---|---|---|
| **Age**<br>**≥60 yrs, =1**<br>**<60 yrs, =0** | 1.06 | 0.89, 1.15 | 0.103 |
| **Gender**<br>**Male=1**<br>**Female=0** | 1.13 | 0.94, 1.31 | 0.228 |
| **Concurrence of active lung TB**<br>**Yes=1**<br>**No=0** | 1.23 | 0.89, 1.56 | 0.130 |
| **Unemployment**<br>**Yes=1**<br>**No=0** | 1.78 | 1.34, 1.90 | 0.021* |
| **Hemoglobin levels**<br>**<12g/L=1**<br>**≥12g/L=0** | 1.97 | 1.23, 2.67 | 0.006 |
| **Previous anti-TB therapy**<br>**Yes=1**<br>**No=0** | 1.21 | 0.89，1.36 | 0.089 |
| **Occupation**<br>**Farmer =1**<br>**Non-farmer=0** | 1.36 | 1.09，1.45 | 0.043* |
| **Education**<br>**Primary school=0**<br>**Junior high school=1**<br>**Senior high school=2**<br>**University or college=3** | 0.77 | 0.69, 0.94 | 0.041* |
| **Immunocompromised status**<br>**Yes=1**<br>**No=0** | 1.98 | 1.38, 2.24 | 0.019* |

*P<0.05 indicated significant significance

Wang C *et al.* established an MDR-TB diagnostic model based on five biomarkers (CD44, KNG1, miR-4433b-5p, miR-424-5p, and miR-199b-5p), provided a group of potential biomarkers for MDR-TB diagnosis, and proposed a new experimental basis to understand the pathogenesis of MDR-TB (14). Nonetheless, these findings require validation in a larger study. Today, the presence of drug resistance in tuberculous pleural effusion is similar to that of pulmonary tuberculosis. Approximately 10% of pleural *Mycobacterium tuberculosis* isolated from patients with PTB are resistant to at least one first-line anti-TB drug while 6-10% are resistant to isoniazid (15-17). The incidence of multidrug-resistant-pleural and extensively drug-resistant-pleural TB is 1-3% and 0-1%, respectively. However, their prevalence is relatively higher in patients with pulmonary tuberculosis complications (16). Although the classical standard anti-TB regimen has been recommended for TPE treatment according to WHO guidelines, epidemiological data and data on the prevalence of drug-resistant bacteria in TPE

patients are relatively insufficient. Moreover, it is difficult to isolate MTB from patients suspected of drug-resistant TPE; this might hamper the possibility of performing drug susceptibility testing to determine the appropriate treatment regimen. For this purpose, to predict MDR-TB occurrence or treatment failure, it is highly helpful to use biomarkers with high sensitivity and specificity based on the measurement of immune cells and soluble cytokines from pleural fluid. In cases where drug-resistant MTB cannot be identified, biomarker levels can be used to predict patients running a high risk of MDR-TB; therefore, anti-TB therapy or second-line anti-TB drugs can be administered during the continuation phase of treatment. Previous studies have shown that the serum levels of certain cytokines can be altered in TB patients (18,19). In fact, a recent study reported that T cell-derived IL-10 impaired host resistance to *Mycobacterium tuberculosis* infection, suggesting that anti-inflammatory cytokines might be immunosuppressive and lead to a worsened infection (20). Over the recent years, investigations have also been conducted regarding the relationship between immune-related gene polymorphisms and the risk of drug resistant tuberculosis. Wu S *et al.* found that IFNG rs1861494 polymorphism was associated with TB, particularly in younger and male subgroups (21). In another study on an Argentinean population, it was observed that the rs1861494 single nucleotide polymorphism could be considered as a biomarker of tuberculosis resistance although no association was detected between the polymorphism and clinical parameters of tuberculosis severity (22). These studies suggest that an immunocompromised status, manifested by a low production of IFN-γ or higher production of anti-inflammatory cytokines such as IL-10, might be related to MDR-TB occurrence and failure of anti-TB treatment. PD-1/PD-L1 is involved in a signaling pathway linked with the immunosuppression of T cells, leading to the impairment of cytotoxic T cells in the tumor or maintained immune homeostasis in autoimmune diseases (23). In MTB-induced infection, PD-L1 blocking enhanced MTB-specific $CD8^+$ T cell killing of $CD14^+$ cells in human tuberculous pleural effusion samples. Previous research has shown that the PD-1/PD-L1 pathway modulates antigen-specific cytotoxicity against M1 targets *in vitro* and indicates the potential of exploring checkpoint blockade as a new adjuvant therapy for TB (24). Therefore, PD-L1 activation during tuberculous infection could entail the impairment of cytotoxic immune cells and the maintenance of *Mycobacteria*, which might in turn be related to poor clinical outcomes and drug resistance in TB patients. Our results suggest that the measurement of soluble PD-l levels in pleural effusions could be a potential predictor for MDR-TB occurrence and failure of anti-TB therapy. In a previous observational study, Pan *et al.* reported that pleural effusion levels of sPD-L1 and PD-L1 on $CD14^+$ monocytes increased in the TPE group as compared with the malignant PE and no-TB non-malignant groups. However, no further investigations have been done regarding the clinical significance of reduction in PD-L1 levels (25). In the present study, we found that the reduced levels of PD-L1 were associated with the occurrence of multi-drug resistant tuberculous pleuritis, which is helpful in the clinical practice to identify patients at a higher risk of MDR-tuberculous pleuritis. We also found that the high ratio of Th2 to Th1 $CD4^+$ T cells and the high concentration of PD-L1 in pleural effusions were both associated with the occurrence of MDR-TB. However, a single detection of either of those factors was not able to efficiently predict MDR-TB occurrence and failure of anti-TB treatment. The use of a single biomarker would lead to a lower specificity for the prediction of MDR-TB and, thus, to a misguided regimen for patients with a lower risk of MDR-TB occurrence. Consistent with the previous

findings, in the multivariable logistic regression analysis, we found that independent risk factors included low education, unemployment, hemoglobin <12 g/L, previous treatment, and being a farmer. Cases of patients that had undergone previous treatment might indicate a relapse of *Mycobacteria tuberculosis* infection and the onset of secondary extrapulmonary tuberculosis infection. Immunocompromised status is an independent risk factor for the occurrence of MDR-TB pleuritis, which might be partly attributed to the fact that the immunosuppression can lead to MTB and failure to control the infection. In conclusion, the combination of Th2/Th1 ratio and the levels of pleural PD-L1 could serve as a useful tool for predicting the risk of MDR-TB occurrence. Our findings also showed that immunosuppression could lead to the occurrence of drug-resistant tuberculous pleuritis, which is associated with poor outcomes and anti-TB treatment failure.

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