

Blood absolute T cell counts may predict 2-month treatment response in patients with pulmonary tuberculosis

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Abstract. *Background and objective:* Little is known about the usefulness of lymphocyte subsets as early predictors of anti-tuberculosis (TB) treatment response in immuno-competent patients.

Methods: Among a total of 64 patients with culture positive pulmonary TB, 29 remained sputum smear/culture positive or had delayed resolution on CXR (slow responders (SR)), and 35 had sputum culture conversion to negative and rapid resolution on CXR (fast responders (FR)) after two months of anti-tuberculosis treatment. Clinical parameters and lymphocyte subsets were investigated.

Results: A larger proportion of patients in the SR group had cavities on CXR, bilateral lung involvement, positive acid-fast bacilli stains, and complaint of cough at diagnosis than those in the FR group. Absolute counts of CD3⁺ T cells ($p = 0.016$) and CD8⁺ T cells ($p = 0.012$) at diagnosis were both significantly higher in the SR group. This trend was present throughout the 6-month treatment course. Absolute T cell counts (odds ratio (OR) 1.002, 95% confidence interval (CI) 1.0–1.004), positive sputum acid fast bacilli stain (OR 6.69, 95% CI 1.37–32.77) and bilateral lung involvement on CXR (OR 13.114, 95% CI 1.87–92.14) at diagnosis were independent predictors for a slow response. Combining these three predictors, a prediction score (PS) could be calculated to display an optimal discrimination for slow response (area under the curve (AUC) = 0.855, $p < 0.001$), whereas absolute T cell counts yielded the highest discriminative value on an individual level (AUC = 0.676, $p = 0.015$).

Conclusions: A higher T cell count at diagnosis in patients with TB may predict a slow response to two months of treatment. The calculation of a PS further increased predictive accuracy and performance.

Keywords: Pulmonary tuberculosis, slow responders, absolute T cell counts, prediction score

1. Introduction

The ultimate success of anti-tuberculosis (TB) treatment is measured by the relapse rate within the first two years after treatment. The presence of both cavitations and a positive culture for *Mycobacterium tuberculosis* (M.tb) after two months of treatment are associated with a higher relapse rate, which can be reduced by prolonging the continuation phase [1–3]. Sputum

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cultures are time consuming, and are often accompanied by contamination of non-tuberculous mycobacterium (NTM). Sputum smears cannot distinguish between *M.tb* and NTM, or between viable and non-viable organisms. Many patients stop expectorating sputum soon after starting anti-TB medication. Immunological biomarkers measurable at diagnosis may shorten clinical trials of new anti-TB drugs and improve clinical management by stratification of patients into different treatment requirements [4,5].

CD4⁺ T cells, CD8⁺ T cells, natural killer (NK) cells, and NK T cells control mycobacterial infection primarily by the production of effective cytokines and apoptosis of infected cells [6]. B cells are present at the site of granulomatous reactions during tuberculosis infection, and severe tuberculosis is associated with hampered antigen-specific B cell responses [7,8]. Several lymphocyte subsets have been shown to be more predominant in active and symptomatic TB patients than in healthy subjects, and might be different at different treatment stages [9–14]. Little is known about the usefulness of lymphocyte subsets as early predictors of 2-month treatment response in immuno-competent patients. Our hypothesis is that these lymphocyte subsets at diagnosis may predict 2-month anti-TB treatment response, defined as sputum culture conversion for *Mycobacterium tuberculosis* (*M.tb*), acid fast bacilli (AFB) stain positivity, or resolution on chest radiography (CXR). Therefore, we prospectively investigated blood lymphocyte subsets in active pulmonary TB patients for a 6-month treatment period. Clinical parameters were included to find independent predictors, and a model formula consisting of a binary logistic regression analysis was constructed to increase predictive accuracy.

2. Study population and methods

A total of 150 patients with a clinical diagnosis of active pulmonary TB were screened for study at Kaohsiung Chang Gung Memorial Hospital from July 2005 to June 2008. The diagnostic criteria were based on the guidelines of American Thoracic Society (ATS) [15]. Patients who were pregnant (3 patients), had true NTM infection (15 patients), multidrug resistant (MDR) TB (4 patients), poor drug compliance (16 patients), and immunocompromised status as a result of human immunodeficiency virus infection (3 patients) or receiving immunosuppressive agent (3 patients), were excluded. Sixty-four patients completed follow-up and were in-

cluded for final analysis. Figure 1 shows the flow chart of how the patients were included and classified into slow responder (SR) and fast responder (FR) groups. The study protocol was reviewed and approved by the Institutional Review Board of Kaohsiung Chang Gung Memorial Hospital. Informed consent was obtained from all patients.

3. Processing of sputum samples for AFB smear and mycobacterial culture

Three sputum samples were obtained from each patient at diagnosis and after two months of anti-TB treatment. Concentrated sputum smears were examined for AFB, using both the Ziehl-Nielsen stain and auramine-rhodamine stain. The culture techniques were undertaken in both solid (LJ) and liquid (MGIT) media following standard procedures. Conventional drug susceptibility tests were done by the agar proportion method for all sputum specimens with positive cultures for *M.tb*, and the results were reported three weeks later to the primary care attending physicians [16].

4. CXR grading of the disease

Standard postero-anterior CXR were taken at diagnosis and after two and six months of anti-TB treatment in all enrolled patients. Text book grading for pulmonary involvement was adopted for disease assessment, including three categories: (I) Minimal lesions: slight-to-moderate density not containing demonstrable cavitation. The total extent should not exceed the lung volume on one side above chondro-sternal junction; (II) Moderately advanced lesions: slight-to-moderate density that extend throughout the total volume of one lung or an equivalent in both lung; dense and confluent density limited to one lung or an equivalent in both lungs; total diameter of cavitation, if present, must be < 4 cm; (III) Far-advanced lesions: more extensive than moderately advanced lesions [17]. Rapid resolution on 2-month CXR was defined as reduction of the lesions (total area of consolidation, infiltrate, nodules, cavity, and pleural effusion) by more than half after two months of treatment compared with the film at diagnosis. Each CXR was reviewed by two independent chest attending physicians.

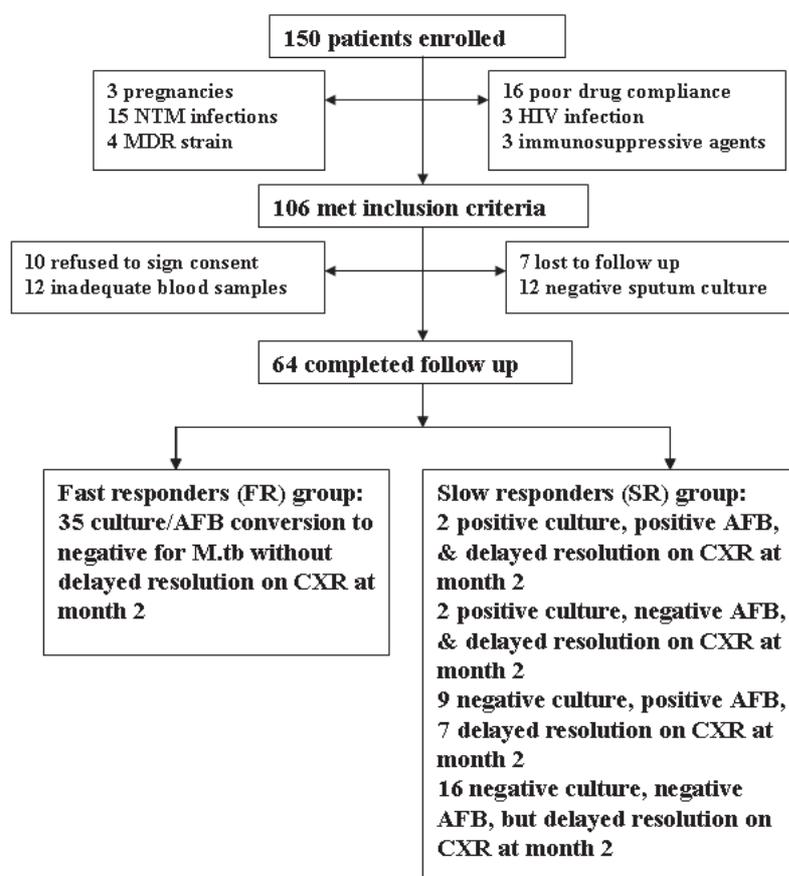


Fig. 1. Flow chart to show classification profiles. M.tb = Mycobacterium tuberculosis; NTM = non-tuberculous mycobacterium; HIV = human immunocompromised virus; MDR = multidrug resistant.

5. Treatment course

All patients were treated in accordance with the American Thoracic Society guideline for management of TB.(15) Briefly, first-line medications were given in the following daily doses in combination: isoniazid (5 mg/kg/day), rifampin (10 mg/kg/day), pyrazinamide (20 mg/kg/day), and ethambutol (15 mg/kg/day). Second-line drugs, including streptomycin and fluoroquinolone, were substituted for one or more of the first-line drugs in special situations, such as drug intolerance or resistance. Every patient received DOTS (direct observed treatment, short-course) strategy and regular follow-up at our Pulmonary Clinic. Patients were treated for at least six months, or longer depending on the presence of cavitations or changing extent on CXR, 2-month sputum smear/culture results, and clinical responses to treatment.

6. Determination of blood lymphocyte phenotypes by flow cytometry

To measure the percentage of lymphocyte subset populations, we used fluorochrome-labeled monoclonal antibodies: anti-CD45- fluorescein isothiocyanate (FITC), CD14-phycoerythrin (PE), CD3-PE, CD4-FITC, CD8-FITC, CD19-FITC, and CD56 + 16-FITC (Beckman Coulter, Marseille, France). Acquisition was performed on a FACScalibur Flow Cytometer (Becton Dickinson, San Jose, CA, USA), and 2×10^4 events were collected with lymphocytes gated in a CD45-FITC versus CD14-PE plot. These were further analyzed for expression of CD3 and CD4 (or CD8, CD19, CD56 + 16) in the FL1 and FL2 channels, respectively. Analysis was performed using SimulSET software. Blood samples at month 0 were obtained in 64 patients (29 SR, 35 FR), month 2 in 46 patients (19 SR, 27 FR), and month 6 in 30 patients (12 SR, 18 FR).

Table 1
Comparisons of clinical baseline characteristics at diagnosis between fast responders and slow responders to 2-month anti-tuberculosis treatment

Characteristics	Fast responders <i>N</i> = 35	Slow responders <i>N</i> = 29	P value
Age, years	61.5 ± 17.2	56.4 ± 18.6	0.255
Male, n (%)	26 (74.3)	23 (79.3)	0.682
Co-morbidity, n (%)			
Diabetes mellitus	12 (34.3)	8 (27.6)	0.618
COPD	5 (14.3)	7 (24.1)	0.29
CRF	3 (8.6)	1 (3.4)	0.415
CHF	1 (2.9)	1 (3.4)	0.876
Chronic hepatitis	1 (2.9)	0 (0)	0.366
Malignancy	7 (20)	5 (17.2)	0.82
Past smoking history, n (%)	18 (51.4)	20 (69)	0.185
Alcoholism, n (%)	9 (25.7)	6 (20.7)	0.682
Drug-resistant M.tb, n (%)	6 (17.1)	6 (20.7)	0.855
Positive acid fast bacilli stain, n (%)	20 (57.1)	26 (89.7)	0.004
1+	5 (14.3)	4 (13.8)	
2+	2 (5.7)	4 (13.8)	
3+	13 (37.1)	18 (62.1)	
Symptoms			
Systemic symptoms, n (%)	23 (65.7)	18 (62.1)	0.7
Hemoptysis, n (%)	6 (17.1)	6 (20.7)	0.678
Chest pain, n (%)	8 (22.9)	7 (24.1)	0.936
Cough, n (%)	26 (74.3)	25 (93.1)	0.047
Dyspnea, n (%)	9 (25.7)	9 (31)	0.589
Chest radiography, n (%)			
Cavity	9 (25.7)	17 (58.6)	0.008
Pleural effusion	6 (17.1)	9 (31)	0.192
Bilateral involvement	21 (60)	27 (93.1)	0.002
Grading			0.018
Minimal lesions	5 (14.3)	0 (0)	
Moderately advanced lesions	9 (25.7)	3 (10.3)	
Far advanced lesions	21 (60)	26 (89.7)	0.007

COPD = chronic obstructive pulmonary disease; CRF = chronic renal failure; CHF = congestive heart failure.

7. Statistical analysis

Values were expressed as mean ± standard deviation (SD). The differences between the FR and SR groups were analyzed by the Student's *t*-test or χ^2 -test, where appropriate. To allow random effects to be properly specified and to handle some missing data in the 6-month longitudinal experiments, mixed model analysis was used to compare lymphocyte subsets between the two groups at three different time points. To investigate independent predictors to identify slow responders, variables that showed significant differences at diagnosis were put into forward stepwise binary logistic regression analysis. To investigate predictive accuracy, the candidate predictor was analyzed by area under the receiver operating characteristic (ROC) curves. To increase predictive accuracy, the independent variables were combined in a mathematical formula based on binary logistic regression analysis, creating a prediction score (PS). Multivariate Cox proportional hazards regression analysis with stepwise forward selec-

tion was used to evaluate independent prognostic factors associated with survival, and the responder group, age, sex, co-morbidity, CXR findings, microbiological yield, and treatment strategy were used as covariates. The Kaplan-Meier method was used to estimate overall survival. Differences in survival among groups were analyzed with the log-rank test. The null hypothesis was rejected at $p < 0.05$. Analyses were performed using the SPSS 15.0 statistical software package (SPSS Corp., Chicago, IL).

8. Results

8.1. Comparisons of clinical characteristics of patients between the FR and SR groups

The clinical baseline characteristics of the two groups are presented in Table 1. There were no significant differences between the two groups in terms of age, gender, co-morbidity, TB pleurisy, smoking sta-

Table 2
Comparison of treatment medication and duration between fast responders and slow responders groups of patients with pulmonary tuberculosis

	Fast responders <i>N</i> = 35	Slow responders <i>N</i> = 29	P value
Duration of medication, months			
Isoniazid	6.8 ± 3	7.1 ± 2.9	0.718
Rifampin	6.6 ± 3.1	6.8 ± 3.4	0.763
Ethambutol	6.5 ± 3.1	5.3 ± 4.1	0.199
Pyrazinamide	3.5 ± 2.6	3.3 ± 2.8	0.778
Use of 2nd line medication* n (%)	4 (11.4)	8 (27.6)	0.184
Treatment duration ≥ 9 month, n (%)	19 (54.3)	17 (58.6)	0.826

*Including Moxifloxacin, Levofloxacin, Ciprofloxacin, and Streptomycin.

tus, alcoholism, and initial symptoms. A larger proportion of patients in the SR group had cavities, bilateral lung involvement, and far-advanced lesions on CXR, as well as positive sputum smear AFB stain and complaint of cough at diagnosis than those in the FR group (Table 1). Durations of individual medication and treatment regimens are presented in Table 2.

8.2. Association of blood absolute counts of lymphocyte subsets with treatment response

The absolute counts of CD3⁺ T cells (1148.7 ± 738.9 vs. 804.4 ± 346.4 cells/μl, *p* = 0.016), and CD8⁺ T cells (488.2 ± 377.1 vs. 311.8 ± 145.4 cells/μl, *p* = 0.012) at diagnosis were both significantly higher in the SR group than those in the FR group, analyzed by the independent t-test. Throughout the 2-month and 6-month treatment course, patients in the SR group still showed persistently higher absolute counts of CD3⁺ T cells (*p* = 0.013), and CD8⁺ T cells (*p* = 0.007) than those in the FR group, but no differences were found between different time points within groups, analyzed by the mixed model analysis (Fig. 2 (A) and (B)). The white cell counts in the SR group also showed a trend toward a higher level throughout the 6 month treatment (*p* = 0.021, Fig. 2 (C)), but did not differ significantly at diagnosis from those in the FR group. The absolute counts of CD4⁺ T cell (606.3 ± 318.3 vs. 464.2 ± 238.0, *p* = 0.044), and CD3⁺CD16⁺56⁺NK T cell (182.8 ± 149.3 vs. 112.4 ± 54.9, *p* = 0.03) were significantly higher at diagnosis in the SR group than those in the FR group, but lack a significant trend toward a higher level throughout the 6 month treatment course (*p* = 0.061 and 0.35 respectively, Fig. 2 (D) and (E)). In contrast, CD3⁻CD16⁺56⁺NK cell, CD19⁺B cell, and lymphocyte counts (Fig. 2 (F), (G), (H)) were not different between the two groups at any time point. In comparisons between the counts at the three time points, only white cell counts consistently depressed after 6-month treatment in both groups (*p* = 0.003).

Absolute counts of CD8⁺ T cell (*p* = 0.036), B cell (*p* = 0.046), and NK cell (*p* = 0.049) significantly elevated after 6-month treatment on in the FR group, whereas absolute counts of B cell (*p* = 0.017) and NK T cell (*p* = 0.039) significantly depressed after 2-month treatment only in the SR group.

8.3. Multivariate analysis models and predictive accuracy

Stepwise multivariate analysis was done using parameters at diagnosis that showed statistical significance in the above comparisons between the two groups. The results showed that absolute T cell counts (odds ratio (OR) 1.002, 95% confidence interval (CI) 1.0–1.004), positive sputum acid fast bacilli stain (OR 6.69, 95% CI 1.37–32.77) and bilateral lung involvement on CXR (OR 13.114, 95% CI 1.87–92.14) at diagnosis were independent predictors for a slow response at month 2. The ROC curve analysis showed that an optimal discrimination between FR and SR could be performed at a cutoff point of 930 cells/μl for the CD3⁺ T cell counts at diagnosis (sensitivity: 69%, specificity: 61.1%) yielding the highest discriminative value on an individual level (AUC = 0.676, 95% CI 0.54–0.81, *p* = 0.015) (Fig. 3(A)). To increase predictive accuracy, the three predictors were combined in a model formula (Fig. 3(B)). The corresponding ROC curves showed that the risk of slow response at month 2 was well captured by the PS (area under the curve (AUC) = 0.855, 95% CI 0.76–0.95, *p* < 0.001) (Fig. 3(A)). Probability at a cutoff value of 0.46 displayed a sensitivity of 75.9% and specificity of 75% for prediction of slow response, dividing the patient population into high-risk and low-risk groups.

8.4. Outcomes

The proportions of patients who received second-line anti-TB medication (17.2 vs. 14.7%, *p* = 0.632)

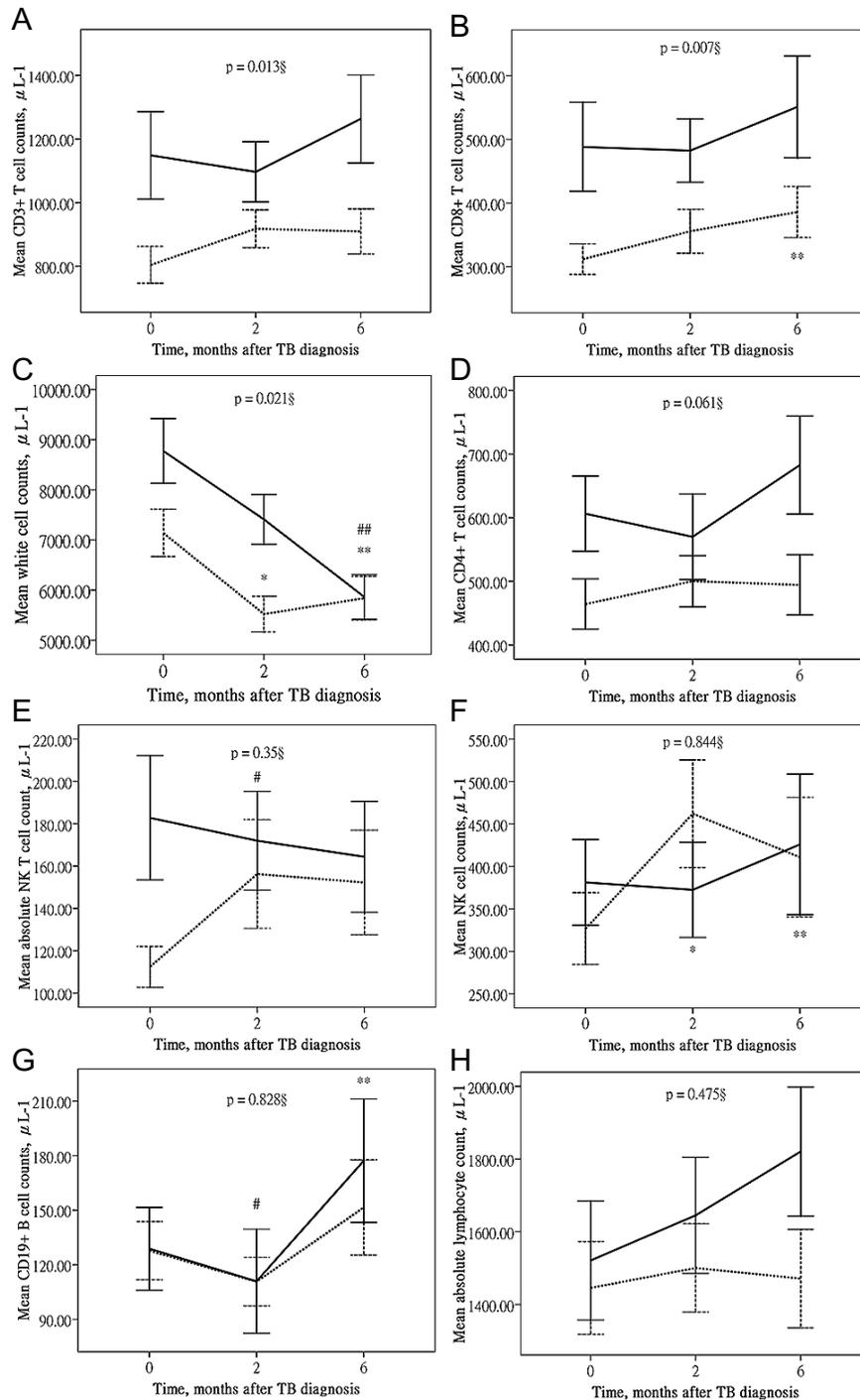


Fig. 2. Relationships between blood absolute counts of T cell subsets, and 2-month response to anti-tuberculosis treatment in the study groups. Data are expressed as the mean values of CD3+ T cell (A), CD8+ T cell (B), white cell (C), CD4+ T cell (D), NK T cell (E), NK cell (F), B cell (G), and lymphocyte (H) counts at 0, 2, and 6 months after treatment. Standard errors are indicated by vertical bars. Results of the fast responder (FR) group are shown in the solid line, and results of the slow responder (SR) group are shown in the dash line. \S p values for the fixed effects of grouping; * $p < 0.05$, month 0 vs. month 2 for the FR group; ** $p < 0.05$, month 0 vs. month 6 for the FR group; # $p < 0.05$, month 0 vs. month 2 for the SR group; ## $p < 0.05$, month 0 vs. month 6 for the SR group.

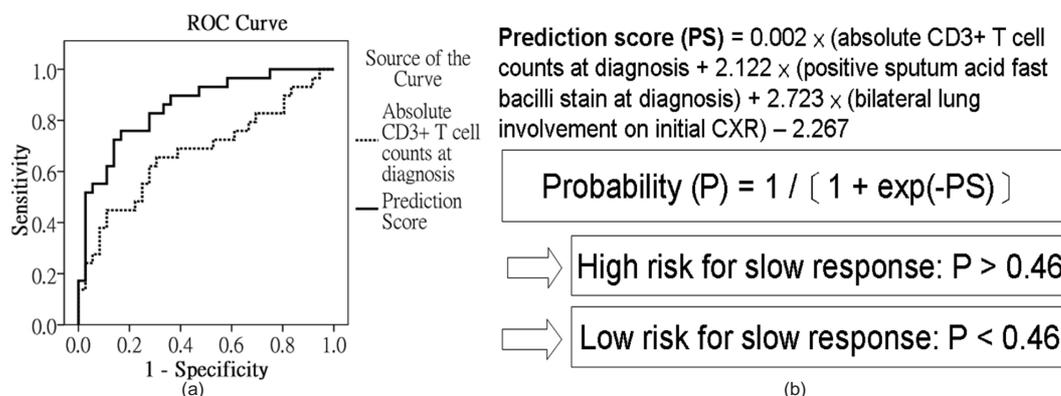


Fig. 3. Predictive accuracy of the T cell counts. (A) Using binary logistic regression analysis, the three independent predictors could be combined in a model formula, resulting in a prediction score (PS). A cutoff for the probability of slow response to 2-month anti-tuberculosis (TB) treatment could be calculated at 0.46, using receiver operating characteristics curves (ROC). (B) Comparison between ROC curves of the PS and absolute CD3⁺ T cell count at diagnosis for the prediction of 2-month anti-TB treatment response (PS: area under the curve (AUC) = 0.855, 95% CI 0.76–0.95, *p* < 0.001; absolute CD3⁺ T cell count at diagnosis: AUC = 0.676, 95% CI 0.54–0.81, *p* = 0.015).

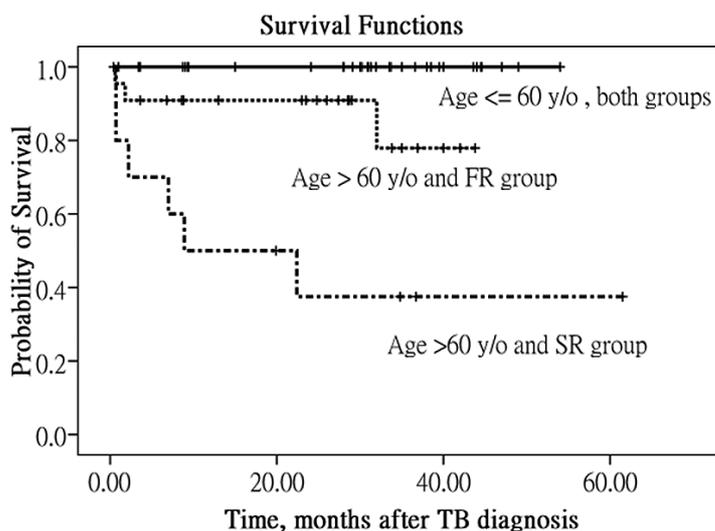


Fig. 4. Kaplan–Meier survival curves for 64 TB patients with separate lines according to age, and response to 2-month anti-tuberculosis treatment. (age ≤ 60 y/o vs. age > 60 y/o & FR group, *p* = 0.024; age > 60 y/o & FR group vs. age > 60 y/o & SR group, *p* = 0.011; age ≤ 60 y/o vs. age > 60 y/o and SR group, *p* < 0.001).

or treatment course over nine months (52.9 vs. 55.2%, *p* = 0.449) did not differ between the two groups. Six patients in the SR group and three in the FR group died during the five-year follow-up period. According to Cox multivariate regression analysis, the SR group (hazards ratio (HR) 8.15, 95% CI 1.46–45.5, *p* = 0.017) and older age (HR 1.17, 95% CI 1.05–1.31, *p* = 0.006) were independently associated with death from any cause. The SR group of patients with an age of more than 60 years had a shorter median overall survival than the FR group of patients with an age of more than 60 y/o (*p* = 0.011), and those with an age of less

than 60 years (*p* < 0.001) (Fig. 4).

8.5. Drug-resistant TB

Six patients in the SR group and six in the FR group were infected with a strain which was resistant to either rifampin, isoniazid, ethambutol, streptomycin, or kanamycin. Two were resistant only to streptomycin, and one only to kanamycin, so that standard first line medication was administered in these three patients. There was no significant difference in terms of clinical symptoms, co-morbidities, sex, age, radiographic pat-

terns, and sputum AFB positivity at diagnosis, as well as resolution on CXR at month 2 and lymphocyte subsets counts at the three time points, between patients with drug-resistant ($N = 12$) and drug-sensitive TB ($N = 52$). A larger proportion of patients with drug-resistant TB had minimal lesions on initial CXR (25 vs. 3.8%, $p = 0.048$), and remained positive culture or smear AFB stain at month 2 (41.7 vs. 13.5%, $p = 0.024$).

9. Discussion

This study was undertaken to identify surrogate biomarkers that can predict response to two months of anti-TB treatment, as defined by sputum smear/culture conversion or delayed resolution on CXR at month 2. We found that blood absolute T cell counts, positive sputum AFB stain, and bilateral lung involvement on CXR at diagnosis were independent predictors for slow responders. We then constructed a model formula combining these three predictors. The calculated PS for month 2 response displayed a sensitivity of 75.9% and specificity of 75% when a cutoff probability value was set at 46%.

Both absolute CD8⁺ and CD4⁺ T cell counts showed significantly higher numbers at diagnosis and remained persistently elevated at months 2 and 6 in the SR group, making a major contribution to the increase of CD3⁺ T cell count. Both CD4⁺ and CD8⁺ T cells are necessary to prevent reactivation and control persistent TB infection, through releasing interferon- γ or lysing heavily infected antigen-presenting cells [18–20]. One mice model showed a steady increase in the percentage of total CD3⁺, CD4⁺, and CD8⁺ cells within lung tissues in the first month after aerosol infection with *M.tb* [21]. In accordance with our findings, Tsao et al. reported a higher percentage of CD8⁺ T cells and a reciprocally lower percentage of bronchioalveolar lavage fluid CD8⁺ T cells in the patients with a higher grade of pulmonary TB [22]. We speculate that immunocompetent patients with high blood T cell counts may mount a lower grade of T cell response in the lung parenchyma infected with *M.tb*, and thus manifest a slow response to anti-TB treatment. Several previous studies showed that blood lymphocyte subsets might change at different time points after anti-TB treatment, but no consistent patterns of their changes were identified [6,8–14]. For example, CD8⁺ T cell at diagnosis have shown to be either elevated, depressed, or no change in different previous studies [9,11,13,14]. We speculate that the

intensity of immune responses mounted in TB patients may reflect the extent of involvement and the burden of *M.tb* bacilli, so we stratified patients according to treatment responses. We found that rebound elevation of CD8⁺ T cell, B cell, and NK cell counts at month 6 occurred only in the FR group, and early depression of B cell and NK T cell counts at month 2 occurred only in the SR group. In Contrast, Veenstra et al. reported that high counts of a population of CD3^{dim}/CD56⁺ NK T cells at diagnosis correlated with a negative sputum culture after 8 weeks of treatment, which was not found in our study [23]. These discrepancies may reflect variations related to the classification criteria for treatment response and the methods used for identifying NK T cells. Anti-CD56 + 16 plus anti-CD3 were used in our study, whereas CD56⁺ was used in the Veenstra et al. study.

Bilateral lung involvement on initial CXR, positive sputum smear AFB, and absolute CD3⁺ T cell count were independently associated with a slow response to two months of treatment in our study. Bilateral lung involvement or cavitations on initial CXR and being sputum culture positive after two months of treatment have been identified as independent risk factors for treatment failure or relapse [3,24–26]. For patients with these risk factors, it is recommended that the continuous phase be prolonged to seven months, making a total treatment period of nine months [15]. Sputum culture conversion after two months of treatment is the only currently accepted biomarker of sterilizing activity, but it is time-consuming and does not parallel with severity grading on CXR [27]. Sputum smear positivity at diagnosis may be a simpler and more rapid marker to predict treatment response. Bilateral lung involvement appear in around half of pulmonary TB patients and implies a major effect on risk of relapse [3,17]. In most patients, initial tuberculosis infection is controlled by host cell-mediated immunity, culminating in granuloma formation [6]. Secondary TB begins as an interaction between granulomatous process and several factors released by virulent *M.tb*, such as trehalose-dimycolate, 19 kilodalton lipoprotein, and heat shock protein. This rapidly produces caseous necrosis leading to cavities, and eventually induces Th1-related cytokines and chemokines release [28,29]. Thus, host immunological markers might be expected to be indicators of disease status or treatment response.

The sequence of pulmonary infection in immunocompromised host, such as HIV-infected individuals, parallels the depletion of CD4⁺ T cell counts. The risk of TB progressively increases with declining immuni-

ty [29] In this study, we demonstrated for the first time that a higher T cell count in the immuno-competent patients with TB may implicate either more extensive involvement of the lung parenchyma or a larger burden of *M.tb* bacilli, which would lead to delayed treatment response at month 2 in the SR group and ultimately higher mortality in the older patients. Thus, either a higher or lower T cell count may be associated with a poorer outcome in patients with pulmonary TB, and require close monitor and extensive treatment strategies.

When assessing various parameters, one encounters a rather large range of standard deviation, making it difficult to predict response for the individual patient. Because several mediators act in a synergic way to control the proliferation of *M.tb*, individual immune parameters may not have sufficient predictive accuracy. One possibility to circumvent this problem is to use a combination of various parameters in a single predictive model. In this way, we raised the predictive power, and provided a simple, objective, and rapid method for clinical judgment. Patients with a risk of slow response above 46% were referred to as high risk, and may need an expanded regimen or prolonged treatment duration. Additionally, the SR group was associated with a shorter overall survival than FR group in patients with an age of more than 60 years.

The present study has some limitations and potential bias. As in most clinical situations, a considerable proportion of patients could not expectorate sputum at diagnosis and at month 2. Although the lack of gold standard for evaluation of disease activity in these patients may have precluded us to detect significant differences between groups, we notified rapid resolution on CXR at month 2 in all the 35 fast responders, and delayed resolution in 27 (93.1%) of the 29 slow responders. The immune markers may provide an indirect indication of mycobacterial load or CXR grading. Secondly, activity status of T cell subsets was not assessed in our study. This may have lead to the lower predictive accuracy of T cell count on an individual level. An additional challenge facing all laboratory techniques relates to NTM colonization, and sputum samples of some TB patients may yield only NTM in the MGIT culture system [30]. A few patients were found to have NTM colonization by one sputum culture yield at month 2. Although true NTM infections were excluded in this study, levels of immune markers may be influenced by NTM colonization. Fourthly, twelve patients were infected with drug-resistant *M.tb*, although those with MDR strains were excluded. This factor may potentially influence the levels of immune markers. Nevertheless, these pa-

tients were treated with effective drugs according to the American Thoracic Society guidelines, and none had treatment failure [15].

In conclusion, blood absolute CD3⁺ T cell count, positive sputum AFB stain, and bilateral lung involvement on CXR at diagnosis were independent predictors of 2-month anti-TB treatment response. The slow responders were characterized by a persistently elevated blood absolute CD3⁺ and CD8⁺ T cell counts throughout the 6-month treatment course. The calculation of a PS further increased predictive accuracy and performance.

Acknowledgements

We gratefully acknowledge Professor HL Eng and the clinical staff of the Department of Pathology of the Kaohsiung Chang Gung Memorial Hospital for performing full blood counts and sputum processing. We would also like to kindly acknowledge the patients who agreed to participate in our study.

Conflict of interest statement

All authors of this paper declare that there is no conflict of interest in this study or in reporting the findings described in this manuscript.

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