A validated nomogram integrating baseline peripheral T-lymphocyte subsets and NK cells for predicting survival in stage I–IIIA non-small cell lung cancer after resection

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Background: Accurately predicting the risk of recurrence in stage I–IIIA non-small cell lung cancer (NSCLC) after resection is critical in the treatment process. This study aimed to establish a novel nomogram to identify patients with a risk of disease progression in stage I–IIIA lung cancer based on clinical characteristics, peripheral T-lymphocyte subsets, and CD16+56 natural killer (NK) cells.

Methods: A total of 306 NSCLC patients from Shanghai Municipal Hospital of Traditional Chinese Medicine between 2010 and 2020 who met the inclusion and exclusion criteria between January 2011 and December 2020 were retrospectively reviewed. Patients were randomly assigned to the training cohort (206 patients) and the validation cohort (100 patients). A nomogram model was developed based on the results of multivariate Cox regression in the training cohort. The optimal cut-off values were determined by X-tile software. The bootstrap method was used to validate the nomogram. Receiver operating characteristics curves (ROC) and the area under the ROC curve (AUC) were used to compare prognostic factors. The concordance index (C-index) was calculated to determine the accuracy of the nomogram in predicting disease-free survival (DFS).

Results: Gender, drinking history, TNM stage, and $CD4^+T/CD8^+T$ were independent factors for DFS and were integrated into the model, while CD16+56 NK cells were not proven to be significant independent factors for DFS. The calibration curves for probability of 3- and 5-year DFS showed excellent agreement between predicted and actual survival. The C-index for the nomogram to predict DFS was 0.839 in the training cohort. The nomogram showed an excellent predictive performance in the training cohort (3-/5-year AUC: 0.860/0.847) and in the validation cohort (3-/5-year AUC: 0.726/0.748).

Conclusions: We developed a prognostic model which provided individual prediction of DFS for stage I–IIIA NSCLC patients after resection. This practical prognostic tool may help oncologists in clinical treatment planning.

Keywords: Non-small cell lung cancer (NSCLC); disease-free survival (DFS); T-lymphocyte subsets; CD16+56 NK cells; nomogram

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Introduction

Lung cancer remains the leading cause of cancer-related death worldwide, with non-small cell lung cancer (NSCLC) representing approximately 85% of all lung cancer cases (1,2). Advances in medical examination techniques have increased detection rates of early-stage lung cancer (3). Surgical resection is the first optimal treatment for non-advanced lung cancer (4,5). In spite of progress in diagnosis and treatment, the 5-year recurrence-free survival (RFS) rate and the 5-year overall survival (OS) rate of clinical stage I NSCLC patients were 73.2% and 79.5%, respectively (6). For patients with stage II–IIIA NSCLC, the 5-year OS rate after resection is estimated to be between 41–65% (7). Although postoperative adjuvant therapy can improve the 5-year OS of NSCLC patients, it has also been recently confirmed that neoadjuvant chemotherapy may achieve similar outcomes as adjuvant chemotherapy (8). For postoperative patients with NSCLC, recurrence and metastasis remain the key factors related to long-term survival (9). The tumor-node-metastasis (TNM) staging system is used to determine prognosis and provide guidance for treatment of NSCLC (10). However, nomograms have been proposed as an alternative standard over the traditional TNM staging system as predicting prognosis via TNM staging appears to be insufficient (11,12).

Nomograms are graphical calculating tools that have been widely applied in clinical practice to predict cancer outcomes by integrating patient factors and relevant hematological parameters (13). For many types of cancer, nomograms have been identified as a reliable approach for predicting a particular endpoint using statistical methods (14-17). There were nomograms which included immune genes as prognostic factors via The Cancer Genome Atlas (TCGA) database and Gene Expression Omnibus (GEO) (18). Besides, numerous studies have explored the influence of T-lymphocyte subsets and natural killer (NK) cells on the prognosis of cancers (19-21). For lung cancer in particular, T-lymphocyte subsets and NK cells are considered to be independent factors related to clinical outcomes such as progression-free survival (PFS) and OS (22,23). The relationship between immunity and lung cancer is complex. The composition of immune cells may predict response and act as an indicator of the ability of the immune system to eliminate residual disease after therapy. It has been found that the absence of lung CD4 cells with an effector-like phenotype ($CD45RA^+/CD27^-$) is a predictor for a favorable outcome (24). NK cells are generally defined as the first line of defense in the fight against infection and circulating cancer cells which can accelerate metastasis (25,26). Functionally, NK cells can produce cytokines that support T helper polarization and T cell activation, as well as stimulate dendritic cell (DC) and B cell maturation to bridge and orchestrate innate and adaptive immune responses (27). Hence, NK cells play an important role in tumor surveillance and can be manipulated by artificial activation techniques to present a highly effective anticancer tool against malignancies, and dependent on successful further rearming and mobilization, against solid tumors in the future (28). However, there are currently few studies on the relationship between the prognosis of stage I–IIIA NSCLC after resection and peripheral T-lymphocyte subsets combined with CD16+56 NK cells.

Several researches have confirmed that T-lymphocyte subsets and NK cells play important roles in the prognosis of NSCLC. The cancer cells may escape from the immune attack in the presence of immune function and pathological changes when aberrant alternation in the quantity and function of the T cell subsets happens (29). The high percentages of peripheral $CD3^+$, $CD4^+$T cells and CD4/CD8 ratio were associated with longer OS in lung cancer patients (30). The increased risk of lung cancer is related to low CD4 cell count and low CD4/CD8 ratio (31). Peripheral $CD8^+$T cells was higher in lung cancer patients compared with the healthy control population, while $CD3^+$, $CD4^+$T cells and CD4/CD8 ratio was significantly lower (32). Moreover, the correlation between NK cell activity and PFS of NSCLC patients was verified (33). The median PFS was significantly better in the high NK group. In addition, CD16+56-NK cell subset is highly enriched in the tumor infiltrate and show activity markers such as CD69, HLA-DR, NKp44, etc. The CD16+56-NK cells enhance protumor neoangiogenesis through secretion of vascular endothelial growth factor (VEGF) (34).

It is essential to construct an efficient clinical model to predict the long-term survival of postoperative NSCLC patients. Hence, this study aimed to establish and validate an easy-to-use nomogram based on an integration of objective baseline clinical characteristics and hematological parameters, including T-lymphocyte subsets and CD16+56 NK cells, that could accurately predict the risk of recurrence and metastasis in patients who have received radical resection of pulmonary carcinoma. We present the following article in accordance with the TRIPOD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6347/rc).

Methods

Study design and patient selection

We performed a retrospective study of stage I–IIIA NSCLC patients who had received radical resection. Patients were collected at Shanghai Municipal Hospital of Traditional Chinese Medicine between 2010 and 2020. This study was approved by an independent ethics committee review board at Shanghai Municipal Hospital of Traditional Chinese Medicine (No. 2020SHL-KY-48). The procedures in this study were carried out in accordance with the principles of the Helsinki Declaration (as revised in 2013). Participants gave informed consent before taking part in this research.

Patients included in the study met the following criteria: (I) patients had undergone radical resection of pulmonary carcinoma; (II) patients had pathological diagnosis of NSCLC; (III) patients had stage I–IIIA disease; (IV) patients agreed to participate in the study, including regular follow-up; and (V) patients with Eastern Cooperative Oncology Group (ECOG) performance ≤2 (35). The exclusion criteria were as follows: (I) patients diagnosed with secondary lung cancer; (II) patients with severe/uncontrolled systemic diseases, including infection, rheumatic immune system disease, cardiovascular disease or liver and kidney insufficiency; (III) patients with other malignant tumors; (IV) patients who were pregnant; (V) patients with severe psychiatric disorders; and (VI) Karnofsky (KPS) score <60.

Assessment of clinical and laboratory parameters

The following data were collected for each patient: (I) basic information: admission number, patient name, age at diagnosis, gender, identity card number, and cellphone number; (II) cancer-related information: date of diagnosis, TNM stage, pathological category, and type of treatment; (III) personal history: smoking and drinking history; (IV) family history: tumor-related family history; and (V) peripheral T-lymphocyte subsets and CD16+56 NK cells.

Clinical outcome assessment and patient follow-up

The patients were followed up via telephone and clinical in-person visits. Disease-free survival (DFS) was defined as the time from the date of first diagnosis to the date of first relapse, cancer-related death, or the last follow-up time. OS was defined as the time from the date of first diagnosis to the date of cancer-related death. Additionally, the date of death was determined from the department of Cancer Control and Prevention via a database maintained by the Shanghai Municipal Center for Disease Control and Prevention of Cancer Patient Registration System. The last follow-up date was December 30, 2020.

X-tile analysis

X-tile software (Yale University School of Medicine, New Haven, USA) can provide a quantitative evaluation of every possible way of dividing a cohort into high- and low-marker expression (36). Further, when separate training and validation cohorts are not available, the software can provide a best P value evaluation by dividing a single cohort into training and validation subsets in order to produce a rigorous statistical estimation. In this study, the optimal cut-off values of $CD3^+T$, $CD4^+T$, $CD8^+T$, $CD4^+T/CD8^+T$, and CD16+56 NK cells were determined by X-tile.

Data and statistical methods

Data were analyzed using IBM SPSS standard version 21.0 (SPSS Inc., Chicago, IL, USA) and R software version 4.1.0 (http://www.R-project.org). All data for continuous variables were presented as mean ± standard deviation (SD), and categorical variables were presented as a rate (%). Normally distributed data were analyzed using either Student's *t*-test for 2 groups or 1-way ANOVA for 3 or more groups. Data with non-normal distribution were analyzed using nonparametric tests. Count data were analyzed with the Chi-square test. Prognostic factors for DFS and OS were assessed by univariate and multivariate analysis. The log-rank test were used to compare survival curves among different groups. All statistical tests were 2-tailed. Statistical significance was set at $P<0.05$.

Receiver operating characteristics (ROC) curves and the area under the ROC (AUC) curve were used to compare prognostic factors. In addition, we calculated the concordance index (C-index) to assess the accuracy of the nomogram for predicting DFS. The bootstrap method was used to validate the nomogram.

Results

Patient clinical characteristics

The baseline characteristics of study population are shown in *Table 1*. A total of 306 NSCLC patients met the inclusion criteria and were enrolled in the retrospective study. There

Table 1 Main clinical characteristics and parameters of patients with NSCLC

Variables	Training cohort (N=206)		Validation cohort (N=100)	
	n	%	n	%
Gender				
Male	60	29.13	30	30.00
Female	146	70.87	70	70.00
Age				
<68 years	176	85.44	79	79.00
≥68 years	30	14.56	21	21.00
TNM stage (AJCC, 8th)				
I	176	85.44	82	82.00
II	18	8.74	9	9.00
IIIA	12	5.83	9	9.00
Pathology				
Adenocarcinoma	196	95.15	86	86.00
Squamous cell cancer	6	2.91	7	7.00
Other types	4	1.94	7	7.00
Family history				
Yes	67	32.52	41	41.00
No	139	67.48	59	59.00
Smoking history				
Yes	33	16.02	20	20.00
No	173	83.98	80	80.00
Drinking history				
Yes	10	4.85	9	9.00
No	196	95.15	91	91.00
$CD3^+T$				
≤75.30%	150	72.82	83	83.00
>75.30%	56	27.18	18	18.00
$CD4^+T$				
≤43.60%	137	66.50	72	72.00
>43.60%	69	33.50	28	28.00
$CD8^+T$				
≤32.00%	181	87.86	84	84.00
>32.00%	25	12.14	16	16.00
$CD4^+T/CD8^+T$				
≤1.3	52	25.24	22	22.00
>1.3	154	74.76	78	78.00
CD16+56 NK cells				
≤15.81%	87	42.23	38	38.00
>15.81%	119	57.77	62	62.00

NSCLC, non-small-cell lung cancer; TNM, tumor-node-metastasis; AJCC, American Joint Committee on Cancer.

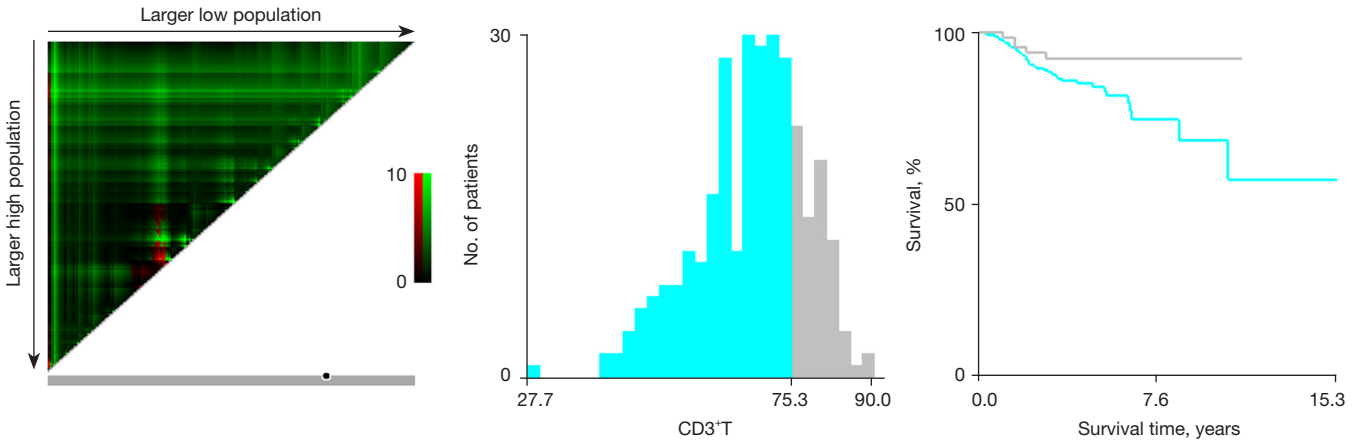

Figure 1 Analysis of CD3$^+$T via X-tile in NSCLC patients. Prognostic significance of CD3$^+$T for NSCLC patients was employed by using the statistical algorithm in X-tile to calculate the best cut-off value. NSCLC, non-small cell lung cancer.

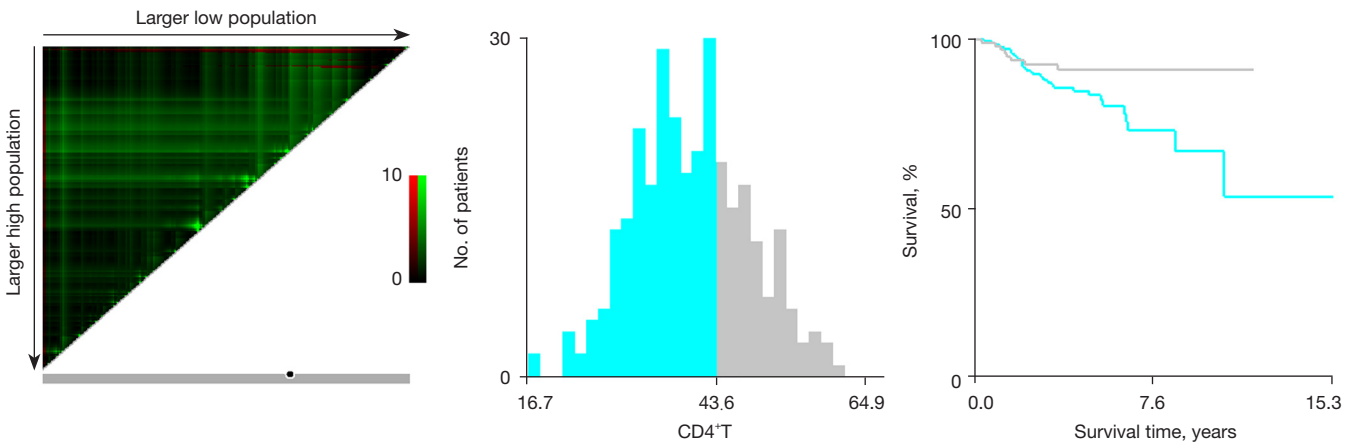

Figure 2 Analysis of CD4$^+$T via X-tile in NSCLC patients. Prognostic significance of CD4$^+$T for NSCLC patients was employed by using the statistical algorithm in X-tile to calculate the best cut-off value. NSCLC, non-small cell lung cancer.

were 206 patients in the training cohort and 100 patients in the validation cohort. In the training cohort, 60 patients (29.13%) were male and 146 patients (70.87%) were female. There were 176 patients (85.44%) at stage I, 18 patients (8.74%) at stage II, and 12 patients (5.83%) at stage IIIA. There were 196 cases (95.15%) of adenocarcinoma and 6 cases (2.91%) of squamous cell cancer. In total, 67 patients (32.52%) had a family history of cancer. Former smokers and former drinkers made up 16.02% and 5.83% of the subjects, respectively. The optimal cut-off values for CD3$^+$T, CD4$^+$T, CD8$^+$T, CD4$^+$T/CD8$^+$T, and CD16+56 NK cells were determined via X-tile and were 75.30% (*Figure 1*), 43.60% (*Figure 2*), 32.00% (*Figure 3*), 1.3 (*Figure 4*), and 15.81% (*Figure 5*), respectively.

Univariate and multivariate Cox analysis of survival time

A univariate regression model was used to investigate DFS prediction based on the baseline demographic, clinical, and laboratory variables, including CD3$^+$T, CD4$^+$T, CD8$^+$T, CD4$^+$T/CD8$^+$T, and CD16+56 NK cells. As shown in *Table 2*, the prognostic factors with P value <0.1 in the training cohort were as follows: gender, TNM stage, smoking history, drinking history, and CD4$^+$T/CD8$^+$T. Further multivariate analysis found that gender, TNM stage, drinking history, and CD4$^+$T/CD8$^+$T were

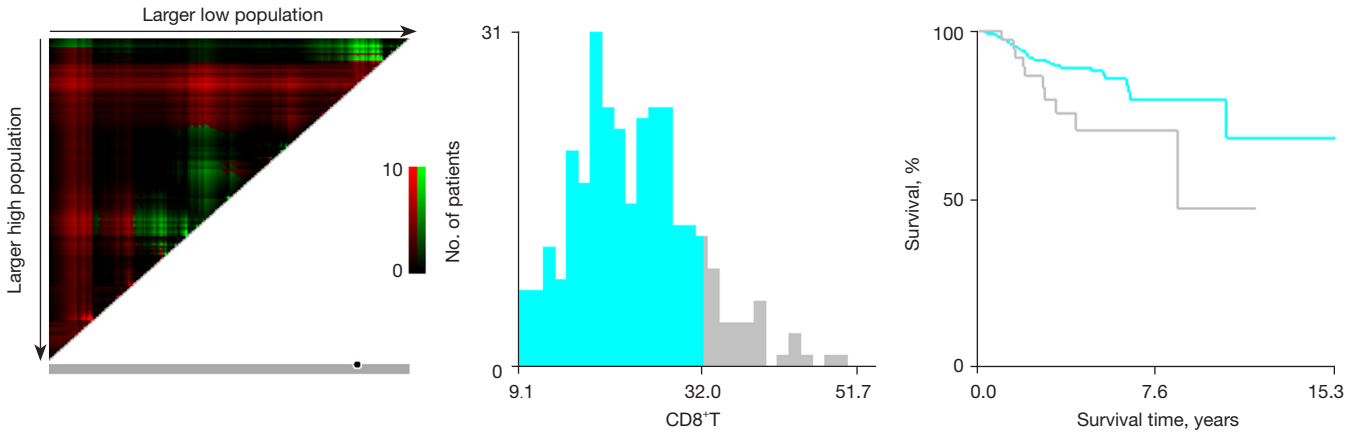

Figure 3 Analysis of CD8⁺T via X-tile in NSCLC patients. Prognostic significance of CD8⁺T for NSCLC patients was employed by using the statistical algorithm in X-tile to calculate the best cut-off value. NSCLC, non-small cell lung cancer.

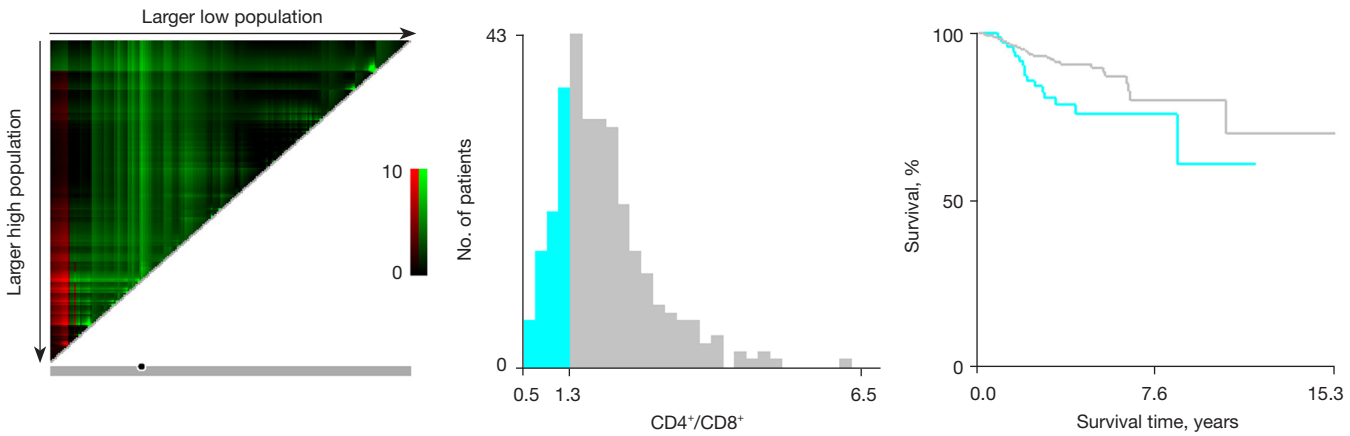

Figure 4 Analysis of CD4⁺T/CD8⁺T via X-tile in NSCLC patients. Prognostic significance of CD4⁺T/CD8⁺T for NSCLC patients was employed by using the statistical algorithm in X-tile to calculate the best cut-off value. NSCLC, non-small cell lung cancer.

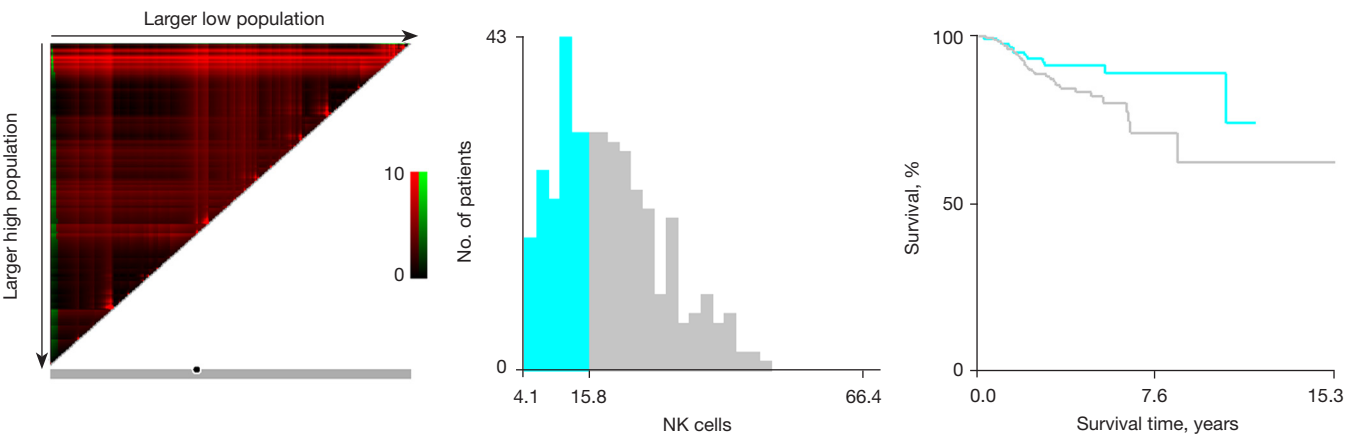

Figure 5 Analysis of CD16⁺56 NK cells via X-tile in NSCLC patients. Prognostic significance of CD16⁺56 NK cells for NSCLC patients was employed by using the statistical algorithm in X-tile to calculate the best cut-off value. NSCLC, non-small cell lung cancer.

Table 2 Univariate and multivariate Cox analysis of the DFS and OS

Variables	DFS				OS	
	Univariate analysis		Multivariate analysis		Univariate analysis	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Gender						
Male	4.232 (1.862–9.616)		3.799 (1.239–11.655)		5.471 (1.796–16.665)	
Female	Reference	0.001*	Reference	0.020*	Reference	0.003*
Age						
<68 years	Reference				Reference	
≥68 years	1.397 (0.520–3.751)	0.507			1.447 (0.404–5.178)	0.570
TNM stage (AJCC, 8th)						
I	Reference		Reference		Reference	
II	4.703 (1.626–13.600)	0.004*	5.792 (1.862–18.012)	0.002*	2.170 (0.460–10.242)	0.328
IIIA	11.944 (4.793–29.765)	<0.001*	14.903 (5.249–42.307)	<0.001*	9.697 (3.096–30.371)	<0.001*
Pathology						
Adenocarcinoma	Reference				Reference	
Squamous cell cancer	1.146 (0.154–8.519)	0.894			NA	0.640
Other types	NA	0.974			NA	0.547
Family history						
Yes	0.492 (0.190–1.270)	0.142			0.757 (0.246–2.333)	0.628
No	Reference				Reference	
Smoking history						
Yes	3.738 (1.628–8.585)	0.002*	0.612 (0.187–2.008)	0.418	2.401 (0.747–7.722)	0.142
No	Reference				Reference	
Drinking history						
Yes	3.663 (1.240–10.819)	0.019*	8.779 (2.222–34.684)	0.002*	4.594 (1.273–16.577)	0.020*
No	Reference		Reference		Reference	
$CD3^+T$						
≤75.30%	Reference				Reference	
>75.30%	0.506 (0.173–1.476)	0.212			0.406 (0.092–1.801)	0.236
$CD4^+T$						
≤43.60%	Reference				Reference	
>43.60%	0.529 (0.198–1.411)	0.203			0.821 (0.260–2.590)	0.737
$CD8^+T$						
≤32.00%	Reference				Reference	
>32.00%	1.390 (0.477–4.053)	0.546			0.537 (0.071–4.087)	0.548
$CD4^+T/CD8^+T$						
≤1.3	Reference		Reference		Reference	
>1.3	0.437 (0.194–0.986)	0.046*	0.312 (0.124–0.790)	0.014*	0.813 (0.255–2.595)	0.727
CD16+56 NK cells						
≤15.81%	Reference				Reference	0.842
>15.81%	1.659 (0.715–3.846)	0.238			0.900 (0.320–2.530)	

*P<0.05. DFS, disease-free survival; OS, overall survival; HR, hazard ratio; TNM, tumor-node-metastasis; AJCC, American Joint Committee on Cancer.

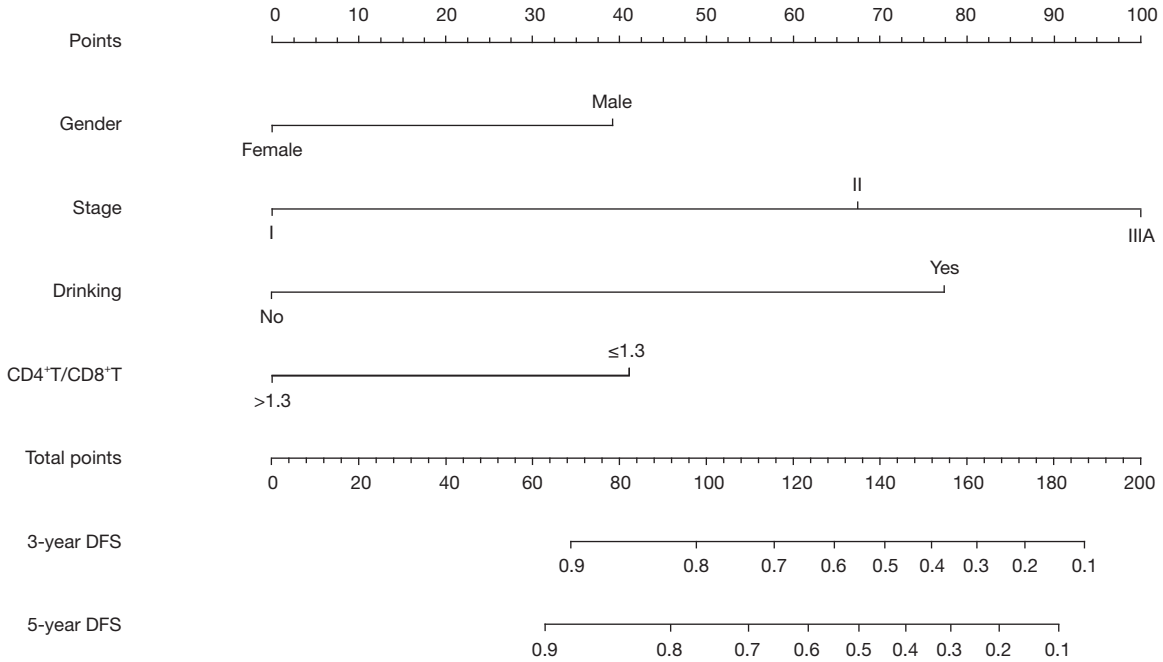

Figure 6 Nomogram predicting 3- and 5-year DFS for NSCLC patients. DFS, disease-free survival; NSCLC, non-small lung cancer cell.

independent factors for DFS. In addition, gender, TNM stage, and drinking history were related to OS.

Development of a prognostic nomogram

Based on the multivariate cox analysis, significant independent factors for DFS were integrated to create the nomogram, including gender, TNM stage, drinking history, and $CD4^+T/CD8^+T$ (*Figure 6*). The nomogram demonstrated that TNM stage and drinking history contributed the most to prognosis, followed by the ratio of $CD4^+T/CD8^+T$. Gender had relatively little impact on survival. By calculating the total score and comparing it to the highest possible score, the predicted 3- and 5-year DFS were easy to obtain.

Calibration and validation of the nomogram

The calibration plot indicated an excellent consistency between the nomogram prediction and the actual survival rate for 3- and 5-year DFS in the training cohort (*Figure 7A*,*7B*). Further, it also showed an acceptable agreement in the external validation cohort (*Figure 8A*,*8B*). In the training cohort, the C-index for the nomogram to predict DFS was 0.839, which indicated good prediction efficiency of the model. In addition, the prediction efficiency of the nomogram model was assessed by ROC analysis. In the training cohort, 3- and 5-year AUC were 0.860 and 0.847, respectively (*Figure 9A*). In the validation cohort, 3- and 5-year AUC were 0.726 and 0.748, respectively (*Figure 9B*). The above findings suggested that our nomogram had more significant potential for the prediction of recurrence compared to the American Joint Committee on Cancer (AJCC) staging system.

Discussion

In this retrospective study, we delivered significantly better results using one of the first nomograms that can predict DFS in stage I–IIIA NSCLC patients based on a relatively large real-world population-based database. We identified gender, drinking history, the ratio of $CD4^+T/CD8^+T$, along with TNM stage as independent factors for prediction via multivariate cox analysis. Most of these independent factors were consistent with a host of previous findings on risk factors for NSCLC (37-39). Subsequently, the above 4 predictors were integrated into a nomogram to calculate the risk probability of disease recurrence or metastasis tailored to each individual patient. In addition, $CD4^+T/CD8^+T$ (≤1.3) was first identified as a risk factor for DFS.

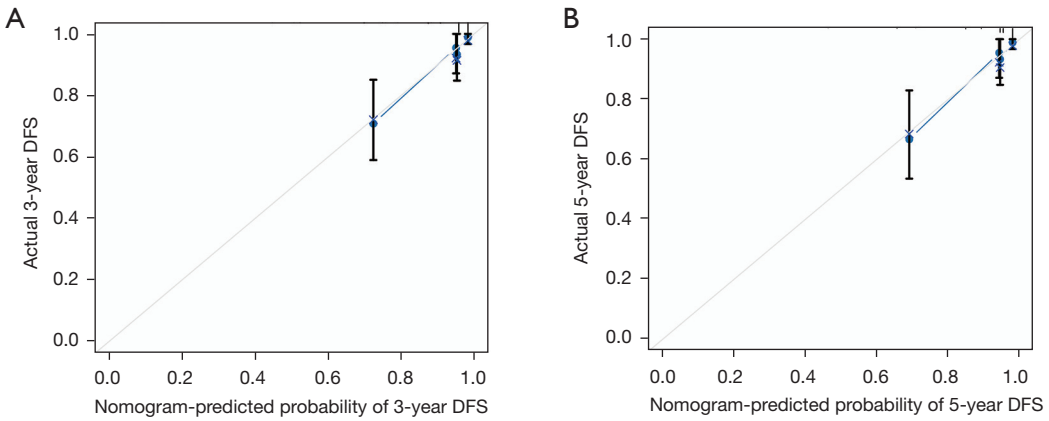

Figure 7 The calibration curves for predicting patient survival at each time interval in the training cohort. DFS, disease-free survival.

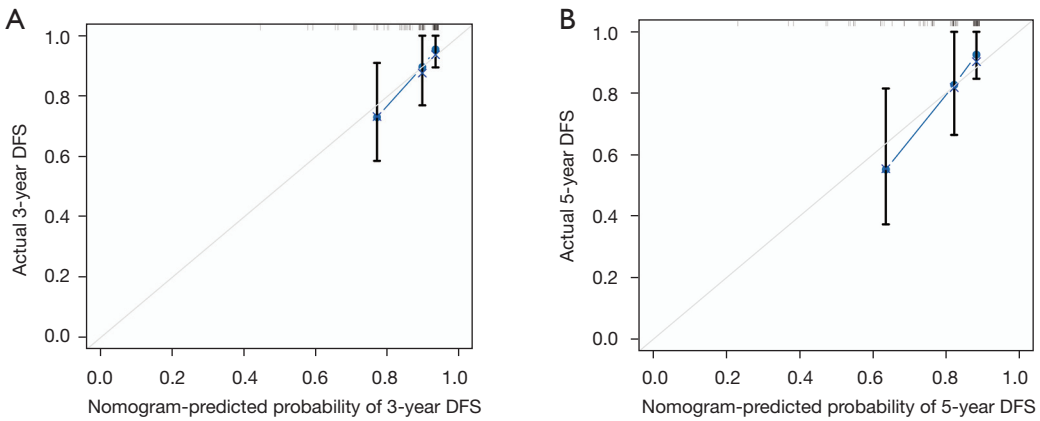

Figure 8 The calibration curves for predicting patient survival at each time interval in the validation cohort. DFS, disease-free survival.

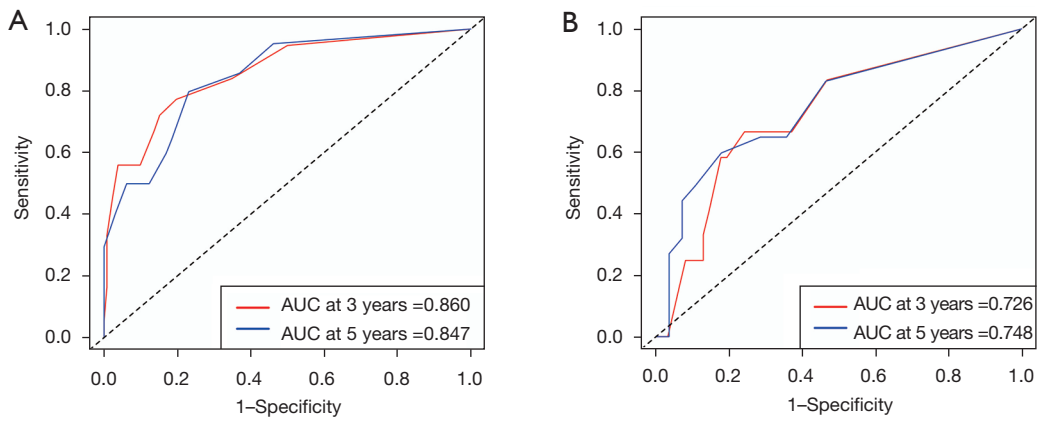

Figure 9 The ROC curves for predicting patient survival according to the nomogram in the training and validation cohorts. ROC, receiver operating characteristics; AUC, area under the curve.

Our nomogram showed that male NSCLC patients with relatively late TNM stage, drinking history, and low ratio of $CD4^+T/CD8^+T$ had a greater risk of disease progression after resection. Regrettably, CD16+56 natural killer (NK) cells were not proven to be significant independent factors for DFS, which may be related to the relatively small sample size of this study. In fact, one of our objectives for developing this new model was to show the correlation between peripheral T-lymphocyte subsets and DFS in a real-world Chinese population.

The use of immunotherapy for cancer treatment has been explored for many years, with numerous patients benefiting from long-term survival. Immune cell infiltration is considered an important determinant of the immune microenvironment of a malignant tumor, of which T cells are the main component of the anticancer immune system. For example, in metastatic castration-resistant prostate cancer, a high proportion of $CD4^+T$, $CD8^+T$, and $CD56^{bright}$ NK cells has been found to be a favorable prognostic factor (34). Similarly, an increase in the proportion of $CD8^+T/CD28^-$ T cells was verified in women with lung cancer (40) and breast cancer (41) during chemotherapy. On the contrary, some types of tumor-infiltrating inflammatory cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), could accelerate angiogenesis and support tumor growth (42). Tregs are abundant in NSCLC and predicted for an increased risk of disease recurrence in early-stage disease (43). As a kind of specialized T-cell subpopulation, Tregs could suppress sterilizing immune response. As a result, it is anticipated that tumor-infiltrating immune/inflammatory cells may be significant hallmarks for immune monitoring of baseline peripheral blood in both treated and non-treated patients after resection (44).

The poor outcomes for NSCLC patients remain a major challenge for oncologists, and a more practical and valuable prognostic marker than TNM classification is urgently needed. Despite an increasing number of new nomograms for lung cancer, none of those combining peripheral T-lymphocyte subsets and CD16+56 NK cells have been established for I–IIIA resected NSCLC patients. It is notable that the Surveillance, Epidemiology, and End Result (SEER) database, which has a considerably large population, has contributed to the construction of numerous nomograms for cancers (45-47). However, the SEER database also has many imperfections, including lack of clinical data on some hematology parameters and missing outcomes such as tumor recurrence. There is no doubt that oncologists should pay more attention to cancer recurrence and metastasis rather than cancer death, particularly in patients who have undergone radical surgery.

The present study oversaw the development of the first prognostic model to predict DFS using a peripheral blood immunological index for stage I–IIIA NSCLC after resection. Nevertheless, a number of inevitable limitations existed in this work. First, this retrospective cohort study was conducted in Shanghai Municipal Hospital of Traditional Chinese Medicine. All enrolled patients received treatment involving traditional Chinese medicine. Previously, we conducted several clinical studies (9,30) confirming that traditional Chinese medicine prolonged DFS in lung cancer patients, especially those after radical resection of non-advanced lung adenocarcinoma. The intervention of traditional Chinese medicine reduced the progressive disease rate, and thus the number of outcome events was reduced correspondingly. Further, the retrospective nature and limited sample may have led to selection bias. There is a high likelihood that some untreated patients were in a worse state than those given the option of treatment, despite this not always being recorded in medical records. In addition, worse baseline clinical parameters could have given rise to longer survival due to more treatment interventions than that reported by blinded clinical trials. Finally, we did not find another independent cohort for external validation.

Conclusions

In this study, gender, drinking history, TNM stage, and $CD4^+T/CD8^+T$ were shown to be independent factors for DFS. To visualize and integrate these 4 factors, we developed a prognostic model which could provide individual prediction of DFS for stage I–IIIA patients after resection. This practical prognostic tool may help oncologists in clinical treatment planning. However, more data from other cohorts are needed to further validate this nomogram.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6347/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by an independent ethics committee review board at Shanghai Municipal Hospital of Traditional Chinese Medicine (No. 2020SHL-KY-48). The procedures in this study were carried out in accordance with the principles of the Helsinki Declaration (as revised in 2013). Participants gave informed consent before taking part in this research.

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