Original Article

A nomogram model based on peripheral blood lymphocyte subsets to assess the prognosis of non-small cell lung cancer patients treated with immune checkpoint inhibitors

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Background: The primary aim of this study was to investigate the prognostic value of peripheral blood lymphocyte subsets in non-small cell lung cancer (NSCLC) patients treated with immune checkpoint inhibitors (ICIs).

Methods: From 2018 to 2019, 82 patients diagnosed with stage IIIB–IV NSCLC at Zhejiang Cancer Hospital were recruited for this study. Peripheral blood lymphocyte subsets of NSCLC patients were analyzed using flow cytometry before and after ICI treatment. The relationship between the percentage of peripheral blood lymphocyte subsets, clinicopathological features, progression-free survival (PFS), and overall survival (OS) was identified by correlation heat map, Kaplan-Meier curve, log-rank test, and Cox regression analysis.

Results: The CD4/CD8 ratio and the percentage of B cells was decreased after ICI treatment. Furthermore, the percentage of $CD3^+$ T cells, natural killer (NK) cells, and natural killer T (NKT) cells before ICI treatment was associated with brain metastases, the proportion of $CD3^+CD4^+$ T cells before ICI treatment was related to epidermal growth factor receptor (EGFR) status, the CD4/CD8 ratio before ICI treatment was correlated to pathology, the ratio of B cells before ICI treatment was related to therapeutic regimen, and the percentage of NKT cells before ICI treatment was associated with use of radiotherapy. Furthermore, univariate survival analysis revealed that low percentage of B cells forecasted a poor OS for NSCLC patients with ICI treatment. In addition, the nomogram developed by percentages of peripheral blood lymphocyte subsets could determine survival probability and survival time of NSCLC patients with immunotherapy.

Conclusions: ICI treatment induced changes in the percentage of peripheral blood lymphocyte subsets, which had prognostic value for brain metastases, radiotherapy, EGFR status, pathology, and therapeutic regimen, along with prognostic value, for NSCLC patients treated with ICIs.

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Keywords: Immune checkpoint inhibitor (ICI); non-small cell lung cancer (NSCLC); peripheral blood lymphocyte subsets

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Introduction

Lung cancer is one of the most common malignant tumors and the major cause of cancer-related death in the world (1). Lung cancer can be divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) according to tissue type, with NSCLC accounting for about 85% of all lung cancer cases (1). Clinically, only a few patients with NSCLC can be diagnosed in the early stage (stage I or II), which allows treatment by surgical resection (2). However, more than 60% of NSCLC patients reach advanced stages or distant metastasis (stage III or IV) at the time of diagnosis, thus surgical resection may no longer be an option (2). Although many advances have been made in the clinical diagnosis and treatment of NSCLC in recent years, such as targeted therapy (3), the 5-year overall survival (OS) rate of NSCLC patients is still less than 20% (3,4). Therefore, it is urgent to improve the prognosis of NSCLC patients with novel therapies.

Immunotherapy is one of the most effective treatments for cancer. It acts through enhancing antitumor immune response and has also been applied to NSCLC treatment (5-7). Immune checkpoint inhibitors (ICIs) have been used in immunotherapy of NSCLC (8,9), with the most commonly used type being, monoclonal anti-programmed death receptor-1 (PD-1) antibody or ligand (PD-L1) antibody (e.g., nivolumab, pembrolizumab, atezolizumab) (10-13). Although no head-to-head clinical trials have been conducted, PD-1 inhibitors appear to be slightly superior to PD-L1 inhibitors in NSCLC (14-17). On the contrary, PD-L1 inhibitors have shown significant advantages over PD-1 inhibitors in SCLC and have been approved for first-line therapy (18). PD-1 is usually expressed on activated T cells and B cells, while numerous cancers including NSCLC express PD-L1 (19,20). The binding of PD-L1 to PD-1 suppresses tumor killing by abolishing effector T cell functioning via restricting T cell proliferation, migration, and release of cytokines (21). Monoclonal anti-PD-1 antibody blocks the association of PD-L1 on the surface of tumor cells with the PD-1 on activated T cells and B cells to enhance tumor cell killing by immune cells (8).

Growing evidence has revealed the predictive and prognostic value of peripheral blood lymphocyte subsets in patients with cancers. For instance, levels of $CD4^+$ and $CD3^+$ lymphocyte subsets in peripheral blood are potent predictive and prognostic indicators in patients with metastatic breast cancer (22). Besides, natural killer (NK) cell percentage in circulating blood has been identified to be a predictor of survival in colorectal cancer patients (23). Moreover, nomogram based on lymphocyte-to-monocyte ratio in peripheral blood could predict survival in patients with stage I NSCLC (24). In addition, PD1-positive $CD4^+$ T cell count in peripheral blood can predict PFS in NSCLC patients receiving treatment with ICIs (25). However, the predictive and prognostic value of other peripheral blood lymphocyte subsets in NSCLC patients treated with ICIs remains unknown.

The primary aim of this study was thus to investigate the predictive and prognostic value of peripheral blood lymphocyte subsets in NSCLC patients treated with ICIs.

We present the following article in accordance with the TRIPOD reporting checklist (available at https://dx.doi.org/10.21037/tlcr-21-899).

Methods

Enrolment to the study

The flowchart was shown in Figure S1. This analysis included 82 patients diagnosed with stage IIIB–IV NSCLC who were treated at Zhejiang Cancer Hospital from August 2018 to November 2020. The brief inclusion criteria were as follows: patients older than 18 but younger than 75 years, an expected survival time of more than 12 weeks, histologically or cytologically confirmed stage IIIB–IV, ECOG performance status (PS) 0–2, and expected treatment with ICIs. The exclusion criteria were the following: patients with symptomatic central nervous system metastasis; lactating women; patients with active infection requiring systemic therapy; patients who could

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receive chest radiotherapy; radiotherapy and chemotherapy performed within 4 weeks before ICI treatment; patients with autoimmune disease that has required systemic treatment or any other disease requiring steroid therapy or immunosuppressive therapy within 7 days prior the first dose of ICIs; and patients who did not comply with the requirements of the study, obviously violated the protocol, or switched to other treatments midway. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All experimental procedures involving human participants were approved by the Ethics Committee of Zhejiang Cancer Hospital. Written informed consent was obtained from all participants enrolled in this study. Clinical data of patients were collected, including pathological classification, tumor-node-metastasis (TNM) staging, pathological diagnosis, survival time, treatment information, and others. Routine follow-up examinations including CT scan was conducted every 3 months.

Treatment

The patients who received any ICI agent as monotherapy or in combination with chemotherapy regardless of treatment line were permitted to enroll to this study. The choice of chemotherapy regimen was based on generally accepted standards of clinical practice.

ICI regimens used of patients were included pembrolizumab (n=23, 28.0%), nivolumab (n=9, 11.0%), sintilimab (n=30, 36.6%), carrelizumab (n=4, 4.9%) and treprizumab (n=16, 19.5%).

The analysis of blood samples

Five mL of peripheral venous blood were collected from 82 patients with advanced NSCLC just 1 day before ICI treatment. Only 47 of the patients were willing to collect another 5 mL of peripheral venous blood at the time of the best curative effect. Next, 50 μL of anticoagulant whole blood was added into the test tube followed by 20 μL of premixed monoclonal antibody (Beckman Coulter, Brea, CA, USA) and homotypic control and incubated for 15 minutes at room temperature (RT) in the dark. Subsequently, the blood samples were treated by 500 μL of hemolysin for 15 minutes at RT in the dark. Next, 1 mL of sheath solution was added to stop hemolysis. Blood samples were then centrifuged for 5 minutes at 1,000 R/min, and supernatants were discarded. After being by phosphate buffer once, cells were resuspended in 500 μL of sheath solution and analyzed by a Beckman Coulter Cytomics FC500 Flow Cytometer.

Flow cytometry

Flow cytometry was performed as described previously (26) to detect lymphocyte subsets, including $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, CD4/CD8 ratio, NK cells ($CD3^-CD56^+$), B cells ($CD19^+$), natural killer T (NKT) cells ($CD3^-CD56^+$), Ts cells ($CD4^+CD45RA^+$), helper T (Th) cells ($CD4^+CD45RA^+$), memory T cells ($CD4^+CD45RO^+$), activated T cells ($CD45RA^+CD45RO^+$). and activated $CD8^+$ cells ($CD8^+CD38^+$). Antibodies used for flow cytometry were obtained from BD Biosciences (San Jose, CA, USA) and included CD3-FITC (#555332), CD4-FITC (#550628), CD8-FITC (#555366), CD19-FITC (#555412), CD56-PE (#55664), CD45RO-APC (#559865), CD45RA-PE (#555489), CD38-PE (#555460), FITC/PE/APC isotype controls (#555748; #555749; #555576). Cell Quest software (BD, Franklin Lakes, NJ, USA) was used to analyze the proportion of peripheral blood lymphocyte subsets.

Statistical analysis

Statistical analyses were performed using SPSS (version 21.0, IBM Corp., Armonk, NY, USA) and R (version 3.5.1, The R Foundation for Statistical Computing). All statistical tests were two-sided, and P values <0.05 were considered statistically significant. A correlation heat map was utilized to identify the association between the percentage of peripheral blood lymphocyte subsets and clinicopathological features. Besides, the compare of continuous variables between two groups was carried out using Mann-Whitney U test and the Student's *t*-test. Moreover, the statistical differences among three or more groups were examined by Kruskal-Wallis test. Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST 1.1) was used to evaluate tumor response. Furthermore, the Kaplan-Meier curve and the log-rank test were performed to determine the differences in the survival rates between two groups. What's more, progression-free survival (PFS) was calculated from the date of advanced disease diagnosis to the date of NSCLC recurrence or metastasis, while OS was determined from the date of advanced disease diagnosis to the date of death or censored at the date of the last follow-up. A univariate Cox regression analysis was performed based on the patients' clinicopathological features and percentage of peripheral blood lymphocyte subsets in NSCLC patients.

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Table 1 Clinicopathological characteristics of 82 NSCLC patients

Characteristics	Number (%)
Age (years)	
<65	46 (56.1)
≥65	36 (43.9)
Gender	
Female	16 (19.5)
Male	66 (80.5)
Smoking	
Yes	55 (67.1)
No	27 (32.9)
Drinking	
Yes	27 (32.9)
No	55 (67.1)
ECOG PS	
0	19 (23.2)
1	62 (75.6)
2	1 (1.2)
Pathology	
Adenocarcinoma	36 (43.9)
Squamous	38 (46.3)
Other	8 (9.8)
Stage	
IIIB–IIIC	11 (13.4)
IV	71 (86.6)
Chemotherapy	
Yes	82 (100.0)
No	0 (0.0)
Radiotherapy	
Yes	42 (51.2)
No	40 (48.8)
Brain metastases	
Yes	19 (23.2)
No	63 (76.8)
Therapeutic regimen for ICI treatment	
Monotherapy	27 (32.9)
Combined therapy	55 (67.1)
First-line therapy	
Yes	28 (34.1)
No	54 (65.9)
EGFR status	
Wildtype	67 (81.7)
Mutation	15 (18.3)

NSCLC, non-small cell lung cancer; PS, performance status; ICI, immune checkpoint inhibitor; EGFR, epidermal growth factor receptor.

Finally, a nomogram was established according to the forward stepwise logistic regression analyses performed to evaluate prognostic factors for survival probability and survival time of NSCLC patients treated with ICIs.

Results

Characteristics of the patients

The clinicopathological features of 82 NSCLC patients are described in *Table 1*. Briefly, 16 (19.5%) female patients and 66 (80.5%) male patients were enrolled with a median age of 61.5 (range, 35–78) years. Among these NSCLC patients, 55 (67.1%) patients and 27 (32.9%) patients had a history of tobacco smoking and alcohol drinking, respectively. Moreover, the ECOG PS of 62 (75.6%) patients was 1. A total of 36 (43.9%) patients were diagnosed with adenocarcinoma while 38 (46.3%) patients were diagnosed with squamous cell carcinoma. Furthermore, 11 (13.4%) patients were diagnosed with stage IIIB–IIIC disease, and 71 (86.6%) with stage IV disease. All patients had received chemotherapy, while 42 (51.2%) patients received radiotherapy before ICI treatment. Additionally, 19 (23.2%) patients had developed brain metastases before ICI treatment. NSCLC patients. Only 28 (34.1%) patients received first-line therapy before ICI treatment. For ICI treatment, 27 (32.9%) patients received monotherapy and 55 (67.1%) patients were treated with combined immunotherapy and chemotherapy.

For epidermal growth factor receptor (EGFR) status, 67 (81.7%) patients had wildtype EGFR while 15 (18.3%) patients had mutated EGFR. As of the last follow-up period on April 5, 2021, 41 (50.0%) patients had died.

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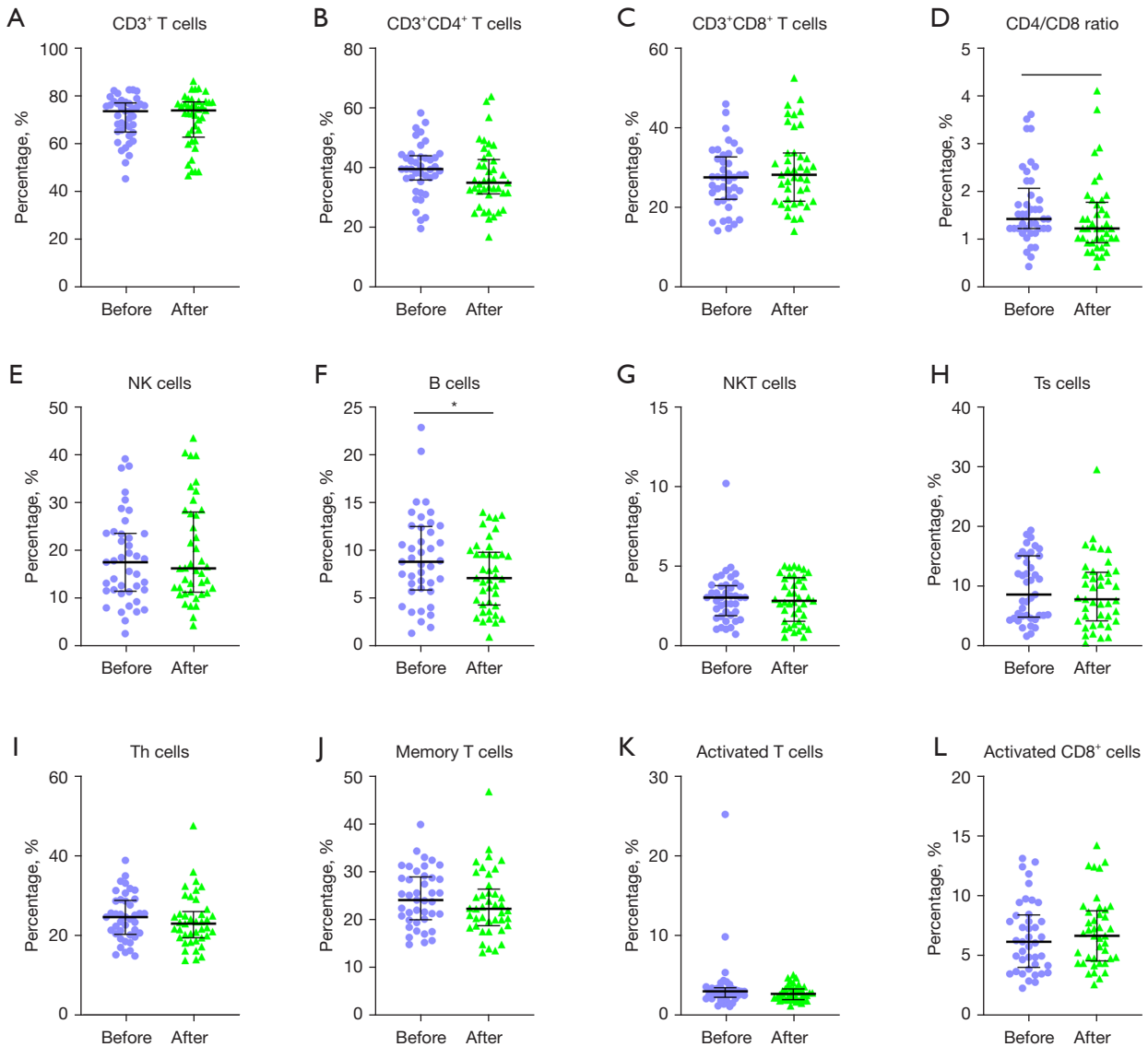

Figure 1 Proportions of peripheral blood lymphocyte subsets. Percentages of peripheral blood lymphocyte subsets before or after ICI treatment were analyzed by flow cytometry, including $CD3^+$ T cells (A), $CD3^+CD4^+$ T cells (B), $CD3^+CD8^+$ T cells (C), CD4/CD8 ratio (D), NK cells ($CD3^-CD56^+$) (E), B cells ($CD19^+$) (F), NKT cells ($CD3^-CD56^+$) (G), Ts cells ($CD4^+CD45RA^+$) (H), Th cells ($CD4^+CD45RA^+$) (I), memory T cells ($CD4^+CD45RO^+$) (J), activated T cells ($CD45RA^+CD45RO^+$), (K) and activated $CD8^+$ cells ($CD8^+CD38^+$) (L). *, P<0.05. ICI, immune checkpoint inhibitor; NK, natural killer; NKT, natural killer T; Th, helper T.

Efficacy of immunotherapy in NSCLC patients

The analysis showed that confirmed objective response occurred in 27 patients NSCLC patients with immunotherapy [partial response (PR), n=27; stable disease (SD), n=41; progressive disease (PD), n=14], and the objective response rate (ORR) was 32.93%. Besides, the disease control rate (DCR) was 82.93% in NSCLC patients treated with immunotherapy.

Changes of peripheral blood lymphocyte subsets in NSCLC patients after ICI treatment

The proportion of peripheral blood lymphocyte subsets is shown in *Figure 1* and *Table 2*. Results indicated that the CD4/CD8 ratio (P=0.036) and the percentage of B cells

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Table 2 The proportion of peripheral blood lymphocyte subsets

Lymphocyte subset	Percentage of NSCLC patients (median)		P value
	Before	After	
$CD3^+$ T cells	71.6	74.0	0.860
$CD3^+CD4^+$ T cells	38.0	35.7	0.122
$CD3^+CD8^+$ T cells	26.5	27.4	0.194
CD4/CD8 ratio	1.3	1.2	0.036
NK cells	17.9	16.7	0.196
B cells	8.3	7.0	0.024
NKT cells	3	2.6	0.807
Ts cells	7.9	7.7	0.143
Th cells	24.3	22.2	0.416
Memory T cells	24.5	23.5	0.275
Activated T cells	3.5	2.8	0.205
Activated CD8 cells	6.2	6.8	0.503

NSCLC, non-small cell lung cancer; NK, natural killer; NKT, natural killer T; Th, helper T.

(P=0.024) was decreased after ICI treatment (*Figure 1* and *Table 2*). The median of CD4/CD8 ratio and B cells decreased within the normal range after ICI treatment (*Figure 1* and *Table 2*). These results suggested that ICI treatment led to changes in the composition of peripheral blood lymphocyte subsets.

Correlation between peripheral blood lymphocyte subset percentage and clinicopathological features before ICI treatment

Correlation analysis demonstrated that the percentage of $CD3^+$ T cells in NSCLC patients was negatively correlated with age (r=–0.36) and positively correlated with brain metastases (r=0.28) before ICI treatment (*Figure 2*). The proportion of $CD3^+CD4^+$ T cells in NSCLC patients was negatively correlated with EGFR status (r=–0.22) before ICI treatment (*Figure 2*). Furthermore, the CD4/CD8 ratio in NSCLC patients was positively correlated to pathology (r=0.24) before ICI treatment (*Figure 2*). The percentage of NK cells in NSCLC patients was positively correlated to age (r=0.48) but negatively correlated to brain metastases (r=–0.24) before ICI treatment (*Figure 2*). Meanwhile, the proportion of B cells in NSCLC patients was negatively correlated to age (r=–0.3), gender (r=–0.27), smoking (r=–0.27), and drinking (r=–0.28) but positively correlated with the therapeutic regimen (r=0.23) before ICI treatment (*Figure 2*). More importantly, the ratio of NKT cells in NSCLC patients was positively correlated to brain metastasis (r=0.24) and radiotherapy (r=0.34). The percentage of activated T cells and activated $CD8^+$ cells was positively correlated to drinking (r=0.22) and age (r=–0.44) before ICI treatment, respectively (*Figure 2*).

The above results suggested that the percentage of $CD3^+$ T cells, NK cells, and NKT cells in NSCLC patients before ICI treatment might be potential predictors for brain metastases, while the proportion of $CD3^+CD4^+$ T cells in NSCLC patients before ICI treatment might be a potential indicator for EGFR status. Moreover, the CD4/CD8 ratio in NSCLC patients before ICI treatment might be correlated with pathology. Furthermore, the ratio of B cells in NSCLC patients before ICI treatment might be affected by the therapy used before ICI treatment, while the percentage of NKT cells in NSCLC patients before ICI treatment may corelate with previous use of radiotherapy.

Correlation between the change of peripheral blood lymphocyte subset percentage and clinicopathological features after ICI treatment

The change of peripheral blood lymphocyte subset percentage after ICI treatment might be affected by clinicopathological features. Results indicated that changes in the percentage of $CD3^+CD8^+$ T cells, B cells, and activated $CD8^+$ cells were related to age (*Figure 3* and *Table 3*). Compared with NSCLC patients over the age of 65 years, the percentage of $CD3^+CD8^+$ T cells and activated $CD8^+$ cells increased, while the ratio of B cells decrease in NSCLC patients under the age of 65 years after ICI treatment (*Figure 3* and *Table 3*). Moreover, the change in the percentage of NKT cells was related to radiotherapy (*Figure 3* and *Table 3*). Compared with NSCLC patients treated without radiotherapy, the percentage of NKT cells decreased in NSCLC patients receiving radiotherapy after ICI treatment (*Figure 3* and *Table 3*). Also, the change in the percentage of $CD3^+CD4^+$ T cells was related to first-line therapy (*Figure 3* and *Table 3*). Compared with NSCLC patients treated without first-line therapy, NSCLC patients receiving first-line therapy after ICI treatment had an increased percentage of $CD3^+CD4^+$ T cells (*Figure 3* and *Table 3*). These results suggested that age, radiotherapy, and first-line therapy might affect the change of peripheral

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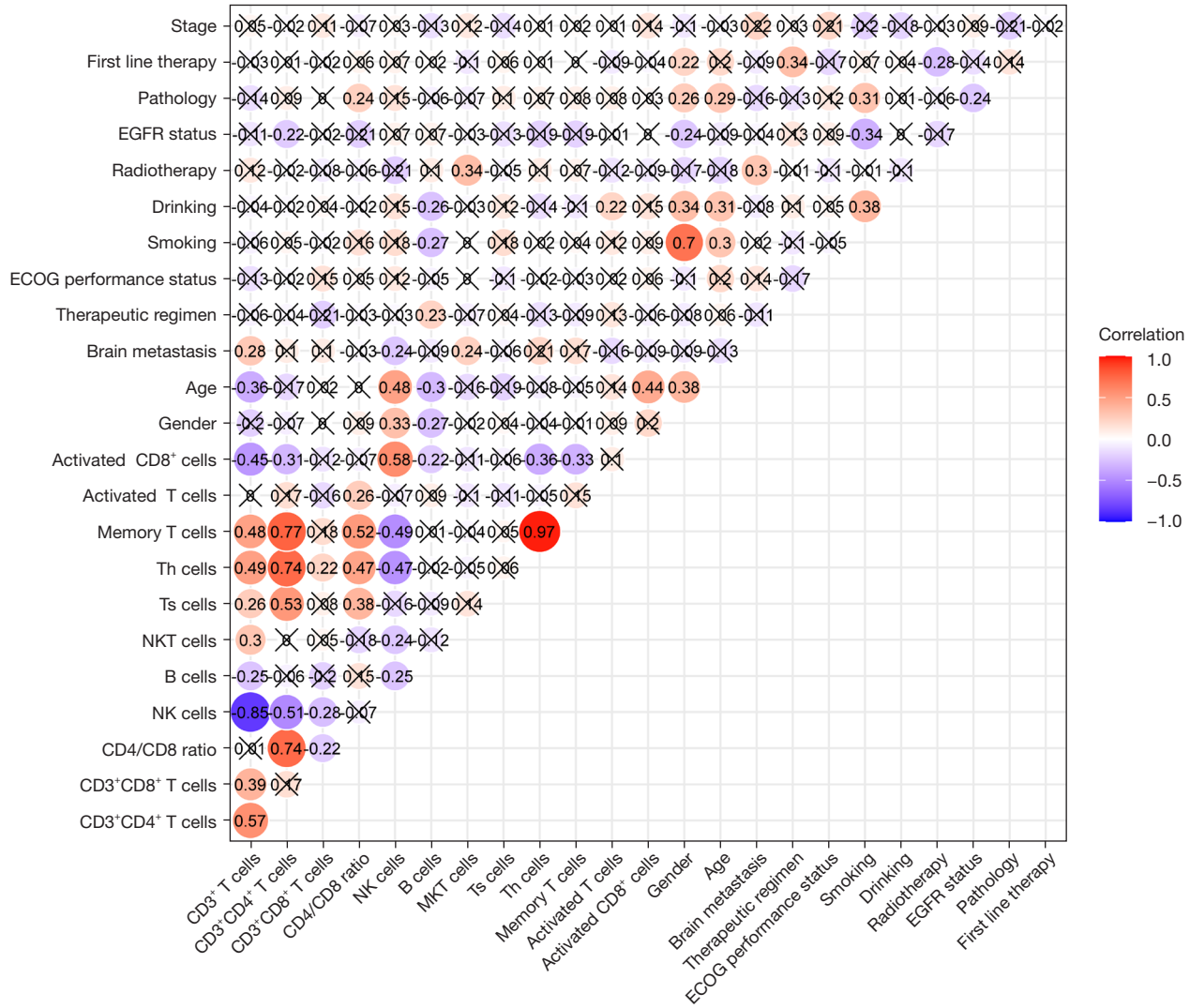

Figure 2 Correlation between peripheral blood lymphocyte subset percentage and clinicopathological features before ICI treatment. The correlation heat map between peripheral blood lymphocyte subset percentage and clinicopathological features before ICI treatment. ICI, immune checkpoint inhibitor; EGFR, epidermal growth factor receptor; NK, natural killer; NKT, natural killer T; Th, helper T.

blood lymphocyte subset percentage after ICI treatment.

Correlation between clinicopathological features or peripheral blood lymphocyte subsets and survival

The median PFS was 8.5 (range, 6.3–10.8) months (*Table 4*). However, all percentages of peripheral blood lymphocyte subsets before ICI treatment were not prognostic factors for PFS in NSCLC patients with ICI treatment (*Table 5*). Analysis found that the median OS in NSCLC patients with immunotherapy was 18.4 (range, 10.8–37.9) months. Univariate analysis indicated that age (P=0.023), physical status score (P=0.006), therapeutic regimen (P=0.004; *Table 6*), and the percentage of B cells (P=0.04) before ICI treatment were independent prognostic factors for OS in NSCLC patients with ICI treatment (*Table 7*). To further determine the prognostic value of peripheral blood lymphocyte subsets in NSCLC patients treated with ICIs, survival analysis was performed. Results revealed that NSCLC patients with a low percentage of B cells had shorter OS than did those with a high percentage of B cells before ICI treatment (*Figure 4*), which was consistent with

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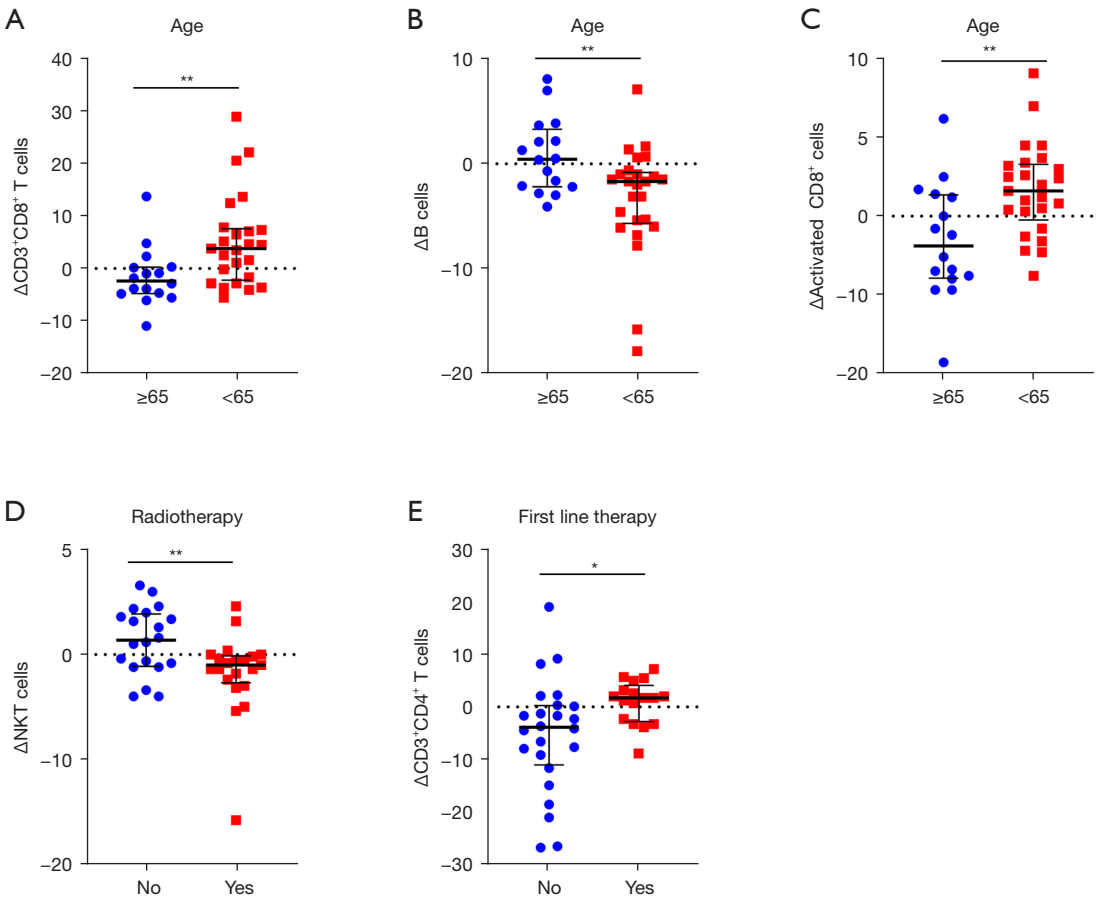

Figure 3 The relationship between changes of peripheral blood lymphocyte subset percentage and clinicopathological features after ICI treatment. (A-C) The correlation between the change in percentage of $CD3^+CD8^+$ T cells (A), B cells (B), activated $CD8^+$ cells (C), and age. (D) The correlation between the change in percentage of NKT cells and radiotherapy. (E) The correlation between the change in percentage of $CD3^+CD4^+$ T cells and first-line therapy. *, P<0.05; **, P<0.01. ICI, immune checkpoint inhibitor; NKT, natural killer T.

the result of univariate Cox regression analyses. Thus, the percentage of B cells before ICI treatment level in tumor tissue were related to long-term survival in NSCLC patients treated with ICIs.

A nomogram model based on peripheral blood lymphocyte subsets

A nomogram model was developed to assess the survival probability and the expected survival time of NSCLC patients treated with ICIs based on age, percentage of B cells, and therapeutic regimen (*Figure 5*). As shown in the nomogram, the prognosis of NSCLC patients with a high percentage of B cells was better after immunotherapy (*Figure 5*). These results were consistent with those of survival analysis, suggesting that the nomogram comprising the percentages of peripheral blood lymphocyte subsets could help to assess the survival probability and the expected survival time of NSCLC patients treated with ICIs.

Discussion

In this study, 82 NSCLC patients who underwent ICI treatment were recruited. Results indicated that the CD4/CD8 ratio and the percentage of B cells were decreased after ICI treatment. In addition, the percentage of $CD3^+$ T cells, NK cells, and NKT cells before ICI treatment was associated with brain metastases; the proportion of $CD3^+CD4^+$ T cells before ICI treatment was related to EGFR status; the CD4/CD8 ratio before ICI treatment was correlated to pathology; the ratio of B cells before

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Table 3 Correlation between the change of peripheral blood lymphocyte subset percentage and clinicopathological features after ICI treatment

Variables	Age			Radiotherapy			First-line therapy		
	z (after-before)		P	z (after-before)		P	z (after-before)		P
	≥65	<65		No	Yes		No	Yes	
ΔCD3$^+$ T cells	−2.20	1.00	0.139	−1.55	1.00	0.179	−1.00	−0.80	0.173
ΔCD3$^+$CD4$^+$ T cells	1.85	−1.70	0.358	−3.30	0.70	0.058	−3.90	1.70	0.014
ΔCD3$^+$CD8$^+$ T cells	−2.35	3.80	0.004	−0.50	1.60	0.845	2.05	0.20	0.171
ΔCD4/CD8 ratio	0.14	−0.26	0.126	−0.17	−0.20	0.732	−0.25	0.06	0.088
ΔNK cells	2.40	0.80	0.756	1.90	0.80	0.366	1.80	0.00	0.297
ΔB cells	0.45	−1.70	0.005	−1.95	−1.20	0.715	−0.80	−2.20	0.057
ΔNKT cells	−0.60	−0.10	0.235	0.70	−0.50	0.010	−0.20	−0.30	0.634
ΔTs cells	0.10	−1.10	0.069	0.05	−1.10	0.824	−0.60	−0.40	0.825
ΔTh cells	−1.00	0.50	0.318	−1.25	0.90	0.282	−1.00	0.70	0.138
ΔMemory T cells	−0.60	0.40	0.268	−1.45	0.80	0.193	−0.85	0.00	0.123
ΔActivated T cells	−0.20	−0.20	0.543	−0.20	−0.20	0.472	−0.40	0.00	0.155
ΔActivated CD8 cells	−1.90	1.60	0.007	0.45	1.00	0.708	1.30	0.40	0.699

ICI, immune checkpoint inhibitor; NK, natural killer; NKT, natural killer T; Th, helper T.

ICI treatment was related to therapeutic regimen; and the percentage of NKT cells before ICI treatment was associated with radiotherapy. Furthermore, univariate analyses and survival analysis revealed that a low percentage of B cells predicted a poor OS for NSCLC patients with ICI treatment.

In tumors, the activity and function of antitumor T cells or B cells are impaired by the association of PD-L1 on the surface of cancer cells with the PD-1 on T cells and B cells (19,20,27). This study indicated that the proportion of peripheral blood lymphocyte subsets were changed after ICI treatment. For example, the CD4/CD8 ratio was decreased after ICI treatment, suggesting that the level of antitumor CD8$^+$ T effect cells was elevated after ICI treatment. These results suggest that ICI treatment might attenuate the impairment of the immune system in NSCLC patients through blocking PD-L1. Similarly, some studies have demonstrated the effects of ICIs on lymphocyte subsets. For example, ICIs restore the function of tissue-resident memory T cells in tumor immune surveillance (28). Besides, PD-1-PD-L1 blockade suppresses the escape of solid cancer cells from B-cell-mediated cytotoxicity (29). Yet, the percentage of B cells was also reduced after ICI treatment in the present study. B cells are usually recognized as the major effector cells in killing cancer cells (30,31). However, some studies have revealed that B cells also play a protumorigenic role. For instance, B cells recruited by hypoxia inducible factor-1 alpha (HIF1α)-suppressed CXCL13 promotes the development of pancreatic neoplasia (32,33). Thus, B cells in NSCLC might exert protumorigenic effects. Furthermore, less activated B cells are required after ICI treatment as the efficiency of B cells is improved.

To date, no studies have revealed the relationship between circulating CD3$^+$ T cells, NK cells, or NKT cells and brain metastases; circulating CD3$^+$CD4$^+$ T cells and EGFR status; CD4/CD8 ratio in peripheral blood and pathology; peripheral blood B cells and therapeutic regimen; or peripheral blood NKT cells and radiotherapy. Thus, this study might provide novel indicators for brain metastases, pathology, therapeutic regimen, and radiotherapy in NSCLC.

In addition, the present study found that the percentage of B cells was negatively correlated with activated CD8$^+$ cells, which exert the immune killing effect on tumor cells (34). The percentage of B cells was also negatively correlated to age. A growing body of evidence indicates that immunity decreases with age (35). Therefore, the above studies suggest that B cells may suppress activated CD8$^+$ cells to kill tumor cells in NSCLC patients with ICI treatment but may decrease with age. This result suggests

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Table 4 Univariate Cox regression analysis of PFS for clinical characteristics

Characteristics	P value	HR	95% CI
Age (years)			
<65	–	–	–
≥65	0.318	1.29	0.78–2.12
Gender			
Female	–	–	–
Male	0.886	0.95	0.51–1.79
Smoking			
No	–	–	–
Yes	0.737	1.09	0.65–1.85
Drinking			
No	–	–	–
Yes	0.20	0.71	0.42–1.20
ECOG PS			
0	–	–	–
1	0.18	1.52	0.82–2.82
2	0.254	3.31	0.42–25.98
Pathology			
Adenocarcinoma	–	–	–
Squamous	0.457	1.22	0.73–2.04
Other	0.593	0.75	0.26–2.15
Stage			
IIIB–IIIC	–	–	–
IV	0.23	0.65	0.32–1.32
Radiotherapy			
No	–	–	–
Yes	0.897	0.97	0.59–1.59
Brain metastases			
No	–	–	–
Yes	0.479	0.82	0.47–1.43
Therapeutic regimen for ICI treatment			
Monotherapy	–	–	–
Combined therapy	0.256	0.74	0.44–1.24
First-line therapy			
No	–	–	–
Yes	0.247	1.37	0.80–2.34
EGFR status			
Wildtype	–	–	–
Mutation	0.741	1.11	0.59–2.09

PFS, progression-free survival; PS, performance status; ICI, immune checkpoint inhibitor; EGFR, epidermal growth factor receptor; HR, hazard ratio; CI, confidence interval.

Table 5 Univariate Cox regression analysis of PFS for peripheral blood lymphocyte subsets

Lymphocyte subset	P value	HR	95% CI
$CD3^+$ T cells			
High	–	–	–
Low	0.50	0.86	0.52–1.41
$CD3^+CD4^+$ T cells			
High	–	–	–
Low	0.20	1.35	0.82–2.22
$CD3^+CD8^+$ T cells			
High	–	–	–
Low	0.80	0.93	0.57–1.53
CD4/CD8 ratio			
High	–	–	–
Low	0.50	1.18	0.72–1.94
NK cells			
High	–	–	–
Low	0.80	0.93	0.56–1.52
B cells			
High	–	–	–
Low	0.90	0.98	0.60–1.61
NKT cells			
High	–	–	–
Low	0.40	0.81	0.49–1.34
Ts cells			
High	–	–	–
Low	0.50	0.86	0.52–1.42
Th cells			
High	–	–	–
Low	0.60	1.15	0.70–1.89
Memory T cells			
High	–	–	–
Low	0.80	1.08	0.66–1.78
Activated T cells			
High	–	–	–
Low	0.50	1.20	0.73–1.98
Activated CD8 cells			
High	–	–	–
Low	0.60	0.87	0.53–1.42

The median percentage of positive cells was used as a cutoff to define low and high level. PFS, progression-free survival; NK, natural killer; NKT, natural killer T; Th, helper T; HR, hazard ratio; CI, confidence interval.

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Table 6 Univariate Cox regression analysis of OS for clinical characteristics

Characteristics	P value	HR	95% CI
Age (years)			
<65	–	–	–
≥65	0.023	0.43	0.21–0.89
Gender			
Female	–	–	–
Male	0.695	1.21	0.46–3.16
Smoking			
No	–	–	–
Yes	0.476	1.34	0.60–3.00
Drinking			
No	–	–	–
Yes	0.254	1.52	0.74–3.10
ECOG PS			
0	–	–	–
1	0.132	2.24	0.78–6.44
2	0.006	25.28	2.51–254.64
Pathology			
Adenocarcinoma	–	–	–
Squamous	0.288	1.52	0.70–3.28
Other	0.176	2.21	0.70–6.98
Stage			
IIIB–IIIC	–	–	–
IV	0.319	0.64	0.26–1.55
Radiotherapy			
No	–	–	–
Yes	0.268	0.67	0.33–1.36
Brain metastases			
No	–	–	–
Yes	0.455	0.71	0.29–1.74
Therapeutic regimen for ICI treatment			
Monotherapy	–	–	–
Combined therapy	0.004	0.35	0.17–0.71
First-line therapy			
No	–	–	–
Yes	0.269	0.63	0.28–1.43
EGFR status			
Wildtype	–	–	–
Mutation	0.392	0.63	0.22–1.81

OS, overall survival; PS, performance status; ICI, immune checkpoint inhibitor; EGFR, epidermal growth factor receptor; HR, hazard ratio; CI, confidence interval.

Table 7 Univariate Cox regression analysis of OS for peripheral blood lymphocyte subsets

Lymphocyte subset	P value	HR	95% CI
$CD3^+$ T cells			
High	–	–	–
Low	0.86	0.94	0.46–1.91
$CD3^+CD4^+$ T cells			
High	–	–	–
Low	0.55	0.8	0.39–1.63
$CD3^+CD8^+$ T cells			
High	–	–	–
Low	0.57	1.23	0.61–2.50
CD4/CD8 ratio			
High	–	–	–
Low	0.82	0.92	0.45–1.87
NK cells			
High	–	–	–
Low	0.09	0.53	0.25–1.11
B cells			
High	–	–	–
Low	0.04	2.15	1.03–4.52
NKT cells			
High	–	–	–
Low	0.99	1.01	0.50–2.04
Ts cells			
High	–	–	–
Low	0.86	0.94	0.46–1.91
Th cells			
High	–	–	–
Low	0.80	0.91	0.45–1.85
Memory T cells			
High	–	–	–
Low	0.48	0.78	0.38–1.58
Activated T cells			
High	–	–	–
Low	0.37	1.38	0.68–2.80
Activated CD8 cells			
High	–	–	–
Low	0.22	0.64	0.31–1.31

The median percentage of positive cells was used as a cutoff to define low and high level. OS, overall survival; NK, natural killer; NKT, natural killer T; Th, helper T; HR, hazard ratio; CI, confidence interval.

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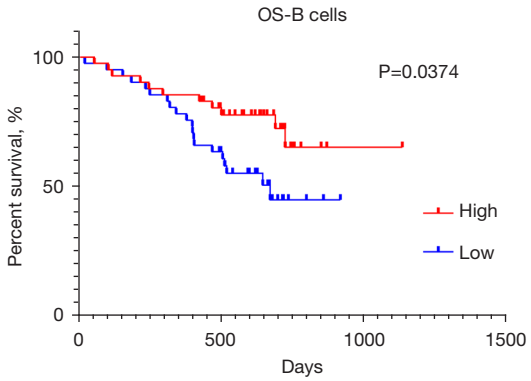

Figure 4 The Kaplan-Meier curve of OS for NSCLC patients with high or low percentage of B cells. OS, overall survival; NSCLC, non-small cell lung cancer.

that B cells may play a protumorigenic role in younger NSCLC patients. By contrast, the percentage of NK cells was positively correlated to activated $CD8^+$ cells and age. Thus, NK cells may collaborate with activated $CD8^+$ cells to kill tumor cells in NSCLC patients with ICI treatment and may increase with age, and the percentage of NK cells might be a potential indicator for the killing efficiency of ICI treatment in older patients.

Numerous studies have indicated the prognostic value of peripheral blood lymphocyte subsets in cancer patients (36-38). However, the prognostic roles of peripheral blood lymphocyte subsets in NSCLC patients treated with ICIs is unclear. This study demonstrated that the percentage of B cells before ICI treatment was an independent prognostic

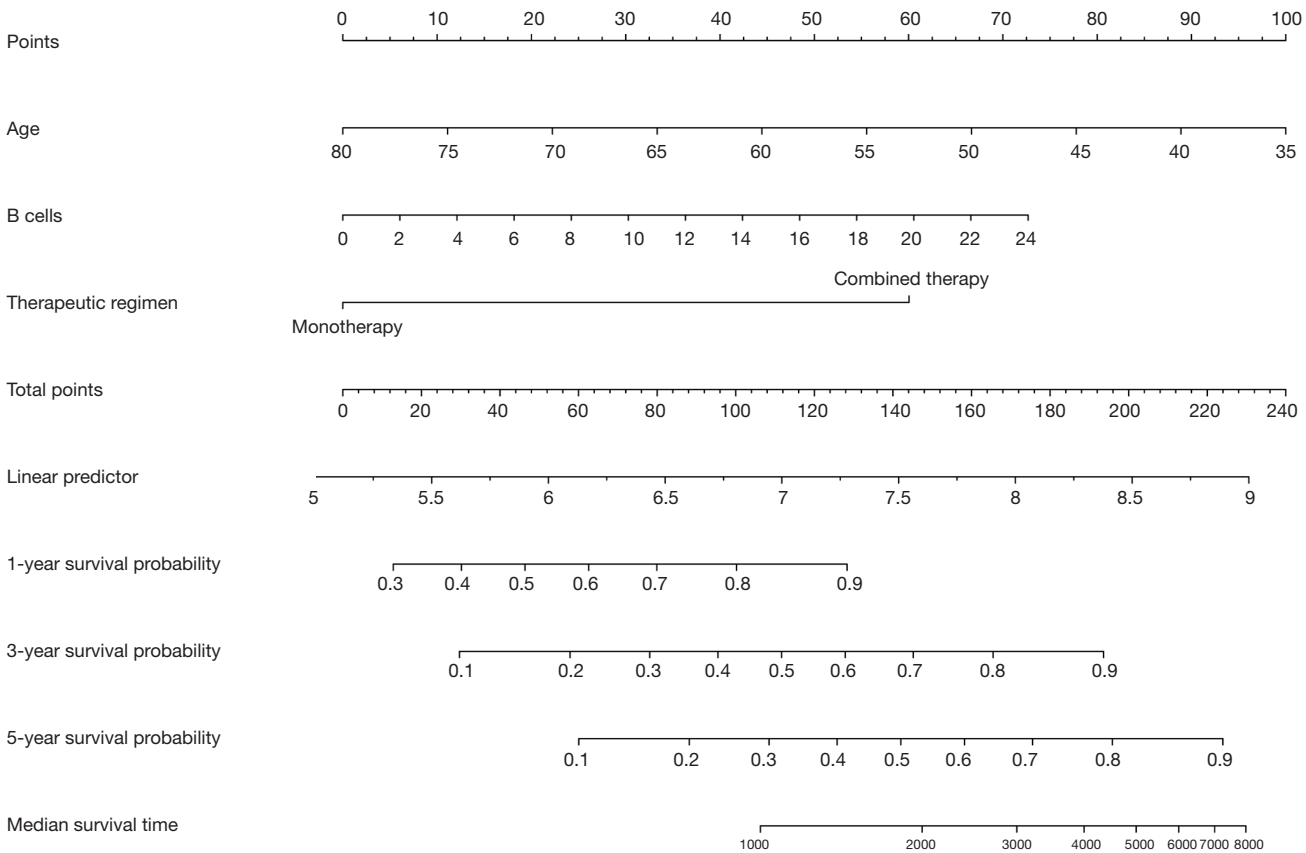

Figure 5 A nomogram for the prediction of survival probability and survival time of NSCLC patients treated with ICIs. NSCLC, non-small cell lung cancer; ICI, immune checkpoint inhibitor.

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factor for OS in NSCLC patients with ICI treatment. Despite this, all percentages of peripheral blood lymphocyte subsets before ICI treatment were not prognostic factors for PFS in NSCLC patients with ICI treatment. A recent study has found that the percentage of NK cells and the CD4/CD8 ratio in peripheral blood before ICI treatment was associated with PFS in lung cancer patients treated with ICIs (39). Therefore, assessing the correlation between peripheral blood lymphocyte subsets and PFS in NSCLC patients treated with ICIs is needed, which requires the recruiting of more NSCLC patients in future study.

Although this study demonstrated the predictive and prognostic value of peripheral blood lymphocyte subsets in NSCLC patients with ICI treatment, it still had a few limitations. First, the follow-up period (median 18.4 months) of this study was relatively short. Second, the correlation between percentages of peripheral blood lymphocyte subsets after ICI treatment and clinicopathological features, PFS or OS, was not identified. Third, the association between peripheral blood lymphocyte subsets and PFS in NSCLC patients' needs to be clarified through an analysis of a greater sample of NSCLC patients in future studies.

Conclusions

ICI treatment induces changes in the percentage of peripheral blood lymphocyte subsets, and this may have prognostic value for brain metastases, radiotherapy, EGFR status, pathology, and therapeutic regimen for NSCLC patients treated with ICIs. However, due to the limitations of our study, further research is needed to verify these findings and explore their implications. In addition, a further study to enlarge the sample size and have more robust results for the validation of the nomogram model will be performed.

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Footnote

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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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All experimental procedures involving human participants were approved by the Ethics Committee of Zhejiang Cancer Hospital. Written informed consent was obtained from all participants enrolled in this study.

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