Research Article

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The GLB, ANC, Bone Metastasis And PD-L1 Are Potential Predictive Markers For The Prognosis Of Advanced Or Metastatic NSCLC Treated With Immune Checkpoint Inhibitors

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Author contributions
Material preparation and data collection were performed by MZ,MH,YL,GW. Data analysis was performed by MZ,XL,YZ. The first draft of the manuscript was written by MZ and TZ, and all authors commented and edited previous version of the manuscript. All authors read and approved the manuscript. The authors have no other conflicts of interest or subject matter of materials discussed in this manuscript.

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1. Abstract

Immune checkpoint inhibitors (ICIs) hold a great promise in treatment of non-small cell lung cancer (NSCLC), while only a portion of patients benefited from the treatment, and others could not achieve optimal therapeutic effects from initial immunotherapy, even for those patients with PD-L1 tested positive. However, the clinical markers for the selection of patients who will benefit from ICIs treatment before hand are largely unknown. In this study, a retrospective analysis was conducted on baseline information of 144 patients with advanced/metastatic NSCLC who received ICIs treatment from the November of 2018 to the January of 2023 in Beijing Chest Hospital. We set up the nomogram model to make quantitative prediction for overall survival (OS) and progression-free survival (PFS) based on different variables, and established a scoring group chart as well as Decision curve analysis (DCA) to assess clinical benefits of ICIs in treatment of patients with advanced/metastatic NSCLC. We found that serum globulin (GLB)>26.6 (g/L) (HR=1.865, P=0.002), absolute neutrophil counts (ANC) (109/L)>5 (HR=2.146, P<0.001), and bone metastasis (HR=2.148, P<0.001) were independent factors affecting the PFS of NSCLC patients. GLB>26.6 (g/L) (HR=1.741, P=0.018), ANC(109/L)>5 (HR=1.807, P=0.008), bone metastasis (HR=1.651, P=0.002), and PD-L1 Negative (HR=2.432,P=0.032) were independent factors affecting the survival of NSCLC patients. These data show that patients with the four favorable factors had better survivals. Thus, in patients with advanced/metastatic NSCLC receiving ICIs treatment, the GLB, ANC, bone metastasis, and PD-L1 may serve as useful predictive markers for the prognosis of NSCLC patients with ICIs treatment.

Keywords:
GLB •ANC •Bone metastasis •ICIs • Advanced NSCLC • Prognosis

2. Introduction

Lung cancer takes the leading position in human cancers in terms of incidence and mortality worldwide [1, 2]. Non-small cell lung cancer (NSCLC) accounts for 80~85% of all lung cancer cases in the Word [3]. Theapplication of immune checkpoint inhibitors (ICIs) to immunotherapy in lung cancerled to striking improvement in progression-free survival (PFS) and overall survival (OS) of NSCLC patients[4-6] . Despite the increasing role of immunotherapy in lung cancer, a minority of patientsstill could not benefit from these therapies. Furthermore, the occurrence of immunotherapy-related toxic effects in some patients diminishes the

quality of life [7-10]. Thus, it becomes imperative todetermine clinical biomarkers, by which the patients who will benefit from ICIs could be hopefully differentiated from those who will not, to avoid unnecessary treatment. PD-L1 was the first FDA-approved predictive biomarker for (NSCLC) in 2015, and series of studies have also obtained this outcome [11, 12]. Moreover, tumor mutational burden (TMB) and microsatellite instability-high (MSI-H) can also serve as effective predictive indicators of prognosis in these patients, andother indicators such as circulating tumor DNA (ctDNA) and circulating tumor cells (CTC) have shown promise in prognostic prediction [13, 14] . However, there are some limitations and disadvantage of using these markers, for example, examination of these markers is expensive, and inconvenient to perform. An easily predictive model is urgently required in clinical practice to accurately guide clinical treatment for NSCLC. Currently, the predictive model of ICIs for NSCLC treatment has much room for improvement. Some investigators suggested the utilization of the Lung Immune Prognostic Index (LIPI), a composite of lactate dehydrogenase (LDH) and neutrophil-associated ratio (dNLR), to predict prognostic outcome[15, 16]. T cell-associated immunoglobulins may be related to the phenomenon of antibody neutralization, where immunoglobulins may bind to immune checkpoint inhibitors (ICIs) or other immunotherapeutic drugs, thus inhibiting their activity and reducing treatment effectiveness [17]. However, the predictive model of ICIs combined therapy was not so effective as expected, and the clinical value of the predictive model that integrates the clinical characteristics and laboratory indicators of patients before treatment deserves further validation. To improve the prognostic survival of patients treated with ICIs and select suitable groups of patients by predictive biomarkers for ICIs treatment, we conducted this study to develop a non-invasive and cost-effective multivariable prognostic model. We speculated that a model that integrated clinicopathological characteristics and laboratory indicators could simultaneously predict the survivals of NSCLC patients that received ICIs combined therapy.

3. Materials and methods

3.1. Study Design and Patients

Total 144 patients with advanced NSCLC (III/IV) who received immune checkpoint inhibitor therapy between November 2018 and January 2023 were retrospectively analyzed. Data were collected from the records of hospitalized. This study was approvedby the Beijing Chest Hospital Medical Ethics Committee (YJS-2023-09), according to the local regulations and the Helsinki declaration. A waiver for patients'notice consent was obtained by the Beijing Chest Hospital Medical Ethics Committee due to the retrospective nature of the study.

3.2. Inclusion and Exclusion Criteria are as follows:

Adult patients aged 18-80 years and diagnosed as advanced NSCLC (IIIB/IIIC/IV) by pathology were included in this study if theydid not receive surgical operation and radical synchronous or sequential radiotherapy and were treated with immune checkpoint inhibitors at least two cycles with complete medical records and follow-up records . Of note, those patients who were diagnosed with other tumors and infectious diseases, treated with steroid hormone for 1 month before treatment, or diagnosed with hematological or autoimmune diseases, were excluded from this study.

3.3. Treatment Regimen

All ICIs were approved by National Medical Products Administration (NMPA) in China. The dosage and frequency of immune checkpoint inhibitors used in clinical trials are recommended according to the manufacturer's instructions.

3.4. Primary Endpoint and Follow-ups

The time of the first ICIs treatment was recorded as the starting point of observation, and each patient was followed up with interviews by telephone, outpatient review, and other forms of visit. Follow-ups were performed until disease progression or decease, and the follow-up study ended on December 25, 2023. Overall survival is the time from the start of immunotherapy to decease or the last follow-up visit. Progression-free survival (PFS) was defined as the time from the start of immunotherapy to clinical or imaging progression.

3.5. Observation Indicators

The clinical indicators collected by electronic medical record system included: Age, gender, smoking history, clinical stage, ECOG PS score, histology, therapeutic regimen, PD-L1 expression, and metastasis for tumor progression. The baselines were defined as the measurements taken within 1 week prior to the treatment with ICIs. The baseline data of peripheral blood included WBC (white blood cell count), ANC(Absolute neutrophil count), LY (Lymphocyte count), NLR (Neutrophil to lymphocyte ratio), PLR (Platelet to lymphocyte ratio), CRP (C-Reactive Protein), SII (Systemic Immune Inflammation Index), MLR (Monocyte to lymphocyte ratio), LDH (lactic dehydrogenase)), GLB (serum globulin), A/G (serum albumin to globulin ratio). A flow chart of sample screening and model constructionwas shown in Figure 1.

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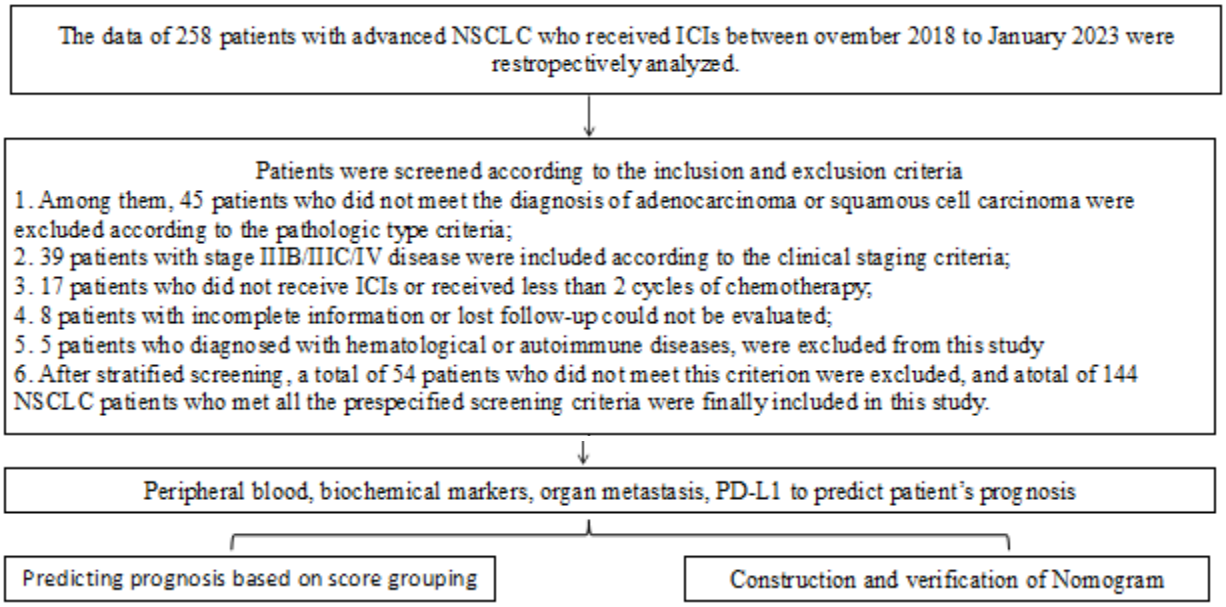
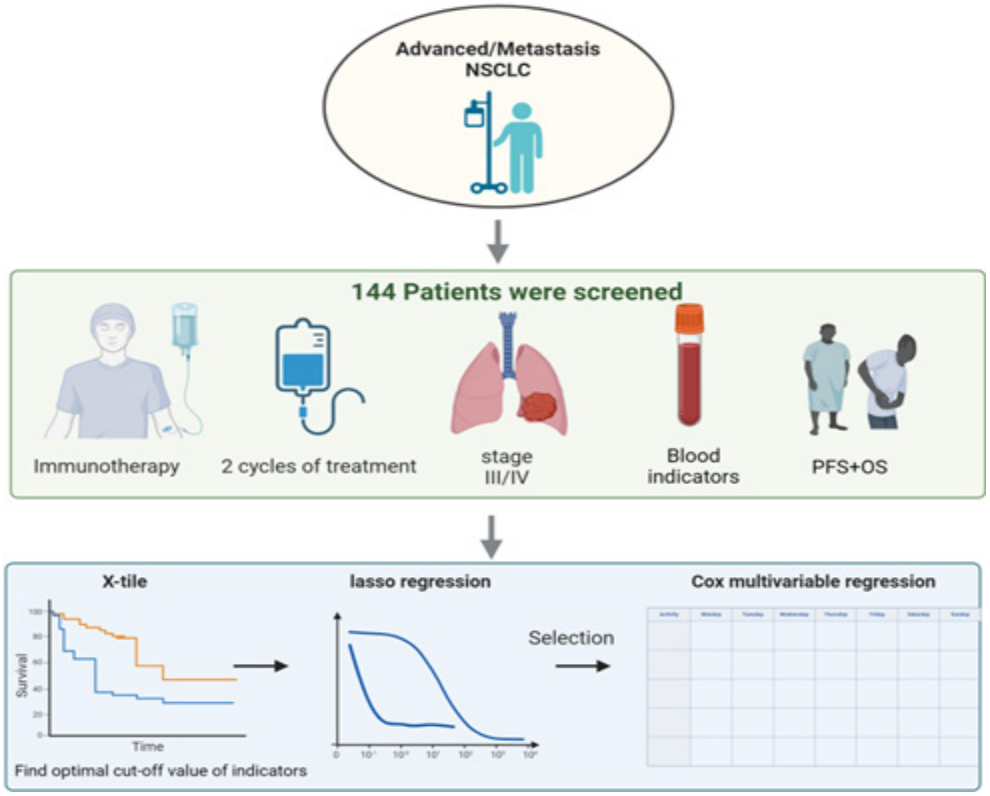


Fig.1: Flow chart of sample and predicted indexes screening

3.6. Statistical Analysis

Data processing was conducted using SPSS 27.0 software. For descriptive analysis, the normal and non-normal distributions of continuous variables were expressed by mean ± standard deviation (SD) and median [interquartile range (IQR)], respectively, while categorical variables were presented as percentages. X-tile softwarewas utilized to determine the optimal cut-off value and divides continuous data into categorical variables. Lasso regression was used to screen for the risk factors. Prediction models including parameters screened by Lasso regression were established based on Cox regression. The predictive abilities of models were compared by C-index. Graphpad prism10.0 for Kaplan Meier method was used to calculate PFS and OS curves, and log-rank test to evaluate differences. The survival, rms, glmnet packages, etc. from Rstudio (version 4.3.2) were used to draw clinical decision curves, column charts for prediction of survivals, and Lasso regression plots. A P value < 0.05 was considered statistically significant. Achart of model construction was shown in Figure 2.



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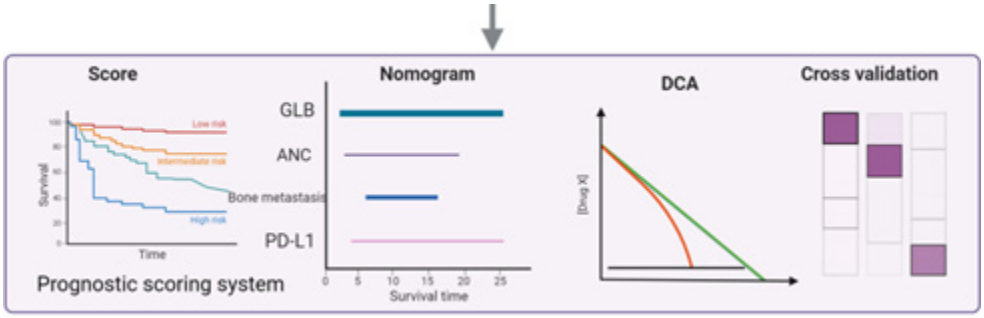

Fig.2: Flow-Chart of model construction

4. Results

This study included a total of 144 patients (109 males, and 35 females) with advanced/metastastic NSCLC (Table 1). Among these patients, 47 were ≤ 60 years old, and 97 > 60 years old. According to clinical stages, 38 patients in stage III and 106 in stage IV. There were 82 cases of adenocarcinoma and 62 cases of squamous cell carcinoma. 100 patients had a smoking history. All patients were treated with PD-1 or PD-L1 immune checkpoint inhibitors. Most people have an ECOG score of 0-1 (137/144, 95.1%). Patients with the distant metastases suffered from bone metastases in most cases (38/144,26.3%).

Table 1: Basics of 144 patients with advanced NSCLC

Variables n=144	Value	Percentage (%)
Gender		
Female	35	24.3
Male	109	75.7
Age		
≤60years	47	32.6
<60years	97	67.3
Smoking status		
Never	44	30.5
Former/current	100	69.4
Clinical stage		
III	38	26.4
IV	106	73.6
ECOG PS		
0-1	137	95.1
2	7	4.9
Histology		
Adenocarcinoma	82	56.9
Squamous	62	43.1
Therapeutic regiemen		
Mono therapy	22	15.2
Combo therapy	122	84.8
PD-L1 TPS%		
Negative (<1%)	53	36.8
Positive (≥1%)	77	53.4
Unknown	14	9.7
Distant metastasis		
Liver metastasis	9	6.2
Brain metastasis	12	8.3
Bone metastasis	38	26.3
Adrenal gland metastases	12	8.3
ICI drug		
Pembrolizumab	19	13.2
Nivolumab	4	2.7
Sintilimab	50	34.7
Toripalimab	19	13.2
Tislelizumab	21	14.6
Camrelizumab	17	11.8
Atezolizumab	1	0.7

Overall, the median PFS and median OS were10.7 and 30.0 months, respectively.Survival curves of immunotherapy for advanced lung cancer were shown in Figure 3a&b.

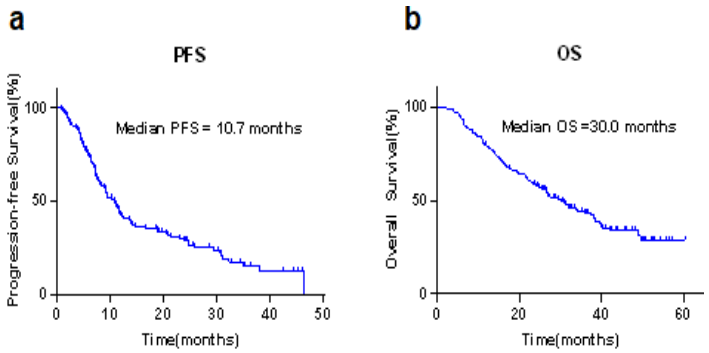

Fig.3: Survival curves of immunotherapy for advanced lung cancer

4.1. Basic clinically pathological characteristics

There were 82 cases of adenocarcinoma and 62 cases of squamous cell carcinoma.As shown in Figure 4a, the median PFS of the two groups of patients were 10.8 and 10.7 months respectively, with the HR=0.878 (95%C1: 0.667-1.156) and P=0.354, suggesting that the difference between the two groups of patents were not significant. As shown in Figure 4b, the median OS were 33.7 and 25.3 months respectively, with the

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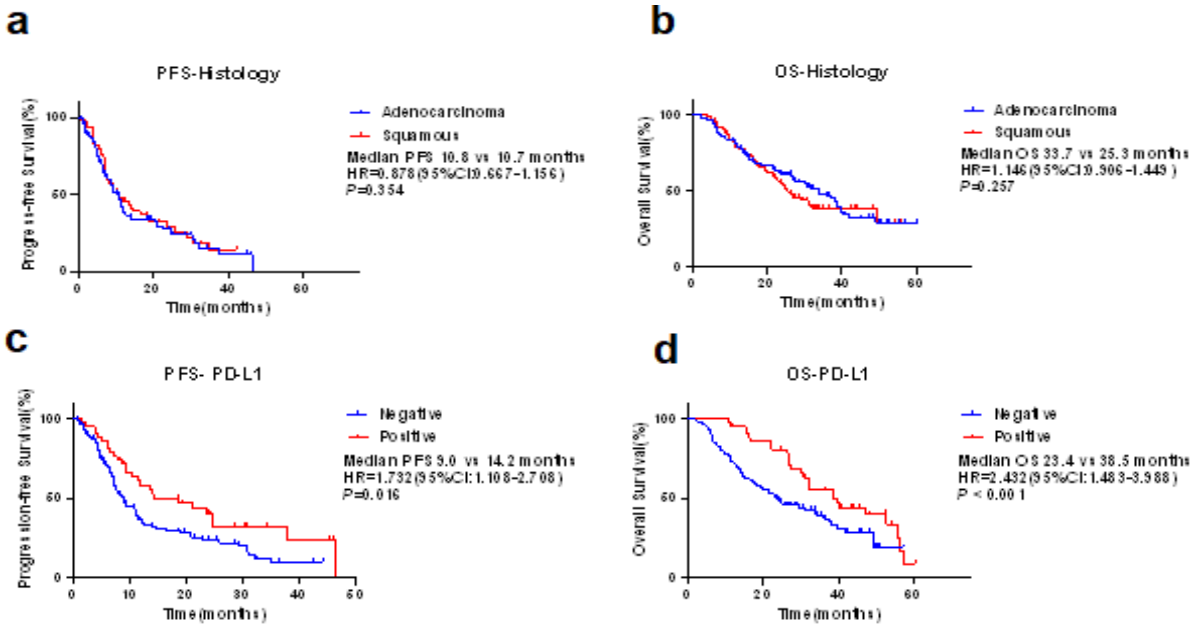
HR=1.146(95%CI:0.906-1.449) and P=0.257, and there was no difference between the two groups of patients. These data indicate the survivals of patients with adenocarcinoma and squamous cell carcinoma is similar. Interestingly, when analyzing102 cases with negative PD-1 expressionand 42 cases with positive PD-1 expression via PFS and OS,we found that the median PFS of the two groups of patients were 9.0 and 14.2 months respectively, with HR=1.732(95%C1:1.108-2.708) and P=0.016 (Figure 4c), suggesting that the PFS of patients with PD1 expression was greater than that of patients without PD1 expression. Consistently, the median OS of the two groups of patients were 23.4 and 38.5 months respectively, with HR=2.432 (95%C1:1.483-3.988) and P<0.001 (Figure 4d), indicating that patients with PD1 expression survived much longer than the ones without PD1 expression.

Fig.4: Survival curves of patients according to histological examination and PD-L1 expression



These data suggest that PD1 expression in patients affects the efficacy of ICIs treatment. (a&b) PFS and OS curves of patients with Adenocarcinoma and Squamous cancers as examined by histology. (c&d) PFS and OS curves of patients without (negative) or with (positive) PD-L1 expression. X-tile software was used to calculate the optimal cut-off value for PFS and OS, dividing peripheral blood markers into high and low groups. Their cut-off values were: NLR>3, LMR>2.4, SII>979, GLB (g/L)>26.6, A/G>1.4, WBC (10 9/L)>6.4, LY (10 9/L)>1.5, and ANC (10 9/L)>5. No optimal cut-off values were determined for PLR,CRP (mg/L), and LDH (U/L).These cut-off values can be used to predict the survivals of the NSCLC patients with ICIs treatment.

Determination of OS and PFS by univariant, lasso, and multivariate analysis of biomarkers. The results of univariate Cox regression analysis show that a total of 10 univariate factors, including clinical stage, ECOG score, bone metastasis, LMR, SII, GLB, A/G, WBC, LY, and ANC, had statistical significance in PFS (P<0.05) , and that a total of 10 single factors, including Histology, ECOG score, treatment methods, bone metastasis, PD-L1 expression in tissues, NLR, SII, GLB, A/G, WBC, and ANC, were statistically significant in OS (P<0.05). Lasso regression was also used to screen parameters related to OS and PFS. As shown in Figure5a, ten variables related to PFS were included in Lasso regression, and when the model fitted well, seven to 10 independent variables were contained in between (Figure5b). For OS, twelve variables were included in Lasso regression (Figure5c), and when the model fitted well, Two to 10 independent variables were contained in between (Figure5d). After Lasso regression ,these variables would subject to, Cox multivariate analysis.

Fig.5: Screening of variables based on Lasso regression (a&b) The variation characteristics of the coefficient of variables for PFS (a) and the selection process of the optimum value of the parameter λ in the Lasso regression model by cross-validation method (b). (c&d)The variation characteristics of the coefficient of variables for OS (c) and the selection process of the optimum value of the parameter λ in the Lasso regression model by cross-validation method (d).

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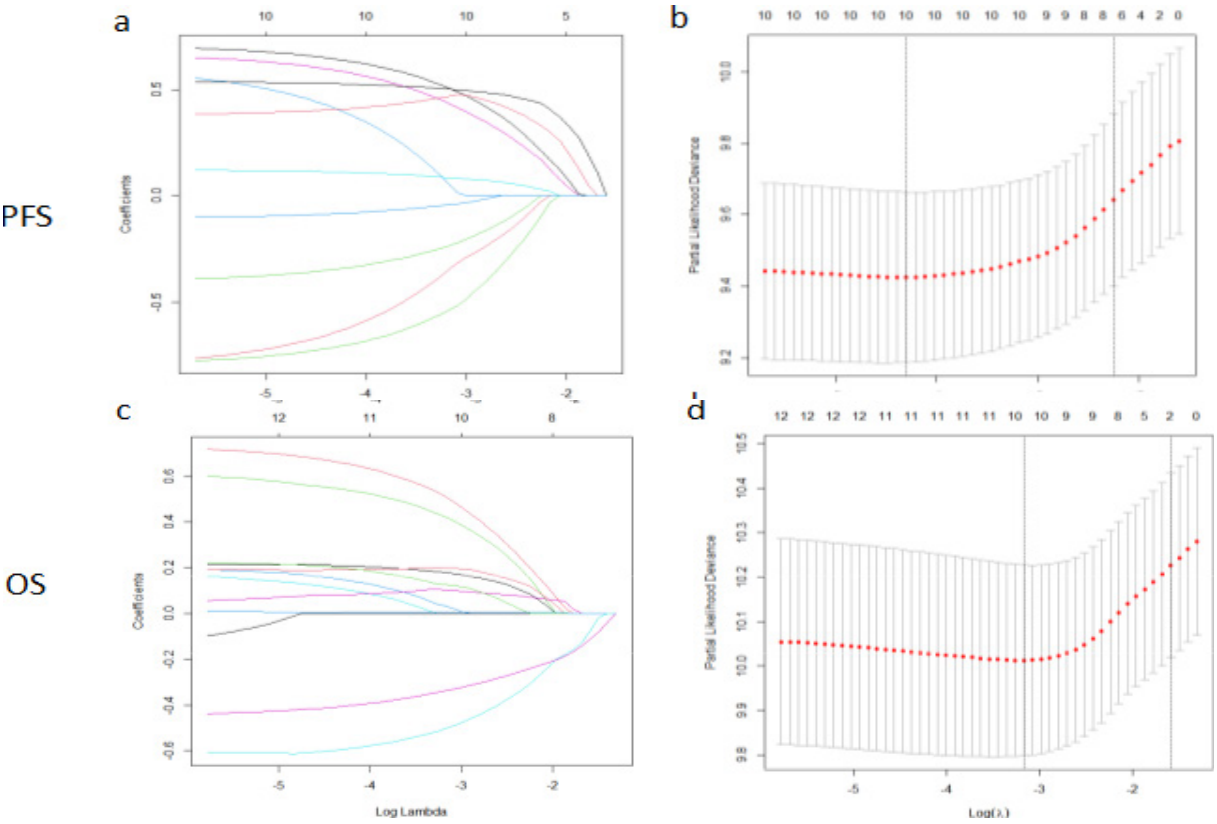

To select significant variables and Identify factors of statistical significance to both PFS and OS, weperformed Cox multivariate regression, and found that GLB (g/L)>26.6, ANC>5(109/L), and bone metastasis were statistically significant in the multiple regression of PFS and OS (P<0.05, Table 2).

Table 2: Univariate and multivariate analyses of PFS and OS

Variable	Category	PFS						OS					
		Univariate			Multivariate			Univariate			Multivariate		
		HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value	HR	95%CI	P value
Gender	Male	1.001	0.637-1.571	0.998				1.14	0.704-1.846	0.594			
Age	Continuous	0.995	0.974-1.018	0.689				1.004	0.982-1.027	0.737			
Histology	Squamous	0.878	0.667-1.156	0.354				1.146	0.906-1.449	0.257			
Clinical	stage IV	1.865	1.142-3.047	0.013*				1.341	0.808-2.226	0.256			
ECOG	2	2.555	1.110-5.880	0.027*				4.095	1.868-8.977	0.003*			
Smoking status	Former/ Current	1.031	0.681-1.561	0.884				0.899	0.630-1.501	0.972			
Therapy	Combo	1.687	0.920-3.091	0.091				2.667	1.275-5.576	0.009*			
Distant metastasis													
	Liver	1.593	0.803-3.163	0.183				0.956	0.418-2.189	0.916			
	Brain	1.098	0.571-2.112	0.779				0.776	0.358-1.679	0.519			
	Bone	2.148	1.420-3.250	<0.001*	2.614	1.703-4.014	<0.001	1.651	1.065-2.559	0.025*	2.012	1.283-3.154	0.002
	Adrenal gland	1.215	0.611-2.414	0.579				1.031	0.450-2.364	0.942			
PD-L1	Negative	1.732	1.108-2.708	0.016*				2.432	1.483-3.988	<0.001*	0.708	0.516-0.971	0.032

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Laboratory parameters													
NLR	>3	1.328	0.897-1.967	0.156				1.597	1.051-2.425	0.028*			
PLR	Not found												
LMR	<2.4	0.644	0.428-0.969	0.035*				0.864	0.556-1.345	0.518			
SII	<979	1.647	1.070-2.525	0.023*				1.645	1.040-2.603	0.034*			
CRP(mg/L)		Not found											
LDH(U/L)		Not found											
GLB(g/L)	<26.6	1.886	1.250-2.848	0.003*	1.865	1.290-2.994	0.002	2.412	1.543-3.759	<0.001*	1.741	1.096-2.766	0.018
A/G	<1.4	0.581	0.389-0.869	0.008*				0.473	0.301-0.725	<0.001*			
WBC (109/L)	<6.4	2.098	1.395-3.155	<0.001*				1.924	1.265-2.931	0.002*			
LY(109/L)	<1.5	1.775	1.192-2.644	0.005*				1.47	0.976-2.217	0.064			
ANC (109/L)	<5	2.146	1.436-3.207	<0.001*	2.185	1.449-3.294	<0.001	1.936	1.268-2.940	0.002*	1.807	1.166-2.799	0.008

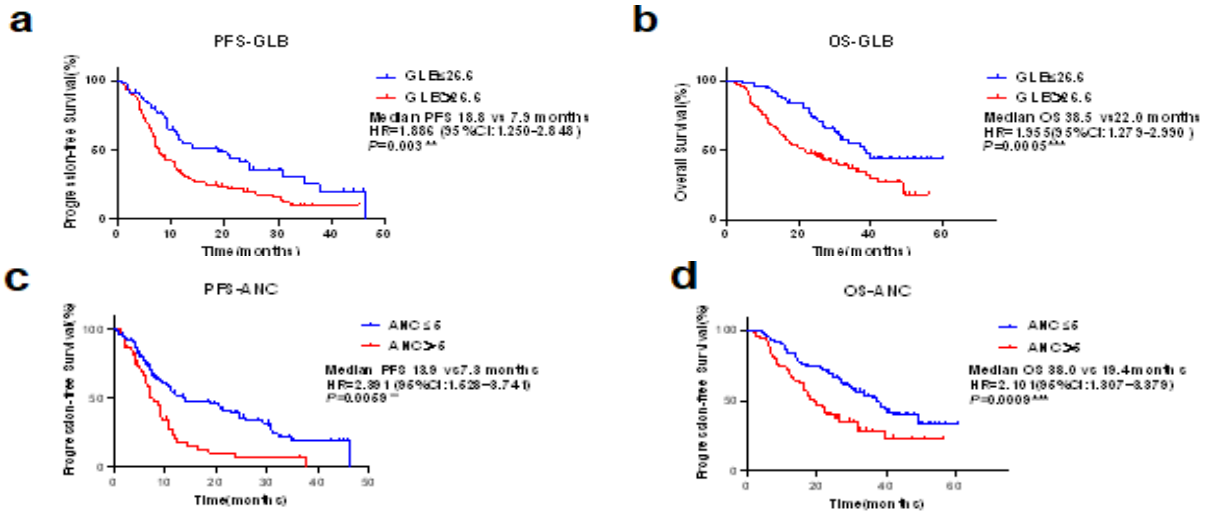
Note:*, P<0.05; Category contains risk factors (Risk factor=1,Protective factors=0); Age was used as a continuous variable

5. Univariate and multivariate analyses of biomarkers for OS and PFS

For the overall population, the medians of OS and PFS demonstrate distinct outcomes based on specific biomarkers. According to the univariate analysis, the factors of statistical significance were as follows: GLB: Individuals with a low GLB (≤26.6) had a markedly longer median of PFS (18.8 months) and OS (38.5 months) compared to those with high GLB (>26.6), which had a median of 7.9 months for PFS and median of 22.0 months for OS (Fig. 6a&b). The hazard ratios for PFS and OS were 1.886 (95% CI: 1.250-2.848, P=0.003) and 1.955 (95% CI: 1.279-2.990, P=0.0005) respectively, indicating that patients with low GLB levels survived much better than the ones with high GLB. These data demonstrate that GLB level of patients could serve as a potential biomarker of prognosis forpatients.

5.1.ANC : Patients with low ANC (≤5) had a longer median of PFS (13.9 months) and OS (38.0 months), whereas those with high ANC (>5) had a median of 7.3 months for PFS and median of 19.4 months for OS (Fig. 6c&d). The hazard ratios were 2.391 (95% CI: 1.528-3.741, P=0.0059) for PFS and 2.101 (95% CI: 1.307-3.379, P=0.0009) for OS. Similar to GLB, ANC level ofpatients might be used as a biomarker of prognosis for patients.

5.2. Bone Metastasis: The presence of bone metastasis significantly affected survival outcomes, with patients having non-bone metastasis showing a median of 12.1 for PFS months and median of 31.9 months for OS, compared to those with bone metastasis, who showed a median of 7.9 months for PFS and median of 19.1 months for OS (Fig. 6e&f). The hazard ratios were 2.548 (95% CI: 1.557-4.169, P=0.0006) for PFS and 2.098 (95% CI: 1.260-3.492, P=0.0047) for OS (Fig. 6g). Thus, bone metastasis ofpatients can be used as a potential biomarker of prognosis for patients.



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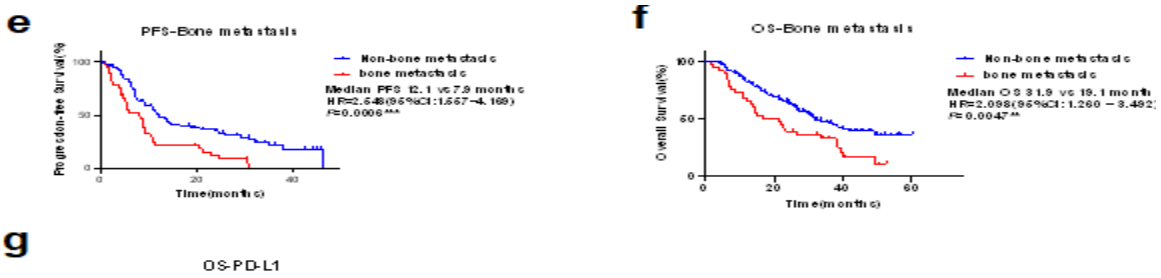


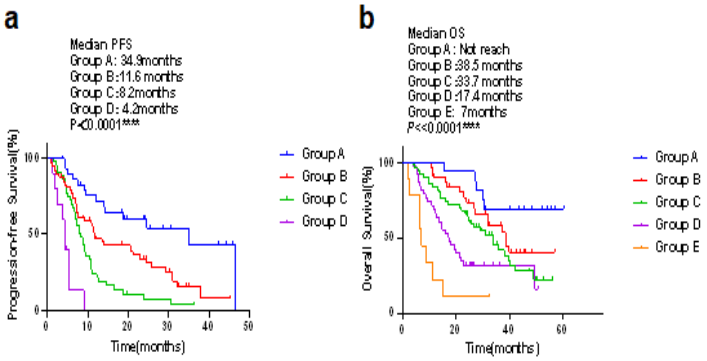
Fig. 6: PFS (a, c, e) and OS (b, d, f, g) curves of patients as analyzed by GLB, ANC, bone metastasis and PD-L1 markers

5.3. PD-L1Expression: Positive PD-L1 expression of patients was associated with a better median of OS (39.8 months) compared to that of patients with negative PD-L1 expression (23.4 months), with a hazard ratio of 1.925 (95% CI: 1.245-2.979, P=0.0009), indicating that PD-L1 expression affected OS of the patients. According to the univariate analysis, the levels of GLB, ANC, bone metastasis and PD-L1 expression are highly correlated to the survival of patients, and could be used as a useful biomarkers of prognosis for patients.

6. Establishment of a prognostic scoring system

Based on the number of advantaged factors (i.e., GLB≤26.6 g/L, ANC≤5 * 109/L, no bone metastasis, and PD-L1 expression positive), We explored the OS and PFS (Fig.7a&b). It was found that there was statistical differences among the groups, and that the OS and PFS of Groups A and B significantly increased compared to that of the other groups (C, D, E) (P< 0.0001), suggesting that the survivals of patients increased depending on the points of the prognostic scoring system.

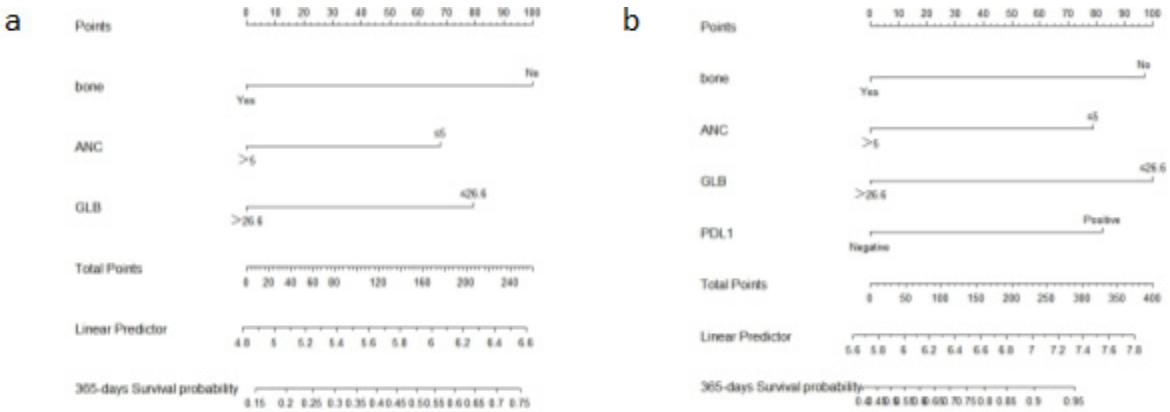
Fig. 7: PFS (a) and OS (b) curves of the multifactor model based on the number of advantage factors at baseline (GLB≤26.6 g/L, ANC≤5 (109/L), non-bone metabolism, PDL1-Positive). Abbreviation: NR, not reached; Group A: 0 point; Group B: 1 point; Group C: 2 points; Group D, 3 points; Group E: 4 points



7. Nomogram diagnostic model construction

To further evaluate the predictive model and the contribution of each variable to survival, we established a 1-year PFS rate and 1-year OS rate nomogram based on multivariate Cox regression (Figure 8a&b). The C index of 1-year PFS rate was 0.731 (0.648-0.814) (P<0.001), and the C index of 1-year OS rate was 0.733 (0.635-0.832) (P<0.001). The results of Decision Curve Analysis (DCA) demonstrate that the nomogram model performed well in clinical practice (Figure 8 c&d).

Fig.8: Data analysis by Nomogram diagnostic model



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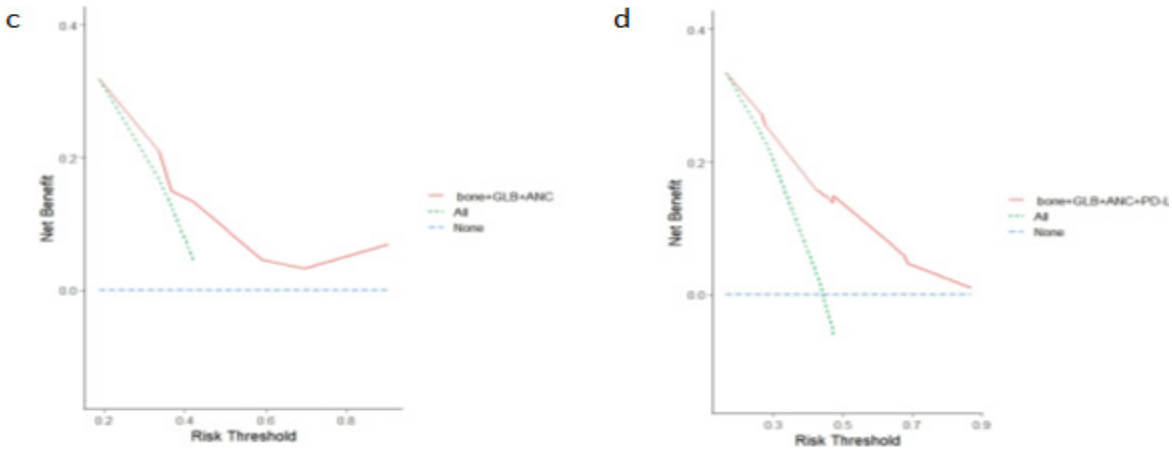

(a&b) Nomogram prediction of 1-year PFS rate and 1-year OS rate in patients with advanced NSCLC.
(c&d) DCA curves for predictive prognostic modeling of Nomogram. Note: Bone: Bone metastasis; ANC: Absolute count of neutrophils; GLB: Serum globulin; PFS, progression-free survival; OS, overall survival; DCA, decision curve analysis.

8. Internal model validation

After internal cross-validation calibration, tested with 5-fold cross-validation, and repeating the procedure 10 times, the C statistic (AUC under the ROC curve of one-year PFS rate) was 0.808 (95% CI: 0.655-0.961), and the C statistic (AUC under the ROC curve area of one-year OS rate) was 0.783 (0.633-0.933) (Figure 9 a&b). These datasuggest that the models were reliable.

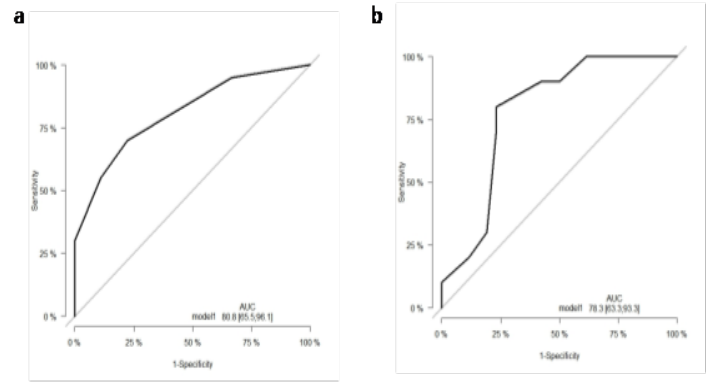

Fig.9: The ROC curve after five-fold cross validation (a) one-year PFS rate. (b) one-year OS rate.

9. Discussion

Although it was reported that noninvasive peripheral blood markers and clinical factors had predictive values in the treatment of advanced NSCLC, such as NLR, SII, there is no universal optimal cut-off value determined in different studies in the treatment of NSCLC patients with ICIs, and application of combined clinical markers is rarely seen.[18, 19].

In this study, 144 patients with advanced/metastatic NSCLC were treated with ICIs and analyzed by combined clinical features, organ metastasis, peripheral blood and biochemical indicators. A prognostic score including GLB, ANC, bone metastasis, and PD-L1 expression was determined and validated. The final cumulative score by grouping the four factors can predict survival prognosis of patients. In both OS and PFS, the higher the score, the worse the prognosis, and the difference between groupswith scores by this subgrouping was highly significant (P< 0.001). Compared with the commonly-used continuous peripheral blood variables without generally-recognized optimal cut-off value in most studies, this scoring system would be a much more valuable alternative to clinical guidance.
In order to further understand the contribution of each factor to the survival of patients and the clinical application value, we constructed one-year PFS and OS rate nomogram for individual survival prognosis prediction. The DCA curves of one-year PFS and OS rate are effective in clinical practice. The whole area of DCA curve of one-year OS rate indicate that this model is of high clinical relevance (Figure 8d). In terms of model validation, due to the limited sample size, we adopted the internal validation, k-fold cross validation (k=5), which divided the sample size into five subsets for mutual validation of the training set and the validation set. The C statistic of 1-year PFS rate was 0.808, and The C statistic of 1-year OS rate was 0.783, supporting the prediction of the model. These data indicate that GLB, ANC, bone metastasis and PD-L1 expression in patients with advanced/metastatic NSCLC are predictive factors closely-related to ICIs treatment.

Neutrophils play a crucial role in the tumor microenvironment. It was reported that neutrophil account and percentage may correlate with the prognosis of ICIs treatment[20, 21] , and that organ metastasis can predict the prognosis of NSCLC patients treated with ICIs [22, 23] . Therefore, organ metastasis was included as a factor in modeling in this study. Up to now, there are no sufficient data regarding the application of the GLB and ANC in the field of cancer immunotherapy. Our data suggest that ANC, GLB and bone metastasis serve as useful biomarkers that can be employed to select the advanced NSCLC population for immunotherapy.
It was reported that serum globulin had prognostic relevance in patients with cancer[24, 25], for example, a retrospective analysis of 186 patients with gastric cancer indicated that a baseline GLB of less than 33.4 (g/L) in

patients was a favorable prognostic factor [24] . In addition, a nomogram prediction model established using the baseline peripheral blood data of 336 patients with oral cancer set the optimal cut-off values of GLB as 26.35g/L, which could predict the prognosis of patients [25], and the multivariate result OR=1.093 (1.014-1.177), P=0.019 was close to the GLB cut-off value in the present study (GLB > 26.6). According to our results, abnormal serum is a poor prognostic factor in patients with tumors. Furthermore, this study focused on the patients with ICIs treatment, and we hypothesized that several mechanisms might be involved. First, Antibody neutralization works: the high level of immunoglobulin may be related to the phenomenon of antibody neutralization, i.e., immunoglobulin may bind to immune checkpoint inhibitors or other immunotherapeutic drugs, thereby neutralizing the activity of these drugs and reducing the effectiveness of treatment[26]. Second, Immune escape occurs: Tumor cells are sometimes able to escape attack by the immune system through a variety of mechanisms. In some cases, high levels of immunoglobulins may be a sign of overactivation of the immune system, and tumor cells may take steps to escape from the immune system[27]. Third, immune related adverse events take place: immunotherapy may cause immune related adverse events, including increased production of immunoglobulins. These adverse events may be related to treatment tolerance and prognosis[28]. More efforts will be required to elucidate the mechanism underlying ICIs-mediated survivals of NSCLC patients.

Accumulating studies have revealed that high ANC will promote tumor progression and deterioration[29-31]A retrospective studyon the analysis of 88 patients with NSCLC treated with PD-1 inhibitors indicated that the lower the patient's ANC at baseline, the better the response to treatment (P=0.017) [32]. In addition, it was found that high ANC before treatment was associated with poor prognosis in lung cancer patients treated with Anlotinib[33]. A descriptive review of immunotherapy for advanced renal cancer also stated that low ANC and NLR were associated with good prognosis of patients[34] . A predictive model for ICIs treatment of NSCLC patients has been established, including the impact of ANC and LDH and some clinical characteristics, ECOG PS score, BMI, etc. on prognosis[23] . Several reports indicated that the prognosis of tumor immunotherapy was related to NLR[35-37] , Although both ANC and NLR are related to immune status and the degree of inflammation, their biological significance may vary. In some cases, ANC may directly indicate immunosuppressive or inflammatory status, while NLR may suggest the balance between neutrophils and lymphocytes, and the prognostic impact of immunotherapy may be relatively complex. In the present study, we found that GLB, ANC, bone metastasis and PD-L1 were closely associated with the survival of NSCLC patients, and could be used as potential biomarkers for diagnosis of NSCLC patients with ICIs treatment. We look forward to following up this study to further investigate the different treatment markers for NSCLC patients, providing a basis for individualized treatment.

In the present study, we took into account the impact of distant organ metastasis on prognosis, and found that only bone metastasis was of statistical significance and predictive value in PFS and OS. We proposed that there might be several reasons. First, there were more patients with bone metastasis and fewer patients with other metastases such as liver, adrenal gland and brain, so the analysis results may be biased. Second, bone metastasis may affect the immune status of patients and the response to immunotherapy, which may lead to immunosuppression, inflammatory response and bone marrow fibrosis, thus affecting the effectiveness of immunotherapy. In addition, bone metastasis may also affect the prognosis of immunotherapy through other mechanisms, such as the change of tumor microenvironment[38]. Third, bone metastasis may affect the prognosis of lung cancer immunotherapy by affecting the bone marrow microenvironment and immune response. Thus, our result supported the previous study that bone metastasis may serve as a reliable indicator of prognosis for the patients with advanced NSCLC treated with ICIs[39].The prognostic factors of immunotherapy obtained by the model established in this study are somewhat different from those of previous publication, because we found that the expression of PD-L1 in tissues has statistical significance in both univariate and multivariate analysis in OS. Although it is still widely recognized standard for evaluating the efficacy of immunotherapy in patients, our data show that PD-L1 in PFS was only significant in univariate analysis, but not in multivariate analysis, indicating that the correlation between PD-L1 and PFS was not as strong as that of OS.

GLB was rarely used in the previous tumor prediction model. However, in this study,we found it was a potential biomarker of statistical significance to the survival of NSCLC patients. Importantly, our model in this study can perform better prediction by the score grouping of advantages and disadvantages factors or nomogram model.Ofnote, there are some limitations to this study, including the number of patients, most of the 123 patients treated with combination drugs, not ICIs monotherapy. Some of these drugs are not used for first-line treatment, and relevant information needs to be supplemented in the follow-up study to continue to analyze the predictive value for the same first-line treatment. Furthermore, the mechanism underlying the prognostic role of nomogram requires further efforts. In conclusion, our data show that the examination of ANC, GLB and bone metastasis can predict the PFS and OS of advanced/metastatic NSCLC patients treated with ICIs, and the optimal cut-off value can provide valuable reference for clinical practice.

Conflict of interest, The authors declare that they have no conflict of interest.

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11. Ethics approval

The current study was conducted with the approval of the Beijing Chest

Hospital Medical Ethics Committee (YJS-2023-09).

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