A Novel Nomogram Based on Immune Scores for Predicting Survival in Patients with Early-Stage Non-Small Cell Lung Cancer (NSCLC)

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: The role of immune parameters in the prognosis of lung cancer has attracted more and more attention. However, studies of the association between immune scores and prognosis of lung cancer are scarce. The goal of our research was to investigate the correlation between immune scores and overall survival (OS) of early-stage non-small cell lung cancer (NSCLC).

Material/Methods: All data regarding patient immune and stromal scores, clinicopathological features, and survival was obtained from the TCGA datasets. Univariable and multivariable Cox regression analyses were utilized to recognize risk factors associated with OS. Afterward, a prognostic nomogram was constructed for predicting 3- and 5-year OS of stage I and II NSCLC patients. Calibration curves and receiver operating characteristic (ROC) were performed to assess the predictive accuracy of the nomogram. Kaplan-Meier methodology was also applied for the survival analysis.

Results: In total, 764 NSCLC (stage I–II) patients were analyzed, and all patients were classified into 3 groups based on immune scores. Results showed that patients with medium-immune scores had significantly worse OS (hazard ratio=1.73, 95% confidence interval: 1.22–2.46) compared with those with low- and high immune scores. Area under the ROC curves (AUC) values for 3- and 5-year OS were 0.65 and 0.64, respectively. Calibration plots demonstrated good consistency in the probability of OS between nomogram predictions and actual observations.

Conclusions: Medium-immune scores are correlated with unsatisfactory prognosis in NSCLC (stage I–II) patients. In addition, the prognostic nomogram may be helpful in predicting OS for stage I and II NSCLC patients.

MeSH Keywords: Carcinoma, Non-Small-Cell Lung • Immunologic Factors • Nomograms • Prognosis

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Background

Lung cancer is one of the most common types of tumors, with a high mortality rate. According to recent data from GLOBOCAN, in 2018, 2,093,876 new lung cancer cases and 1,761,007 deaths have been estimated worldwide [1]. In terms of the histological type, lung cancer can be categorized into 2 main subtypes: non-small cell lung cancer (NSCLC) and small cell lung cancer [2]. Furthermore, NSCLC can also be classified as 2 major subtypes, namely squamous cell carcinoma (SCC) and adenocarcinoma (AC) [3]. Patients with NSCLC can be potentially cured by surgery if the disease is detected at an early stage, but there is no cure for postoperative metastatic recurrence [4,5]. At present, it is confirmed that the immunosuppressive microenvironment has developed even in stage I and stage II disease compared with normal lung tissue [6]. In recent years, immunotherapies have been increasingly used in lung cancer patients, and recent research revealed that immunotherapies are correlated with improved survival in NSCLC patients [7]. In addition, previous studies indicated that immunotherapy can potentially be applied to treat early-stage lung cancer patients [6,8]. Thus, investigating the correlation between immune system and prognosis of stage I and II NSCLC is essential for the effective use of immunotherapies [9].

Recently, the relationship between cancer microenvironments and prognosis of NSCLC has received increasing attention [10,11]. Tumor microenvironments comprise tumor, immune, and stromal cells, etc.[12] In addition, a previous study has reported that immune scores calculated by gene expression data can be applied to infer the level of infiltrating stromal and immune cells in cancer tissues [13]. Also, a recent study showed that immune infiltration is related to the prognosis of patients with NSCLC [14]. These research findings, however, have not yet been introduced into regular clinical practice of stage I or II NSCLC. Nowadays, nomograms are extensively applied for predicting the prognosis of patients with cancers, including colorectal cancer [15], liver cancer [16], and NSCLC [17]. As far as we know, nomogram integrated immune scores for early-stage (stage I and II) NSCLC has not been reported. In this study, the correlation between immune scores and prognosis was assessed, and a prognostic nomogram based on immune scores for patients with stage I and II NSCLC was established.

Material and Methods

Data collection and preprocessing

Clinical data of the NSCLC's The Cancer Genome Atlas (TCGA) datasets was downloaded from cBio Cancer Genomics Portal (*http://www.cbioportal.org/*) [18,19]. In addition, the NSCLC dataset consisted of 2 subsets: lung AC and SCC. The cBio Cancer Genomics Portal open access database was designed to make the raw data generated by large-scale cancer genomic projects more easily and directly available, and it contains several provisional TCGA datasets [18]. Immune and stromal score of each sample in the TCGA datasets were extracted from ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data, *https://bioinformatics.mdanderson.org/estimate/disease.html*). Immune and stromal scores of each sample reflected the gene signature enrichment of stromal and immune cells [20] and were calculated, as previously described [13]. The previous study has described an algorithm to calculate immune scores and stromal scores to deduce the degree of infiltrating immune and stromal cells, which can help to predict tumor purity [13]. In ESTIMATE, a 141-gene expression signature of immune cell infiltrating samples are applied to estimate immune scores, and the same algorithm is used to obtain stromal scores. After that, immune and stromal scores were matched to clinical data using sample ID codes. In addition, samples with missing clinicopathological information or survival data would be removed.

Associations between immune scores and clinicopathological characteristics

The optimal cut-off values for immune and stromal scores were determined with the X-tile software (Yale University, Version 3.6.1) [21]. X-tile can help researchers to obtain the best grouping of variables, and reveal the significance of variables in tumor prognosis and treatment scientifically [9,22]. In the present study, TCGA samples were classified into low-, medium- and high immune/stromal scores groups. Comparisons among subgroups of immune scores between different clinicopathological characteristics were carried out by the Fisher exact test or χ^2 (Chi-square) test (as appropriate) using IBM SPSS Statistics 22 (Chicago, IL, USA). P-values less than 0.05 were considered statistically significant.

Construction and assessment of the immune scores-based nomogram

Before the establishment of the nomogram, stromal and immune scores, and the clinicopathological factors, including age, histological type, sex, TNM stage, T stage, and lymph node metastasis status, were subjected to univariate and multivariate Cox regression analyses, respectively. Then, the immune scores-based nomogram was constructed for predicting survival probabilities in patients with early-stage NSCLC. Calibration plots were used to evaluate the performance of the nomogram, and internal validation was performed using 1000 bootstrap resamples. Furthermore, the time-dependent receiver operating characteristic (ROC) curves were built and the area under the ROC curves (AUC) values were calculated
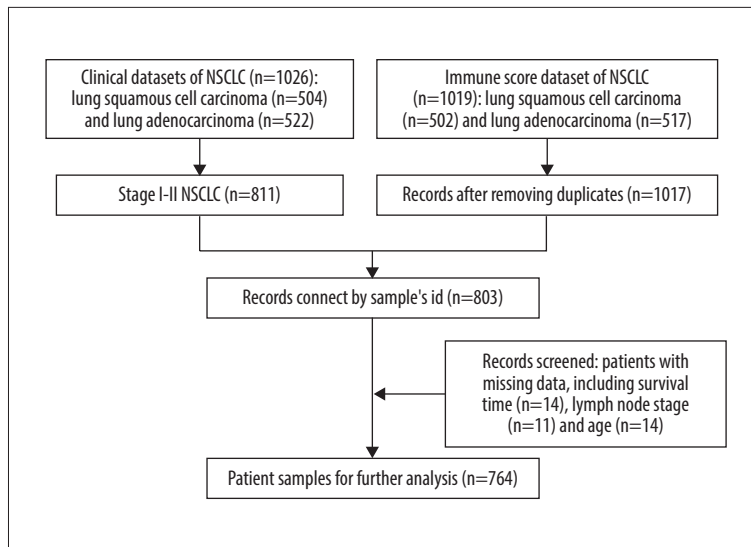
Figure 1. Workflow detailing process of samples selection.

to measure the predictive ability of the immune scores-based nomogram. Predictive accuracy of the nomogram was quantified by AUC, categorized as: no better than a random guess (AUC 0.5 to 0.6), general (AUC 0.6 to 0.7); moderate (AUC 0.7 to 0.9) and excellent (AUC 0.9 to 1.0). The nomogram and calibration plots were carried out with "survival" and "rms" packages of R version 3.6.0 (*http://www.r-project.org*), and ROC curves were done by using "timeROC" package of R version 3.6.0. Besides, results of multivariable Cox analysis were visualized by a forest plot using "survminer" package of R version 3.6.0.

Statistical analysis

The principal outcome of our study was overall survival (OS) defined as death from any cause. Continuous data with normal distribution were shown as mean±standard deviation (SD), continuous data with non-normal distribution were presented as median (quartile range), and noncontinuous data were expressed in percentage. Hazard ratios (HR) with their corresponding 95% confidence intervals (95% CI) were calculated based on univariate and multivariate Cox regression models. Besides, Kaplan-Meier (KM) method was applied to analyze differences in survival among immune scores groups by "survival" and "survminer" packages of R version 3.6.0. All the statistical tests were 2-sided, and statistical significance was set at P-value <0.05.

Results

Characteristics of stage I–II NSCLC patients

After matching the sample ID and filtering for missing data, 764 patients with stage I–II NSCLC were retained for further analysis, and the detailed screening process is shown in Figure 1.

In our analysis datasets, each immune score corresponds to a NSCLC patient. The median age (quartile range) of the NSCLC patients was 68 (60–73), ranging from 38–88 years, of which 595 (77.88%) patients were greater than 60 years old. The optimal cut-off values of immune scores were 122.6 and 687.9, and then patients were divided into low-, medium-, and high immune scores subgroups (Suppplementary Figure 1). The best cut-off values of stromal scores were −719.2 and −819.7, and patients were classified into low-, medium- and high stromal scores subgroups (Suppplementary Figure 2). Of all the patients with stage I–II NSCLC, 77.49% patients had lymph node metastasis, 58.51% were T2 stage, and 64.14% were TNM stage I. Median immune score (quartile range) of stage I–II NSCLC patients was 714.36 (72.39–1396.48), and the median OS time (quartile range) of the patients in this study was 22.01 (13.06–39.08) months, ranging from 0.07 to 238.11 months. The clinicopathological features of the 3 different immune scores groups are shown in Table 1. Medians (quartile range) of the 3 immune scores groups were -397.97 (–639.92 to −147.32), 403.67 (260.77 to 563.89) and 1381.48 (1061.32 to 1849.46), respectively. As for histological type, the proportion of SCC was lower than AC in high immune scores group. In addition, the proportion of males was higher than females in the low- and medium-immune scores group. Furthermore, patients in the high immune scores group tended to have high stromal scores compared with those in the low-immune scores group. All of the above results are detailed in Table 1.

Results of univariate and multivariate Cox regression analyses for OS

Table 2 and Figure 2 show the results of the univariate and multivariate analyses, respectively. In the univariate analysis, oldest age group (≥80), high T stage (T2 and T3), high TNM stage (stage II), high stromal scores (>819.7), and medium-immune

Table 1. Associations between immune scores and clinicopathological features in 764 NSCLC patients.

Features	Total	Immune scores			χ²	P
		≤122.6	>122.6, ≤687.9	>687.9		
Sample sizes	764	201 (26.30)	174 (22.77)	389 (50.91)	–	–
Age					17.84	0.22
≤50	41	17 (8.46)	9 (5.17)	15 (3.86)		
51–60	158	47 (23.38)	37 (21.26)	74 (19.02)		
61–70	282	83 (41.29)	63 (36.21)	136 (34.96)		
71–80	247	50 (24.88)	55 (31.61)	142 (36.50)		
>80	36	4 (1.99)	10 (5.75)	22 (5.66%)		
Sex					23.32	0.000
Female	308	56 (27.86)	65 (37.36)	187 (48.07)		
Male	456	145 (72.14)	109 (62.64)	202 (51.93)		
Histological type					61.18	0.000
AC	374	58 (28.86)	75 (43.10)	241 (61.95)		
SCC	390	143 (71.14)	99 (56.90)	148 (38.05)		
T stage					17.85	0.001
T1	251	43 (21.39)	58 (33.33)	150 (38.56)		
T2	447	138 (68.66)	102 (58.62)	207 (53.21)		
T3	66	20 (9.95)	14 (8.05)	32 (8.23)		
Lymph node*					1.64	0.440
Negative	592	153 (76.12)	141 (81.03)	298 (76.61)		
Positive	172	48 (23.88)	33 (18.97)	91 (23.39)		
TNM stage					5.32	0.70
I	490	116 (57.71)	119 (68.39)	255 (65.55)		
II	274	85 (42.29)	55 (31.61)	134 (34.45)		
Stromal scores						
Low	169	127 (63.18)	28 (16.09)	14 (3.60)	326.1	0.000
Medium	499	74 (36.82)	141 (81.03)	284 (73.01)		
High	96	0 (0.00)	5 (2.87)	91 (23.39)		

AC – adenocarcinoma; SCC – squamous cell carcinoma. * Lymph node metastasis status.

scores (>122.6, ≤819.7) were found to be statistically correlated with poor OS, respectively (P<0.05). Table 2 summarizes the detailed results of univariate analysis.

The forest plot in Figure 2 demonstrates the results of the multivariate Cox regression analysis. Stage I–II NSCLC patients in medium-immune scores group had significantly worse OS (HR: 1.73, 95% CI: 1.22–2.46) compared with those in low-, and high

immune scores groups. In addition, the high stromal scores group was significantly associated with poor OS (HR: 1.70, 95% CI: 1.05–2.75). Interestingly, patients with positive lymph node metastasis had significantly improved OS (HR: 0.52, 95% CI: 0.29–0.92). However, the samples in the positive lymph node metastasis group were dramatically less than those in the negative lymph node metastasis group, which may weaken the result. Compared with stage II patients, patients in stages I

Table 2. Univariate analysis of clinicopathological factors, stromal scores, and immune scores with OS in NSCLC patients.

Characteristics	Total	Death	Survival	HR (95% CI)		P
Age						
≤50	41	11	30	Reference		
51–60	158	46	112	1.22	(0.63–2.37)	0.553
61–70	282	101	181	1.45	(0.77–2.72)	0.251
71–80	247	100	147	1.68	(0.89–3.17)	0.106
>80	36	17	19	2.39	(1.11–5.14)	0.026
Sex						
Female	308	100	208	Reference		
Male	456	175	281	1.14	(0.89–1.47)	0.284
Histological type						
AC	374	116	258	Reference		
SCC	390	159	231	1.22	(0.96–1.56)	0.107
T stage						
T1	251	76	175	Reference		
T2	447	175	272	1.31	(1.00–1.72)	0.049
T3	66	24	42	1.84	(1.16–2.91)	0.010
Lymph node*						
Negative	592	200	392	Reference		
Positive	172	75	97	1.27	(0.97–1.65)	0.082
TNM stage						
I	490	161	329	Reference		
II	274	114	160	1.56	(1.23–1.99)	0.0003
Immune scores						
≤122.6)	201	71	130	Reference		
>122.6, ≤687.9	174	81	93	1.79	(1.30–2.47)	0.0003
>687.9	389	123	266	1.08	(0.80–1.44)	0.621
Stromal scores						
≤−719.2)	169	64	105	Reference		
>−719.2, ≤819.7	499	172	327	1.21	(0.90–1.61)	0.205
>819.7	96	39	57	1.55	(1.03–2.32)	0.034

AC – adenocarcinoma; SCC – squamous cell carcinoma. * Lymph node metastasis status.

had significantly better OS. Moreover, patients aged greater than or equal to 80 years have a worse OS (HR: 2.20, 95% CI: 1.01–4.78) compared with other age groups. Other variables, including sex, histological type, and T stage, were not statistically significant.

Establishment and evaluation of the immune scores-based nomogram

After taking variables related to immune scores and the Cox regression analysis results together into consideration, the prognostic nomogram for predicting OS of stage I and II NSCLC was established (Figure 3). The calibration plots exhibited good consistency
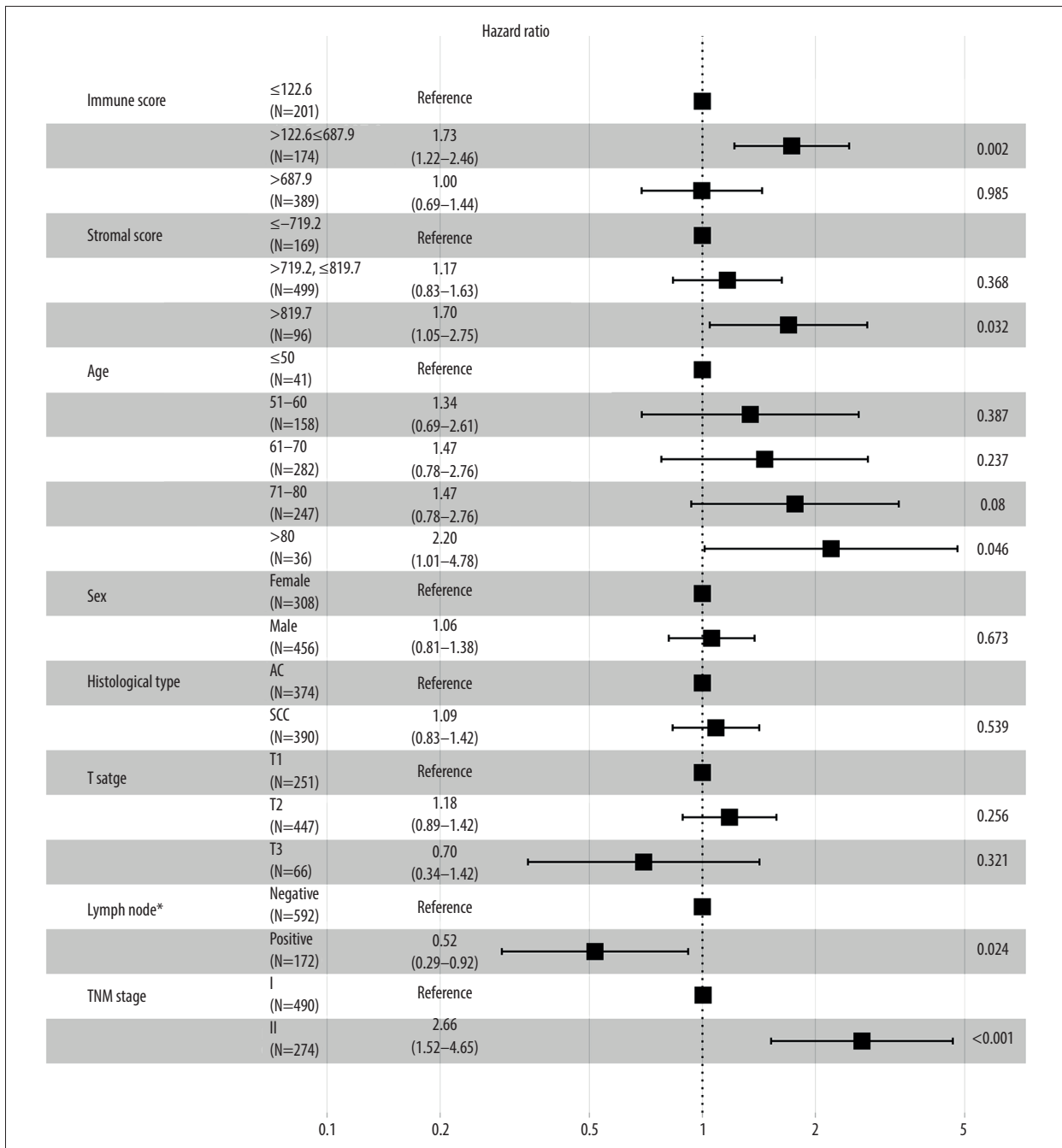
Figure 2. Forest plot of multivariate Cox regression analysis of clinicopathological factors, stromal scores and immune scores on patient survival in NSCLC. AC – Adenocarcinoma; SCC – squamous cell carcinoma. * Lymph node metastasis status.

between the predicted probability and actual probability, and the results are presented in Figure 4A and 4B. The AUC of ROC curves for 3- and 5-year survival were 0.65 and 0.64, respectively (Figure 4C), which revealed general predictive performances of 3- and 5-year OS. In addition, the result of the KM survival curves demonstrated that the prognosis of stage I–II NSCLC patients in the medium-immune scores group was poorer compared with that in the low- and high immune scores groups (Figure 4D).

Discussion

In this study, using TCGA datasets of NSCLC, we assessed the prognostic value of immune scores in patients with stage I–II NSCLC. Through Cox regression and KM survival analyses, we discovered that the OS time of medium-immune scores group was significantly worse than the low- and high immune score groups. Furthermore, we established a prognostic nomogram
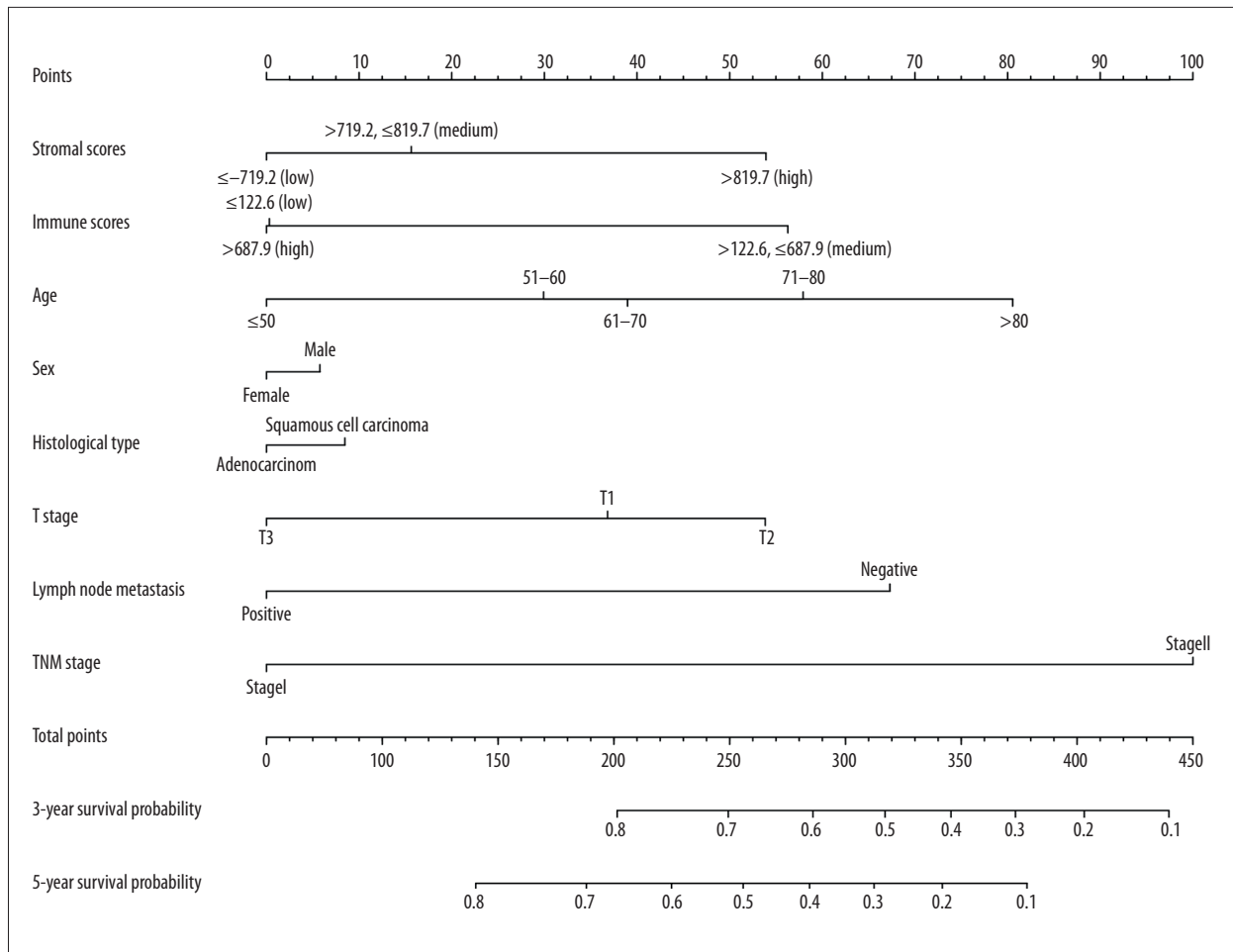
Figure 3. Nomogram for predicting OS of stage I–II NSCLC patients. For using this nomogram, each variable axis represents a risk factor, and a vertical line drawn upward is used to identify the points of each factor. Total points can be obtained by the sum of 8 variables and could be utilized to predict the survival probabilities (3- and 5-year) of stage I–II NSCLC patients.

based on immune scores to predict the OS of patients with stage I–II NSCLC.

Nomogram is a kind of graphical calculator based on regression analysis and has become a frequently used tool for constructing predictive models [23]. Recently, there have been a significant number of studies on the prognostic nomograms of NSCLC [24–26]. Furthermore, there are also several studies regarding the predictive nomograms for prognosis in patients with early-stage NSCLC [27–29]. However, these studies on prognostic nomograms for early-stage NSCLC did not involve immunological parameters.

Immune cells within the tumor microenvironment serve a crucial role in tumor progression [30]. Previous studies reported that lung cancer immunological parameters (e.g., CD68$^+$ macrophages, tumor-infiltrating lymphocytes, and interleukin-17) have a good ability to predict the clinical prognosis [31–33]. Moreover, a study suggested that immunoscore based on

immunological analysis has a prognostic value that should add to the TNM-classification [34]. Thus, immune parameters of cancer may be beneficial to predict prognosis and personalized therapies. In addition, previous research has established an immune scores-based nomogram for breast cancer patients and found a significant correlation between medium-immune scores and better OS time in breast cancer patients [9]. Our results demonstrated that medium-immune scores were significantly related to poor OS time in patients with stage I–II NSCLC. One possible reason is that immune cells of medium-immune scores group may be anergic with reduced functions in the NSCLC microenvironment [35]. However, the specific mechanism needs further study. Besides, we found that stage I and II NSCLC patients with low- and medium-immune scores tended to be males and lung SCC, and patients in T1 stage tended to have high immune scores. Preceding literature reported that females have stronger immune responses than males [36]. As for T stage, large tumor size has been proved to be a predictive and prognostic negative factor of Immune
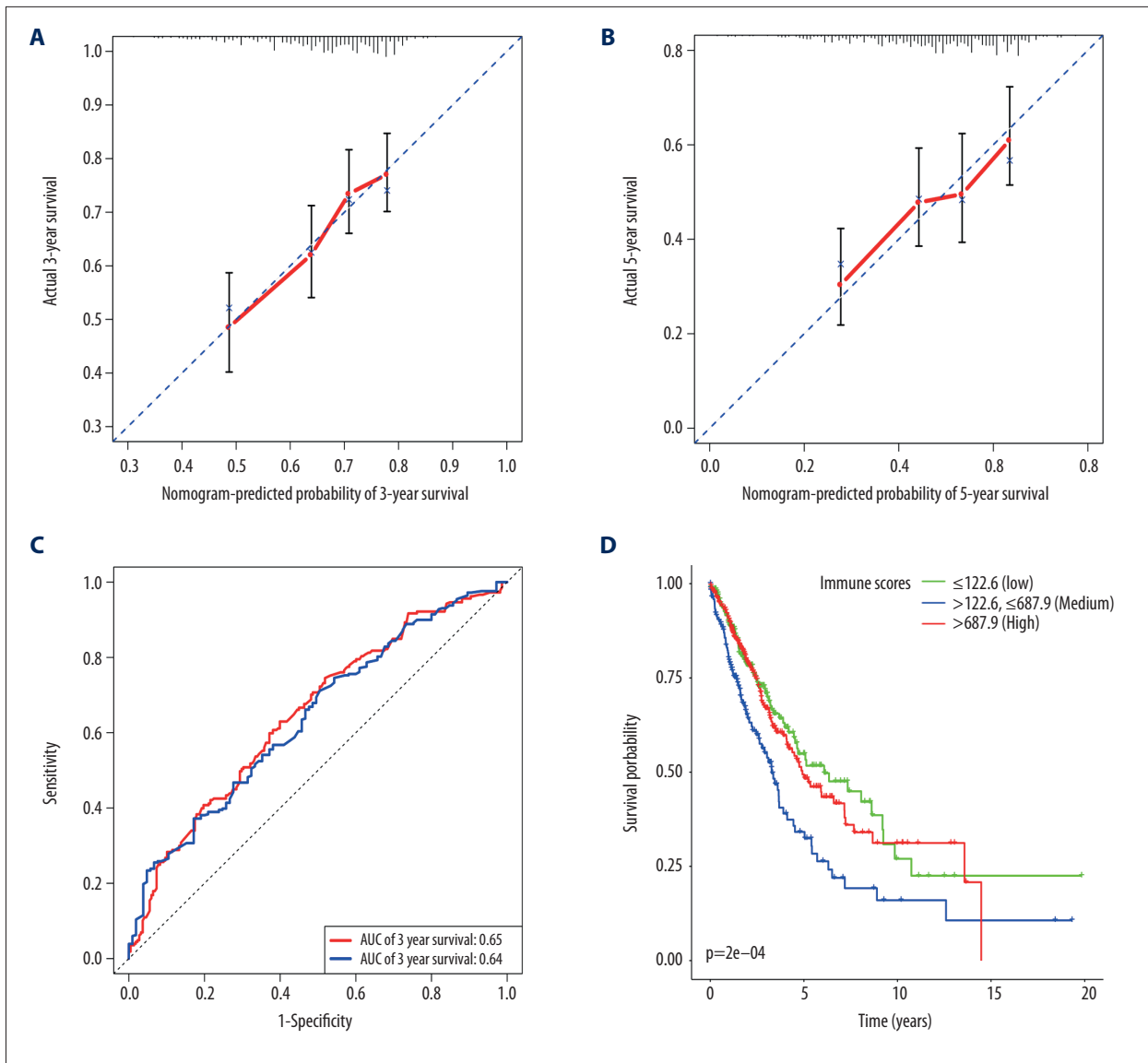
Figure 4. Assessment of the nomogram and Kaplan-Meier (KM) survival curves for immune scores. (**A**) 3-year and (**B**) 5-year overall survival (OS) nomogram calibration curves. Calibration plots report predicted outcome probabilities (on the x-axis) against observed outcome (on the y-axis), and the 45-degree blue dotted line represents the perfect prediction. (**C**) ROC curves of immune scores-based nomogram. The area under the ROC curves (AUC) values for 3- and 5-year survival were 0.65 and 0.64, respectively. (**D**) Comparison of OS time between the immune scores groups by KM curves.

checkpoint inhibitors therapy for NSCLC [37]. Our result also suggested that patients with low-immune scores tended to have low-stromal scores. Current studies showed that stromal cells are well recognized to play important roles in tumor growth and disease progression [38,39].

Univariate and multivariate Cox regression analysis revealed an increased risk of death for patients with high stromal scores. It has been reported that stroma-rich tumors are correlated with worse prognosis than are stroma-poor tumors in patients with NSCLC [40]. In addition, both univariate and multivariate

analyses revealed that age >80 years and TNM stage II was significantly correlated with worse OS. A previous study has shown that age is independent risk factors for survival of patients with early (T1N0M0) NSCLC, and survival probabilities decrease as age increases [27]. The multivariate Cox analysis in this study suggested that T stage may not be an independent prognostic factor in patients with stage I and II NSCLC.

As far as we know, this is the first nomogram integrated immune scores and clinicopathological factors for predicting the OS of early-stage (I–II) NSCLC patients. Through this easy-to-use

scoring model, physicians may predict an individualized survival. Although our study has evaluated the correlation between immune scores and prognosis of NSCLC patients, there were several limitations in the present study. Firstly, our research lacked external validation, because fewer datasets containing gene expression data that could be applied to obtain immune and stromal scores are currently available. In addition, some subgroup samples were relatively small, which might influence the robustness of our results. Furthermore, due to the lack of detailed information about treatment in the TCGA dataset, our study could not compare the effects of different treatments on prognosis. Last but not least, future studies should validate our model using other samples, and further immunological parameters are encouraged to improve our nomogram.

Conclusions

The results obtained in the present study show that that medium-immune scores are associated with worse OS in stage I and II NSCLC patients. Moreover, using the TCGA datasets, we have constructed and evaluated an immune scores-based nomogram for predicting the prognosis of patients with stage I–II NSCLC. The immune scores-based nomogram may be useful for easily estimating OS rate and choosing appropriate treatment strategies in patients with early-stage NSCLC.
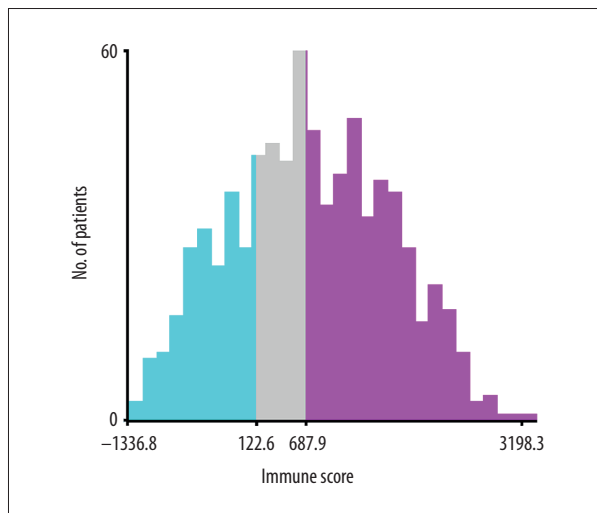
Acknowledgements

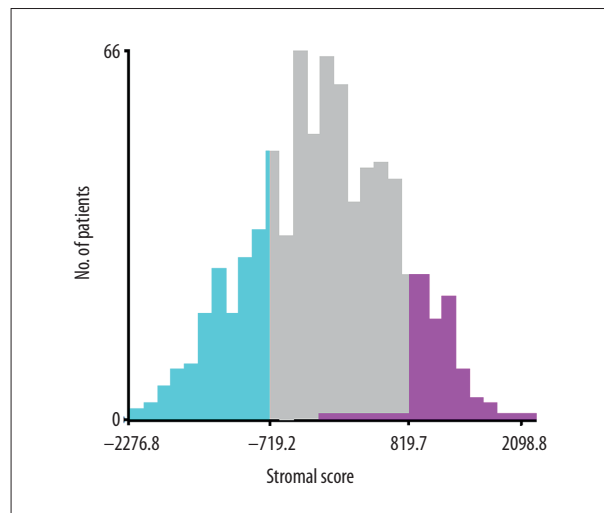We would like to thank the cBio Cancer Genomics Portal, ESTIMATE, and TCGA database for providing open access.

Conflict of interest

None.

Supplementary Data

Supplementary Figure 1. Identifying the best immune scores cut-off values.

Supplementary Figure 2. Identifying the optimal stromal scores cut-off values.

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DATABASE ANALYSIS

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