Development of Prognostic Nomogram Based on Immune Scores in Lung Adenocarcinoma Patients

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Abstract

Background: Immunotherapy has significantly altered the treatment landscape for non-small cell lung cancer (NSCLC).However, there is no report on the prediction of overall survival (OS) of lung adenocarcinoma (LDAC) based on immune score. In this study,we aim to investigate the immune scores of the LADC and the prognosis-related factors and construct a nomogram for prognosis prediction.

Methods:A total of 407 cases were included in the study.And the clinicopathological characteristics of patients with LADC and immune scores were download from TCGA database.We used Cox proportional hazards regression models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs).Nomograms were framed from Cox models and internally validated by use of 1000 bootstrap.Model discrimination was assessed by using the concordance index (c-index) and the calibration curve.

Results：Patients were divided into groups with low, moderate, or high Subgroups based on immune scores.This study shows that compared with patients with low and intermediate immune scores, only those with high immune scores had significantly improved OS (HR and 95% confidence interval[CI]:0.488 [0.327-0.730]).The C-index for OS prediction was 0.707(95% CI, 0.664-0.750) .The calibration curves for prediction of 3-years and 5-years OS probabilities demonstrated good calibration and discrimination.

Conclusions：High immune scores Subgroup is very significantly correlated with better OS in patients with LADC. Moreover, the nomograms for predicting prognosis may help to assess the survival of patients with LADC.

1 Introduction

Updated statistics of lung cancer in China (2019.1) shows that the morbidity and mortality the highest rate of lung cancer both rank the first in all kinds of malignant tumors throughout a whole nation.There was estimated to 787,000 new cases and 631,000 deaths from lung cancer in China in 2015[1].Recently released data for the global cancer burden in 2017 show that incidence rate is ranked second among all cancer types and the highest mortality rate worldwide[2].

The standard approach to lung cancer treatment is multidisciplinary and includes traditional surgery, chemotherapy, radiotherapy are still effective treatments in the treatment of lung cancer.

Recently, there have been notable developments in molecular targeted therapy and immunotherapy for the treatment of lung cancer.These new methods led to major changes in the treatment of lung cancer, which have improved the quality of life and survival rate of patients[3].Non-small cell lung cancer(NSCLC) accounts for ~ 80% of all lung cancers. Lung adenocarcinoma (LADC) accounts for 40% -50% of NSCLC.It has been reported that lung adenocarcinoma is often heterogeneous with certain subtypes of invasiveness.And in LADC with epidermal growth factor receptor (EGFR) mutations who can respond to anti-EGFR targeted therapy[5]. Recently, Japanese scholars have studied the immunophenotype in the tumor microenvironment (TME) of EGFR mutant LADC[6]. Patients with EGFR-mutated lung

adenocarcinoma in the analysis were treated with anti-PD-1 antibody monotherapy. However, this method are largely ineffective. Therefore, targeted therapy is only effective in a subset of appropriately selected patients with LADC [7].

Considering the advantages of recognizing the immune response in solid tumors and the immunological changes of immunotherapy in the process of LADC, immunotherapy shows an advantage in the treatment of lung cancer. Among them, PD-1 inhibitors that block the PD-1 / PD-L1 pathway have long been the first-line treatment drugs for advanced non-small cell lung cancer with positive PD-L1 expression.This therapy has dramatically improved the outcome and raised the 5-year survival rate to 15.5%-23% for patients with NSCLC [8].In a clinical trial, 305 patients with high expression of PD-1 were randomly selected and given Pembrolizumab 200 mg fixed dose every 3 weeks for 35 cycles or until disease progression compared with chemotherapy drugs. The progression-free survival rate (10.3 vs 6 months) and overall response rate (44.8% vs 27.8%) of chemotherapy drugs were significantly improved[9]. Recent studies have shown that PD-1 is highly expressed in non-small cell lung cancer[10].

Therefore, understanding of the relationship between the immune system and prognosis is of vital importance.Help us to more effectively use immuno-oncology and promote the development of this discipline to improve the efficacy of tumors. The Li Guoxin group in China first proposed a model based on immune score to predict the survival of patients with gastric cancer after surgery and the effect of adjuvant chemotherapy, predicting patients with stage II and III gastric cancer who can benefit from adjuvant chemotherapy[11]. Ju Wang et al. studied the application of immune score in prognostic of breast cancer[12]. However, there have been no reports on studies that assess the prognosis of LADC based on immune scores. In this study, we tried to evaluate the relationship between immune score and prognosis, and based on this relationship, we established a clinical nomogram to predict the survival of LADC patients.

2 Material And Methods

2.1 Materials

The data used in this study were collected from The Cancer Genome Atlas database(TCGA)[13]. TCGA is a project supported by the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI), has generated comprehensive, multi-dimensional maps of the key genomic changes in various types of cancers. TCGA is currently the largest public data set available for tumor genome analysis, including more than 200 cancer and clinical information as well as DNA methylation, gene expression, somatic mutation,etc[14].We downloaded the LADC raw data from the TCGA database (http://www.cbioportal.org/) on April, 2020.

The immune score data, from a public data platform ~ https://cancergenome.nih.gov/. The immune score is based on the number of lymphocytes in the central area of the tumor and the infiltration junction area. The score indicates that the degree of immune cell invasion in the tumor is large or small[15].

2.2 Data preprocessing

Pre-process the downloaded LADC case data and the corresponding immune score data. Remove incomplete information cases, and finally get 411 LADC case data. Use R language software (version 3.6.3) to merge the two types of data. Then remove the duplicate cases and finally get 407 case data for analysis, where each immune score corresponds to a patient.

Process the case data studied as follows. The survival status is divided into two categories ~ 0 means survival, 1 means death. Age is classified into 3 categories ~ 0 represents "≤50 years old", 1 represents "51–70 years old", and 2 represents "70 years old < ". The tumor TNM comprehensive stage is divided into 4 categories ~ 0 represents "□", 1 represents "II", 2 represents "III", and 3 represents "IV". Immune scores were classified into 3 categories ~ 0 for "low score (≤ 698.1)", 1 for "medium score (698.1 < and ≤ 1246.3)" 2 for "high score (1246.3 <)". See Sect. 2.3 for the specific grouping basis of the immune score. The outcome is whether or not new tumor is present after the start of treatment is divided into two categories ~ 0 stands for "present new tumor ", 1 stands for "no new tumor ". There are two categories of gender ~ 0 for "male" and 1 for "female". There are two categories of whether to perform radiation therapy ~ 0 represents "radiation therapy has been performed", 1 represents "no radiation therapy". In order to show our research ideas more intuitively, Fig. 1 lists the details of each stage of data preprocessing and the sample size of each stage in the form of a flowchart.

2.3 Statistical analysis

The downloaded data provides the overall survival time (OS) of each patient. OS was defined as the time from pathology diagnosis to death[16]. In this study, X-tile software (version 3.6.1 ,Yale University School of Medicine, New Haven, CT, USA) was used to get the cut-off point of the immune score[17]. In this study, X-tile was used to evaluate the immune score and divided into the best three groups, as shown in Fig. 2.

Univariate cox analysis was performed by R package survival. In the multivariate Cox regression model, all variables with p values < 0.15 in the univariate analysis were included using R software with package survival. In this study, we assessed the risk of the immune score after incorporating the tumor TNM comprehensive stage (□, □, □, □) and the factors of new tumor after treatment were also considered into the modeal. Evaluation of the adjusted hazard ratio (HR) and 95% confidence intervals(CI).And forest analyses were performed using the survminer package implemented in the R software. On the basis of multivariate COX regression analysis, use R softwar with package survival and rms to built the nomogram. Bootstrap test (1000 replicates) was performed to validate the nomogram. The prognostic accuracy was measured by calculating the concordance index (c-index) ,use R softwar with package survival .The ROC curves were calculated using the R software with package survival and timROC.The “rms and survival” package was used to generate a prognostic nomogram for 3 years and 5 years OS. Use R softwar with package survival and survminer.The Kaplan-Meier method was utilized to draw the survival curve, and the log-rank test was employed for survival analysis.

Using SPSS 21.0 to perform chi-square test on categorical variables.The continuous variables were tested for normal distribution and variance homogeneity, using one-way ANOVA.And in all statistical tests, $P < 0.05$ is considered statistically significant.

3 Results

3.1 Patients'characteristics

After the case data downloaded from the TCGA ,and database was processed, at last 407 patients were included(The specific data preprocessing is shown in Fig. 1).The mean age of the patients was 65.17 years(SD = 10.09,Range 39–88 years).33 patients (8.1%) were younger than 50 years of age, 237 patients (58.2%) were 51 to 70 years old, and 137 patients (33.7%) were older than 70 years of age.Of the 407 patients, 183(45.0%) and 224(55.0%) were men and women, respectively.TNM staging of tumors, I 223 (54.8%), Ⅱ 105 (25.8%), Ⅲ 62 (15.2%), Ⅳ 17 (4.2%).Patients were divided into subgroups with high, medium, and low immune scores (X-tile diagram, shown in Fig. 2).154 (37.8%) had an immune score less than or equal to 698.1 (low score group), 155 (38.1%) had an immune score greater than 1246.3 (high score group), and had an immune score greater than 698.1 (less than or equal to 1246.3 (medium score group) Of 98 (24.1%).There were 156 new tumors (38.3%) after the first treatment, and 251 (61.7%) did not find new tumors; 58 patients (14.3%) had received radiotherapy, and 349 had not received radiotherapy (85.7%).

Table 1

lists the clinical pathology and other characteristics of the research subjects according to the subgroups of immune scores.The average ages of the high, middle and low subgroups of the immune score were: 62.27 (SD = 10.48) years, 68.05 (SD = 9.39) years, 66.24 (SD = 9.42) years.As for the new lesions found after treatment, they are more likely to occur in the low immune score group.In the "≤50" age group, the number of people in the low immune score group is the largest. This situation is similar to the "51–70" age group.Patients who had received radiotherapy were average in the immune score subgroup.But among the patients who did not receive radiotherapy, the highest proportion of the high immune score group.Female patients have the highest proportion in the high immune score subgroup and male patients have the opposite situation.In the staging of TNM tumors, high immune score groups and are more likely to appear.

Table 1 Correlation between clinicopathological characteristics and immune score in 407 patients with LADC

		Immune scores				
Characteristics	Total	≤ 698.1	698.1to1246.3	> 1246.3	x^2 value	*P* value
Sample size	407	154(37.8%)	98(24.1%)	155(38.1%)		
Age					19.36	0.001
≤ 50	33	22(14.3)	3(3.1)	8(5.1)		
51–70	237	95(67.1)	56(57.1)	86(55.5)		
> 70	137	37(24.0)	39(39.8)	61(39.4)		
New_tumor					0.70	0.71
Yes	156	63((40.9)	36(36.7)	57(36.8)		
No	251	91(59.1)	62(63.3)	98(63.2)		
Radiotion					0.910	0.64
Yes	58	23(14.9)	16(16.3)	19(12.3)		
No	349	131(85.1)	82(83.7)	136(87.7)		
Sex					8.330	0.015
Male	183	83(53.9)	41(41.8)	59(38.1)		
Female	224	71(46.1)	75(58.2)	96(61.9)		
TNM stage					21.370	0.002
	223	80(51.9)	45(45.9)	98(63.2)		
	105	41(26.6)	24(24.5)	40(25.8)		
	62	22(14.3)	26(26.5)	14(9.1)		

Table 1 Correlation between clinicopathological characteristics and immune score in 407 patients with LADC

□	17	11(7.1)	3(3.1)	3(1.9)

3.2 Univariate and multivariate analyses for OS

The correlation between the OS of LADC patients and clinicopathological characteristics was explored using a univariate analysis, see Table 2.As shown in Table 2 and Fig. 3,Patients with low, medium, and high scores based on immune score group have significant differences in OS ($P < 0.001$).In addition,there is a statistically significant difference in OS in the TNM stage of tumor ($p = 0.002$).There were also significant differences in the occurrence of new tumors after the initial treatment ($p < 0.001$) .There is also a difference in OS whether receiving radiotherapy ($p = 0.003$).However, there is no difference between age subgroup and gender subgroup ($p > 0.05$).

Table 2
Univariate analyses of OS among LADC patients according to clinic pathological characteristics and immune scores

		OS			
Characteristics	total	survival	Deadth	HR(95%CI)	*P* value
Age					
≤ 50	33	20(60.6)	13(39.4)	1.0	
51–70	237	164(69.2)	73(30.8)	0.768 (0.424–1.391)	0.384
> 70	137	78(56.9)	59(43.1)	1.199 (0.653-2.200)	0.558
Sex					
Male	183	117(63.9)	145(64.7)	1.0	
Female	224	66(36.1)	79(35.3)	0.917(0.661–1.273)	0.605
New-tumor					
Yes	156	61(39.1)	95(60.9)	1.0	
No	251	201(76.7)	50(34.5)	0.381(0.270–0.536)	< 0.001
Radiation					
Yes	58	26(9.9)	32(22.1)	1.0	
No	349	236(90.9)	113(77.9)	0.548(0.370–0.812)	0.003
TNM stage					
	223	167(74.9)	56(38.6)	1.0	
	105	60(57.1)	45(42.9)	2.190(1.472–3.258)	< 0.001
	62	27(43.5)	35(56.5)	3.110(2.029–4.767)	< 0.001
	17	8(47.1)	9(52.9)	2.645(1.304–5.365)	0.007
Immune scores					
≤ 698.1	175	86(32.8)	68(46.9)	1.0	
698.1to1246.3	129	59(22.5)	39(26.9)	0.837(0.564–1.241)	0.375
1246.3>	182	117(44.7)	38(26.2)	0.457(0.308–0.683)	0.0001

Multivariate Cox proportional hazards regression analysis results are shown in Table 3.Compared with patients with low immune score and middle subgroup, patients with high immune score subgroup significantly improved OS(HR and 95% CI:0.488 [0.327–0.730]).In tumor staging, the OS of patients with stage II, III, and IV was significantly worse than those with stage I (HR and 95% CIfor stages II, III, and IV

were 2.117 [1.415–3.168], 3.077 [1.939–4.883] and 1.914 [0.937–3.911]).After the first treatment, patients without new lesions had better OS than those with new lesions, and their HR and 95% CI were 0.387 [0.273–0.550]).However, whether or not to receive radiotherapy did not show differences in multiple Cox risk regression analysis.Therefore, was not included in the multivariate model.

Table 3
Multivariate analyses of OS among LADC patients according to immune scores and clinic pathological characteristics

	OS				
Characteristics	total	survival	Deadth	HR(95%CI)	*P* value
Radiation	486				
Yes	58	26(9.9)	32(22.1)	1.0	
No	349	236(90.9)	113(77.9)	0.815(0.533,1.246)	0.345
New_tumor					
Yes	156	61(39.1)	95(60.9)	1	
No	251	201(76.7)	50(34.5)	0.387(0.273 ,0.550)	< 0.001
TNM stage					
	223	167(74.9)	56(38.6)	1	
	105	60(57.1)	45(42.9)	2.117(1.415,3.168)	< 0.001
	62	27(43.5)	35(56.5)	3.077(1.939,4.883)	< 0.001
	17	8(47.1)	9(52.9)	1.914(0.937,3.911)	0.075
Immune scores					
≤ 683.7	175	86(32.8)	68(46.9)	1	
683.7to1246.3	129	59(22.5)	39(26.9)	0.821(0.547,1.233)	0.342
1246.3>	182	117(44.7)	38(26.2)	0.488(0.327,0.730)	< 0.001

3.3 Prognostic nomogram for OS

The associations between potential prognostic factors and OS were evaluated by univariate and multivariate analyses using a Cox regression model. The potential prognostic factors: immune score, tumor staging and whether or not occurrence of new tumors after the initial treatment .Eliminate whether or not receiving radiotherapy(p = 0.345).From the results of the multivariate Cox regression analysis, the forest curves were plotted. The forest plot visualize the relationship between the potential prognostic factors and OS, see Fig. 4.

The prognostic nomogram that integrated all significant independent factors from multivariate analysis for OS was shown in Fig. 5.The C-index for OSpredictions were 0.707(95%CI,0.66–0.75). On the basis of above analysis, a receiver-operator-characteristics (ROC) curve was drawn, and the area under the curve (AUC) was calculated. AUC of 3 years and 5 years are 0.74 and 0.736, respectively (see Fig. 6).The reliability of the prediction model is proved by C index and ROC curve.

Calibration curves for 3- and 5-year survival showed good consistency in the probability between the actual observation and the nomogram prediction (Fig. 7A,B).

A

B

4 Discussion

In this study, we used publicly available data from the TCGA repositories to identify immune score associated with OS of LADC. Also after adjusting for possible confounding factors, we found that a high immune score subgroup was significantly associated with OS in patients with LADC. At the same time, we have also established a nomogram, which can easily predict the survival time of patients with LADC.

A significant contribution of immune cells to LADC has been widely accepted[18],and immune gene was thinked as a biomarker for immune response in immunotherapy[19]. Earlier studies also showed that some immune genes are significantly related to the prognosis of LADC[20]. In addition, other studies have found that immune gene expression should be included in the current multi-gene test to improve the prognosis of patients with LADC[21, 22]. However, these studies have not been used to predict the probability of OS in clinical studies. In addition, there are few studies that take the immune score into the nomogram. In the current study, based on the TCGA data set, the clinicopathological information and immune score of LADC patients were used to explore the relationship and prognosis. Furthermore, a nomogram has been established to easily evaluated the prognosis of patients with LADC.

Several possible confounders were adjusted in this study, the higher the immune score, the higher the OS of LADC patients. Similar results were observed in the Pagès F, et al[23],Study.The possible reason is that the high immune score indicates that the immune system and immune function are enhanced, which can improve the anti-tumor immunity of the tumor microenvironment, thereby controlling and eliminating the tumor[24]. In addition, related studies have found that in patients with relatively long-lived tumors, genes related to immune cell activation are significantly increased[25]. Moreover, some important genes, such as CD302, are used to calculate immune characteristics and play a vital role in immune function[26]. Furthermore, a study of T cell-related markers, CD3 + and CD8 + LADC patients with higher expression and patients receiving related neoadjuvant chemotherapy has a good prognosis[27]. Therefore, the immune score can not only be used as a prognostic biomarker for patients with LADC, but also has potential clinical value in the selection of treatment strategies.

In this study, we found that whether a new tumor occurs after the first treatment is an important independent prognostic factor for patients with lung adenocarcinoma ($p < 0.001$). Patients who did not develop new tumors after the first treatment had the highest proportion in the high immune score subgroup, and also had better OS, HR ~ 0.387; 95% CI ~ 0.273–0.550. With the increasing awareness of cancer early screening among citizens, and the improvement of medical and health level. The early detection rate of cancer has increased significantly(Similar to our study, more than 50% of the patients enrolled were stage I patients). However, these results were different from previous studies. Earlier studies have confirmed that LADC is at an advanced stage the majority of LADC patents are at advanced stages (□ / □) when initially diagnosed, missing the operation opportunity[28, 29]. Early detection greatly increases the success of patient treatment and prolongs patient survival. For the early stage LADC patients, the main treatment is surgery, with adjuvant radiotherapy and chemotherapy. However, there have been reports that chemical / radiotherapy has the possibility of long-term carcinogenesis[30]. Postoperative radiochemotherapy followed, can reduce the patient's own immunity to weaken the defense ability against the tumor, and also benefit the tumor regeneration. If the LADC patients with high immune scores before and after surgery, take immunotherapy or combined immunotherapy on the basis of precise gene sequencing can reduce the harm caused by radiotherapy and chemotherapy[31]. Studies have confirmed that postoperative immunotherapy generally reduces the local recurrence rate by about 30% and significantly reduces distant metastases. Even if there is a relapse, it will obviously move backwards in time, and it can increase the 5-year survival rate by about 20% [32]. It was confirmed ~ as for surgically resectable non-small cell lung cancer, neoadjuvant immunotherapy, high safety does not affect surgery, and the pathological significant remission rate is 45%, and the 18-month relapse-free survival rate is 73% [33]. Studies have also shown that early non-small cell cancer administration of immune drugs to block multiple molecular tags can kill cancer cells with fewer side effects[34]. Therefore, in addition to the traditional treatment of lung cancer patients, taking immunotherapy can improve the survival rate of patients[35].

The prognostic model of LADC constructed based on immune cell infiltration score and clinicopathological characteristics illustrates the relationship between immune cell infiltration and its occurrence and development. The prognostic model we established can effectively predict the 3-year and 5-year survival rates of LADC patients, it also suggests the role of different immune score subgroups in the development of LADC. This discovery provides new ideas for the treatment and prognosis of LADC from the perspective of immune cell infiltration. However, in our study, we found that there are relatively few data sets involving gene expression data that can be used to calculate immune scores. So, our prognostic model is effectively limited, and no external data verification is performed but performed effective internal verification. Therefore, there is still a lot of work to be done in the future. In addition to including clinical pathological factors, further efforts will be made to collect case data related to immune gene expression to update and develop our model.

5 Conclusion

This study indicate that those patients with LADC, high immune score are significantly associated with better OS. In addition, we build and effectively use nomogram to predict prognosis. This practical prognostic model can easily evaluate the OS of patients and determine subgroups of patients who need active treatment .

Abbreviations

NSCLC,non-small cell lung cancer;OS, overall survival;LDAC, lung adenocarcinoma;HR, hazard ratios;CI, confidence intervals; c-index,concordance index;EGFR,epidermal growth factor receptor; TME,the tumor microenvironment;TCGA,The Cancer Genome Atlas; NCI, National Cancer Institute; NHGRI, National Human Genome Research Institute;ROC,receiver-operator-characteristics;AUC,areaunder the curve.

Declarations

Availability of data and materials

Not applicable.

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Authors' contributions

Xie Hui designed the study, searched, analyzed and interpreted the literature and was a major contributor in writing the manuscript. Zhang Jianfang collect the case data and Li Qing revised the manuscript.

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Figures

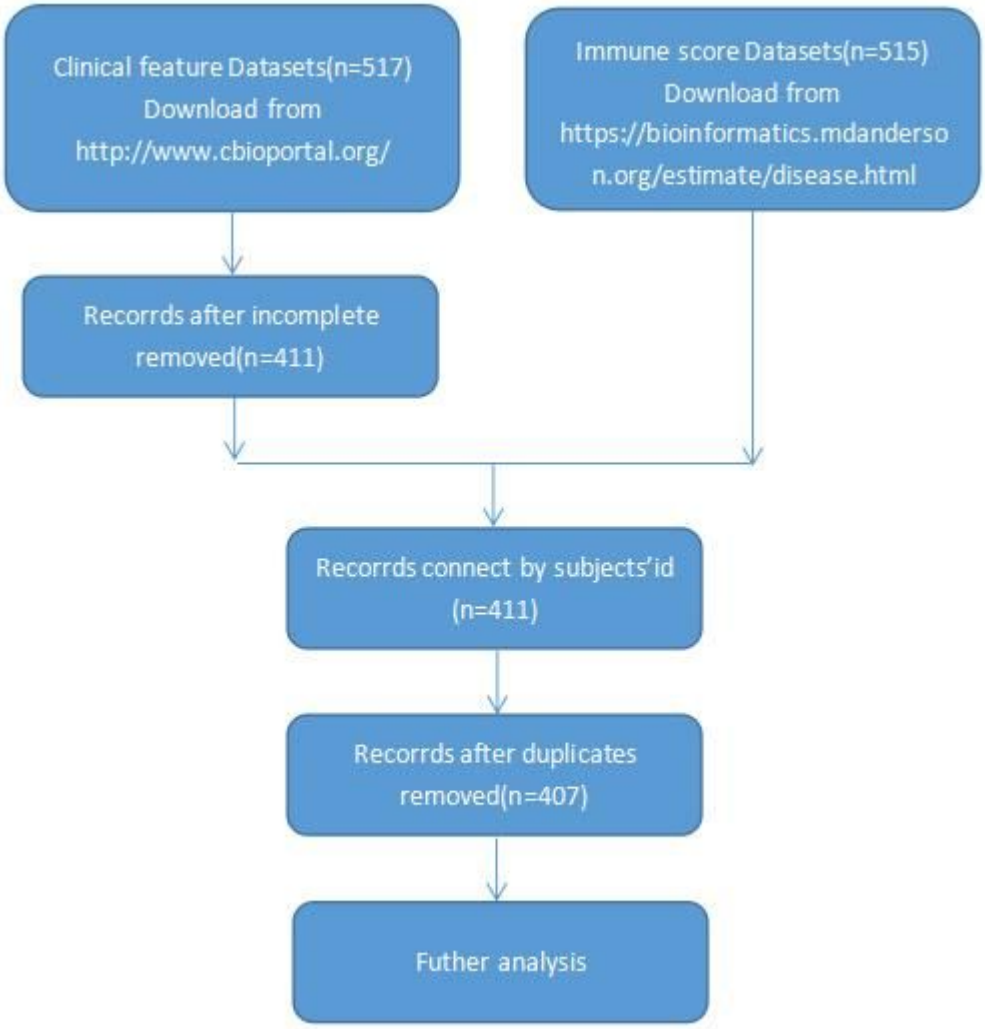

Figure 1

Research flow chart details the sample flow for each analysis stage

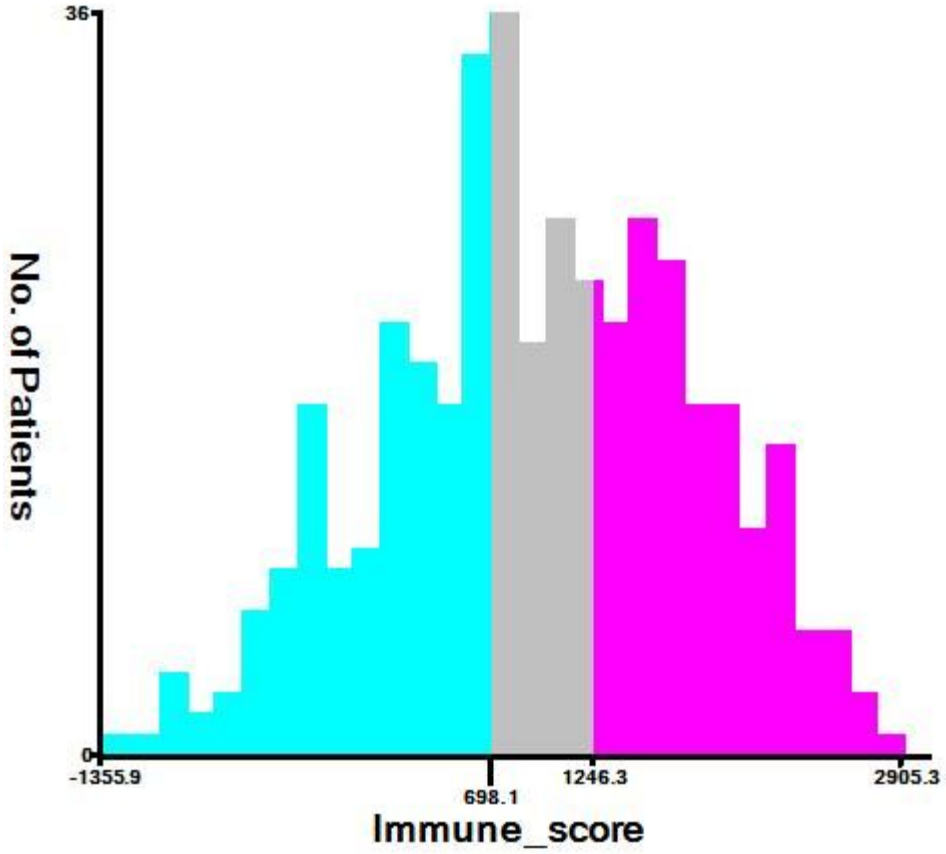

Figure 2

Cutoff values for high, medium, and low immune scores, as determined by X-tile software(X-axis represents immune score, Y-axis represents number of people).

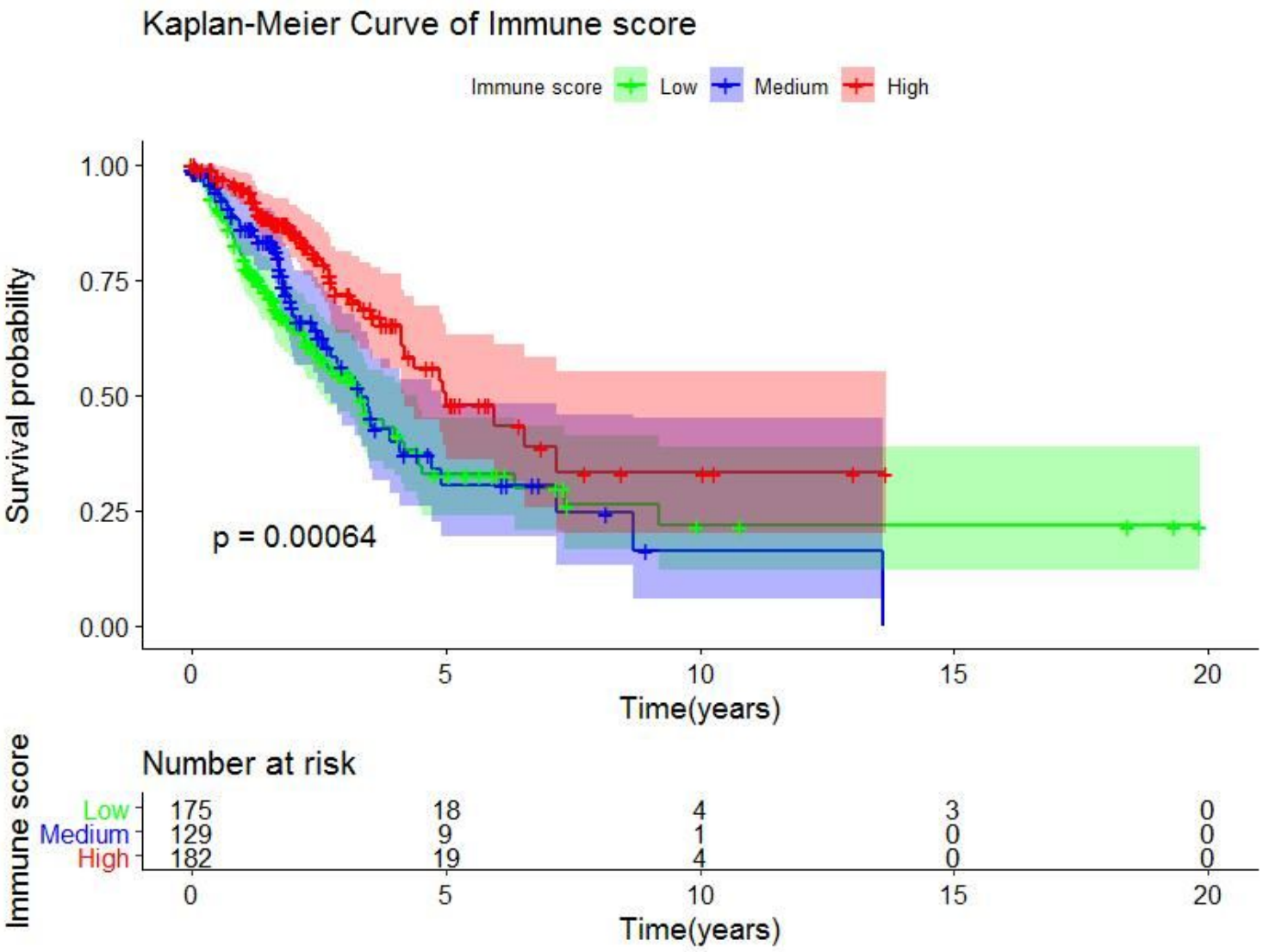

Figure 3

Kaplan-Meier curves are displayed associations of immune scores subgroups with overall survival (OS) for patients with LADC. Comparison of OS among patients with ≤698.1 immune scores (group low), patients with immune scores between 698.1 and 1246.3 (group intermediate), and patients with>1246.3 immune scores (group high).

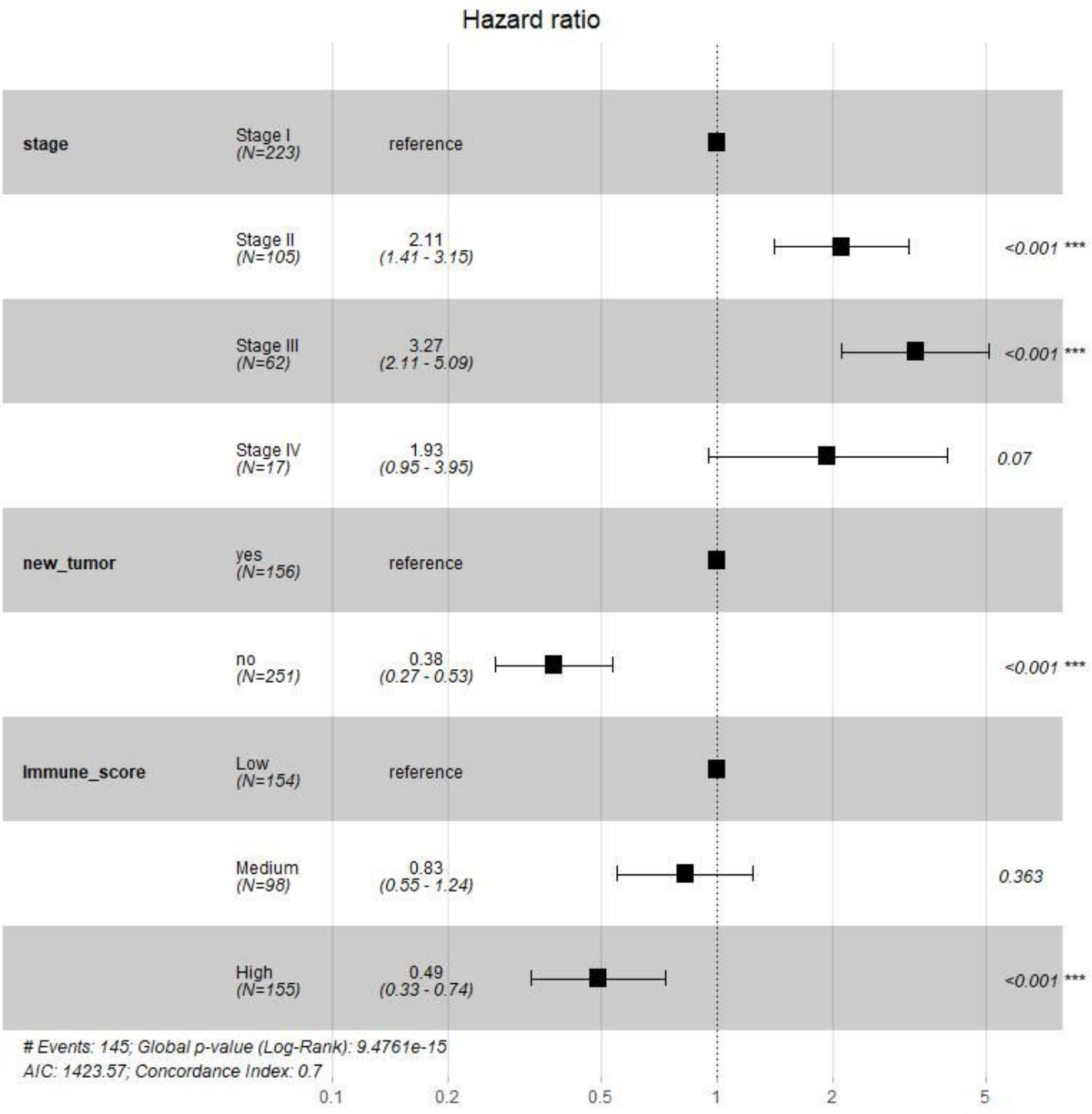

Figure 4

Forest plot of overall survival (OS).Subgroups analyses of OS performed using patient multivariate analyses characteristices.

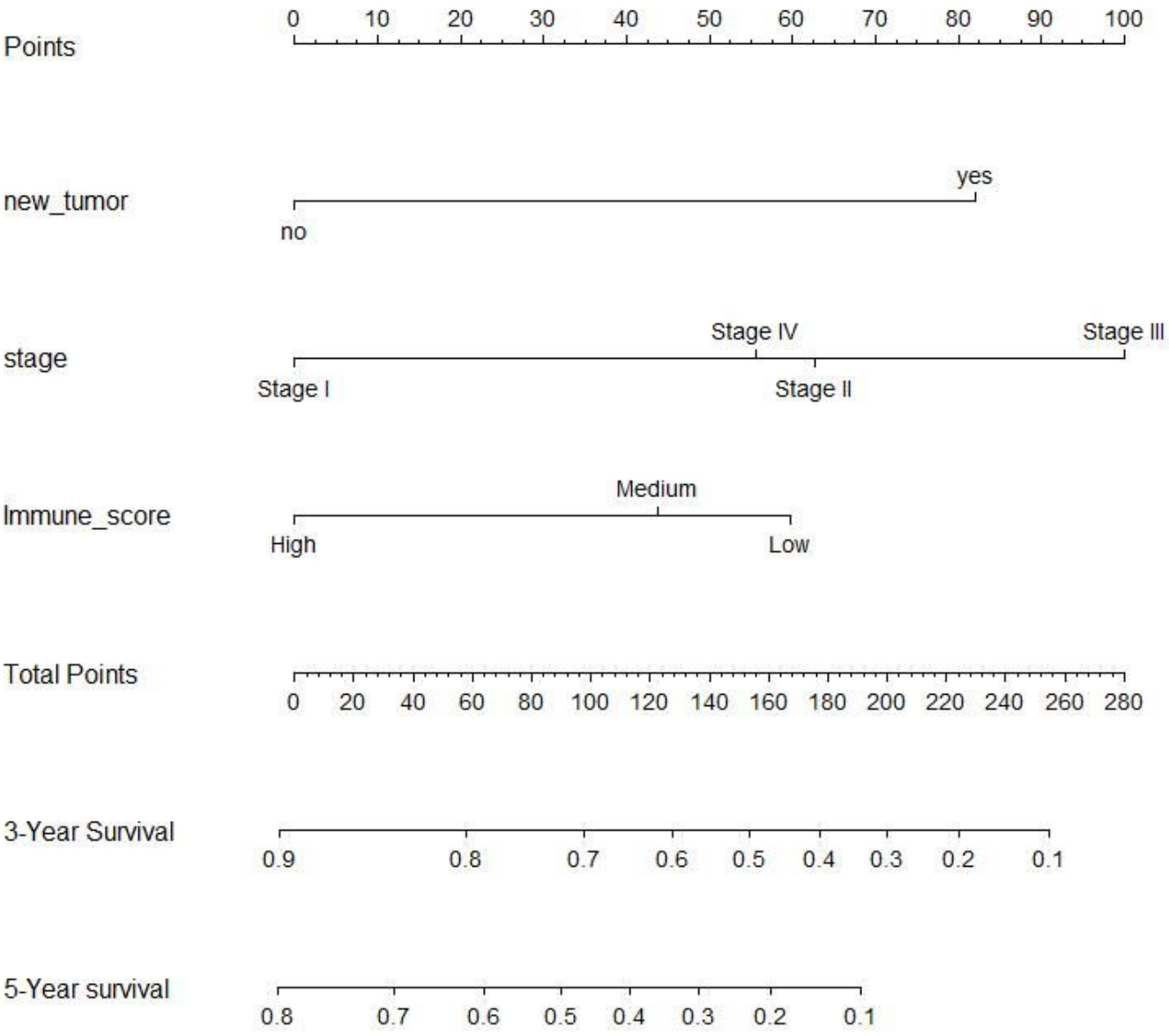

Figure 5

Nomograms for predicting survival of LADC.

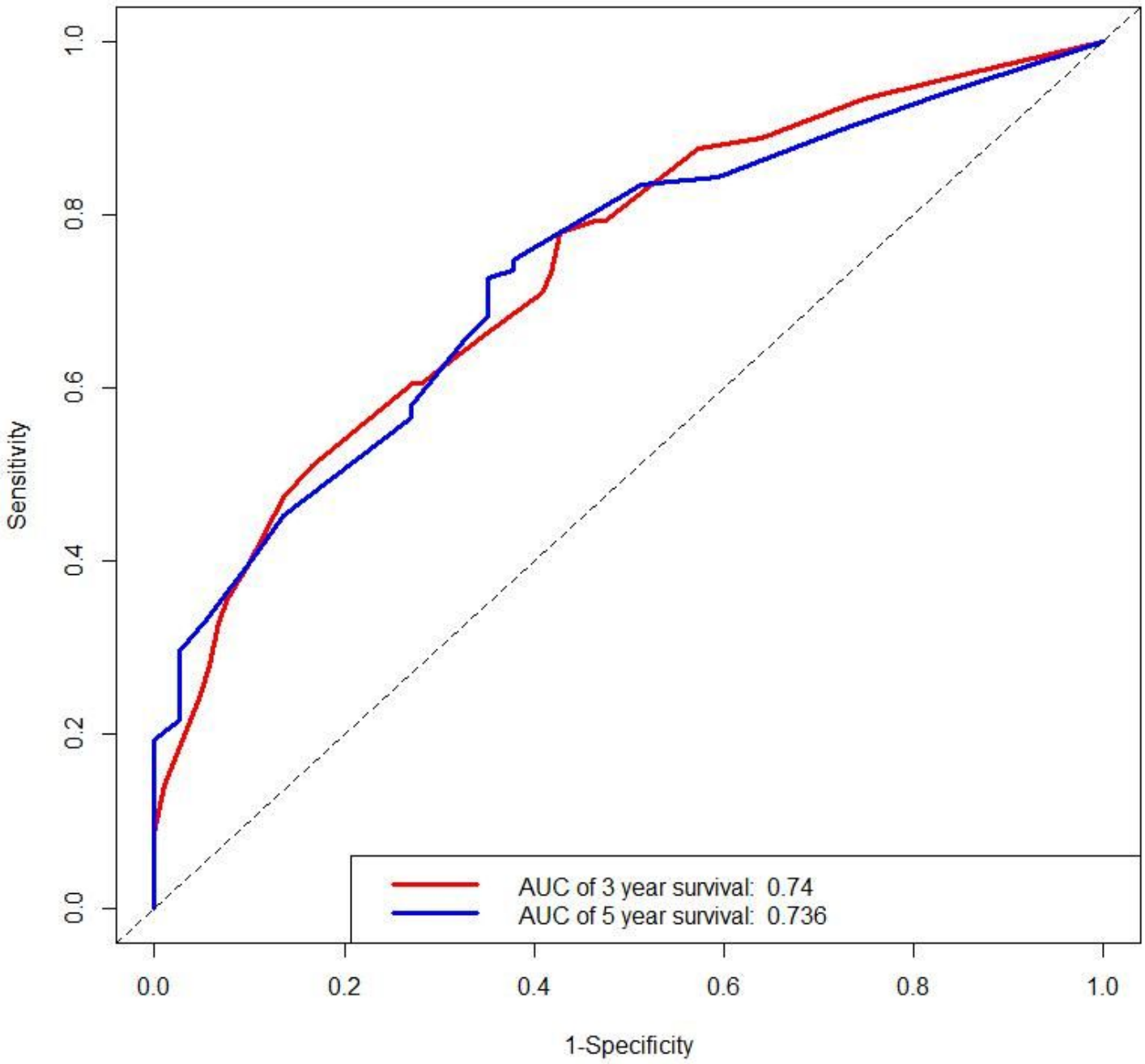

Figure 6

The ROC curve of overall survival (OS) at 3 and 5 years for the LADC .

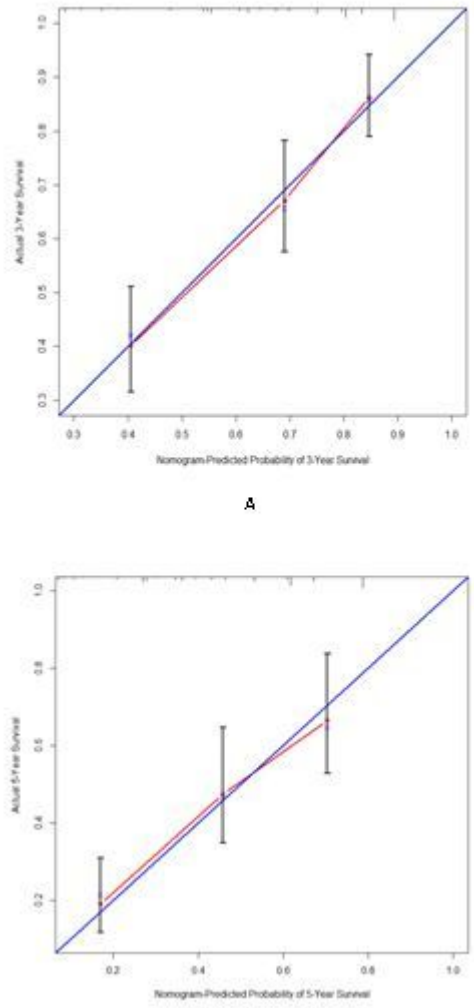

Figure 7

Calibration curve for 3 and 5 years overall survival (OS) to the LADC . Nomogram-predicted probability of OS is plotted on the x-axis; actual OS is plotted on the y-axis.