

Prognostic Nomogram Incorporating Immunohistochemical Results for the Overall Survival of Patients with Bladder Cancer.

Tianwei Wang (✉ 1634489391@qq.com)

Huaian First People's Hospital

Yunyan Wang

Huaian First People's Hospital

Research article

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Abstract

Objectives: In this study, we want to combine GATA3, VEGF, EGFR and Ki67 with clinical information to develop and validate a prognostic nomogram for bladder cancer.

Methods: A total of 188 patients with clinical information and immunohistochemistry were enrolled in this study, from 1996 to 2018. Univariable and multivariable cox regression analysis was applied to identify risk factors for nomogram of overall survival (OS). The calibration of the nomogram was performed and the Area Under Curve (AUC) was calculated to assess the performance of the nomogram. Internal validation was performed with the validation cohort., the calibration curve and the AUC were calculated simultaneously.

Results: Univariable and multivariable analysis showed that age (HR: 2.229; 95% CI: 1.162-4.274; P=0.016), histology (HR: 0.320; 95% CI: 0.136-0.751; P=0.009), GATA3 (HR: 0.348; 95% CI: 0.171-0.709; P=0.004), VEGF (HR: 2.295; 95% CI: 1.225-4.301; P=0.010) and grade (HR: 4.938; 95% CI: 1.339-18.207; P=0.016) remained as independent risk factors for OS. The age, histology, grade, GATA3 and VEGF were included to build the nomogram. The accuracy of the risk model was further verified with the C-index. The C-index were 0.65 (95% CI, 0.58-0.72) and 0.58 (95% CI, 0.46-0.70) in the training and validation cohort respectively.

Conclusions: A combination of clinical variables with immunohistochemical results based nomogram would predict the overall survival of patients with bladder cancer.

Introduction

With increasing incidence and mortality, bladder cancer (BCa) has become one of the most important public health problems. With over 549,000 new cases and 200,000 deaths estimated worldwide, bladder cancer ranking in 10th place for incidence and 14th place for mortality[1]. In respect to disease management, surgery, chemotherapy and immunotherapy are widely used clinically[2]. However, prognosis after these therapies is unsatisfactory, with a high incidence of recurrence and mortality. Therefore, the prognostication and risk assessment is crucial for treatment decisions.

The American Joint Committee on Cancer (AJCC) TNM staging system is used widely to predict disease recurrence. To predict the probabilities of disease recurrence and progression, the European Organization for Research and Treatment of Cancer (EORTC) also developed a scoring system and risk tables. What's more, the Bladder Cancer Research Consortium (BCRC) and the International Bladder Cancer Nomogram Consortium (IBCNC) also constructed risk models respectively[3]. On the one hand, these models show advantages in estimating prognosis in a certain population. On the other hand, they never include novel information such as biomarkers, genetic markers and molecular classifications and so on.

Risk models often include p53[4], SMAD6[5], MMP11[6], Ki67[7] and so on to predict prognosis in recent studies. Although GATA3 has been validated associated with bladder cancer prognosis and shows a

protective effect[8, 9], which has not been involved in risk models ever. In this study, we combine GATA3, VEGF, EGFR, Ki67 and clinical information to develop and validate a prognostic nomogram for bladder cancer.

Material And Methods

Patients

This retrospective study was approved by the Institutional Review Board and the patient informed consent was obtained before surgery. We retrospectively identified 4362 bladder cancer patients in the pathology database from January 1996 to July 2018. A total of 188 patients with clinical information and immunohistochemistry were enrolled in this study finally according to the following inclusion criteria: (1) Pathology confirmed bladder cancer; (2) The pathological results showed immunohistochemical information; (3) Clinical information available; (4) The patients with follow up data. In this study, patients were distributed into training cohort and validation cohort according to a ratio of 7:3. The flowchart is shown in Fig. 1.

Recorded data

We collected clinical information from medical records, pathological reports and telephone follow-up. Variables which had been widely studied in bladder cancer were included as explanatory variables in our study. These factors include:

- Age (≤ 65 years vs. >65 years);
- Gender (male vs. female);
- Smoking (no vs. yes);
- The number of tumor (solitary vs. multiple);
- Tumor size (≤ 3 cm vs. >3 cm);
- Tumor grade (high vs. low);
- Tumor stage (superficial vs. invasive);
- Histology (papillary vs. others);
- Lymph node metastasis (no vs. yes);
- Microwave invasion (no vs. yes);
- Nerve invasion (no vs. yes);
- Radical cystectomy (no vs. yes);
- Systemic chemotherapy (no vs. yes);
- GATA3 (low vs. high);
- VEGF (low vs. high);

- EGFR (low vs. high);
- Ki67(low vs. high)

The superficial stage included pTa, pT1 and TIS and the invasive stage incorporated pT2, pT3 and pT4. Squamous carcinoma, adenocarcinoma, sarcomatoid carcinoma, neuroendocrine carcinoma and so on were defined as other histology. Cases with no, focal or suspicious GATA3 expression were defined as low expression, meanwhile cases with exact GATA3 expression(include “+,++–+++–++++”) were defined as high expression, the same with VEGF, EGFR and Ki67. The study response variable was the overall survival, which was defined as from diagnosis of bladder cancer to death or censoring.

Construction of the clinical factor model

Univariable cox regression analysis was used to identify factors that affect overall survival. Multivariable cox regression analysis was applied to develop the risk models by using the significant factors ($P < 0.1$) from the univariable Cox regression analysis as inputs. Hazard ratio (HR) and 95% confidence intervals (CI) were calculated for every dependent variable.

Validation of the nomogram

The calibration of the nomogram was performed and the Area Under Curve (AUC) was calculated to assess the performance of the nomogram. Internal validation was performed with the validation cohort., the calibration curve and the AUC were calculated simultaneously.

Statistical analysis

Statistical analysis was performed with SPSS (version 22.0, IBM). Univariable and multivariable cox regression analysis were used to screen risk factors to build nomogram. Figures were plotted using R statistical software (version 3.5.3, <https://www.r-project.org>). The nomogram development and calibration plots were performed using the “rms” package and the receiver operating characteristic curves (ROC) was plotted using the “pROC” package. The Kaplan–Meier survival curve was calculated using the “survival” package. The $p < 0.1$ was considered statistical significance in univariate analysis, while $p < 0.05$ was considered statistical significance in the multivariate analysis.

Results

Clinicopathological characteristics of patients

188 patients met our inclusion criteria, who were divided into training (124 cases) and validation (64 cases) sets. The clinical variables of the patients in the training and validation cohorts are provided in

Table 1. All factors were found no difference between two groups. The mean overall survival was 44.07 months and 36.69 months in the two groups, which show no difference ($P= 0.262$).

Table 1
Baseline characteristics of the study population

Characteristics	Training cohort (n = 124)	Validation cohort (n = 64)	<i>P</i> value
Age(years), <i>n</i> (%)			
≤65	63(50.81)	23(35.94)	
>65	61(49.19)	41(64.06)	0.052
Gender, <i>n</i> (%)			
Male	91(73.39)	43(67.19)	
Female	33(26.61)	21(32.81)	0.373
Smoking, <i>n</i> (%)			
No	112(90.32)	54(84.38)	
Yes	12(9.68)	10(15.62)	0.383
Multifocality, <i>n</i> (%)			
Solitary	72(58.06)	38(59.38)	
Multiple	52(41.94)	26(40.62)	0.753
Size(cm), <i>n</i> (%)			
≤3	79(63.71)	38(59.38)	
>3	45(36.29)	26(40.62)	0.561
Grade, <i>n</i> (%)			
Low	29(23.39)	15(23.44)	
High	95(76.61)	49(76.56)	0.994
Tumor stage, <i>n</i> (%)			
Superficial(pTa,pT1,TIS)	52(41.94)	29(45.31)	
Invasive(pT2 or more)	72(58.06)	35(54.69)	0.658
Histology, <i>n</i> (%)			
papillary	81(65.32)	45(70.31)	
Others(squamous carcinoma,adenocarcinoma,sarcomatoid carcinoma et al)	43(34.68)	19(29.69)	0.490
Lymph node metastasis, <i>n</i> (%)			

Characteristics	Training cohort (n = 124)	Validation cohort (n = 64)	<i>P</i> value
No	106(85.48)	58(90.63)	
Yes	18(14.52)	6(9.37)	0.317
Microwave invasion, <i>n</i> (%)			
No	101(81.45)	54(84.37)	
Yes	23(18.55)	10(15.63)	0.618
Nerve invasion, <i>n</i> (%)			
No	98(79.03)	56(87.50)	
Yes	26(20.97)	8(12.50)	0.153
Radical cystectomy, <i>n</i> (%)			
No	68(54.84)	37(57.81)	
Yes	56(45.16)	27(42.19)	0.697
Systemic chemotherapy, <i>n</i> (%)			
No	99(79.84)	55(85.94)	
Yes	25(20.16)	9(14.06)	0.303
GATA3			
Low	76(61.29)	45(70.31)	
High	48(38.71)	19(29.69)	0.221
VEGF			
Low	60(48.39)	23(35.94)	
High	64(51.61)	41(64.06)	0.103
EGFR			
Low	59(47.58)	30(46.88)	
High	65(52.42)	34(53.12)	0.927
Ki67			
Low	53(42.74)	26(40.63)	
High	71(57.26)	38(59.37)	0.781

Prognostic variables associated with overall survival

Univariable and multivariable COX regression analysis were performed in the training cohort, results of which are showed in Table 2. Univariable COX regression analysis indicated that age (HR: 1.884; 95% CI: 1.021–3.478; P = 0.043), grade (HR: 5.700; 95% CI: 1.760-18.454; P = 0.004), tumor stage (HR: 2.277; 95% CI: 1.182–4.385; P = 0.014), histology (HR: 0.247; 95% CI: 0.109–0.559; P = 0.001), VEGF (HR: 2.107; 95% CI: 1.141–3.890; P = 0.017) and EGFR (HR: 2.150; 95% CI: 1.121–4.123; P = 0.021) were significant. Besides, GATA3 (HR: 0.538; 95% CI: 0.280–1.031; P = 0.062) showed marginal significance for predicting overall survival.

Table 2
Univariable and multivariable COX regression analysis of variables associated with tumor overall survival.

Variables	Univariable HR (95% CI)	P value	Multivariable HR (95% CI)	P value
Age	1.884(1.021–3.478)	0.043	2.229(1.162–4.274)	0.016
Gender	0.702(0.365–1.350)	0.289		
Smoking	1.198(0.666–2.156)	0.546		
Multifocality	0.912(0.676–1.231)	0.549		
Size	0.833(0.439–1.583)	0.577		
Grade	5.700(1.760-18.454)	0.004	4.938(1.339–18.207)	0.016
Tumor stage	2.277(1.182–4.385)	0.014	0.900(0.429–1.886)	0.779
Histology	0.247(0.109–0.559)	0.001	0.320(0.136–0.751)	0.009
Lymph node metastasis	1.749(0.835–3.665)	0.138		
Microwave invasion	1.639(0.825–3.257)	0.159		
Nerve invasion	1.168(0.575–2.375)	0.667		
Radical cystectomy	1.363(0.748–2.483)	0.311		
Systemic chemotherapy	1.262(0.582–2.738)	0.556		
Ki67	1.332(0.717–2.477)	0.364		
GATA3	0.538(0.280–1.031)	0.062	0.348(0.171–0.709)	0.004
VEGF	2.107(1.141–3.890)	0.017	2.295(1.225–4.301)	0.010
EGFR	2.150(1.121–4.123)	0.021	1.621(0.834–3.153)	0.155

These variables were included in the multivariable analysis, which showed that age (HR: 2.229; 95% CI: 1.162–4.274; P = 0.016), histology (HR: 0.320; 95% CI: 0.136–0.751; P = 0.009), GATA3 (HR: 0.348; 95%

CI: 0.171–0.709; $P = 0.004$), VEGF (HR: 2.295; 95% CI: 1.225–4.301; $P = 0.010$) and grade (HR: 4.938; 95% CI: 1.339–18.207; $P = 0.016$) remained as independent risk factors (AS shown in Table 2).

The nomogram construction and assessment

The age, histology, grade, GATA3 and VEGF were included to build the nomogram (Fig. 2). The accuracy of the risk model was further verified with the C-index and calibration curve. The C-index were 0.65 (95% CI, 0.58–0.72) and 0.58 (95% CI, 0.46–0.70) in the training and validation cohort respectively. Figure 3a, c shows the calibration curve for the 3-year survival of the nomogram both in training and validation groups. Figure 3b, d shows the calibration curve for the 5-year survival of the nomogram both in training and validation groups. The diagnostic performance for the nomogram was using ROC curves that are shown in Fig. 4. The area under curve (AUC) for time points of 36 and 60 months were 0.689 and 0.813 respectively in the training cohort.

Discussion

The EORTC risk tables and the Club Urológico Español de Tratamiento Oncológico (CUETO) scoring model are most widely used and validated tools for prognosis prediction in bladder cancer. However, they all overestimate the recurrence and progression risks in patients[10]. There are limited studies that have incorporated biomarkers into predictive models. What's more, GATA3 has never been included in nomogram. Thus, we tried to combine clinical factors and pathological immunohistochemical results to construct a practical predictive model.

The high heterogeneity of bladder cancer makes it difficult to predict the prognosis. Previous studies have suggested that many factors influence the prognosis of patients with bladder cancer, such as age, grade, histology, Ki67, VEGF, GATA3 and so on. In our study, we found that age, histology, grade, GATA3 and VEGF were independent risk factors for overall survival, which is consistent with previous studies[11–15]. A new nomogram model was constructed based on these five variables.

Many researchers have found that the indicators of immunohistochemistry are associated with the prognosis of the tumor. For example, Nakahara K, et al reported that PAR-2 is associated with the recurrence of bladder cancer based on immunohistochemical analysis[16]. March-Villalba JA, et al believed that Ki-67, Cyclin D1, p16INK4a and Survivin are predictive indicators for bladder cancer[17]. However, such a few studies have combined the immunohistochemical indicators and clinical factors to build risk models to predict the prognosis of bladder cancer.

Our study has several limitations. Firstly, as a retrospective single-center design, there was unavoidable selection bias. Hence, we need to cooperate with other agencies and incorporate more cases. Secondly, because of immunohistochemical indicators are limited, we cannot include more indicators to optimize the model. We will collect pathological sections from the department of pathology to further fulfill the pathological data. Finally, an external analysis was not performed with an independent validation cohort

to further verify the model. In summary, we need to validate and optimize the risk model with a larger, multicenter, prospective study.

In conclusion, we have shown that a combination of clinical variables with immunohistochemical results based nomogram would predict the overall survival of patients with bladder cancer. This kind of model may serve as an effective tool to make clinical decisions.

Declarations

Guarantor

The scientific guarantor of this publication is Tianwei Wang.

Conflict of interest

The authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

Ethics approval and consent to participate

The study received the approval from Ethics Committee of The affiliated Huaian No.1 people's Hospital of Nanjing Medical University(Approval No. YX-P-2020-001-01).

Statistics and biometry

One of the authors have significant statistical expertise (Tianwei Wang).

Methodology

- Retrospective
- Observational
- Performed at one institution

Author's Contribution

TW Wang: Manuscript writing and Data collection

YY Wang: Protocol/project development

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Figures

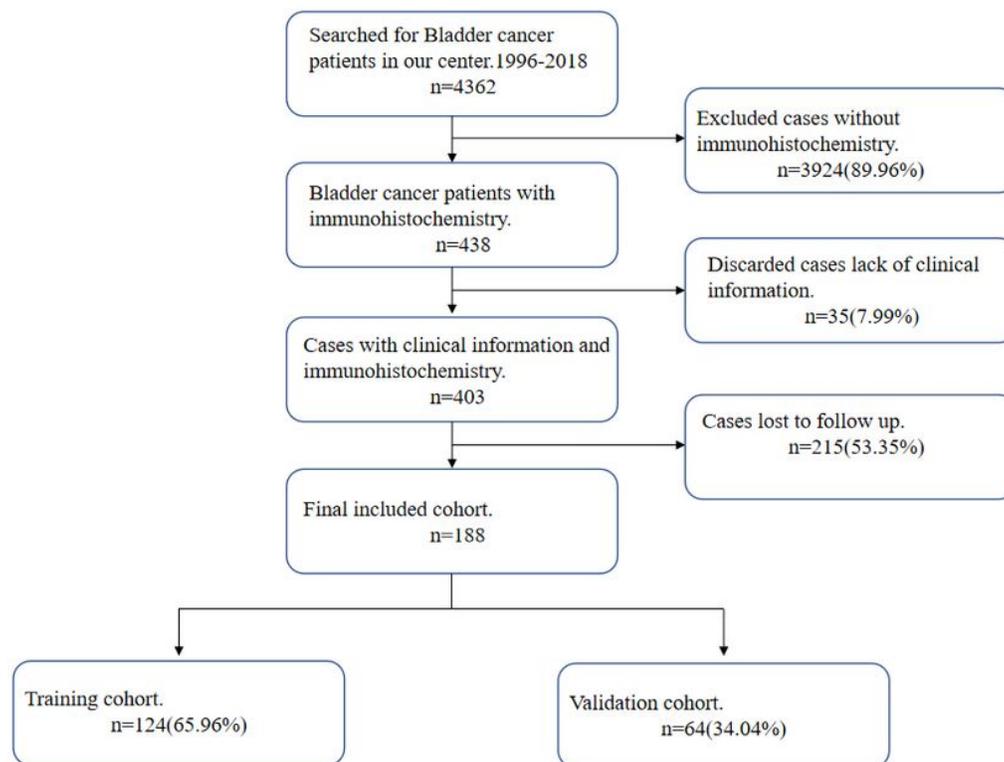


Figure 1

The flowchart of incorporating patients.

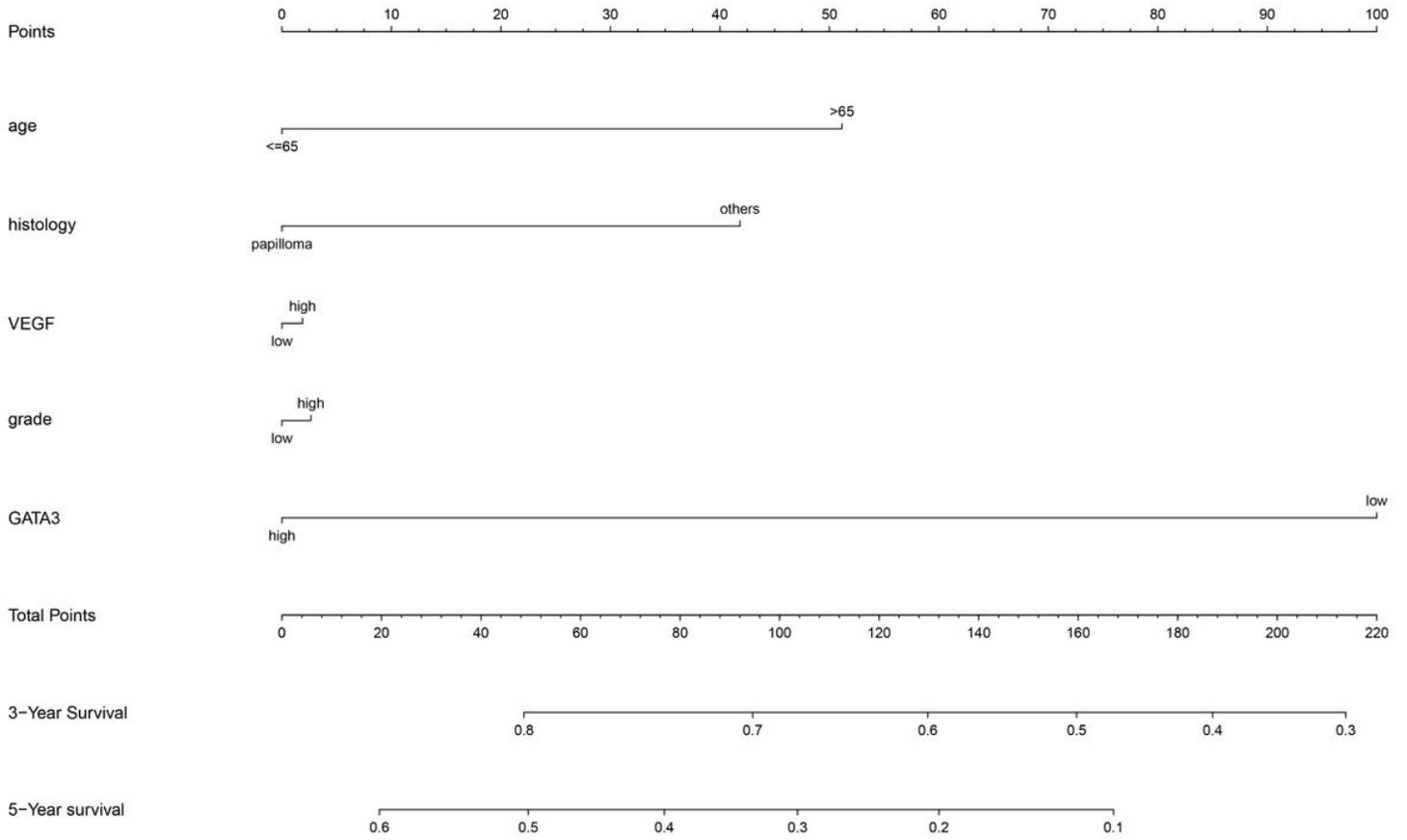


Figure 2

Nomogram for the prediction of the overall survival of the patients with bladder cancer, where age, histology, grade, GATA3 and VEGF define the overall survival probability.

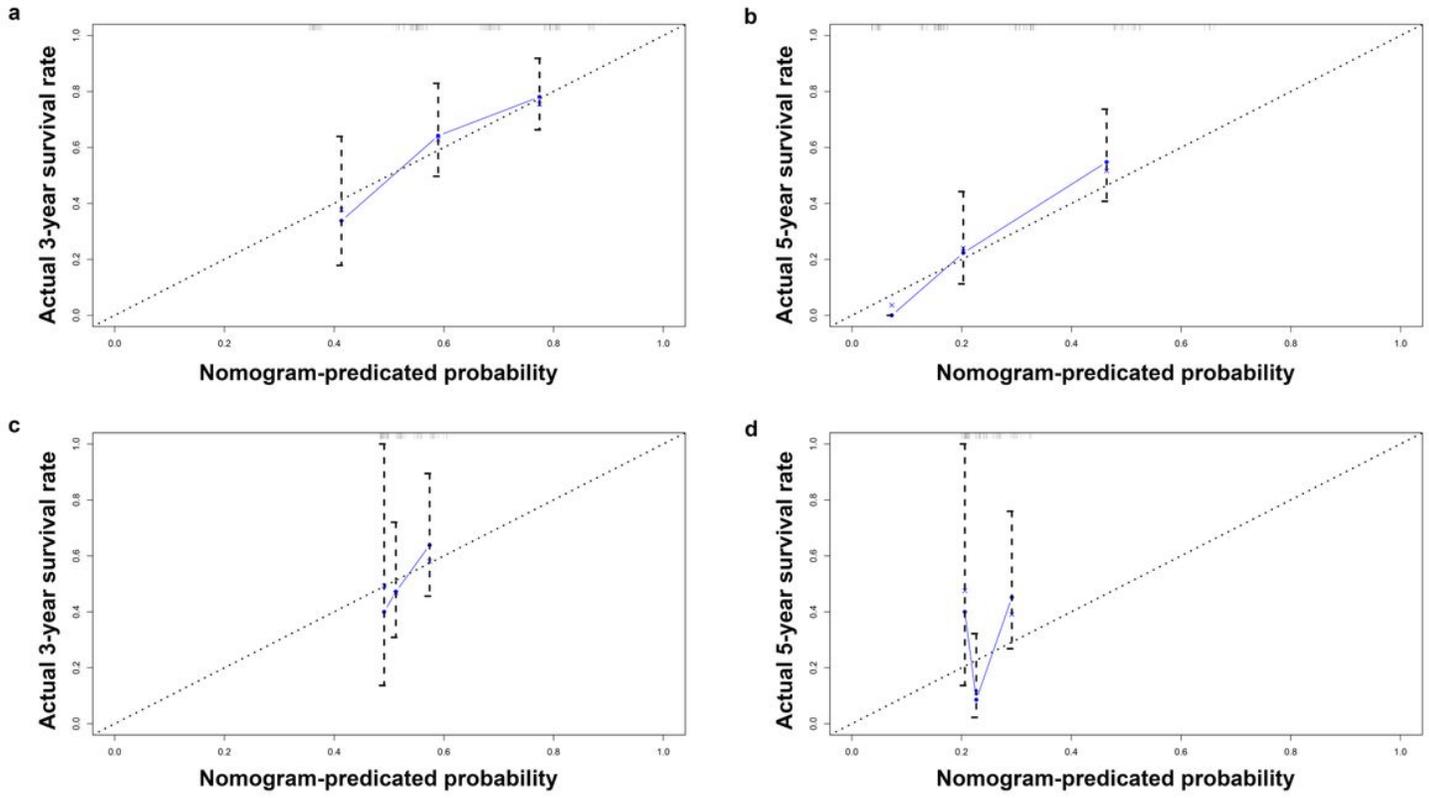


Figure 3

Calibration plot, where the x-axis represents the predicted probability and the y-axis represents the actual survival rate. The 45° dashed line represents ideal predictions, the solid line (bias-corrected) represents the predictive performance of the nomogram. The scatter plot at the top of the figure shows the distribution of the individual nomogram-predicted probabilities. (a) and (b) represent the 3-year and 5-year survival calibration curve of training cohort. (c) and (d) show the 3-year and 5-year survival calibration curve of validation cohort.

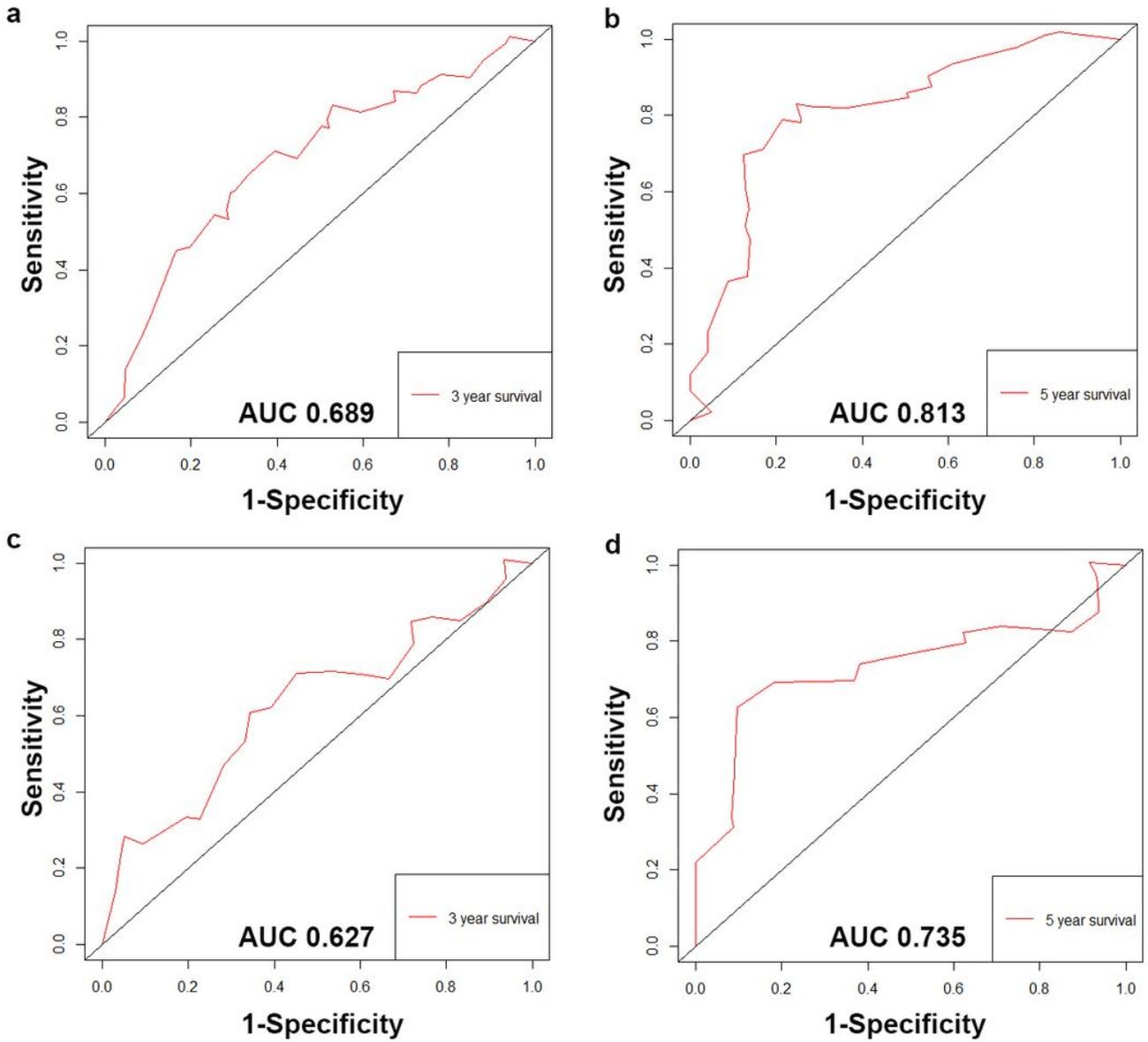


Figure 4

Area under the receiver operating characteristic (ROC) curve for performance in predicting the overall survival of patients with bladder cancer. (a) and (b) represent the 3-year and 5-year ROC curve of training cohort. (c) and (d) show the 3-year and 5-year survival ROC curve of validation cohort.