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Research Article

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Posted Date: November 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11665354/v1

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Additional Declarations: No competing interests reported.

Exploration of Prognostic Factors and Development of a Predictive Model for HER2-Positive Non-Muscle-Invasive Bladder Cancer

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Abstract

Objective: High expression of human epidermal growth factor receptor 2 (HER2) in non-muscle invasive bladder cancer (NMIBC) is typically associated with a poor prognosis. This study aimed to collect clinical data from HER2-positive NMIBC patients, analyze factors influencing postoperative recurrence-free survival (RFS), and construct a prognostic nomogram.

Methods: Patients diagnosed with HER2-positive NMIBC who underwent transurethral resection of bladder tumor (TURBT) and intravesical therapy between 2019 and 2024 were retrospectively enrolled based on strict inclusion/exclusion criteria. The cohort was randomly divided into training (n=106) and validation (n=53) sets at a 2:1 ratio using random sampling without replacement. Multivariate Cox regression analysis was performed on the training set to identify independent risk factors for RFS and develop a prognostic model. The model’s performance was evaluated using the area under the receiver operating characteristic curve (AUC-ROC), calibration curves, and decision curve analysis (DCA) to assess predictive accuracy, consistency, and clinical utility.

Results: A total of 159 eligible patients were included. Multivariate analysis identified maximum tumor diameter, pathological T stage, pathological grade, and HER2 expression level as independent prognostic factors for RFS. A nomogram incorporating these factors was developed. In the training set, the 1- and 2-year AUCs for RFS prediction were 0.837 and 0.927, respectively. In the validation set, the corresponding AUCs were 0.866 and 0.878. Calibration curves demonstrated concordance between predicted and observed survival probabilities. DCA confirmed the model’s clinical utility.

Conclusion: Maximum tumor diameter, pathological T stage, pathological grade, and HER2 expression level are independent prognostic factors for RFS in HER2-positive NMIBC patients. The validated nomogram provides a reliable tool for urologists to assess prognosis and optimize clinical decision-making.

Keywords: Non-muscle invasive bladder cancer; Human epidermal growth factor receptor 2; Nomogram; Recurrence-free survival

1 Introduction

Bladder cancer, as a malignant tumor disease originating from the urothelial tissue of the bladder, can be classified into two types based on the depth of tumor invasion according to international consensus: non-muscular-invasive (NMIBC) and muscular-invasive (MIBC) [1]. NMIBC mainly affects the mucosal layer and the lamina propria tissue. The preferred treatment method for NMIBC is transurethral resection of bladder

tumor (TURBT), followed by combined intravesical chemotherapy [2].However, the risk of tumor recurrence and progression in postoperative NMIBC patients is a dual challenge that needs to be addressed at present.This significantly increases the complexity of subsequent treatment plans and the difficulty of clinical management [3].Chemotherapy drugs and Bacillus Calmette-Guérin (BCG) are two main types of intravesical perfusion agents. They exert anti-tumor effects through cytotoxicity and immune regulation mechanisms respectively [4].For the special patient group that does not respond to BCG treatment or experiences disease progression, radical cystectomy (Radical Cystectomy, RC) is included in the clinical pathway as the final treatment option. However, the urinary diversion complications associated with this surgical procedure and the sexual function impairment caused by pelvic autonomic nerve injury often impose a double burden on the patient's physiological functions and social psychological state. How to strike a balance between tumor control and quality of life has become a consideration in clinical decision-making [5][6].

The human epidermal growth factor receptor 2 (HER2), as a component of the epidermal growth factor receptor family, participates in the core regulatory mechanism of bladder cancer progression.This tyrosine kinase-type transmembrane protein drives malignant phenotypes such as abnormal cell proliferation, anti-apoptosis, and mesenchymal transformation through the cascade activation of downstream signaling pathways. Its abnormal high expression not only enhances the invasive and metastatic potential of tumors but also accelerates the formation of new blood vessels by inducing the secretion of pro-angiogenic factors (Vascular Endothelial Growth Factor, VEGF), thereby establishing a microenvironmental homeostasis that supports the continuous progression of tumors [7][8].In urinary system tumors, the histological specimens of bladder cancer tissues show a higher positive rate of HER2 protein expression than normal urothelium [9].Molecular typing studies on NMIBC indicate that cases with positive HER2 expression at a staining intensity of 2+ or 3+ in immunohistochemical detection account for approximately 18.3% to 21% of the overall population, and this proportion remains relatively stable across different regions and detection platforms [10][11].HER2 positive expression in NMIBC patients has clear prognostic warning value:

such patients not only have a lower efficiency of immune response to BCG instillation therapy [12],have a shorter disease recurrence interval, and have a higher probability of progressing to muscle-invasive cancer compared to patients with negative HER2 expression [10].The latest clinical research data show that for high-risk NMIBC patients with HER2 positive expression, the effect of HER2-targeted therapy is partially superior to traditional BCG instillation treatment [12].

This study aims to collect and utilize clinical data to analyze the risk factors related to disease recurrence or progression in HER2-positive NMIBC patients and build a nomogram prediction model based on the risk factors. To provide a useful tool for urologists to assess the prognosis of HER2-positive NMIBC patients.

2 Methods

2.1 Study Design

The flowchart of the research design is shown in **Figure 1**.

Inclusion criteria: (1) Primary NMIBC, with postoperative pathological stage of Ta or T1; (2) Age ≥ 18 years; (3) No lymph node or distant metastasis of the tumor; (4) Postoperative regular perfusion treatment in accordance with the guidelines; (5) Postoperative tissue pathological immunohistochemical detection

Exclusion criteria: (1) Having other types of tumors; (2) Having carcinoma in situ (TIS); (3) Patients who did not undergo or did not follow-up regularly with perfusion therapy after surgery; (4) Those without regular follow-up or short-term follow-up (less than 3 months).

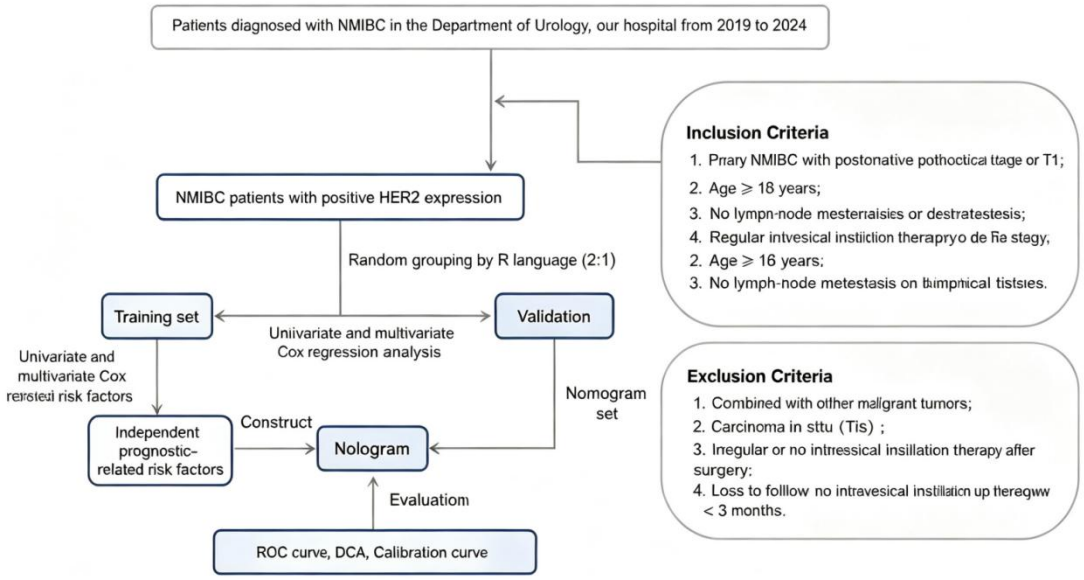


Figure 1. Flowchart of Research Design

2.2 Clinical Data

The information of patients with NMIBC who underwent TURBT surgery was collected from this research unit. The patients received regular perfusion treatment and follow-up at our hospital from January 1, 2019 to December 31, 2024. The follow-up period ended on April 1, 2025 or when the patients experienced tumor recurrence or progression.

This study is a retrospective study. It utilizes the pathological and clinical data obtained from previous clinical diagnoses for research. It poses no risk to the enrolled patients and will not have any adverse effects on the rights and health of the subjects. The data collected in this study can ensure the confidentiality and protection of the privacy and identity information of the subjects. The research methods of this study follow the standards outlined in the Helsinki Declaration (revised in 2013). The study has passed the project ethics review of the relevant unit, and the project ethics review approval number is (2024ZDSYLL108-P01).

2.3 Data Collection

The clinical, pathological and demographic characteristics of the patients were obtained by querying the electronic pathology system of our hospital. The following variables were obtained: gender, age, hypertension, diabetes, smoking history, maximum tumor

diameter, whether the tumor was multiple, pathological grade, pathological T stage, HER2 expression level, and perfusion drugs. In terms of HER2 expression level, it was classified into three categories based on the immunohistochemical results in the pathological report: IHC 1+; IHC 2+; IHC 3+. In terms of drug administration, it is classified into three categories based on the type of drug: anthracycline drugs (epirubicin/epirubicin); pyrimidine drugs (gemcitabine); immunotherapy drugs (BCG). The recurrence or progression of NMIBC (non-muscle-invasive bladder cancer) or its absence was regarded as the endpoint for observation. The recurrence-free survival (RFS) was defined as the time from the end of TURBT surgery to the end of the follow-up, which was the time when the patient's tumor recurred or progressed. The follow-up plan was to conduct a re-examination every 3 months in the first two years after the surgery, and every 6 months after that.

2.4 Statistical Approach

In this study, a combined method of digital coding and descriptive analysis was adopted for categorical variables, and the chi-square test was used to evaluate the differences between groups of categorical variables. The data were divided using simple random sampling, and the samples were allocated in a 2:1 ratio to the training set and the validation set. The training set was used for variable selection and model construction of the nomogram, while the validation set was responsible for the validation of model validity.

During the statistical analysis stage, the single-factor Cox proportional hazards model (Cox Proportional-Hazards Model, Cox) was initially employed to preliminarily screen potential predictors related to recurrence-free survival (Recurrence-Free Survival, RFS). Subsequently, the multi-factor Cox regression model was used to further identify independent risk factors. Based on the significant results of the multi-factor Cox regression, a nomogram model was constructed to predict the 1-year and 2-year RFS of patients. Model validation was conducted using a three-repetition composite evaluation system: ① Receiver Operating Characteristic Curve (ROC): The area under the curve (Area Under Curve, AUC) was calculated to quantify the model's

predictive accuracy; ② Decision Curve Analysis (DCA): This was used to evaluate the clinical practicability of the prediction model; ③ Calibration curve: This was used to verify the calibration degree of the prediction.

This study employed SPSS 30.0 IBM for univariate and multivariate Cox regression statistical analysis. Additionally, using the R language version 4.4.3 (http://www.r-project.org/), the software packages such as car, rms, survival, pROC, timeROC, and ggDCA were utilized to develop and validate the nomogram. In this study, a P value less than 0.05 was considered statistically significant.

3 Results

3.1 Baseline Demographic and Clinical Characteristics

This study included a total of 159 patients with HER2 positive expression of NMIBC from 2019 to 2024. These patients were randomly divided into two groups: the training group with 106 cases, accounting for 50.0%, and the validation group with 53 cases, accounting for 50.0%. The average age of the included patients was 68 years, and the average follow-up time was 18 months. 83% of them were male. The number of patients with hypertension and diabetes was relatively small, accounting for 47.8% and 13.2% respectively; the population with a smoking history accounted for only 9.4%. The average maximum tumor diameter was 2.6 cm. 10.8% of the patients had multiple tumors, and 89.2% had single tumors. In terms of pathological T stage, 64.2% of the patients were in T1 stage, and 35.8% were in TA stage. Among the pathological grades, 32.7% were G1/G2 patients and 67.3% were G3 patients. Regarding HER2 expression levels, based on the hospital data, only the immunohistochemical results of HER2 were collected in this study, with 46.5% being IHC 1+, 42.8% being IHC 2+, and 10.7% being IHC 3+. In terms of perfusion therapy, it was divided into three groups based on different drugs, with anthracycline drugs (epirubicin/epirubicin) accounting for 44.0%, pyrimidine drugs (gemcitabine) accounting for 39.0%, and immunotherapy drugs (BCG) accounting for 17.0%. In terms of outcomes, 54.1% of the patients had no recurrence or progression, while 45.9% had recurrence or progression.

The specific baseline data and clinical pathological characteristics are shown in **Table 1**.

Table 1. Baseline Data and Clinical Pathological Characteristics of NMIBC Patients

		AverageValue	Count	ColumnN%
Age		68		
Sex	**Woman**		27	17.00%
	Man		132	83.00%
Hypertension	**No Hypertension**		83	52.20%
	Has Hypertension		76	47.80%
Diabetes	**No Diabetes**		138	86.80%
	Has Diabetes		21	13.20%
Smoking History	**No History of Smoking**		144	90.60%
	Has a History of Smoking		15	9.40%
The Maximum Diameter of the Tumor（cm）		2.6		
Multiple Occurrence of Tumors	**Single**		140	89.20%
	Multiple		17	10.80%
Pathological T Stage.	**Ta**		57	35.80%
	T1		102	64.20%
Pathological Grading.	**G1/G2**		52	32.70%
	G3		107	67.30%
HER2 Expression Level	**IHC 1+**		74	46.50%
	IHC 2+		68	42.80%
	IHC 3+		17	10.70%
Intravenous Administration of Medication	**Aryl hydrocarbon Drugs (epirubicin/epoetaxel)**		70	44.00%
	Pyrimidine-based Drugs (Gemcitabine)		62	39.00%
	Immunotherapy Drugs (BCG)		27	17.00%
Ending	**No**		86	54.10%
	Yes		73	45.90%
Follow-up Month (month)		18		

The tests were conducted on each group of data in the training set and the validation set respectively, and the results are shown in **Table 2**.

According to **Table 2**, the comparison results of the differences between the training set and the validation set show that there is no significant statistical difference in the baseline data between the two groups ($P > 0.05$).Comparison of baseline characteristics between patients in the training set (n = 106) and the validation set (n = 53) showed that the two groups were generally well balanced: both were predominantly male (85.8% vs

77.4%), with similar mean ages (67.73 ± 11.67 years vs 69.68 ± 11.82 years), comparable proportions of hypertension, diabetes, and smoking history, and essentially similar maximum tumor diameters (2.56 ± 1.21 cm vs 2.58 ± 1.21 cm). Most lesions were solitary, most were pathologically staged as T1, most were pathologically graded as G3, and HER2 expression was predominantly IHC 1+/2+ in both groups. Regarding drug therapy, the validation set had a higher proportion of anthracycline use (56.6% vs 39.6%), whereas the training set had slightly higher proportions of pyrimidine use (42.5% vs 32.1%) and immunotherapy use (17.9% vs 11.3%). All P values for the compared variables were greater than 0.05 (0.122–0.912), and none of the differences were statistically significant, indicating that the baseline characteristics of the training and validation sets were comparable and that the grouping did not result in significant feature shift.

Table 2. Comparison of inter-group differences between the training set and the validation set

Variate		TrainingSet	ValidationSet	PValue
Sex	Woman	15 (14.2%)	12 (22.6%)	0.262
	Man	91 (85.8%)	41 (77.4%)	
Age (years)		67.73 ± 11.67	69.68 ± 11.82	0.232
Hypertension	No	53 (50%)	30 (56.6%)	0.368
	Yes	53 (50%)	23（43.4%）	0.412
Diabetes	No	93 (87.7%)	45 (84.9%)	0.803
	Yes	13 (12.3%)	8 (15.1%)	
Smoking History	No	94 (88.7%)	50 (94.3%)	0.387
	Yes	12 (11.3%)	3 (5.7%)	
Maximum diameter of the tumor (cm)		2.56 ± 1.21	2.58 ± 1.21	0.912
Multiple Occurrence of Tumors	Single	91 (87.5%)	49 (92.5%)	0.501
	Multiple	13 (12.5%)	4 (7.5%)	
Pathological Stage	TA	42 (39.6%)	15 (28.3%)	0.219
	T1	64 (60.4%)	38 (71.7%)	
Pathological Grade	G1/G2	36 (34%)	16 (30.2%)	0.765
	G3	70 (66%)	37 (69.8%)	
HER2 Expression Level	IHC 1+	51 (48.1%)	23 (43.4%)	0.723
	IHC 2+	43 (40.6%)	25 (47.2%)	

Variate		TrainingSet	ValidationSet	PValue
	IHC 3+	12 (11.3%)	5 (9.4%)	
Intravenous Administration of Medication	Aryl hydrocarbon Drugs (epirubicin/epoetaxel)	42 (39.6%)	30 (56.6%)	0.122
	Pyrimidine-based Drugs (Gemcitabine)	45 (42.5%)	17 (32.1%)	
	Immunotherapy Drugs (BCG)	19 (17.9%)	6 (11.3%)	

3.2 Univariate and Multivariate Regression Analyses of RFS in Patients with NMIBC

To identify independent prognostic risk factors, we conducted univariate and multivariate Cox regression analyses of RFS for NMIBC patients in the training set. The following variables were included in the study: gender, age, hypertension, diabetes, smoking history, tumor maximum diameter, tumor multiplicity, pathological grade, pathological T stage, HER2 expression level, and perfusion drugs. The corresponding p-values of specific variables in the univariate and multivariate regression analyses of RFS are summarized in **Table 3**.

Table 3. Univariate and multivariate Cox regression analysis of RFS in patients with NMIBC

Variate	UnivariateCOXregressionanalysis HR（95%CI）	PValue	MultivariateCOXregression analysis HR（95%CI）	PValue
Sex				
Woman	Reference			
Man	0.706（0.340-1.464）	0.349		
Age	1.016（0.992-1.040）	0.207		
Hypertension				
No	Reference			
Yes	0.787(0.445-1.392)	0.411		
Diabetes				
No	Reference			
Yes	0.965(0.427-2.181)	0.931		
Smoking History				
No	Reference			
Yes	1.550(0.721-3.331)	0.262		

Variate	UnivariateCOXregressionanalysis HR（95%CI）	PValue	MultivariateCOXregressionanalysis HR（95%CI）	PValue
The maximum diameter of the tumor	1.713(1.419-2.067)	＜0.001	1.364（1.077-1.729）	0.01
Multiple Occurrence of Tumors				
Single	Reference			
Mutiple	1.398（0.667-2.930）	0.375		
Pathological T Staging				
TA	Reference		Reference	
T1	9.719（4.105-23.011）	＜0.001	4.344（1.465-12.885）	0.008
Pathological Grade				
G1/G2	Reference		Reference	
G3	20.794（5.006-89.374）	＜0.001	7.109（1.535-32.935）	0.012
HER2 Expression Level				
IHC 1+	Reference	＜0.001	Reference	0.035
IHC 2+	3.504（1.744-7.039）	＜0.001	2.408（1.174-4.941）	0.017
IHC 3+	8.218（3.550-19.025）	＜0.001	2.563（1.080-6.084）	0.033
Intravenous Administration of Medication				
Aryl hydrocarbon Drugs (epirubicin/epoetaxel)	Reference	0.274		
Pyrimidine-based Drugs (Gemcitabine)	0.711（0.370-1.366）	0.307		
Immunotherapy Drugs (BCG)	1.336（0.621-2.874）	0.458		

The results of the regression analysis indicated that the maximum diameter of the tumor, pathological T stage, pathological grade, and HER2 expression level were independent risk factors for RFS.

3.3 Construction of the Line Graph Prediction Model

Based on the independent risk factors for RFS in NMIBC patients, a corresponding nomogram was constructed (**Figure 2**).

The nomogram, as a visual predictive tool, is interpreted based on a weight-integration system of variable parameters: each prognostic variable is assigned a specific score according to the intensity of its impact on the outcome. By adding up the corresponding scores of all variables, the predicted survival probability of the patient can be directly read on the coordinate axis. During implementation, the top variable axis sets independent scale lines for each covariate, and the contribution score of the variable is determined through vertical projection; the sum of the scores of each parameter corresponds to the position on the total score axis, and thus forms a mapping relationship with the survival probability axis at the bottom, achieving a direct conversion from clinical characteristics to quantitative prognosis. As shown in **Figure 2**, the nomogram constructed in this study is specifically used to predict the 1-year and 2-year RFS of patients with NMIBC. This model integrates four independent prognostic factors that have been verified by multivariate analysis: the maximum diameter of the tumor, pathological stage, pathological grade, and HER2 expression level. The score range of each variable is determined by the transformation of multivariate Cox regression coefficients, where the pathological stage shows a significant stratification feature: patients in the TA stage receive the lowest score (corresponding to the most favorable prognosis), and the score increases significantly in the T1 stage, which is consistent with the mechanism in the biological behavior of bladder cancer where the depth of invasion determines the risk of recurrence. The maximum diameter of the tumor is assigned a nonlinear scoring strategy, and when the diameter exceeds 4 cm, the score increases more rapidly, reflecting the clinical pattern of increased invasiveness of large-volume tumors. Different expression levels of HER2 have different prognostic values.

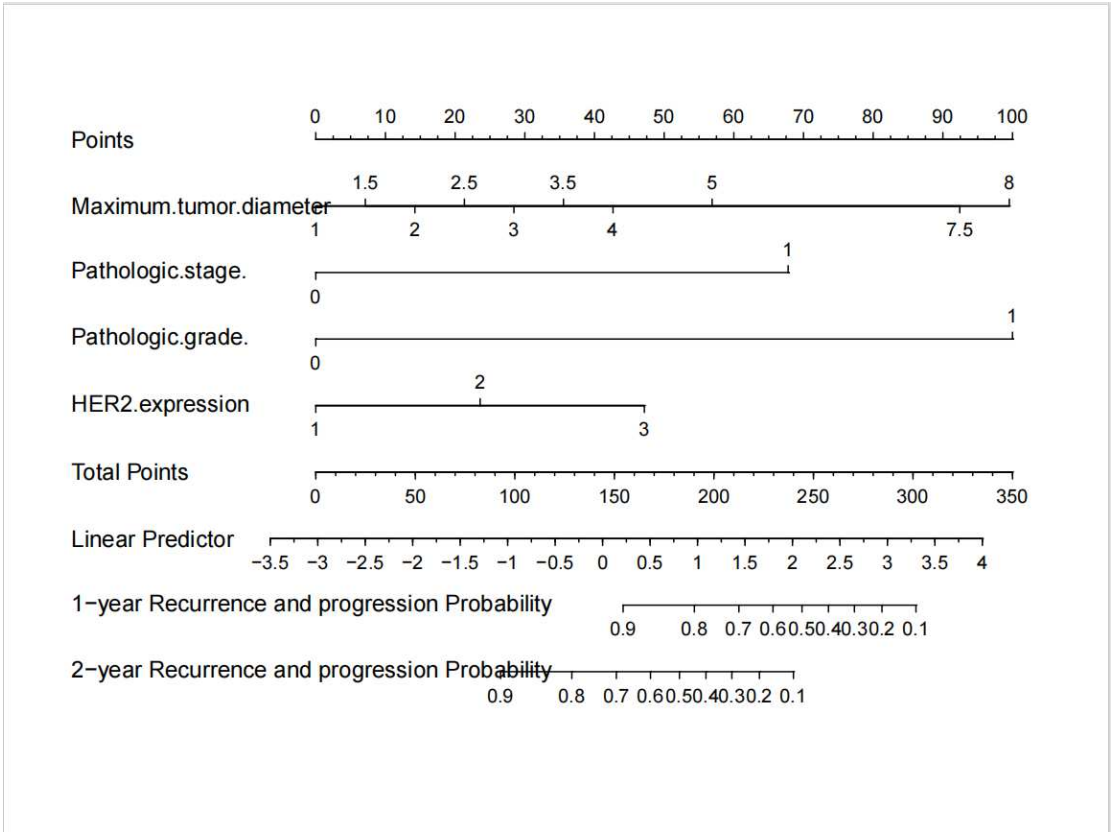


Figure 2. Predictive nomogram for 1-year and 2-year RFS in NMIBC patients in the training set. In this figure, TA = 0, T1 = 1; in pathological grading, G1/G2 = 0, G3 = 1; in HER2 expression level, IHC 1+ = 1, IHC 2+ = 2, IHC 3+ = 3.

3.4 Validation of the Line Graph Prediction Model

3.4.1 The ROC curve confirmed that the nomogram has good accuracy and robustness in predicting the RFS of patients with NMIBC.

ROC is often used to evaluate the event discrimination ability of predictive models. Its core principle is to dynamically adjust the risk determination threshold and calculate the corresponding relationship between the true positive rate (sensitivity) and the false positive rate (1 - specificity) at different cut-off values. AUC, as a quantitative indicator, reflects the overall discriminative efficacy of the model: when AUC = 0.5, it indicates that the model has no discriminative power, equivalent to a random result; AUC ranging from 0.7 to 0.9 suggests that the model has moderate clinical reference value; when AUC > 0.9, it achieves a high level of diagnostic accuracy. Traditional ROC analysis is limited to cross-sectional study designs and is difficult to adapt to the characteristics of time-dependent variables and censored data in survival analysis. Therefore, time-

dependent ROC (Time-dependent ROC) introduces the concept of dynamic risk windows to evaluate the predictive accuracy of the model at specific time points, and has now become the standard method for validating survival models.

From the ROC curve analysis shown in Figure 3, we can observe that the areas under the ROC curves for 1-year and 2-year RFS in the training set are 0.837 and 0.927 respectively. This indicates its excellent predictive ability in the validation set. In the validation set, the areas under the ROC curves for 1-year and 2-year RFS are 0.866 and 0.878 respectively, indicating that the model has higher efficacy in predicting 1-year and 2-year RFS for NMIBC patients in the validation set compared to the training set, and has good accuracy. These analysis results verify the superiority of the nomogram in accurately predicting the survival probability of NMIBC patients, especially in the 2-year RFS prediction results, where the model shows robustness and accuracy.

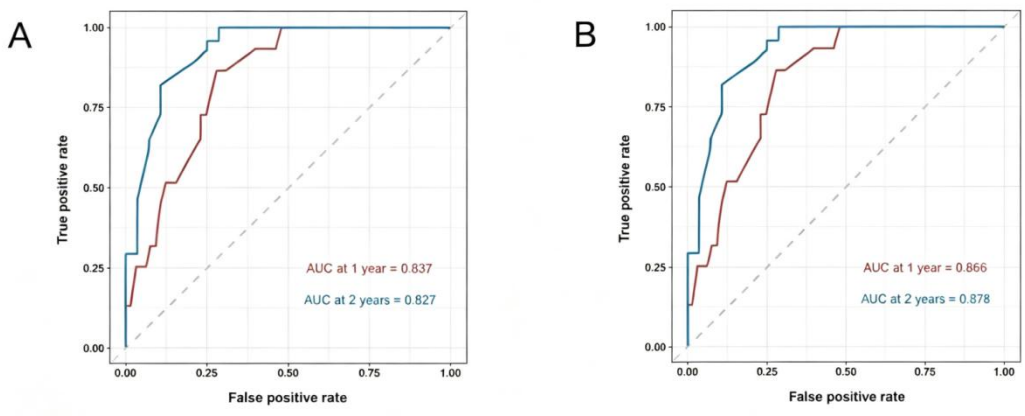


Figure 3. (A) ROC curves of 1-year and 2-year RFS in the training set; (B) ROC curves of 1-year and 2-year RFS in the validation set

3.4.2 The calibration curve confirmed the consistency between the predicted survival rate and the actual survival rate as predicted by the nomogram.

Figure 4 shows the calibration curves for the 1-year and 2-year RFS used for validating the predictions in the training and validation sets. The curves in the figure are close to the ideal reference line, and the data points on the curves are also close to or even overlap with the ideal reference line, indicating that the nomogram demonstrates the consistency between the predicted survival rate and the actual survival rate in both the training set and the validation set, showcasing the prediction accuracy.

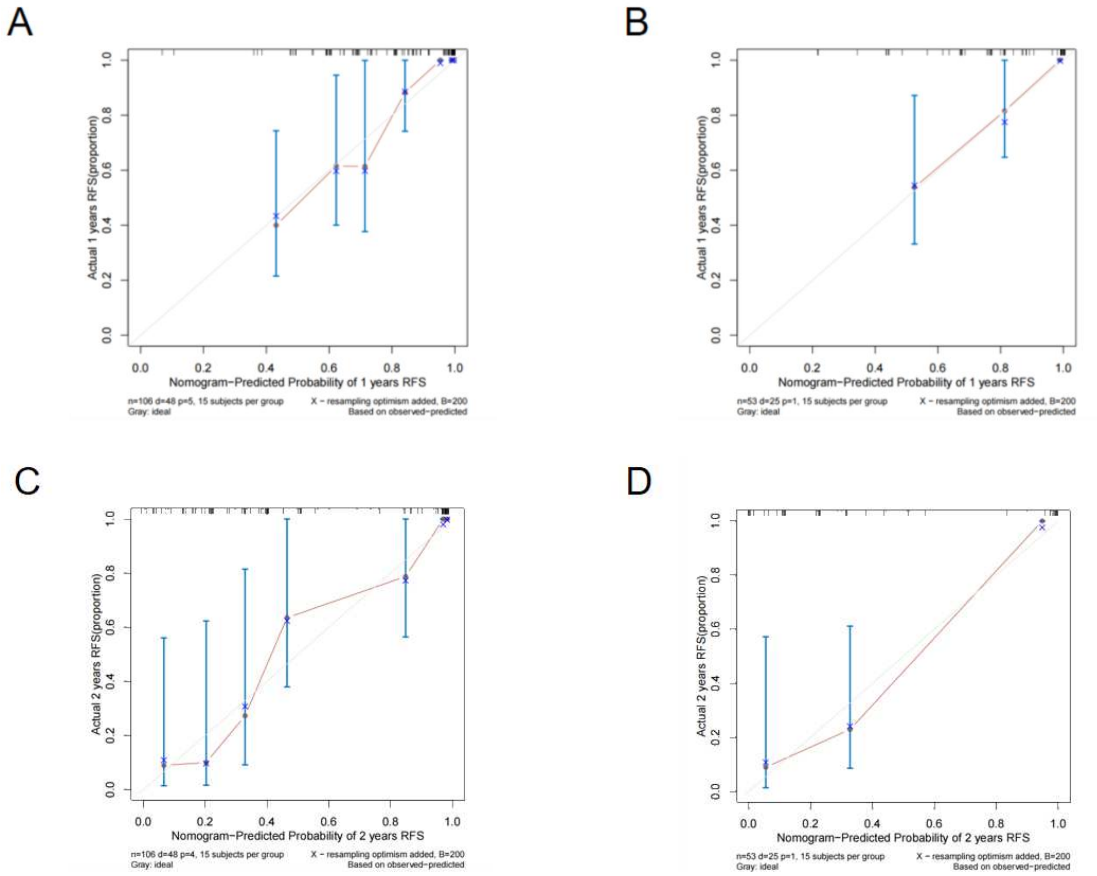


Figure 4. (A) Calibration curve of 1-year RFS in the training set; (B) Calibration curve of 1-year RFS in the validation set; (C) Calibration curve of 2-year RFS in the training set; (D) Calibration curve of 2-year RFS in the validation set

3.4.3 The decision curve confirmed the effectiveness of the nomogram in clinical decision-making for high-risk patients.

DCA, as an important tool for evaluating the clinical utility of predictive models, its core value lies in quantifying the net benefit level of the predictive model for patient management under different risk thresholds by simulating real clinical decision-making scenarios. Unlike traditional ROC curves and calibration curves, DCA innovatively incorporates the calculation of "clinical decision consequences" weights, converting the potential health damages caused by false positives (over-treatment) and false negatives (delayed treatment) into comparable standardized indicators. This method sets a continuous risk threshold probability (Threshold Probability, Pt) to simulate the decision-making tendencies of doctors in different clinical situations - when the

patient's risk prediction value exceeds Pt, specific intervention measures are initiated. The net benefit (Net Benefit, NB) is the core quantitative parameter of DCA, and its calculation formula is: NB = (TP - FP × Pt/(1-Pt))/N, where TP is the number of true positive cases, FP is the number of false positive cases, and N is the total sample size. This algorithm essentially reflects the dynamic balance between the clinical benefits of correctly identifying high-risk patients and the costs of mistakenly intervening in low-risk patients at a specific Pt.

Net benefit is the key metric of DCA, which takes into account the benefits when the prediction is correct and the costs when the prediction is incorrect. In **Figures 5**A to B, whether for the training set or the validation set, the ROC curve shows a certain net benefit at most high-risk thresholds, especially in the medium to high-risk threshold range. This indicates that it has greater clinical efficacy in the treatment decisions for high-risk patients and supports its use in clinical practice.

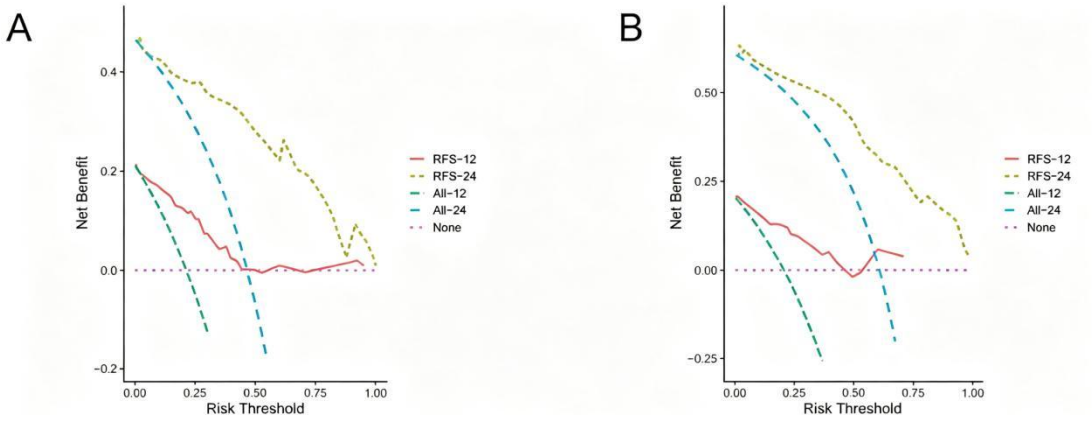


Figure 5. (A) DCA curves of RFS for the training set over 1 year (12 months) and 2 years (24 months); (B) DCA curves of RFS for the validation set over 1 year (12 months) and 2 years (24 months).

4 | Discussion

The European Urological Guidelines state that NMIBC encompasses Ta, T1 and high-risk Tis lesions, and the pathological stage and grade directly influence clinical decisions and prognosis [13]. Although NMIBC can be controlled early through TURBT combined with bladder instillation, the risk of recurrence and progression is high; existing prediction models mostly only include traditional clinical pathological

indicators and lack key information such as surgical quality, instillation execution, and patient compliance, making it difficult to accurately identify high-risk patients. This study focused on HER2-positive NMIBC as the research object. Through Cox regression, the maximum tumor diameter, pathological stage, pathological grade, and HER2 expression level were identified as independent risk factors for RFS, and a nomogram prediction model was constructed. The AUC of 1-year and 2-year RFS in the training set was 0.837 and 0.927, respectively, and 0.866 and 0.878 in the validation set. The calibration curve and DCA confirmed the consistency and clinical practicability of the model prediction. This study suggests that HER2 overexpression (IHC 2+/3+) indicates a poor prognosis; smoking history and tumor multifocality did not become independent prognostic factors for this cohort. It is speculated that regular instillation can weaken the prognostic effect of multifocality, and there is no statistical difference in the efficacy of BCG and conventional chemotherapy instillation. HER2 expression may reduce the efficacy of BCG, and HER2 is an independent predictor of poor BCG response [12].Generally speaking, clinical guidelines suggest that the efficacy of BCG infusion is superior to traditional chemotherapy and recommend that low-risk NMIBC patients undergo infusion chemotherapy while high-risk NMIBC patients receive BCG infusion treatment [14]. Considering that all the patients included in this study had HER2 expression, it is possible that the expression of HER2 led to a decrease in the efficacy of BCG infusion treatment but did not affect the efficacy of chemotherapy. Further data from patients without HER2 expression need to be included to support this claim.

Previous studies have suggested that tumor diameter, T1 stage, and G3 grade are high-risk factors for NMIBC [20]. The EORTC, CUETO model, and Alexandre et al.'s research also regarded stage and grade as core predictive indicators for recurrence and progression. High-risk patients in T1 stage can benefit from a second TURBT [21]; Irene et al. found that the pathological grade may have a greater weight on prognosis than the stage [22], which is consistent with the result in this model that the G3 score is higher than the T1 stage. The conclusion of this study that HER2 positive indicates poor prognosis is consistent with the results of Xuwei and Francesca [23][24]. Animal

experiments and clinical studies have also suggested that HER2-targeted drug RC48-ADC bladder instillation has therapeutic potential and may serve as an alternative or adjunctive regimen to BCG [25]. Previous studies have confirmed that smoking and multifocal tumors increase the risk of recurrence [26][27], and Lu et al. proposed that regular instillation can counteract the adverse prognostic effect of multifocality [28], which can explain the negative results of this study; the value of nomograms in individualized prognosis assessment of urinary system tumors has also been supported by multiple studies [15][16][17][18][19].

The classification of HER2-targeted drugs includes antibodies, TKIs, and ADCs. The mechanisms of these three types of drugs are different. Antibodies bind to the extracellular domain of the HER2 protein, preventing the formation of HER2-containing heterodimers, regulating ERBB2 signal transduction, and exerting cytotoxic effects [29].TKIs can catalyze intracellular kinases to compete with adenosine triphosphate (ATP) to inhibit the downstream signal transduction of the HER2 family [30].ADCs, due to their special composition of antibody, linker, and cytotoxic drug, can bind to the HER2 protein through internalization and release sufficient small molecule cytotoxic drugs in the cytoplasm after entering tumor cells to destroy substances such as tumor cell DNA and microtubulin, thereby precisely killing tumor cells [31].In other malignant tumors such as breast cancer, the introduction of HER2-targeted therapy has led to a change in the treatment landscape. Currently, the approved HER2 monoclonal antibodies for treating breast cancer include trastuzumab and pertuzumab. In studies such as NeoSphere and APHINITY, the pertuzumab combined with trastuzumab regimen can improve the prognosis and prolong the survival of patients with HER2-positive breast cancer. Lapatinib, tukastinib, niraparib, and pyrrolopyrimidine are TKI drugs that have been used in clinical treatment of breast cancer. The Food and Drug Administration (FDA) has approved ADC drugs for treating breast cancer, including Trastuzumab Emtansine (T-DM1) and Trastuzumab (T-DXd) [32].In bladder cancer, HER2 monoclonal antibodies have not been approved by the FDA for the treatment of urothelial carcinoma, but the results of Hussain's trial indicate that trastuzumab combined with chemotherapy can significantly improve the prognosis and prolong the

survival of patients with HER2-positive advanced urothelial carcinoma [33].Drug research on TKI treatment of bladder cancer includes lapatinib, afatinib, and niraparib, and the clinical trials of these drugs have shown certain efficacy [34].RC48 is currently the ADC drug approved for the treatment of urothelial carcinoma and recommended by the guidelines. In the treatment of high-risk NMIBC, a phase II clinical trial indicates that RC48 combined with tislelizumab has good efficacy in treating high-risk NMIBC and the adverse reactions are controllable, indicating an encouraging application prospect of HER2-targeted immunotherapy in high-risk NMIBC patients [35].

Overexpression of HER2 can promote the malignant biological behavior of tumors and drive recurrence, serving as the molecular basis for the poorer prognosis of HER2-positive NMIBC; at the same time, HER2 can regulate the anti-tumor immune response mediated by Bacillus Calmette-Guérin (BCG), resulting in a decline in the response to BCG treatment [12]. This provides a theoretical basis for HER2-targeted perfusion therapy. Higher pathological grade indicates stronger tumor proliferation and invasion ability, and is more capable of reflecting the inherent malignant phenotype of the tumor compared to the simple T stage [22]; bladder perfusion clears residual tumor cells through cytotoxicity or immune activation, counteracting the recurrence risk brought by multifocality [28]. Smoking can promote the progression of bladder cancer through mutagenesis and oxidative stress. The negative results of this study mainly stem from reporting bias caused by patient self-reported information, and do not negate the carcinogenic effect of smoking itself [26][27].

This study is a single-center retrospective study. The routine detection rate of HER2 is low, and the sample size for standardized perfusion and long-term follow-up is limited, which restricts the extrapolation of the model; some potential prognostic variables such as tumor location, adverse reactions to perfusion, and molecular classification have data missing, and the variables included are not comprehensive. The retrospective design can only indicate the correlation but cannot establish a causal relationship, and it is prone to selection bias. In addition, the model is constructed based on Cox regression, and the clinical application threshold is high. There is no simple visualization tool developed, it has not been embedded in the electronic medical record system, and there

is a lack of external validation with multiple centers and different populations, as well as evidence of net benefit under composite endpoints.

In conclusion, the nomogram model constructed in this study for predicting the recurrence-free survival (RFS) of HER2-positive NMIBC patients has reliable predictive efficacy. It can achieve individualized prognostic stratification, assist in identifying high-risk populations, optimize perfusion and targeted treatment plans, avoid excessive intervention or insufficient treatment, and optimize the allocation of medical resources. At the same time, it supports the addition of HER2 testing in the routine pathological assessment of NMIBC, providing a basis for the selection of perfusion schemes, the formulation of standardized TURBT and perfusion processes, and the optimization of health policies. Subsequently, a multicenter prospective cohort study will be conducted to integrate new markers such as genomic and tumor microenvironmental factors, combined with machine learning to improve model accuracy. Based on the biological sample bank, long-term follow-up will be carried out to clarify the causal relationship of risk factors, and targeted and immunotherapy clinical trials will be conducted based on risk stratification. A web-based nomogram tool will be developed to promote the embedding of the model in electronic medical records for practical application. With the development of interdisciplinary technologies, it is expected to further improve the risk stratification system for NMIBC and improve the long-term prognosis of HER2-positive NMIBC patients.

5 Conclusion

This study found that the maximum tumor diameter, pathological T stage, pathological grade, and HER2 expression level were independent prognostic factors for HER2-positive expressing patients with NMIBC; based on these factors, a nomogram model for the prognosis of HER2-positive expressing NMIBC patients was constructed; after verification, this model has good scientificity, accuracy and practicability in clinical prognosis assessment.

Declarations

Ethical Approval and Accordance

“ The study protocol was approved by [IEC for Clinical Research of Zhongda Hospital,affiliated to Southeast University, Protocol No.2024ZDSYLL108-P01], in accordance with the Declaration of Helsinki and relevant national ethical regulations. This study adopted prospective enrollment of participants, and the requirement for informed consent was waived by the ethics committee for this retrospective analysis of fully anonymized clinical data.”

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Clinical trial number

Not applicable.

Author Contributions

Xinyang Peng:Investigation, obtaining funds, writing-initial draft, writing-review and revision.Dakun Zhang: Investigation,writing-initial draft.Chunhui Liu: Investigation, writing-initial draft, obtaining funds. Zejun Wang:Conceptualization, investigation. Zhicheng Ye:Investigation.Jiangang Chen: Conceptualization, investigation, resources. Jianping Wu:obtaining funds, Conceptualization, investigation, guidance.

Acknowledgements

Jiangsu Provincial Key Discipline and Laboratory Construction Funds of Urology, 2023YXZDXK02; National clinical key discipline construction funds, CZXM - ZK - 47.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used in this study are not publicly available but can be shared in de-identified form upon reasonable request to the corresponding autho.

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