Development and Validation of a Nomogram for Preoperative Prediction of Perineural Invasion in Colorectal Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
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Background:
In colorectal cancer (CRC), perineural invasion (PNI) is usually identified histologically in biopsy or resection specimens and is considered a high-risk feature for recurrence of CRC and is an indicator for adjuvant therapy. Preoperative identification of PNI could help determine the need for adjuvant therapy and the approach to surgical resection. This study aimed to develop and validate a nomogram for the preoperative prediction of PNI in patients with CRC.

Material/Methods:
A total of 664 patients with CRC from a single center were classified into a training dataset (n=468) and a validation dataset (n=196). The least absolute shrinkage and selection operator (LASSO) regression model was used to select potentially relevant features. Multivariate logistic regression analysis was used to develop the nomogram. The performance of the nomogram was assessed based on its calibration, discrimination, and clinical utility.

Results:
The nomogram consisted of five clinical features and provided good calibration and discrimination in the training dataset, with an area under the curve (AUC) of 0.704 (95% CI, 0.657–0.751). Application of the nomogram in the validation cohort showed acceptable discrimination, with the AUC of 0.692 (95% CI, 0.617–0.766) and good calibration. Decision curve analysis (DCA) showed that the nomogram was clinically useful.

Conclusions:
The nomogram developed in this study might allow clinicians to predict the risk of PNI in patients with CRC preoperatively. The nomogram showed favorable discrimination and calibration values, which may help optimize preoperative treatment decision-making for patients with CRC.

MeSH Keywords:
Colorectal Neoplasms • Decision Support Techniques • Nomograms

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Background

Worldwide, colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer-related death [1]. In the US, it has been estimated that 140,250 new cases and 50,630 deaths due to CRC occurred in 2018 [2]. Histopathological grade and clinical stage are the main prognostic factors that are used to select patients who should undergo adjuvant therapy. However, recently, perineural invasion (PNI) has also been recognized to be associated with poor prognosis.

The definition of PNI is tumor cell invasion in, around, and through the nerves [3]. In CRC, the prevalence of PNI is up to 33% of cases at the time of surgical resection [4]. Several studies have demonstrated that PNI is a strong prognostic indicator in CRC, and studies have shown a significant association between PNI and a decreased survival, and an increased rate of tumor recurrence, using univariate and multivariate analysis [4]. Also, PNI has been shown to predict the progression or recurrence of the Union for International Cancer Control (UICC) stage II CRC [5], and to predict the local recurrence of rectal cancer, which may allow clinicians to stratify patients for intensive follow-up [6].

Also, PNI is considered to be a site-specific prognostic indicator in CRC, according to the eighth edition of the American Joint Committee on Cancer (AJCC) Staging Manual [7]. The clinical practice guidelines of the National Comprehensive Cancer Network (NCCN) also includes PNI as a high-risk feature for recurrence of CRC, with adjuvant therapy recommended for patients with stage II CRC and PNI [8]. However, despite its strong prognostic role, PNI status can only be assessed in the postoperative setting, and it would be useful to identify PNI preoperatively to improve pretreatment decision-making, including the need for neoadjuvant therapy and the adequacy of surgical resection. In this context, PNI status has been reported to be associated with the CpG island methylator phenotype-high (CIMP-H) status [9], as well as the expression levels of nuclear CK2a and serpin B5 [10,11], although these biomarkers have limited practical use.

Nomograms have recently begun to attract increasing attention as an easy to use clinical tool to predict clinical events and outcome. Each nomogram is a graphical prediction model that combines several prediction factors and allows the user to assign scores for each factor using a scale, with the total score subsequently being used to predict the risk of a specific event. Since the first report in 1928 regarding the clinical application of the nomogram [12], nomograms have been developed to diagnose or predict the prognosis of various malignancies, and some nomograms have been considered to have better predictive value than the TNM staging system [13].

Therefore, the present study aimed to develop and validate a nomogram for predicting the presence of PNI based on the preoperative clinical features of patients with CRC. The predictive value of the nomogram was evaluated based on the goodness of fit, discrimination, and clinical utility in separate training and validation cohorts.

Material and Methods

Patients

The protocol of this retrospective study was approved by the Ethics and Human Subject Committee of the Affiliated Tumor Hospital of Guangxi Medical University. All experiments and methods were performed according to relevant guidelines and regulations. There were 989 patients with colorectal cancer (CRC) included in the analysis who underwent surgery at the Affiliated Tumor Hospital of Guangxi Medical University between August 2013 and April 2018. The inclusion criteria were: patients with histologically confirmed CRC; cases that underwent primary tumor resection; a postoperative histopathology report that contained information regarding the status of perineural invasion (PNI).

The exclusion criteria were: preoperative therapy, including radiotherapy, chemotherapy, or chemoradiotherapy); the diagnosis of other tumors during the same period; and cases with a history of familial adenomatous polyposis (FAP) or hereditary non-polyposis colon cancer.

Patient medical records were retrospectively reviewed to obtain data regarding demographic and preoperative clinical features. These features included age, gender, body mass index (BMI), past and present medical history, family history, routine blood test results, serum immunoglobulin level, computed tomography (CT)-based T-status and N-status, preoperative histological grade, and gross tumor type. Self-reported weight change during the last three months was also considered. The T and N status were determined according to the 8th edition of the American Joint Committee on Cancer (AJCC) Staging Manual. Based on the inclusion and exclusion criteria, 664 patients were finally included in the study, who had complete information, and these patients were randomized to training (468 cases) and validation (196 cases) datasets in a 7: 3 ratio based on computer-generated random numbers.

Feature selection

The least absolute shrinkage and selection operator (LASSO) logistic regression algorithm is useful for regression analysis of high-dimensional data [14]. The LASSO logistic regression algorithm was used to select the most significant predictive

features in the training dataset. Categorical variables were transformed into dummy variables, and PNI status was defined as the dependent variable. The suitable tuning parameter (λ) for the LASSO logistic regression was determined using cross-validation.

Nomogram construction and performance assessment

The features selected in the training dataset using the LASSO algorithm were subjected to multivariate logistic regression analysis to develop the nomogram. The goodness of fit between the observed values and the predicted values was assessed using a calibration curve and Spiegelhalter's Z-test, based on the fact that an ideal calibration curve would perfectly fit the 45-degree reference line. The predictive discrimination of the nomogram was evaluated using the receiver operating characteristic (ROC) curve and the area under the curve (AUC). In the logistic regression model, the AUC value was the same as the concordance index (C-index), and an AUC of 1.0 indicated that the nomogram provides total discrimination.

Validation of the nomogram

The performance of the nomogram was evaluated using the validation dataset. Predicted values were calculated for each patient in the validation dataset according to the formula that was constructed using the training dataset. The ROC and AUC values were then used to evaluate the predictive discrimination of the nomogram. The goodness of fit in the validation dataset was also evaluated using the calibration curve and Spiegelhalter's Z-test.

Clinical utility

Decision curve analysis (DCA) was used to evaluate the clinical utility of the nomogram based on net benefits at different threshold probabilities in the entire dataset, the training and validation datasets [15].

Statistical analysis

Statistical analysis was performed using R software (version 3.4.0). The LASSO logistic regression analysis was performed using the glmnet package. The logistic regression analysis, nomogram construction plot, and nomogram calibration plots were performed using the rms package. The DCA was performed using the dca.R function and Spiegelhalter's Z-test was performed using the val.prob function in the rms. Differences were considered statistically significant at two-sided P-values of <0.05.

Results

Clinicopathological characteristics of the patients with colorectal cancer (CRC)

This study included 664 patients, and data collection included >200 clinical parameters. Patient clinicopathological characteristics are summarized in Table 1. The training set consisted of 468 cases (265 men and 203 women), and the validation set consisted of 196 cases (124 men and 72 women). There were 314 patients with rectal cancer and 350 patients with colon cancer. More than half of the patients had perineural invasion (PNI) (335 cases) based on their postoperative pathology reports.

There were no significant differences in age, gender, body mass index (BMI), and tumor location between PNI-positive and PNI-negative group in the training set and validation set (all P>0.05). However, significant differences were found in weight loss during the three months before diagnosis, first-degree relative (FDR) tumor history, computed tomography (CT) T-stage, CT N-stage, tumor differentiation (grade), and gross tumor type between the PNI-positive and the PNI-negative group (P=$9.65×10^{-8}$–0.042).

Although carcinoembryonic antigen (CEA) is a recognized biomarker associated with recurrence and prognosis of CRC, no significant difference was found in CEA level between PNI-positive and PNI-negative group in the training set (P=0.28) and the validation set (P=0.68).

Feature selection

The training dataset and the LASSO logistic regression algorithm were used to select the most significant predictive features, which were then used to construct the prediction model. A total of 114 features were used for the LASSO logistic regression, and five features with non-zero coefficients were subsequently selected, with an optimal lambda value of 0.054 (Figure 1A, 1B). The model ultimately included five features: differentiation, CT-based N-status, FDR tumor history, gross tumor type, and weight loss during the three months before diagnosis.

Nomogram construction and performance

The five features selected using the LASSO logistic regression algorithm were included in the multivariate logistic regression modeling. Table 2 shows that multivariate logistic regression showed that PNI was independently influenced by moderate tumor differentiation (grade) (P=0.013), poor tumor differentiation (grade) (P=$1.36×10^{-4}$), no FDR tumor history (P=0.018), weight loss during previous three months before diagnosis

Table 1. Clinicopathologic characteristics of the patients with colorectal cancer (CRC).

Characteristics	Training set (n = 468)			Validation set (n = 196)		
	PNI-positive n (%)	PNI-negative n (%)	P-value	PNI-positive n (%)	PNI-negative n (%)	P-value
Age			0.61			0.65
Median (IQR) (year)	59 (49.75, 67)	60 (51, 67)		63 (53, 70.5)	62 (52, 68)	
Gender			0.83			0.11
Male	137 (51.7)	128 (48.3)		63 (50.8)	61 (49.2)	
Female	107 (52.7)	96 (47.3)		28 (38.9)	44 (61.1)	
BMI			0.30			0.86
Median (IQR) (kg/m²)	21.54 (19.89, 23.75)	22.08 (19.98,2 4.14)		21.87 (19.65, 24.05)	22.31 (20.49, 23.71)	
Primary site			0.88			0.73
Rectum	116 (51.8)	108 (48.2)		43 (47.8)	47 (52.2)	
Colon	128 (52.5)	116 (47.5)		48 (45.3)	58 (54.7)	
Weight loss#						
Median (IQR) (kg)	0 (0, 3)	0 (0, 2)	0.0047*	0 (0, 5)	0 (0, 3)	0.042*
FDR tumor history			0.009*			0.62
Yes	33 (39.3)	51 (60.7)		24 (43.6)	31 (56.4)	
No	211 (54.9)	173 (45.124)		67 (47.5)	74 (52.5)	
CT T-stage			0.031*			0.082
T1–T2	23 (39.0)	36 (61.0)		6 (28.6)	15 (71.4)	
T3–T4	221 (54.0)	188 (46.0)		85 (48.6)	90 (51.4)	
CT N-stage			0.004*			0.001*
N0	120 (46.2)	140 (53.8)		40 (36.0)	71 (64.0)	
N1–N2	124 (59.6)	84 (40.4)		51 (60.0)	34 (40.0)	
Differentiation (endoscopic biopsy)			9.65×10^{-8}*			0.002*
Well	2 (10.0)	18 (90.0)		0 (0.0)	5 (100.0)	
Moderately	185 (49.6)	188 (50.4)		72 (43.9)	92 (56.1)	
Poorly	57 (76.0)	18 (24.0)		19 (70.4)	8 (29.6)	
Tumor gross type			0.028*			0.195
Ulceration	136 (58.9)	95 (41.1)		56 (52.8)	50 (47.2)	
Infiltrative	16 (53.3)	14 (46.7)		4 (30.8)	9 (69.2)	
Ulceration and Infiltrative	11 (42.3)	15 (57.7)		6 (42.9)	8 (57.1)	
Protruded	78 (44.1)	99 (55.9)		24 (38.7)	38 (61.3)	
Other	3 (75.0)	1 (25.0)		1 (100.0)	0 (0.0)	
CEA						
Median (IQR) (ng/ml)	3.14 (1.82, 7.91)	2.88 (1.64, 6.11)	0.28	2.90 (1.71, 6.30)	2.60 (1.71, 6.61)	0.69

Weight loss during the three months before diagnosis; * P<0.05. IQR – interquartile range; FDR – first-degree relative; BMI – body mass index; CEA – carcinoembryonic antigen.
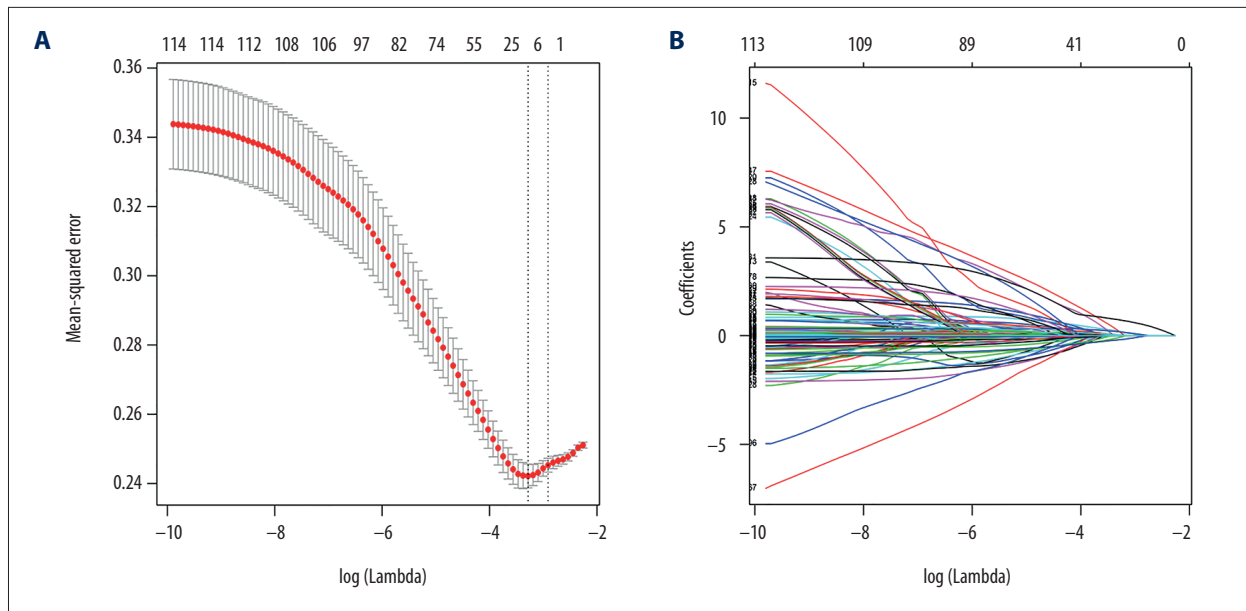
Figure 1. Feature selection using least absolute shrinkage and selection operator (LASSO) logistic regression. (**A**) Tuning parameter (λ) selection in the LASSO logistic regression performed using 10-fold cross-validation via the minimum criteria. The binomial deviance is plotted versus log (λ). The black vertical lines are plotted at the optimal λ based on the minimum criteria and 1 standard error for the minimum criteria. (**B**) The LASSO coefficient profiles of the 114 clinical features. A coefficient profile plot is produced versus the log (λ).

Table 2. Multivariate logistic regression analysis of the selected clinical features in the training set.

Variable	Odds ratio (95% CI)	P-value
Differentiation (biopsy)		
Well	1	
Moderately	6.66 (1.85–42.73)	0.013*
Poorly	21.42 (5.35–145.17)	1.36×10^{-4}*
CT N-stage		
N0	1	
N1/N2	1.46 (0.99–2.16)	0.058
FDR tumor history		
Yes	1	
No	1.85 (1.11–3.11)	0.018*

Variable	Odds ratio (95% CI)	P-value
Gross tumor appearance		
Ulcerating	1	
Infiltrative	0.58 (0.25–1.32)	0.19
Ulceration & infiltration	0.40 (0.16–0.96)	0.042*
Polypoid	0.55 (0.36–0.84)	0.0054*
Other	1.58 (0.17–33.58)	0.70
Weight loss (kg)	0.088 (0.013–0.34)	0.013*

FDR – first-degree relative; CRC – colorectal cancer.

(P=0.013) and a gross tumor appearance that was ulcerative and infiltrative tumor (P=0.042), and a protruding or polypoid tumor type (P=0.0054).

The nomogram was constructed using five main features (Figure 2). Each feature corresponded to a specific point by drawing a line straight upward to the Points axis. After the sum of the points was located on the Total Points axis, the sum represented the probability of PNI by drawing straight down

to the Diagnostic axis. For example, a patient with CRC with a 4 kg weight loss during the three months before diagnosis (11 points), no first-degree relative tumor history (20 points), CT-based N-stage of N0 (0 points), an ulcerative tumor appearance (29 points), and a moderately differentiated (grade) (62 points) tumor, the total points equal 122 and the PNI probability would be approximately 60%. Based on a 50% threshold, this patient would be high-risk and neoadjuvant chemotherapy might be considered.
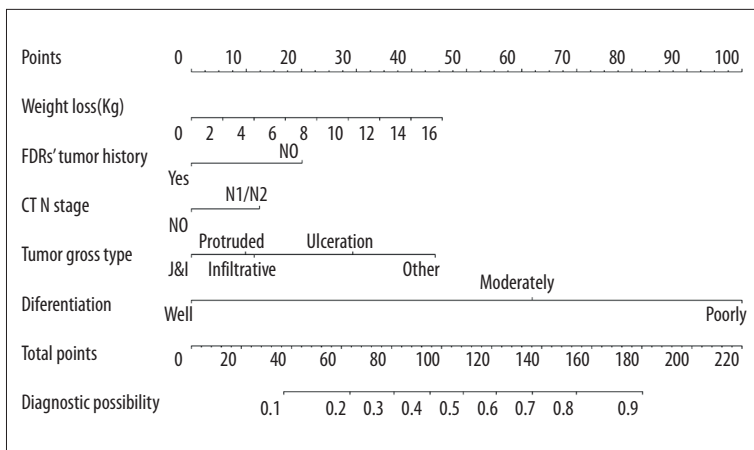
Figure 2. The nomogram for preoperative prediction of perineural invasion (PNI) in colorectal cancer (CRC). Points are assigned for differentiation, computed tomography-based N status, first-degree relative (FDR) tumor history, weight loss during the three months before diagnosis and gross tumor type. The score for each value is assigned by drawing a line upward to the Points line, and the sum of the five scores is plotted on the Total points line (probability of PNI).
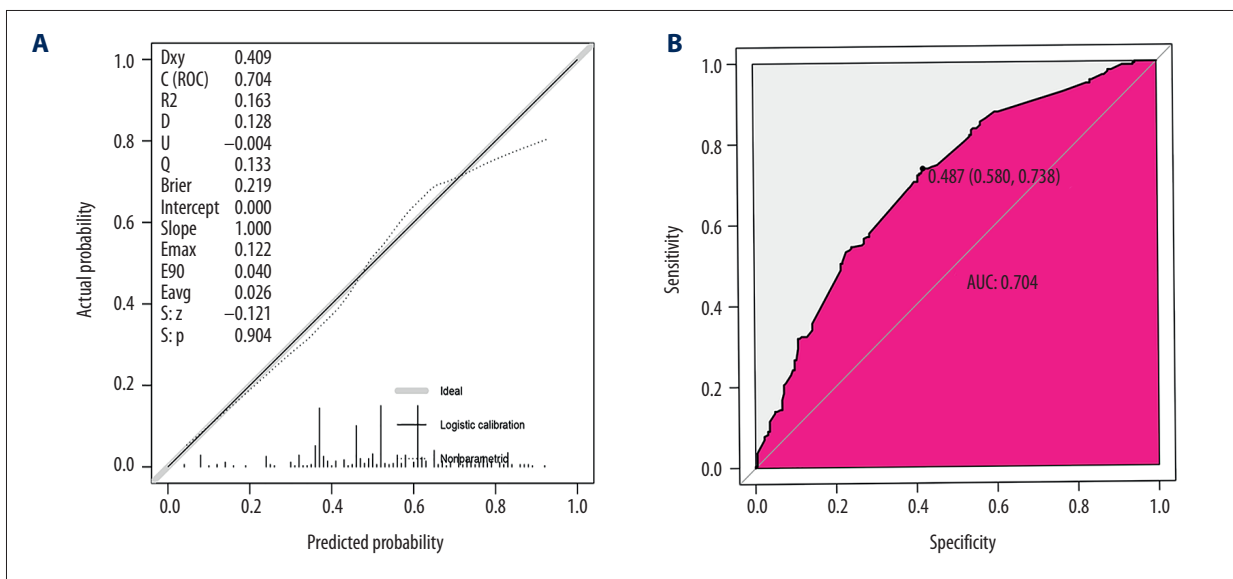

Figure 3. The performance of the nomogram in the training dataset. (**A**) The calibration plot of the nomogram in the training dataset. The x-axis is the nomogram-predicted probability of perineural invasion (PNI) and the y-axis is the actual rate of PNI. The reference line is 45° and indicates perfect calibration. (**B**) The receiver operating characteristic (ROC) curves of the nomogram in the training dataset.

The calibration plot showed good agreement between the predicted and observed rates in the training dataset (Figure 3A). The p-value for Spiegelhalter's Z-test was 0.90, which indicated that there was no significant departure from a perfect fit. The area under the curve (AUC) for the prediction nomogram in the training dataset was 0.704 (95% CI: 0.657–0.751) (Figure 3B).

Validation of the nomogram

The validation dataset (196 patients) was also used to evaluate the nomogram. The calibration curve showed good concordance between the predicted and actual probabilities, and Spiegelhalter's Z-test gave a non-significant result (P=0.921) (Figure 4A). In the validation dataset, the nomogram provided an AUC of 0.692 (95% CI: 0.617–0.766) (Figure 4B).

Clinical utility of the nomogram

The decision curve analysis (DCA) was performed to evaluate the clinical utility of the nomogram using the data from all 664 patients. Figure 5 shows that when the nomogram-predicted probability of PNI was >30% and <75%, the nomogram provided additional value relative to the treat-all-patients scheme or the treat-none scheme. For example, based on a 50% probability of PNI, the nomogram added a net benefit of 15.1% relative to the treat-all-patients scheme or the treat-none scheme, which suggested that the nomogram was clinically useful.
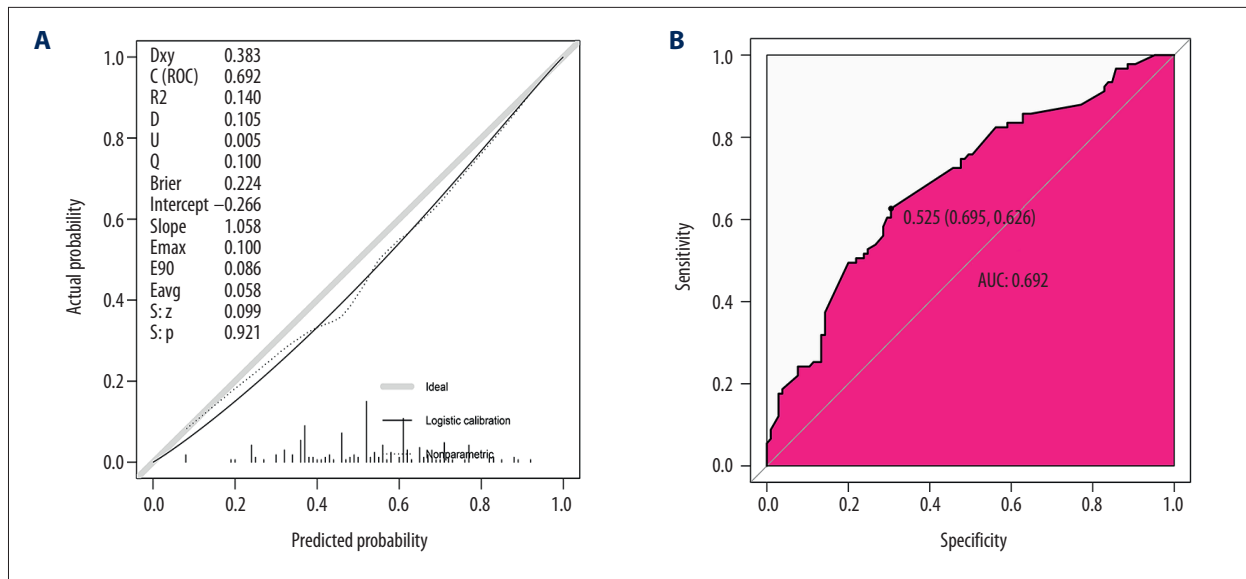
Figure 4. The performance of the nomogram in the validation dataset. (**A**) The calibration plot of the nomogram in the validation dataset. The x-axis is the nomogram-predicted probability of perineural invasion (PNI) and the y-axis is the actual rate of PNI. The reference line is 45° and indicates perfect calibration. (**B**) The receiver operating characteristic (ROC) curves of the nomogram in the validation dataset.
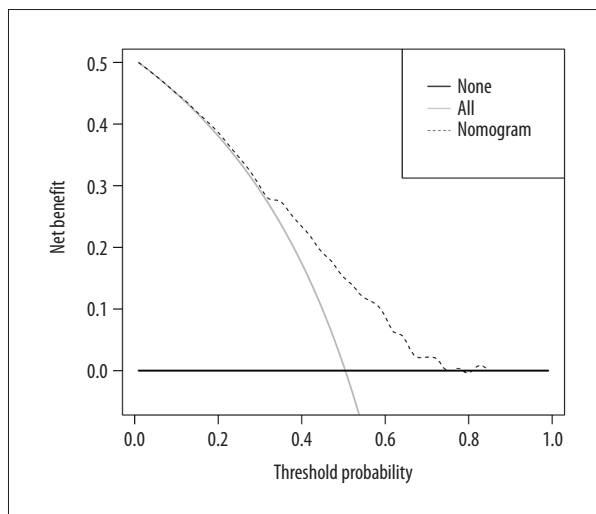

Figure 5. The decision curve analysis (DCA) for the nomogram. The net benefit was plotted versus the threshold probability. The dotted line represents the nomogram. The gray line represents the treat-all-patients scheme and the black line represents the treat-none scheme.

Discussion

In the present study, a nomogram was developed and validated to predict perineural invasion (PNI) based on the preoperative clinical features of patients with colorectal cancer (CRC). The nomogram provided favorable discrimination and calibration values. The results of this nomogram may help to optimize the preoperative management of patients with CRC.

In CRC, PNI is a strong prognostic indicator, and several studies have shown associations between PNI positivity and a poor prognosis with increased risk of tumor progression or recurrence [16]. Therefore, to construct a nomogram for the preoperative predictive value of PNI in patients with CRC, useful features were selected using the LASSO logistic regression algorithm. This algorithm has previously been widely used for selection of key features in multidimensional data analysis, as it is assumed to be a suitable selection method based on the strength of the univariate associations with outcome [17]. The nomogram was constructed using five features with non-zero coefficients: differentiation, computed tomography (CT)-based N-status, first-degree relative (FDR) tumor history, gross tumor type, and weight loss during the three months before diagnosis. The nomogram provided favorable discrimination and calibration values in the training dataset, with an AUC of 0.704 and Spiegelhalter's Z-test p-value of 0.90. The nomogram was tested using the validation dataset, which showed reasonable discrimination and acceptable calibration based on an AUC of 0.692 and Spiegelhalter's Z-test p-value of 0.921. The decision curve analysis (DCA) indicated that when the predicted probability of PNI was >30% and <75%, using the nomogram added more benefit relative to the treat-all-patients scheme or the treat-none scheme. Thus, the nomogram could serve as an easy-to-use preoperative predictor of PNI in CRC.

PNI is a recognized high-risk feature for recurrence in CRC. In patients with PNI-positive stage II CRC, curative surgery followed by adjuvant chemotherapy has become the standard of care [8]. However, about 20–30% patients with CRC with stage

II/III non-metastatic tumors who underwent radical surgical tumor dissection and received adjuvant chemotherapy suffered from local or distant recurrence, which reflects the limitation of such a therapeutic strategy in preventing the risk of locoregional tumor dissemination or eradicating distant micrometastases [18,19]. Several reasons may account for this limitation. The start of postoperative chemotherapy may be delayed for months after the initial diagnosis, and cancer may progress during this time interval [20]. Also, the increasing growth factors and immunosuppression induced by surgery may facilitate the dissemination and metastasis of tumor cells [21]. To overcome these limitations, the use of neoadjuvant chemotherapy has attracted increasing attention for the treatment of patients with high-risk CRC [22]. Neoadjuvant chemotherapy has advantages in controlling the potential high-risk factors and eradicating circulating micrometastases. Also, neoadjuvant chemotherapy contributes to a more likely achieved tumor-free resection margins [23].

Two randomized controlled trials (RCTs) involving patients with stage II/III CRC indicated that patients with stage III disease receiving neoadjuvant chemotherapy had lower mortality, recurrence, and rates of liver metastasis [24,25]. The FoxTROT Collaborative Group phase II clinical trial also demonstrated the feasibility of preoperative chemotherapy in patients with high-risk stage II CRC [22]. However, risk evaluation by CT may lead to undertreatment in a significant proportion of high-risk patients, with a sensitivity that can be as low as 42.9% [26]. The nomogram developed in the present study not only included feature based on CT, but also contained features related to the pathology, family history, and clinical symptom, which achieved favorable discrimination and calibration values. This nomogram might provide a novel strategy for identifying patients with CRC who are high-risk and who may benefit from neoadjuvant chemotherapy.

The nomogram developed in the present study incorporated five features that included tumor differentiation (grade), CT-based lymph node (N) status, first-degree relative (FDR) tumor history, tumor gross tumor type, and weight loss during the three months before diagnosis. In this context, tumor grade, lymph node metastasis, and the depth of invasion are well-established prognostic factors for CRC [27]. A previous study also showed that PNI was associated with lymph node metastasis and depth of invasion [28]. Given that tumor grade relates to

malignant behavior, it would be reasonable to predict that PNI reflects the degree of tumor differentiation or grade.

Preoperative CT imaging is an effective and convenient modality for evaluating lymph node metastasis, while endoscopic biopsy can easily provide data regarding the grade of CRC. Therefore, data regarding these features is readily and easily accessible. In the present retrospective study, it was shown that a first-degree relative (FDR) tumor history independently influenced the risk of PNI, which suggests that patients without FDR tumor history are more likely to develop PNI. The FDRs of CRC patients have a two-fold to four-fold increased risk for developing CRC. However, the role of heredity in PNI remains unclear and should be addressed in further studies. Weight loss has also been reported to be associated with a high risk of CRC recurrence and a poor prognosis [29]. In the present study, weight loss during the three months before diagnosis was significantly associated with the risk of developing PNI. Ulceration, infiltration, and protrusion are common gross forms of CRC, and a previous study has indicated that the gross appearance of CRC was associated with PNI [30], which supported the findings of the present study.

This study had two important limitations. First, the nomogram was developed using retrospectively collected data from a cohort of patients at a single center, and further research is needed to validate the performance of the nomogram in a larger external validation cohort. Second, the present study did not consider genomic characteristics, despite genomic classifiers now being regarded as promising predictive tools.

Conclusions

A nomogram was developed in this study that might allow clinicians to predict the risk of perineural invasion (PNI) in patients with colorectal cancer (CRC), preoperatively. The nomogram showed favorable discrimination and calibration values, which may help optimize preoperative treatment decision-making for patients with CRC.

Conflict of interest

None.

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