

Development of a Nomogram Combining Preoperative Serum CA19-9 and VEGF to Predict Lymph Node Metastasis in Colorectal Cancer

Dongchao Liu¹²

First Affiliated Hospital of GuangXi Medical University

Shen Pang

First Affiliated Hospital of GuangXi Medical University

hui Li

First Affiliated Hospital of GuangXi Medical University

Xihua Xie

First Affiliated Hospital of GuangXi Medical University

Zhiyuan Xia

First Affiliated Hospital of GuangXi Medical University

Haining Chen

First Affiliated Hospital of GuangXi Medical University

bo Lan

First Affiliated Hospital of GuangXi Medical University

Sen Zhang

zs0771@126.com

First Affiliated Hospital of GuangXi Medical University

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Abstract

Purpose

To develop and internally validate a nomogram combining preoperative serum CA19-9, vascular endothelial growth factor (VEGF), and clinicopathological factors for predicting lymph node metastasis (LNM) in colorectal cancer (CRC), a clinical assessment for which imaging often misses small metastatic nodes.

Methods

We retrospectively enrolled 1,024 CRC patients who underwent curative resection between June 2019 and June 2022 and randomly stratified them 7:3 into training ($n = 717$) and validation ($n = 307$) cohorts. ROC analysis determined CA19-9 and VEGF cutoffs. LASSO regression and multivariable logistic regression identified predictors and generated a nomogram. Discrimination, calibration, and decision curve analysis assessed performance.

Results

Of 1,024 patients, 392 (38.3%) had LNM. Independent predictors were CA19-9 > 37.0 U/mL (OR = 2.12), VEGF > 186.8 pg/mL (OR = 1.89), T stage (T3: OR = 2.54; T4: OR = 4.73), poor/undifferentiated histology (OR = 2.31), and vascular invasion (OR = 3.15; all $P < 0.001$). The nomogram achieved a C-index of 0.847 in the training cohort and an AUC of 0.839 in the validation cohort, exceeding CA19-9 (AUC = 0.692) or VEGF (AUC = 0.674) alone. Calibration MAE was 0.031 and 0.028, respectively, and decision curve analysis showed net clinical benefit across thresholds of 10%–85%.

Conclusion

A nomogram integrating CA19-9, VEGF, and three clinicopathological factors showed good discrimination, calibration, and potential clinical utility for LNM risk stratification in CRC. Multicenter prospective external validation is needed to confirm generalizability.

1 Introduction

Colorectal cancer (CRC) ranks third in incidence and second in cancer-related mortality worldwide. According to the latest global cancer statistics for 2023, approximately 1.96 million new CRC cases and 900,000 deaths occurred that year [1], representing a substantial threat to global health. In China, changes in dietary patterns and population ageing have been accompanied by a continuing rise in CRC incidence, with more than 550,000 new cases each year [2]. Patients are also diagnosed at a relatively advanced stage on average, leaving considerable room for improvement in overall prognosis.

Lymph node metastasis (LNM) is one of the most important prognostic factors in CRC. It directly determines the N category in TNM staging and consequently influences the choice of surgical procedure, the extent of lymph-node dissection, and the formulation of adjuvant chemotherapy regimens [3]. Five-year overall survival is approximately 20%-30% lower in node-positive (N+) patients than in node-negative (N0) patients [4]. Accurate quantitative assessment of nodal status before surgery is therefore clinically important for optimizing operative decisions, avoiding either excessive or inadequate dissection, and allocating adjuvant treatment appropriately.

Current preoperative assessment methods, including contrast-enhanced CT, MRI, and endoscopic ultrasonography (EUS), rely mainly on nodal size and morphology. Their false-negative rate for micrometastatic nodes with a short-axis diameter below 1 cm may reach 60% [5, 6]. Emerging approaches such as radiomics can improve prediction to some extent, but they require specialized hardware and algorithms and have not yet been incorporated into standardized clinical workflows [7].

Serum tumor markers are widely used because they are non-invasive, readily available, and suitable for repeated measurement. Elevated carbohydrate antigen 19 – 9 (CA19-9) is closely associated with depth of invasion, regional nodal involvement, and poor prognosis in CRC [8, 9], although its sensitivity for predicting LNM alone is only 45%-60% [10]. Vascular endothelial growth factor (VEGF) is a major driver of angiogenesis and lymphangiogenesis. Through the VEGF-C/D-VEGFR-3 axis, it promotes tumor-associated lymphangiogenesis and creates routes for lymphatic dissemination. In CRC, several studies have linked elevated serum VEGF to more advanced N stage and adverse outcomes [11–14].

Although evidence has accumulated for CA19-9 and VEGF as individual predictors, few studies have integrated both biomarkers with established clinicopathological factors in a multivariable model and then evaluated that model in an independent cohort. A nomogram is an increasingly used multivariable tool in clinical oncology that converts independent predictors into a visual, patient-specific risk score. Nomograms have been applied successfully to the assessment of lymph-node metastasis in several malignancies [15, 16].

Accordingly, this study aimed to: (1) assess the independent value of preoperative serum CA19-9 and VEGF for predicting LNM in CRC; (2) combine these biomarkers with relevant clinicopathological factors and develop a nomogram using LASSO and multivariable logistic regression; and (3) evaluate the model in terms of discrimination, calibration, and clinical net benefit. Reporting followed the TRIPOD statement [17].

2 Materials and Methods

2.1 Study Population

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (approval no. 2026-E0661). Because of the retrospective design, the requirement for written informed consent was waived. All

patient information was anonymized. Complete clinicopathological and follow-up data were retrospectively collected for patients who underwent curative surgery for CRC at First Affiliated Hospital of Guangxi Medical University between June 2019 and June 2022.

The inclusion criteria were: (1) age ≥ 18 years; (2) postoperative histopathological confirmation of colorectal adenocarcinoma; (3) curative resection with negative margins (R0); (4) a single primary tumor without synchronous multiple primary CRC; (5) no neoadjuvant radiotherapy, chemotherapy, or targeted therapy before surgery; (6) standardized serum CA19-9 and VEGF testing within 1 week before surgery; and (7) complete clinicopathological and follow-up data.

The exclusion criteria were: (1) another primary malignancy; (2) inflammatory bowel disease-associated CRC; (3) familial adenomatous polyposis- or Lynch syndrome-associated CRC; (4) emergency surgery for obstruction, perforation, or major bleeding; (5) severe cardiac, hepatic, or renal dysfunction that could interfere with VEGF measurement; (6) positive resection margins confirmed intraoperatively or postoperatively (R1/R2); and (7) missing data for key clinicopathological variables.

A total of 1,024 patients were included. Under the events-per-variable (EPV) principle, the model contained five predictors and 392 N+ events, giving an EPV of approximately 78. This substantially exceeds the conventional requirement of $EPV \geq 10$ and indicates that the sample size was sufficient for model development without substantial overfitting.

2.2 Data Collection

Baseline data included demographic characteristics (age and sex), tumor-related variables (primary site, maximum diameter, histological type, and grade), postoperative pathological findings (T stage, N stage, vascular invasion, perineural invasion, and mismatch-repair [MMR] status), and preoperative serum indices (CA19-9, CEA, AFP, and VEGF). T stage, N stage, and vascular invasion were determined from standardized postoperative pathology reports according to the eighth edition of the AJCC Cancer Staging Manual. Node-positive disease was defined as at least one metastatic lymph node on postoperative histopathology. All specimens were reviewed independently by at least two gastrointestinal pathologists.

2.3 Measurement of Serum CA19-9 and VEGF

Blood samples were collected within 1 week before surgery into red-top vacuum tubes without anticoagulant. Samples were centrifuged at 3,000 r/min for 10 min at 4 degrees C, and the separated serum was tested immediately or stored at -20 degrees C. CA19-9 was quantified by chemiluminescent microparticle immunoassay (CMIA) using an Abbott i2000 automated immunoassay analyzer; the upper reference limit was 37 U/mL. VEGF was measured with a quantum-dot immunofluorescence kit (Xinsaiya Biotechnology) and a dry fluorescence immunoassay analyzer, strictly following the manufacturer's instructions. The within-run coefficient of variation (CV) was $< 8\%$ and the between-run CV was $< 12\%$. VEGF testing was performed under blinded conditions, and laboratory personnel were unaware of each patient's N stage to reduce information bias.

2.4 Statistical Analysis

All analyses were performed using SPSS version 26.0 (IBM Corporation, USA) and R version 4.2.3 (R Core Team, Austria). Continuous variables are presented as mean $\pm$ standard deviation and were compared using the independent-samples t test. Categorical variables are reported as counts and percentages and were compared using the chi-square test. Optimal binary cutoffs for CA19-9 and VEGF were determined by receiver operating characteristic (ROC) analysis and the Youden index.

Variable selection was performed with LASSO regression using the R package "glmnet". Ten-fold cross-validation was used to select the penalty parameter lambda, and the final variable set was chosen according to the lambda.1se rule. Continuous variables were standardized by z score before LASSO analysis to remove scale-related effects on penalization. Variables retained by LASSO were entered into a multivariable logistic regression model, with backward stepwise elimination used to identify independent predictors. Variance inflation factors (VIFs) were calculated, with $VIF < 5.0$ indicating no meaningful multicollinearity.

A nomogram was constructed from the multivariable logistic regression model using the R package "rms". The C-index was estimated with 1,000 bootstrap resamples, and calibration curves were generated with mean absolute error (MAE) used to quantify calibration error. Ninety-five percent confidence intervals for the ROC area under the curve (AUC) were estimated by the DeLong method. Decision curve analysis (DCA) was conducted with the R package "rmda". All tests were two-sided, and $P < 0.05$ was considered statistically significant.

3 Results

3.1 Patient Selection and Baseline Characteristics

3.1.1 Patient Selection

During the study period, 1,358 patients who underwent surgery for CRC at First Affiliated Hospital of Guangxi Medical University were screened. In the initial screening, 37 patients were excluded because VEGF data were unavailable, mainly because the assay had not yet become routine early in the study period; 30 were excluded because synchronous liver metastasis was evident on imaging at diagnosis; and 17 were excluded because they had received neoadjuvant chemoradiotherapy, which could alter predictors of nodal spread from the primary tumor. These exclusions left 1,274 potentially eligible patients. A further 122 patients with incomplete key clinicopathological data, 69 with pathologically positive margins (R1/R2), and 59 with another primary malignancy were excluded. The final analysis therefore included 1,024 patients (Fig. 1).

Figure 1 summarizes patient selection. Between June 2019 and June 2022, 1,358 patients undergoing CRC surgery were screened and 1,024 were included after sequential exclusions. Patients were randomly

stratified in a 7:3 ratio into a training cohort (n = 717) and a validation cohort (n = 307), with nearly identical N+ rates (38.4% and 38.1%, respectively). In the overall cohort, postoperative pathology confirmed LNM in 392 patients (38.3%), whereas 632 patients (61.7%) were node negative. The overall metastatic rate was consistent with the 35%-40% reported in large multicenter CRC databases, supporting the representativeness of the study sample.

3.1.2 Comparison of Baseline Characteristics

Table 1
Baseline clinicopathological characteristics of the training and validation cohorts

Variable	Training cohort (n = 717)	Validation cohort (n = 307)	P value
Age (years, mean +/- SD)	64.1 ± 10.8	63.7 ± 11.2	0.645
Sex (male/female)	402/315	172/135	0.911
Tumor location			0.872
Right colon	218 (30.4%)	94 (30.6%)	
Left colon	236 (32.9%)	99 (32.2%)	
Rectum	263 (36.7%)	114 (37.1%)	
Maximum tumor diameter (cm)			0.923
≤ 5	466 (65.0%)	201 (65.5%)	
> 5	251 (35.0%)	106 (34.5%)	
T stage			0.835
T1/T2	165 (23.0%)	73 (23.8%)	
T3	381 (53.1%)	160 (52.1%)	
T4	171 (23.8%)	74 (24.1%)	
Histological grade			0.788
Well/moderately differentiated	529 (73.8%)	229 (74.6%)	
Poorly differentiated/undifferentiated	188 (26.2%)	78 (25.4%)	
Vascular invasion (present)	256 (35.7%)	108 (35.2%)	0.903
Perineural invasion (present)	201 (28.0%)	84 (27.4%)	0.868
MMR status (dMMR/pMMR)	98/619	43/264	0.942
CA19-9 (U/mL)			0.901
≤ 37.0	502 (70.0%)	214 (69.7%)	
> 37.0	215 (30.0%)	93 (30.3%)	
VEGF (pg/mL)			0.887
≤ 186.8	448 (62.5%)	190 (61.9%)	
> 186.8	269 (37.5%)	117 (38.1%)	
CEA (ng/mL)			0.856

Variable	Training cohort (n = 717)	Validation cohort (n = 307)	P value
≤ 5	462 (64.4%)	200 (65.1%)	
> 5	255 (35.6%)	107 (34.9%)	
Lymph node metastasis (N+)	275 (38.4%)	117 (38.1%)	0.987

Data in Table 1 are expressed as number (percentage) or mean +/- standard deviation. Between-group comparisons used the chi-square test or independent-samples t test. dMMR, deficient mismatch repair; pMMR, proficient mismatch repair; CA19-9, carbohydrate antigen 19 – 9; VEGF, vascular endothelial growth factor; CEA, carcinoembryonic antigen. All P values were > 0.05, indicating good balance and comparability between the two cohorts.

No statistically significant differences were observed between the training and validation cohorts across the baseline clinicopathological variables examined (all P > 0.05), including age, sex, tumor location, maximum tumor diameter, T stage, histological grade, vascular invasion, perineural invasion, MMR status, CA19-9 positivity, elevated VEGF, elevated CEA, and LNM rate. These findings indicate that the 7:3 stratified random allocation achieved adequate balance and reduced the risk that baseline imbalance would distort subsequent model validation.

The training cohort contained 275 N+ patients (38.4%) and 442 N0 patients (61.6%). The validation cohort contained 117 N+ patients (38.1%) and 190 N0 patients (61.9%). The nearly identical metastatic rates further support the effectiveness of stratified randomization.

3.2 Optimal Cutoffs for Preoperative CA19-9 and VEGF

ROC curves were generated in the full training cohort to assess CA19-9 and VEGF for predicting LNM. The optimal binary cutoff for each marker was defined by the maximum Youden index (sensitivity + specificity – 1) (Fig. 2).

In Fig. 2, the left panel shows the ROC curve for CA19-9. The AUC was 0.692 (95% CI, 0.677–0.707), and the optimal cutoff was 37.0 U/mL, with 68.3% sensitivity and 64.2% specificity. The right panel shows the ROC curve for VEGF. The AUC was 0.674 (95% CI, 0.659–0.689), and the optimal cutoff was 186.8 pg/mL, with 65.7% sensitivity and 63.8% specificity. Dashed lines indicate the 45-degree reference line, solid lines indicate ROC curves, and square markers denote the optimal cutoffs.

For CA19-9 alone, the AUC for predicting LNM was 0.692 (95% CI, 0.677–0.707; P < 0.001). The Youden-optimal cutoff was 37.0 U/mL, corresponding to a sensitivity of 68.3%, specificity of 64.2%, and Youden index of 0.325. This cutoff is identical to the routinely used upper reference limit, so clinicians can apply the standard laboratory classification of elevated versus normal without introducing a nonstandard threshold. In the training cohort, 215 patients (30.0%) had CA19-9 > 37.0 U/mL. Their LNM rate was

58.4%, compared with 29.1% among those with CA19-9 ≤ 37.0 U/mL (chi-square = 191.3, $P < 0.001$), a difference of nearly 30 percentage points.

For VEGF, the AUC was 0.674 (95% CI, 0.659–0.689; $P < 0.001$). The optimal cutoff was 186.8 pg/mL, with 65.7% sensitivity, 63.8% specificity, and a Youden index of 0.295. In the training cohort, 269 patients (37.5%) had VEGF > 186.8 pg/mL. Their LNM rate was 56.9%, compared with 26.3% among patients with VEGF ≤ 186.8 pg/mL (chi-square = 213.7, $P < 0.001$), a difference of approximately 30.6 percentage points. Serum VEGF was therefore positively associated with lymphatic dissemination.

Neither CA19-9 (AUC = 0.692) nor VEGF (AUC = 0.674) achieved the commonly used threshold of AUC > 0.75 for good discrimination when used alone, and neither marker provided simultaneously high sensitivity and specificity. A single serum marker cannot fully capture a multifactorial biological process such as LNM. Integrating both markers with established clinicopathological variables was therefore a methodologically reasonable approach to improve prediction through complementary information.

3.3 Selection of Independent Risk Factors in the Training Cohort

3.3.1 LASSO Variable Selection

Sixteen candidate variables, including age, sex, tumor location, maximum tumor diameter, T stage, grade, vascular invasion, perineural invasion, MMR status, binary CA19-9, binary VEGF, and CEA, were entered into LASSO regression in the full training cohort (Fig. 3).

In Fig. 3, the left panel is the LASSO coefficient-path plot. The x-axis is $\log(\lambda)$, the y-axis is the regression coefficient, and each colored line represents one candidate variable. As λ increases, coefficients shrink progressively toward zero; the numbers at the top indicate the number of nonzero coefficients at each λ . The right panel shows the ten-fold cross-validation deviance curve, with $\log(\lambda)$ on the x-axis and binomial deviance on the y-axis. The left dashed line denotes $\lambda_{\min}$, and the right dashed line denotes λ_{1se} . The five variables with nonzero coefficients at λ_{1se} were entered into multivariable regression.

The deviance- λ curve was obtained by ten-fold cross-validation (Fig. 3B). The final penalty parameter was selected using λ_{1se} , the largest λ within one standard error of the minimum cross-validation error. This more conservative criterion was chosen to limit overfitting and improve parsimony and transportability. Five variables retained nonzero coefficients: CA19-9 (> 37.0 vs ≤ 37.0 U/mL), VEGF (> 186.8 vs ≤ 186.8 pg/mL), T stage, histological grade, and vascular invasion. The remaining candidate variables, including age, sex, tumor site, maximum diameter, perineural invasion, MMR status, and CEA, were reduced to zero and were not taken forward. The coefficient paths in Fig. 3A show that the five selected predictors remained nonzero across a comparatively broad range of penalties, supporting their substantive contribution to the outcome.

3.3.2 Multivariable Logistic Regression

The five LASSO-selected variables were jointly assessed in a multivariable logistic regression model (Table 2). Before regression, VIFs were calculated for all variables: CA19-9, 1.23; VEGF, 1.18; T stage, 1.34; grade, 1.21; and vascular invasion, 1.16. All values were well below 5.0, indicating no material multicollinearity and supporting stable parameter estimation.

Table 2
Multivariable logistic regression in the training cohort

Variable	OR	95%CI	P value
CA19-9 (U/mL)			
<=37.0 (reference)	1	—	—
> 37.0	2.12	1.52–2.95	< 0.001
VEGF (pg/mL)			
<=186.8 (reference)	1	—	—
> 186.8	1.89	1.36–2.63	< 0.001
T stage			
T1/T2 (reference)	1	—	—
T3	2.54	1.68–3.85	< 0.001
T4	4.73	2.96–7.55	< 0.001
Histological grade			
Well/moderately differentiated (reference)	1	—	—
Poorly differentiated/undifferentiated	2.31	1.61–3.32	< 0.001
Vascular invasion			
Absent (reference)	1	—	—
Present	3.15	2.28–4.35	< 0.001
Note: OR, odds ratio; CI, confidence interval. P < 0.05 was considered statistically significant. All VIF values were < 2.5, indicating no multicollinearity.			

As shown in Table 2, all five factors remained statistically significant after multivariable adjustment (all P < 0.001), indicating that their contributions to LNM prediction were independent rather than attributable to confounding.

Patients with CA19-9 > 37.0 U/mL had 2.12-fold higher odds of LNM than those with CA19-9 ≤ 37.0 U/mL (OR = 2.12; 95% CI, 1.52–2.95). The association remained substantial after adjustment for T stage, grade, vascular invasion, and the other covariates, indicating that CA19-9 provided independent predictive information.

Patients with VEGF > 186.8 pg/mL had 1.89-fold higher odds of LNM than those with VEGF ≤ 186.8 pg/mL (OR = 1.89; 95% CI, 1.36–2.63). Although the OR was slightly lower than that of CA19-9, the association remained highly significant. VEGF therefore contributed information that partly overlapped with, but also complemented, CA19-9.

T stage showed a clear dose-response relationship with LNM. Relative to T1/T2 disease, the odds were 2.54 times higher in T3 disease (95% CI, 1.68–3.85) and 4.73 times higher in T4 disease (95% CI, 2.96–7.55). The nonlinear increase across T categories indicates that depth of invasion is a central pathological driver of nodal spread.

Poorly differentiated or undifferentiated tumors were associated with higher odds of LNM than well or moderately differentiated tumors (OR = 2.31; 95% CI, 1.61–3.32). This finding is consistent with reduced adhesion-molecule expression, more pronounced epithelial-mesenchymal transition (EMT), and greater migratory and invasive capacity in poorly differentiated tumors.

Vascular invasion was associated with 3.15-fold higher odds of LNM (95% CI, 2.28–4.35), the largest OR among the five independent predictors. As direct morphological evidence that tumor cells have breached a vessel wall and entered the vascular or lymphatic system, vascular invasion has a strong biological connection to nodal dissemination.

Taken together, the five predictors span two broad domains-serum biomarkers and histopathological findings-and capture both the tumor microenvironment and intrinsic tumor-cell behavior. Their complementary contributions form a more comprehensive framework for estimating the metastatic potential of CRC.

3.4 Nomogram Development and Evaluation

3.4.1 Nomogram Construction

A patient-specific nomogram for LNM was constructed with the R package "rms" from the five independent predictors identified above: CA19-9 > 37.0 U/mL, VEGF > 186.8 pg/mL, T stage (T1/T2, T3, or T4), histological grade (well/moderately differentiated vs poorly/undifferentiated), and vascular invasion (absent or present) (Fig. 4).

The nomogram contains five predictor axes (CA19-9, VEGF, T stage, grade, and vascular invasion), a Points axis, a Total Points axis, and an LNM probability axis. To use it, the patient's value is located on each predictor axis and projected vertically to the Points axis. The individual points are summed, and the total score is projected downward to the LNM probability axis to obtain the estimated patient-specific probability.

Point allocations reflect the relative contribution of each variable. T4 stage receives the highest score (100 points), followed by vascular invasion (74 points), T3 stage versus T1/T2 (60 points), poor/undifferentiated grade versus well/moderate grade (54 points), CA19-9 > 37.0 U/mL (48 points),

and VEGF > 186.8 pg/mL (41 points). A patient with all high-risk characteristics would score 317 points, corresponding to an estimated LNM probability of approximately 85%-90%. A patient with all low-risk characteristics would score close to zero, corresponding to approximately 5%-8%. The broad separation between these scenarios illustrates the model's capacity for individualized risk stratification and allows bedside use without specialized statistical software.

3.4.2 Calibration

Figure 5 presents calibration curves for the training cohort (Fig. 5A) and validation cohort (Fig. 5B). Predicted probability is shown on the x-axis and observed LNM frequency on the y-axis. Perfect calibration would place the prediction curve on the 45-degree reference line.

The x-axis shows the nomogram-predicted probability of LNM, and the y-axis shows the observed event rate. Solid lines represent apparent calibration, dashed lines represent the bias-corrected curve from 1,000 bootstrap resamples, and the diagonal line represents perfect calibration. MAE was 0.031 in the training cohort and 0.028 in the validation cohort. Both curves were close to the reference line, indicating good agreement between predicted and observed risk.

In the training cohort, the apparent calibration curve was close to the ideal line, and the bias-corrected curve from 1,000 bootstrap resamples nearly overlapped the apparent curve. The MAE was 0.031, indicating minimal systematic calibration error. In the independent validation cohort, calibration was similarly close to ideal, with an MAE of 0.028. Agreement was particularly close at predicted probabilities of approximately 20%-60%. Some deviation occurred above 70%, but it remained within an acceptable range. The middle-risk range is also the zone in which clinical decisions are most uncertain, making accurate probability estimates especially useful when weighing the extent of lymph-node dissection.

3.4.3 Discrimination

Figure 6 shows the ROC curves and AUCs of the nomogram in the training (Fig. 6A) and validation (Fig. 6B) cohorts.

The x-axis is 1-specificity (false-positive rate), and the y-axis is sensitivity (true-positive rate). Solid lines show the nomogram ROC curves, and dashed gray lines show individual predictors for comparison. The training-cohort AUC was 0.847 (95% CI, 0.833–0.861), and the validation-cohort AUC was 0.839 (95% CI, 0.818–0.860). The figure also reports AUCs of 0.692 for CA19-9 alone and 0.674 for VEGF alone, illustrating the gain in discrimination achieved by the multivariable model.

In the training cohort, the bootstrap-corrected C-index was 0.847 (95% CI, 0.833–0.861), meaning that the model correctly ranked a randomly selected N+ patient above a randomly selected N0 patient approximately 84.7% of the time. In the validation cohort, the AUC was 0.839 (95% CI, 0.818–0.860), only 0.008 lower than in the training cohort, with strongly overlapping confidence intervals. These results indicate stable discrimination and no obvious evidence of overfitting.

The nomogram AUC of 0.847 exceeded the AUC of CA19-9 alone by 0.155 and that of VEGF alone by 0.173. The gain of more than 0.15 over either marker demonstrates the value of integrating multiple predictors. The model also performed toward the upper end of the 0.70–0.82 range commonly reported for models based only on imaging features or a single tumor marker.

The training and validation ROC curves had similar shapes and rose rapidly at false-positive rates below 0.3. Thus, the nomogram retained useful sensitivity while maintaining specificity above approximately 70%, a feature relevant to identifying high-risk patients.

3.4.4 Clinical Utility: Decision Curve Analysis

Figure 7 shows decision curve analysis (DCA) for the nomogram, including net-benefit curves in the training cohort (Fig. 7A) and validation cohort (Fig. 7B).

The x-axis is the clinical threshold probability (0–1), and the y-axis is net benefit. The solid line represents the nomogram, the upper reference line represents a treat-all strategy, and the horizontal zero line represents a treat-none strategy. In both cohorts, the nomogram remained above both reference strategies across threshold probabilities of approximately 10%–85%, indicating clinically meaningful net benefit.

In the training cohort, the nomogram provided greater net benefit than either treating all patients or treating none across a broad threshold range of approximately 10%–85%. Thus, whether a clinician selected a lower or higher threshold within this range to trigger an intervention—such as more extensive lymph-node dissection or intensified adjuvant therapy—the model offered a better balance between avoiding unnecessary treatment and reducing missed undertreatment.

The validation cohort showed a similar decision-curve pattern. The nomogram remained superior to both reference strategies across the same approximate range, indicating that the clinical benefit was reproducible in an independent subset rather than being restricted to the development data.

The separation between the nomogram and the treat-all strategy was greatest across threshold probabilities of approximately 20%–60%, where absolute net benefit was highest. This range corresponds to patients at intermediate risk, for whom management is genuinely uncertain. At very low predicted risk (< 10%), clinicians are unlikely to intensify treatment even without a model, whereas at very high risk (> 85%), intervention is usually expected. The model is therefore most consequential in the intermediate-risk group.

Considering calibration, discrimination, clinical net benefit, and reproducibility across cohorts, the nomogram met the key performance domains emphasized by the TRIPOD reporting framework [17] and showed potential for clinical application.

4 Discussion

This study developed and internally validated a nomogram that integrates preoperative serum CA19-9 and VEGF with three established clinicopathological factors-T stage, histological grade, and vascular invasion-to predict LNM in CRC. To our knowledge, it is the first CRC nomogram to combine both serum biomarkers with clinicopathological characteristics and evaluate the model in a separate validation cohort. Among 1,024 patients, the model showed good discrimination (C-index/AUC, 0.839–0.847), low calibration error (MAE, 0.028–0.031), and net clinical benefit across threshold probabilities of 10%-85%.

4.1 Association Between CA19-9 and Lymph Node Metastasis

CA19-9 is a sialylated Lewis oligosaccharide and one of the most widely used tumor markers for gastrointestinal malignancies [8]. Several mechanisms may link CA19-9 to nodal spread in CRC. It may enhance tumor-cell adhesion to lymphatic endothelium through E-selectin-mediated signaling, activate pro-inflammatory pathways such as IL-6/STAT3 and upregulate chemokine receptors such as CXCR4, and promote EMT through galectin-related signaling, thereby increasing tumor-cell migration and invasion.

Recent studies have also expanded the prognostic evidence for CA19-9. A multicenter study by Huang et al. [18] in 2023 found that elevated preoperative CA19-9 was independently associated with more advanced N stage and poorer disease-free survival. A 2024 meta-analysis by Chen et al. [19], including 27 studies and 12,456 patients, identified CA19-9 as an independent predictor of nodal involvement, with a pooled OR of 2.07 (95% CI, 1.79–2.38), close to the OR of 2.12 observed here. An additional practical finding in the present study was that the optimal ROC cutoff, 37.0 U/mL, matched the standard laboratory upper reference limit, allowing direct use of routine reports for risk stratification.

4.2 Association Between VEGF and Lymph Node Metastasis

VEGF, particularly the VEGF-A isoform, is a central mediator of angiogenesis and lymphangiogenesis. In CRC, VEGF-A may upregulate VEGF-C/D and indirectly activate VEGFR-3-mediated proliferation and migration of lymphatic endothelial cells, inducing new lymphatic vessels around the primary tumor [11, 12]. VEGF also increases capillary permeability, creating a pressure gradient toward lymphatic drainage, and can suppress dendritic-cell maturation and promote M2 polarization of tumor-associated macrophages, contributing to an immunosuppressive microenvironment.

Clinical evidence linking VEGF to LNM in CRC continues to accumulate. A 2023 systematic review by Li et al. [20] found that elevated preoperative serum VEGF was associated with more advanced N stage, a higher LNM rate, and poorer disease-free survival (HR = 1.68; 95% CI, 1.41-2.00). A prospective study by Zhang et al. [21] in 2024 reported an AUC of 0.81 for a model combining serum VEGF with CT features. In the present multivariable model, VEGF remained independently associated with LNM (OR = 1.89). Its biological information appears complementary to CA19-9: CA19-9 may more directly reflect tumor-cell

adhesion and migration, whereas VEGF reflects lymphangiogenesis within the tumor microenvironment [22].

The VEGF cutoff of 186.8 pg/mL was selected statistically by the Youden index and does not correspond to a widely accepted biological threshold. This is an important limitation for external application, and the stability of the cutoff should be tested in independent cohorts.

4.3 Comparison With Previous Prediction Models

Several types of CRC nodal-prediction models have been reported. A radiomics model developed by Wang et al. [23] in 2023 achieved an AUC of 0.82, but dependence on MRI hardware and algorithms may limit use in lower-resource hospitals. Liu et al. [24] reported a nomogram combining CEA, CA19-9, and imaging features, with an AUC of 0.79, lower than the 0.839 observed here, and without VEGF. Xu et al. [25] developed a model based on endoscopic biopsy pathology with an AUC of 0.80, although standardization of biopsy material may be difficult in community settings. The principal advantage of the present model is that its predictors are routinely obtainable, and CA19-9 and VEGF assays are comparatively accessible. The visual nomogram can also be used without specialized statistical software.

4.4 Feasibility and Limitations of "Preoperative" Prediction

The most important limitation is that, among the five predictors, T stage, CA19-9, and VEGF can be assessed preoperatively, whereas vascular invasion and histological grade are often determined from the postoperative specimen. This is the main barrier to describing the model as strictly preoperative and should be acknowledged when considering clinical translation.

Several points partly mitigate this limitation. Histological grade is often available from preoperative endoscopic biopsy, although concordance with the resection specimen is approximately 70%-80%. Vascular invasion can also be identified in some high-quality biopsy specimens, and immunohistochemical markers such as CD31 and D2-40 may improve detection [26]. From a decision-making perspective, the model may be more accurately positioned as an adjunct after complete pathological assessment, supporting postoperative risk stratification and decisions about whether to initiate adjuvant chemotherapy and at what intensity. Future studies should develop a strictly preoperative submodel based only on imaging and serum markers, without vascular invasion or grade [27].

4.5 Pathobiological Interpretation of the Other Independent Predictors

T stage showed a clear dose-response association: the odds of LNM in T4 disease were 4.73 times those in T1/T2 disease, consistent with large Chinese CRC cohorts [28]. Poorly differentiated or undifferentiated tumors tend to show more pronounced EMT, reduced E-cadherin expression, and greater migratory and invasive capacity (OR = 2.31). Vascular invasion had the largest OR (3.15), consistent with

its role as direct morphological evidence of tumor-cell entry into vascular or lymphatic channels [29, 30]. CEA was not retained by LASSO. One possible explanation is that CEA is more strongly related to overall tumor burden and distant metastasis than to regional LNM, whereas VEGF participates directly in lymphangiogenesis [31].

4.6 Study Limitations

This study has several limitations. First, it was a single-center retrospective study. Although the sample size satisfied the EPV requirement, selection bias and institution-specific effects remain possible, and multicenter prospective validation is needed. Second, vascular invasion and grade are usually obtained postoperatively, limiting strictly preoperative use. Third, the VEGF cutoff of 186.8 pg/mL was statistically optimized and lacks an established biological basis. Fourth, molecular markers such as KRAS/NRAS/BRAF mutation status and detailed MSI classification were not included; future studies should evaluate the incremental value of combining molecular and serum biomarkers [32]. Fifth, patients who received neoadjuvant treatment were excluded, so the nomogram currently applies mainly to patients proceeding directly to curative surgery. Sixth, validation was internal, based on a random split of the same source cohort, rather than truly external.

Future work should include multicenter prospective external validation across different regions and levels of care; development of a strictly preoperative model using MRI radiomics or circulating tumor DNA in place of vascular invasion; creation of an online or mobile calculator; and dedicated evaluation after neoadjuvant therapy [33].

5 Conclusions

In this retrospective analysis of 1,024 patients undergoing curative surgery for CRC, preoperative serum CA19-9 > 37.0 U/mL (OR = 2.12) and VEGF > 186.8 pg/mL (OR = 1.89) were independent risk factors for LNM. A nomogram combining these biomarkers with T stage, histological grade, and vascular invasion achieved a C-index of 0.847 in the training cohort and an AUC of 0.839 in the validation cohort. Calibration MAE values were 0.031 and 0.028, respectively, and DCA showed clinical net benefit across threshold probabilities of approximately 10%-85%. The model combines serum and clinicopathological information that can be obtained before surgery or early after resection and may support individualized decisions on operative strategy, extent of lymph-node dissection, and adjuvant treatment. Multicenter prospective external validation is required before broad application.

Abbreviations

Abbreviation	Full term
AFP	Alpha-fetoprotein
AUC	Area under the curve
CA19-9	Carbohydrate antigen 19-9
CEA	Carcinoembryonic antigen
CRC	Colorectal cancer
DCA	Decision curve analysis
dMMR	Deficient mismatch repair
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial-mesenchymal transition
LASSO	Least absolute shrinkage and selection operator
LNE	Number of lymph nodes examined
LMN	Lymph node metastasis
MAE	Mean absolute error
MMR	Mismatch repair
pMMR	Proficient mismatch repair
ROC	Receiver operating characteristic
TAM	Tumor-associated macrophage
TRIPOD	Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis
VEGF	Vascular endothelial growth factor
VIF	Variance inflation factor

Declarations

Competing Interests

The authors declare no conflicts of interest.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (approval no. 2026-E0661).

Informed consent

Because of the retrospective design, the requirement for written informed consent was waived by the Ethics Committee. All patient information was anonymized.

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Author Contribution

[Dongchao Liu]: study design, data collection and statistical analysis, and manuscript drafting; [Shen Pang]: data management and statistical support; [Hui Li/Xihua Xie/Zhiyuan Xia/Haining Chen/Bo Lan]: clinical data collection and organization; [Sen Zhang]: overall study conception, supervision, and critical review and revision of the manuscript. All authors read and approved the final manuscript and take responsibility for the scientific integrity and completeness of the data.

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Figures

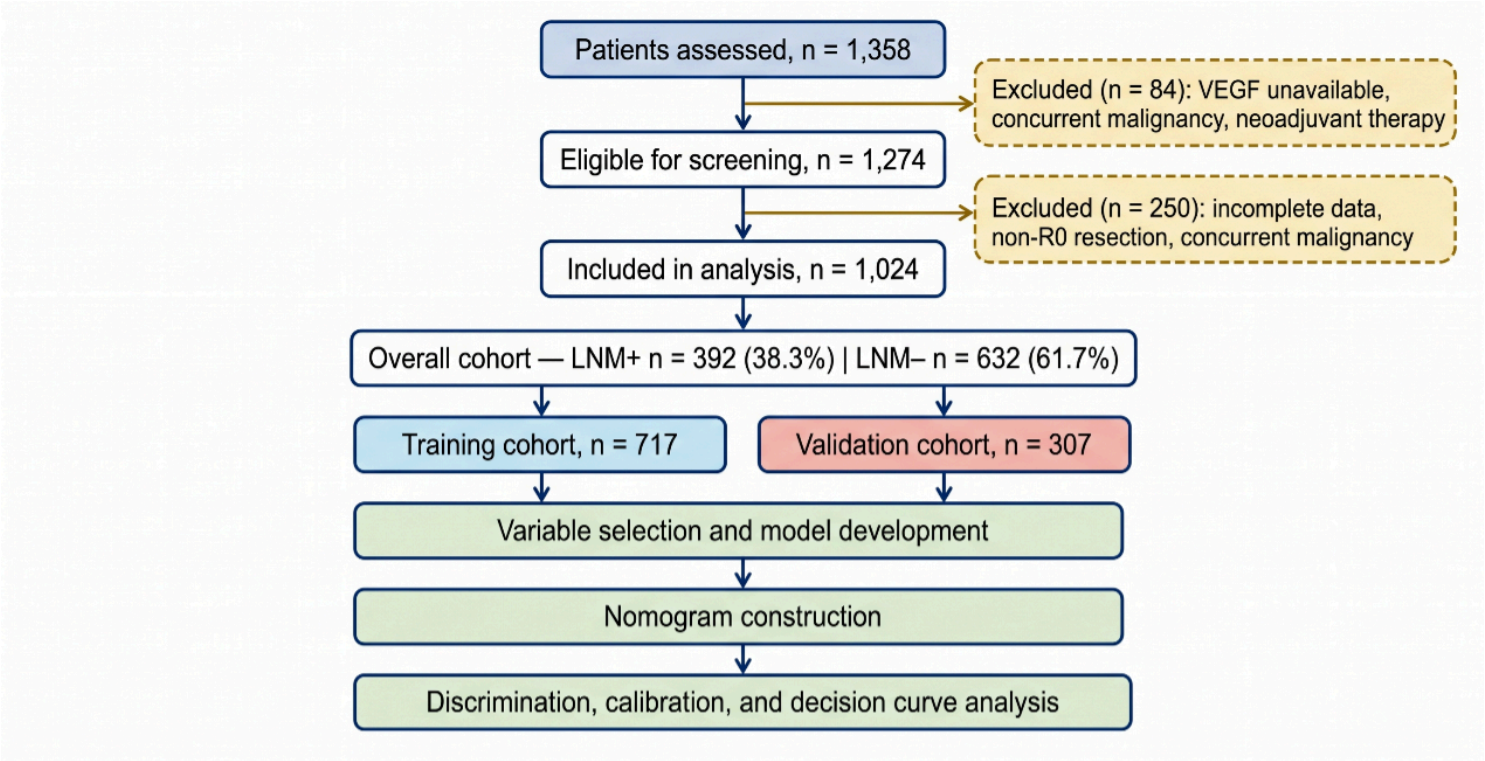


Figure 1

Flowchart of patient inclusion and exclusion

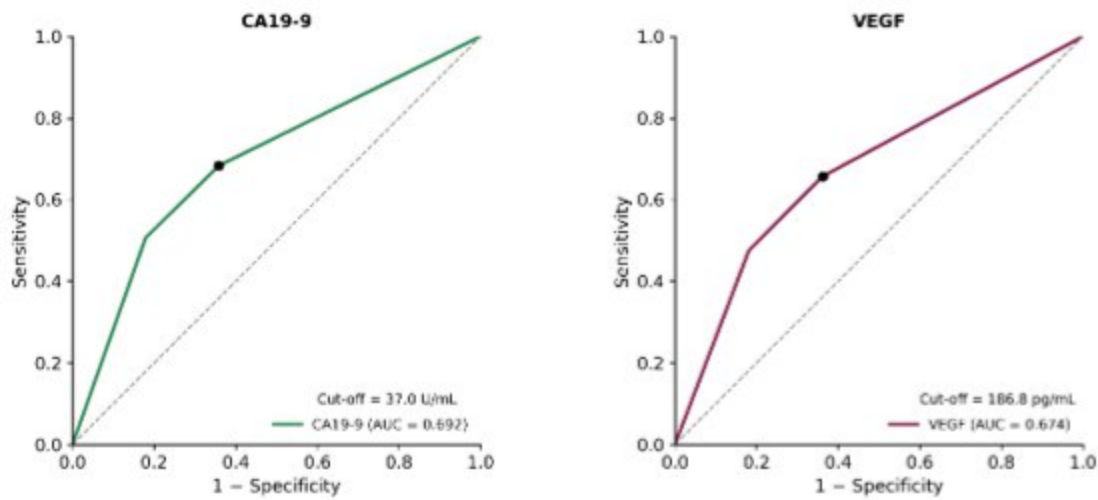


Figure 2

ROC curves and optimal cutoffs for CA19-9 and VEGF in the training cohort

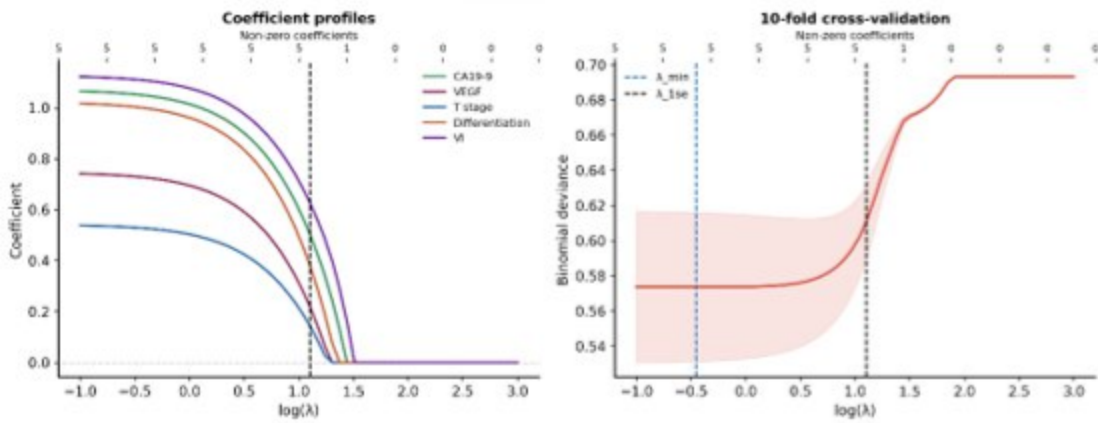


Figure 3

Variable selection by LASSO regression

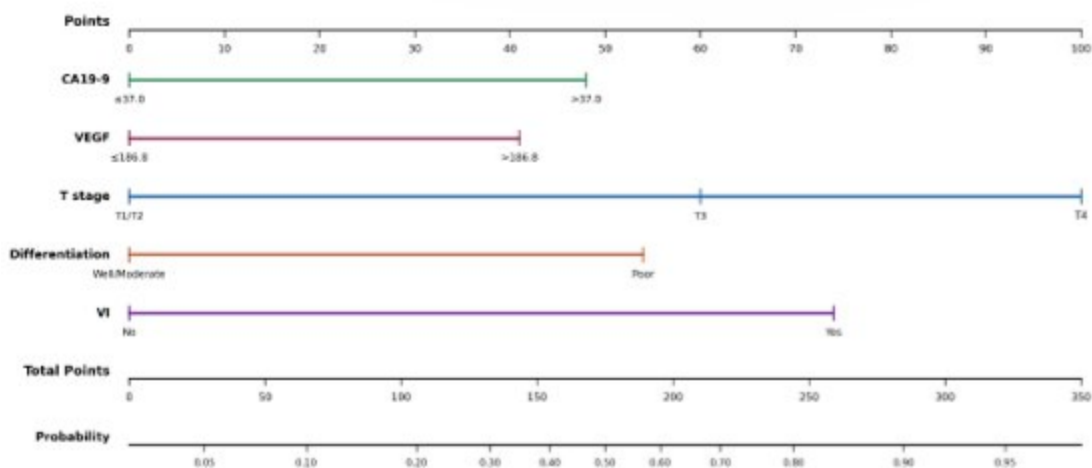


Figure 4

Nomogram for predicting lymph node metastasis in colorectal cancer

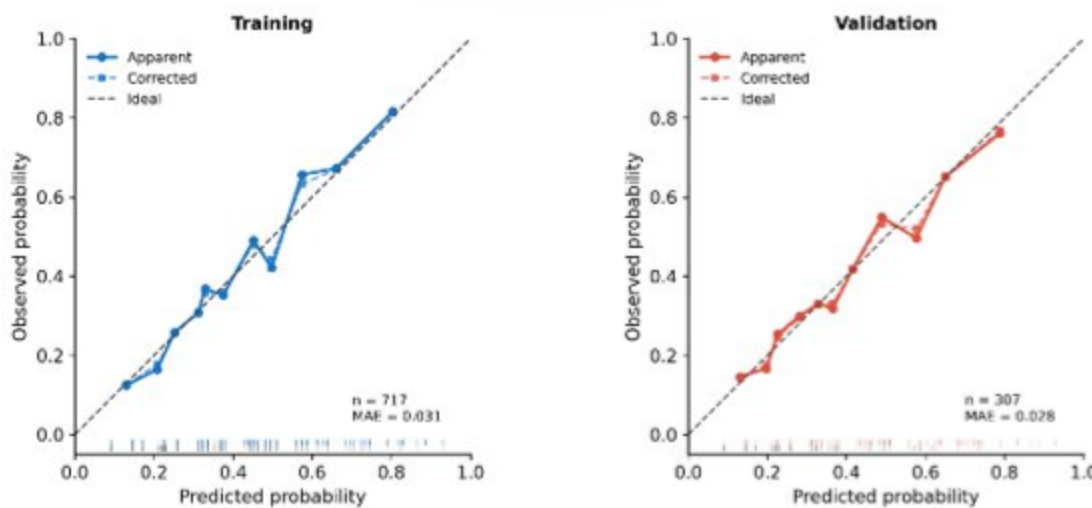


Figure 5

Calibration curves of the nomogram in the training and validation cohorts

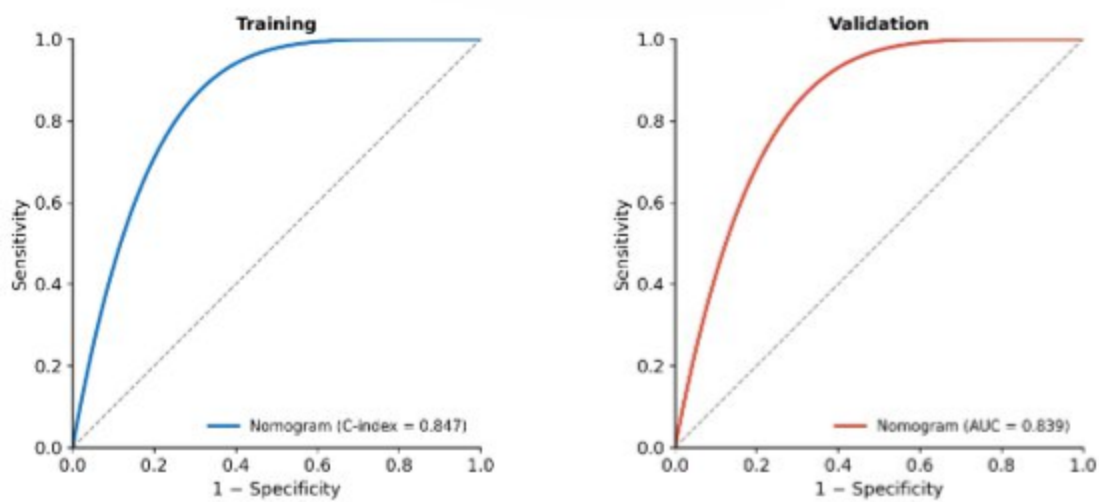


Figure 6

ROC curves of the nomogram in the training (A) and validation (B) cohorts

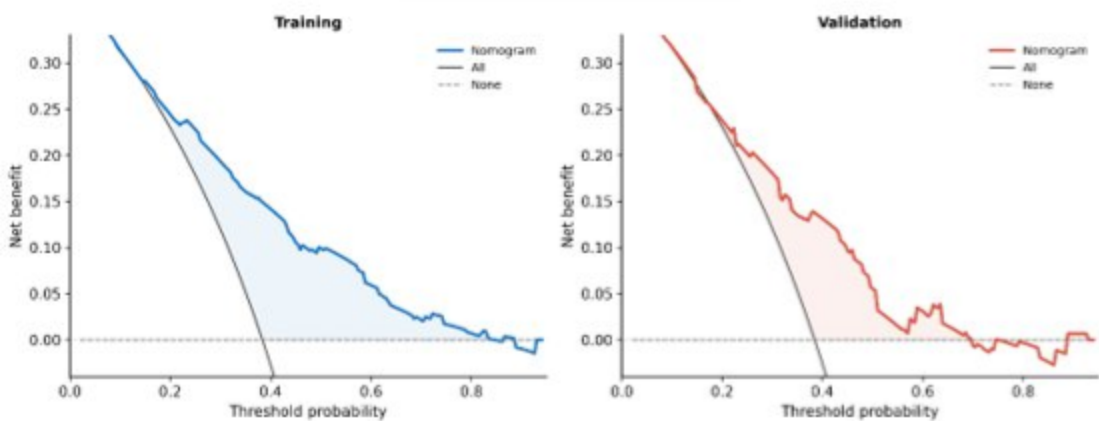


Figure 7

Decision curves of the nomogram in the training (A) and validation (B) cohorts