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ARTICLE

Combining Circulating Tumour DNA with Clinical Pathological Risk Factors for Developing Peritoneal Metastasis Prediction Model in Patients with Colorectal Cancer

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Abstract

Aims/Background Peritoneal metastasis in colorectal cancer (CRC) indicates a poor prognosis for patients. Circulating tumour DNA (ctDNA) effectively predicts recurrence and metastasis. Therefore, this study aims to construct a predictive model for peritoneal metastasis by integrating ctDNA with clinicopathological factors in stage I–III CRC patients.

Methods We conducted a retrospective analysis of 299 CRC patients who underwent ctDNA detection at The Sixth Affiliated Hospital, Sun Yat-sen University between January 2010 and December 2022. Patients were randomly divided into training (n = 209) and validation (n = 90) sets in a 7:3 ratio using a random number table method. The least absolute shrinkage and selection operator (LASSO) regression model optimized feature selection, and multivariable logistic regression constructed the predictive model.

Results Among the study cohort, 59 patients were ctDNA-positive. Postoperative ctDNA positivity was associated with an 8.522-fold increased risk of peritoneal metastasis ($p < 0.001$, odds ratio (OR) 8.522, 95% confidence interval (CI) 4.371–16.615). The model included preoperative carbohydrate antigen 125 (CA-125), pathological lymph node staging, perineural invasion, and ctDNA levels, achieving an area under the curve (AUC) of 0.808 (95% CI 0.727–0.888) in the training set and 0.784 (95% CI 0.658–0.910) in the validation set.

Conclusion This model can accurately identify high-risk patients for peritoneal metastasis in postoperative CRC, facilitating early detection and timely intervention.

Key words: colorectal cancer; circulating tumour DNA; peritoneal neoplasms; risk factors; nomogram

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Introduction

Colorectal cancer (CRC) ranks as the third most prevalent cancer worldwide, with approximately 1.9 million new cases and 935,000 deaths recorded in 2020 (Sung et al, 2021). Advances in diagnostic and screening approaches have facilitated earlier detection, with many patients diagnosed before metastasis and primarily treated through surgical interventions.

Adjuvant therapy for CRC patients primarily relies on risk stratification based on clinical and pathological factors. While only 15–20% of patients benefit from a combination of surgical and adjuvant chemotherapy, about 60% can be cured with surgery alone, indicating that many patients do not require adjuvant chemotherapy (Kotani et al, 2023). Nonetheless, recurrence or metastasis still occurs in 15–30% of patients who endure the toxic effects of chemotherapy despite standard treatment regimens (Böckelman et al, 2015). Metastasis most commonly occurs in the liver, followed by the lung and peritoneum, all of which significantly impact long-term survival. Furthermore, peritoneal metastasis occurs in 4%–19% of CRC patients, posing significant diagnostic challenges due to the limitations of imaging methods for early detection (Bang et al, 2023). Moreover, peritoneal metastasis is associated with a poor prognosis, with a median overall survival (mOS) of merely 6 to 9 months following diagnosis, where greater metastatic spread links to shorter survival times (Foster et al, 2022).

Circulating tumour DNA (ctDNA) has become an exceptionally promising biomarker for prognosis and prediction due to its non-invasive, real-time, and rapid detection characteristics. It can effectively identify minimal residual disease (MRD), a significant cause of cancer recurrence. Studies indicate that nearly all MRD-positive patients may experience relapse if left untreated (Tie et al, 2016; Tie et al, 2019). Using ctDNA for risk stratification in CRC patients receiving adjuvant therapy has shown better accuracy than traditional clinical-pathological factors (Mo et al, 2023; Tie et al, 2021). Furthermore, several observational studies have revealed that ctDNA can detect recurrence months earlier than imaging or tumour markers (Henriksen et al, 2022; Tie et al, 2016; Tie et al, 2019).

The current prediction models for peritoneal metastasis of CRC are mainly based on imaging and clinicopathological factors, primarily targeting advanced-stage patients (Song et al, 2022; Yuan et al, 2020). This study used a tumour-agnostic fixed-panel assay to assess postoperative ctDNA and its clinical significance in predicting recurrence. We employed ctDNA and clinicopathological factors and developed an internally validated predictive model for peritoneal metastasis in individuals with stage I–III CRC. This model enhances predictive accuracy and makes peritoneal metastasis earlier than imaging, offering a valuable visual tool for clinical decision-making.

Methods

Study Design and Patients

This retrospective observational study included 299 CRC patients (at stage I–III) who underwent curative surgery without neoadjuvant chemotherapy at The Sixth Affiliated Hospital, Sun Yat-sen University, China, between January 2010 and December 2022. Patients were randomly assigned into training and validation sets in a 7:3 ratio using a random number table method, with 209 patients in the training set for model construction and 90 patients in the validation set for internal validation. Fig. 1 depicts the inclusion and exclusion criteria along with the study flowchart. Tumour tissue samples were collected during surgery, and blood sam-

ples were taken between postoperative days 14 and 28. Detailed methodological information is provided in the **Supplementary Material ctDNA**.

Clinical data collected included age at diagnosis, gender, primary tumour location, preoperative carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA-125), carbohydrate antigen 199 (CA-199), tumour pathology, pathological T and N stages, presence of perineural or vascular invasion, tissue gene mutations or amplifications (consistent with the ctDNA monitoring panel), pathological tumour node metastasis (TNM) staging, and the history of adjuvant treatment received.

Patient follow-up data were collected through outpatient visits, hospital medical records, and telephone follow-ups, with the last follow-up date recorded in January 2024. They were followed at an average follow-up interval of three months, with a median follow-up duration of 65.8 months.

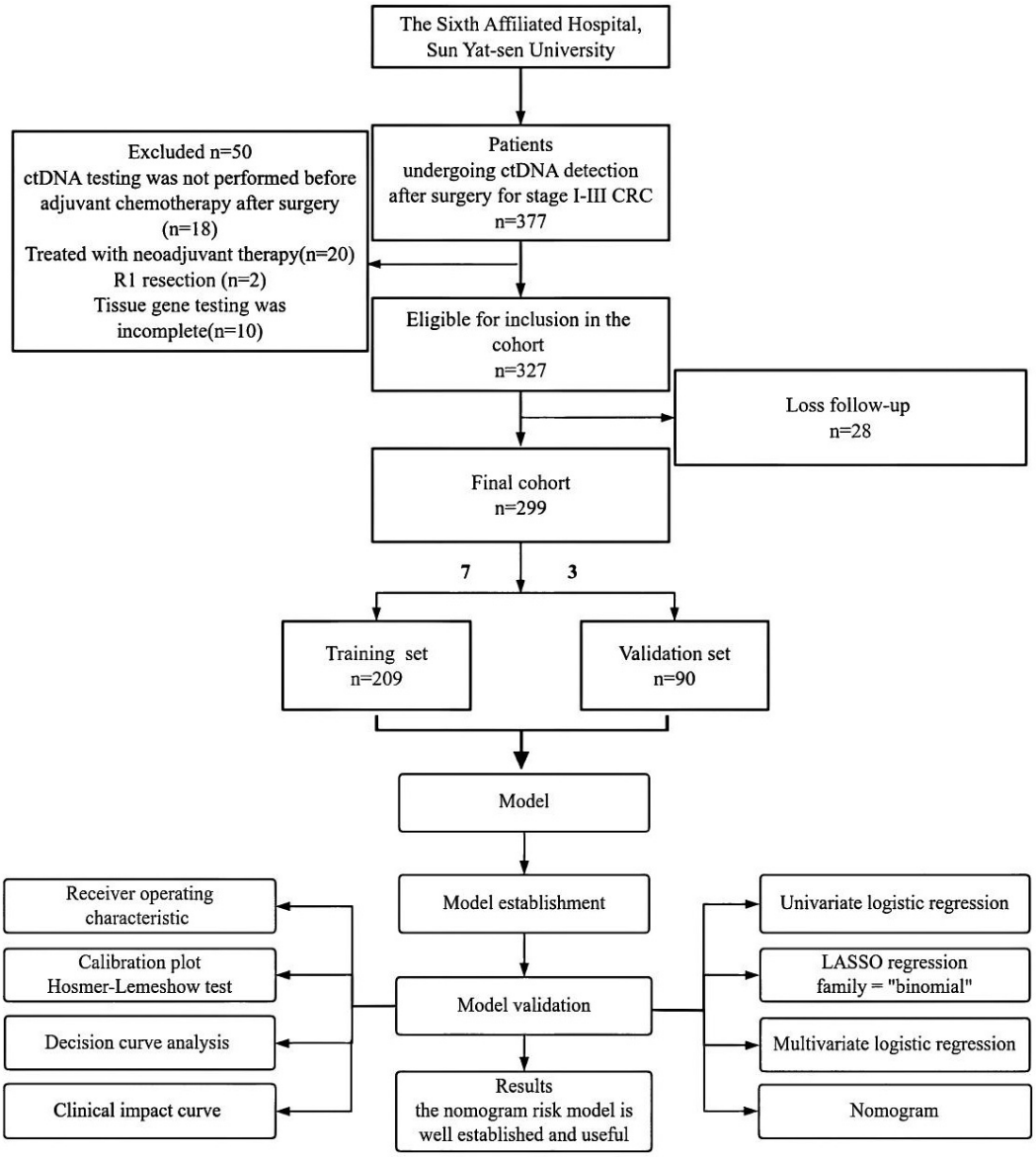

Fig. 1. A flowchart for patient selection and model development. CRC, colorectal cancer; ctDNA, circulating tumour DNA; LASSO, least absolute shrinkage and selection operator.

Definition of Variables

ctDNA positivity is characterized by class I or II mutations, indicating at least one detected somatic mutation, while ctDNA negativity is defined by class III mutations or the absence of detectable somatic variations in the ctDNA sample.

Primary tumour location: The left half of tumours include the splenic flexure, descending colon, sigmoid colon, and rectum, whereas the right half comprises the cecum, ascending colon, hepatic flexure, and transverse colon. Tumour recurrence: It is defined as the recurrence of cancer at the primary site or in lymph nodes adjacent to the primary tumour following the surgical intervention or adjuvant treatment, as well as the occurrence of distant metastasis at a site away from the original tumour site. Tumour recurrence was assessed using computed tomography (CT) or magnetic resonance imaging (MRI) by two experienced senior radiologists and oncologists. Peritoneal metastasis: This is defined as the first detection of metastasis in the peritoneum post-surgery, regardless of the presence of extraperitoneal metastasis.

The study design was approved by the Ethics Committee of The Sixth Affiliated Hospital, Sun Yat-sen University (Approval ID: 2023ZSLYEC-686) and complied with the principles outlined in the Declaration of Helsinki. Furthermore, the Sixth Affiliated Hospital, Sun Yat-sen University waived the requirement for informed consent.

Detection of ctDNA

Plasma ctDNA testing was conducted 2–8 weeks postoperatively before the initiation of adjuvant therapy. This study utilized an 88-gene Next-Generation Sequencing (NGS) panel provided by Guangzhou Kingmed Diagnostics Group Co., Ltd., to detect single nucleotide variations, insertions/deletions, and copy number variations in the exonic regions of 22 genes associated with colorectal and lung cancers. These genes included epidermal growth factor receptor (*EGFR*), erb-B2 receptor tyrosine kinase 2 (*ERBB2*), fibroblast growth factor receptor 1 (*FGFR1*), fibroblast growth factor receptor 2 (*FGFR2)*, fibroblast growth factor receptor 3 (*FGFR3*), mesenchymal epithelial transition factor (*MET*), phosphoinositide-3-kinase catalytic subunit alpha (*PIK3CA*), v-akt murine thymoma viral oncogene homolog 1 (*AKT1*), anaplastic lymphoma kinase (*ALK*), B-Raf proto-oncogene (*BRAF*), catenin beta 1 (*CTNNB1*), discoidin domain receptor tyrosine kinase 2 (*DDR2*), erb-B2 receptor tyrosine kinase 4 (*ERBB4*), F-Box and WD repeat domain containing 7 (*FBXW7*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), mitogen-activated protein kinase kinase 1 (*MAP2K1*), neurogenic locus notch homolog protein 1 (*NOTCH1*), neuroblastoma RAS viral oncogene homolog (*NRAS*), phosphatase and tensin homolog (*PTEN*), SMAD family member 4 (*SMAD4*), serine/threonine kinase 11 (*STK11*), and tumour protein P53 (*TP53*). The NGS panel achieved a sequencing depth of 10,000× and exhibited an effective sensitivity of 0.25%.

Detailed information on the DNA detection method is provided in the **Supplementary Material ctDNA**.

Statistical Analysis

Statistical analyses were conducted using R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria), and the data were processed and graphically presented through SPSS version 25.0 (International Business Machines Corporation, Armonk, NY, USA).

Initially, the data were assessed for normality using the Kolmogorov-Smirnov test. Variables following a normal distribution were presented as mean ± standard deviation, and group comparisons were performed using the student's *t*-test. For continuous variables that did not meet the normality assumptions, descriptive statistics were reported as the median and interquartile range, employing the Wilcoxon rank-sum test as a non-parametric alternative for group comparisons. Categorical variables were expressed as counts and percentages, and group comparisons were conducted using the chi-square test or Fisher's exact test, depending on the expected frequencies.

The dataset was randomly grouped into training and validation sets in a 7:3 ratio using the Caret package (https://cran.r-project.org/web/packages/caret/index.html) in R. Variables with a $p < 0.05$ in univariate analysis were included in the least absolute shrinkage and selection operator (LASSO) regression. LASSO was conducted with ten-fold cross-validation, applying the minimum lambda criterion to select the optimal combination of influencing factors.

The cross-validation process involved dividing the dataset into 10 equal subsets and iteratively applying LASSO regression across the training set. The smallest lambda value, obtained based on cross-validated mean squared error (MSE), was selected to balance model complexity. Subsequently, multiple backward stepwise logistic regression was performed for further variable selection, using the smallest Akaike Information Criterion (AIC) to identify the most significant factors. These factors were then used to construct the peritoneal metastasis nomogram.

The receiver operating characteristic (ROC) and calibration curves were plotted to evaluate the nomogram's discriminatory power and calibration accuracy. Model calibration was further evaluated using the Hosmer-Lemeshow test, with a significant result indicating poor fit. For internal validation, Harrell's concordance index (C-index) was calculated, and bootstrapping with 500 replicates was applied to ensure robustness. Decision curve analysis (DCA) was performed to assess the net benefit of the nomogram across varying threshold probabilities, while the clinical impact curve (CIC) provided additional insights into its clinical significance. All statistical analyses were performed using R software (Version 4.3.1; https://www.R-project.org), the R software packages used include:

- survival (https://cran.r-project.org/web/packages/survival/index.html),
- pROC (https://cran.r-project.org/web/packages/pROC/index.html),
- ComplexHeatmap (https://bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html),
- calibrate (https://cran.r-project.org/web/packages/calibrate/index.html),
- MASS (https://cran.r-project.org/web/packages/MASS/index.html),
- rms (https://cran.r-project.org/web/packages/rms/index.html),
- foreign (https://cran.r-project.org/web/packages/foreign/index.html),

- nricens (https://cran.r-project.org/web/packages/nricens/index.html),
- glmnet (https://cran.r-project.org/web/packages/glmnet/index.html),
- Matrix (https://cran.r-project.org/web/packages/Matrix/index.html),
- ggplot2 (https://cran.r-project.org/web/packages/Matrix/index.html),
- lattice (https://cran.r-project.org/web/packages/lattice/index.html),
- Caret (https://cran.r-project.org/web/packages/caret/index.html).

Results

Analysis of Clinicopathological Characteristics Across the Study Cohort

We analyzed 299 stage I–III CRC patients who underwent curative-intent surgery without receiving neoadjuvant chemotherapy, identifying 59 (19.7%) as ctDNA-positive. Detailed clinicopathological characteristics of these patients are shown in **Supplementary Fig. 1**. The heatmap analysis revealed that ctDNA-positive individuals exhibited a higher prevalence of clinical and pathological risk factors compared to ctDNA-negative patients, which are linked to significantly higher rates of recurrence, metastasis, and mortality. Postoperative ctDNA-positive patients had a 32.729-fold higher risk of recurrence and an 11.244-fold higher risk of mortality compared to ctDNA-negative patients. Furthermore, ctDNA positivity was linked to an 8.522-fold higher risk of peritoneal metastasis ($p < 0.001$, Table 1). The study cohort was divided into a training set (n = 209) and a validation set (n = 90) in a 7:3 ratio, with no statistically significant differences found in the majority of baseline characteristics between the two sets (**Supplementary Table 1**).

Peritoneal Metastasis Model Construction

In the training set, univariate logistic regression analysis was conducted to evaluate factors associated with peritoneal metastasis. Factors with $p < 0.05$ were selected for inclusion in the subsequent LASSO logistic regression analysis (**Supplementary Table 2**). Using LASSO cross-validation, 9 factors were identified for multivariable regression analysis, including ctDNA, CA-125, pathological N stage (N1 and N2), perineural invasion, tissue TP53, plasma TP53, plasma PIK3CA, and plasma SMAD4 (**Supplementary Fig. 2**). Subsequently, multiple stepwise regression (backward method) was conducted, with the smallest AIC used to identify the most significant predictors. The final model included CA-125, ctDNA, pathological N stage, and perineural invasion (**Supplementary Table 2**). To improve clinical utility, a nomogram was developed to provide an accurate estimation of a patient's probability of peritoneal metastasis. By summing the scores assigned to each variable and aligning the total point on the vertical axis, the nomogram facilitates precise risk assessment (Fig. 2).

Table 1. Clinicopathological features and the association between ctDNA status and related parameters.

Factors	Levels	Overall study cohort (n = 299, %)	ctDNA positive (n = 59, 19.7%)	ctDNA negative (n = 240, 80.3%)	*p*-value	OR	95% CI
Sex	Male Female	189 (63.2%) 110 (36.8%)	34 (57.6%) 25 (42.4%)	155 (64.6%) 85 (35.4%)	0.332	1.341	0.751–2.395
Age, Median (Q1–Q3), (years)		58.0 [50.0; 65.0]	60.0 [45.0; 68.0]	58.0 [51.0; 64.0]	0.750	0.996	0.972–1.020
Primary tumour location	Left Right	225 (75.3%) 74 (24.7%)	44 (74.6%) 15 (25.4%)	181 (75.4%) 59 (24.6%)	0.893	1.046	0.543–2.014
Pre-CEA	≤5 ng/mL >5 ng/mL	176 (58.9%) 123 (41.1%)	28 (47.5%) 31 (53.5%)	148 (61.7%) 92 (38.3%)	0.049	1.781	1.004–3.160
Pre-CA-199	≤37 u/mL >37 u/mL	243 (81.3%) 56 (18.7%)	41 (69.5%) 18 (30.5%)	202 (84.2%) 38 (15.8%)	0.011	2.334	1.214–4.487
Pre-CA-125	≤35 u/mL >35 u/mL	268 (89.6%) 31 (10.4%)	47 (79.7%) 12 (20.3%)	221 (92.1%) 19 (7.9%)	0.007	2.970	1.350–6.532
Pathological T staging	1 2 3 4	8 (2.6%) 19 (6.4%) 217 (72.6%) 55 (18.4%)	1 (1.7%) 3 (5.1%) 36 (61.0%) 19 (32.3%)	7 (2.9%) 16 (6.7%) 181 (75.4%) 36 (15.0%)	Ref 0.826 0.765 0.237	 1.313 1.392 3.694	 0.115–14.927 0.166–11.664 0.423–32.284
Pathological N staging	0 1 2	131 (43.8%) 100 (33.4%) 68 (22.7%)	17 (28.8%) 20 (33.9%) 22 (37.3%)	114 (47.5%) 80 (33.3%) 46 (19.2%)	Ref 0.152 0.002	 1.676 3.207	 0.827–3.399 1.562–6.586
Cancerous node	No Yes	219 (73.2%) 80 (26.8%)	31 (52.5%) 28 (17.4%)	188 (78.3%) 52 (21.7%)	<0.001	3.266	1.799 5.927
Perineural invasion	No Yes	201 (67.2%) 98 (32.8%)	33 (55.9%) 26 (44.1%)	168 (70.0%) 72 (30.0%)	0.041	1.838	1.026–3.295

Table 1. Continued.

Factors	Levels	Overall study cohort (n = 299, %)	ctDNA positive (n = 59, 19.7%)	ctDNA negative (n = 240, 80.3%)	*p*-value	OR	95% CI
Vascular invasion	No	202 (67.6%)	35 (59.3%)	167 (69.6%)	0.133	1.569	0.872–2.824
	Yes	97 (32.4%)	24 (40.7%)	73 (30.4%)			
Pathology	Adenocarcinoma	248 (82.9%)	49 (83.1%)	199 (82.9%)	0.980	0.991	0.464–2.115
	Non-adenocarcinoma	51 (17.1%)	10 (16.9%)	41 (17.1%)			
Pathological TNM staging	1	13 (4.35%)	1 (1.7%)	12 (5.0%)	Ref		
	2	106 (35.5%)	11(18.6%)	95 (39.6%)	0.763	1.389	0.165–11.732
	3	180 (60.2%)	47 (79.7%)	133 (55.4%)	0.171	4.241	0.537–33.503
Chemotherapy	No	37 (12.4%)	11 (18.6%)	26 (10.8%)	0.107	0.530	0.245–1.147
	Yes	262 (87.6%)	48 (91.4%)	214 (89.2%)			
Recurrence	No	173 (57.9%)	4 (6.8%)	169 (70.4%)	<0.001	32.729	11.428–93.730
	Yes	126 (42.1%)	55 (93.2%)	71 (29.6%)			
Dead	No	251 (83.9%)	30 (50.8%)	221 (92.1%)	<0.001	11.244	5.623–22.482
	Yes	48 (16.1%)	29 (49.2%)	19 (7.9%)			
Peritoneal metastasis	No	248 (82.9%)	31 (52.5%)	217 (90.4%)	<0.001	8.522	4.371–16.615
	Yes	51 (17.1%)	28 (47.5%)	23 (9.6%)			

ctDNA, circulating tumour DNA; OR, odds ratio; CI, confidence interval; Q1, the first quartile; Q3, the third quartile; Pre-CEA, preoperative carcinoembryonic antigen; Pre-CA-199, preoperative carbohydrate antigen 199; Pre-CA-125, preoperative carbohydrate antigen 125; TNM, tumour node metastasis; Ref, reference.

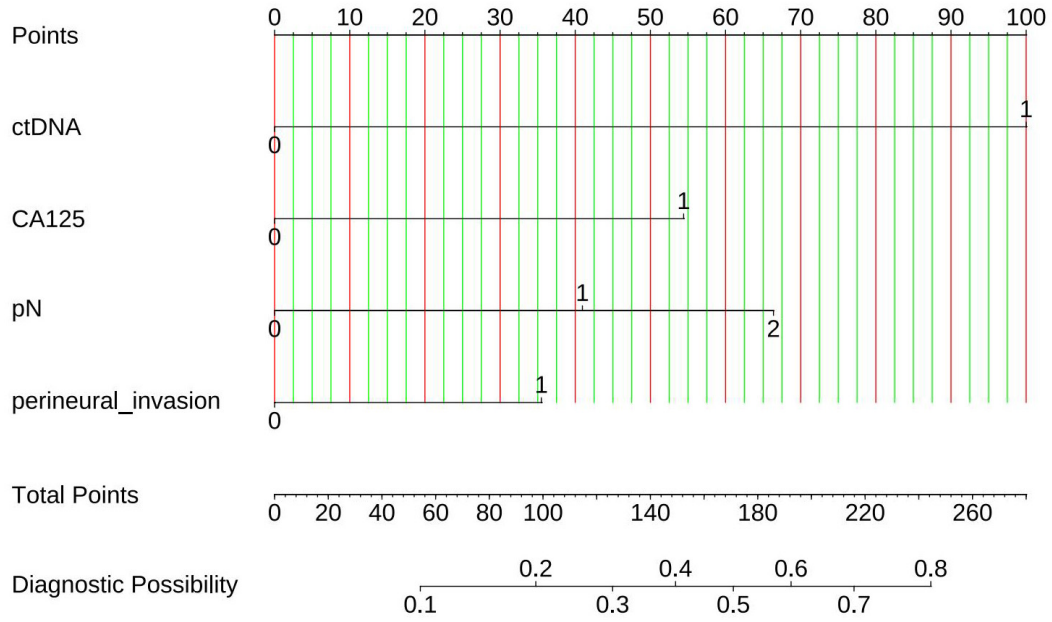

Fig. 2. Predictive nomograms for peritoneal metastasis. ctDNA, circulating tumour DNA, "0" represents "negative", "1" represents "positive"; CA-125, carbohydrate antigen 125, "0" represents "≤35 μ/mL", "1" represents ">35 μ/mL"; pN, pathological N stage, "0" represents "pN0", "1" represents "pN1", "2" represents "pN2"; perineural invasion, "0" represents "No", "1" represents "Yes".

Performance and Validation of the Nomogram

The area under the curve (AUC) for the training and validation sets were 0.808 and 0.784, respectively. Bootstrap validation with 500 replications yielded a C-index of 0.809 for the training set and 0.776 for the validation set, indicating the strong discriminatory ability of the model (Fig. 3A–D). The Hosmer-Lemeshow test results were 0.394 and 0.261 for the training and validation sets, respectively, suggesting good model calibration. Calibration curves for both study cohorts exhibited high consistency between predicted and observed outcomes (Fig. 3E,F).

Furthermore, the clinical efficacy of the model was assessed using DCA and CIC. DCA indicated that the model provided maximum net benefits when threshold probabilities ranged between 10% and 90% in both training and the validation sets, demonstrating high diagnostic accuracy within this range (Fig. 4A,B). The CIC further demonstrated the superior overall net benefit of the nomogram across a wide range of threshold probabilities (Fig. 4C,D).

Two predictive models were constructed to assess the risk of peritoneal metastasis using logistic regression: one model excluding the ctDNA element and the other based on the TNM staging system. The AUC of our model surpassed both the model and the conventional TNM staging system (Fig. 5A,B). Comparative DCA of these models also demonstrated that our model has substantial clinical applicability (Fig. 5C,D).

These results highlight the significant predictive value of our model. Analysis of the nomoscore revealed that patients predicted to develop peritoneal metastasis

in both the training and validation sets exhibited significantly higher nomoscore values than those without peritoneal metastasis ($p < 0.001$, Fig. 5E,F).

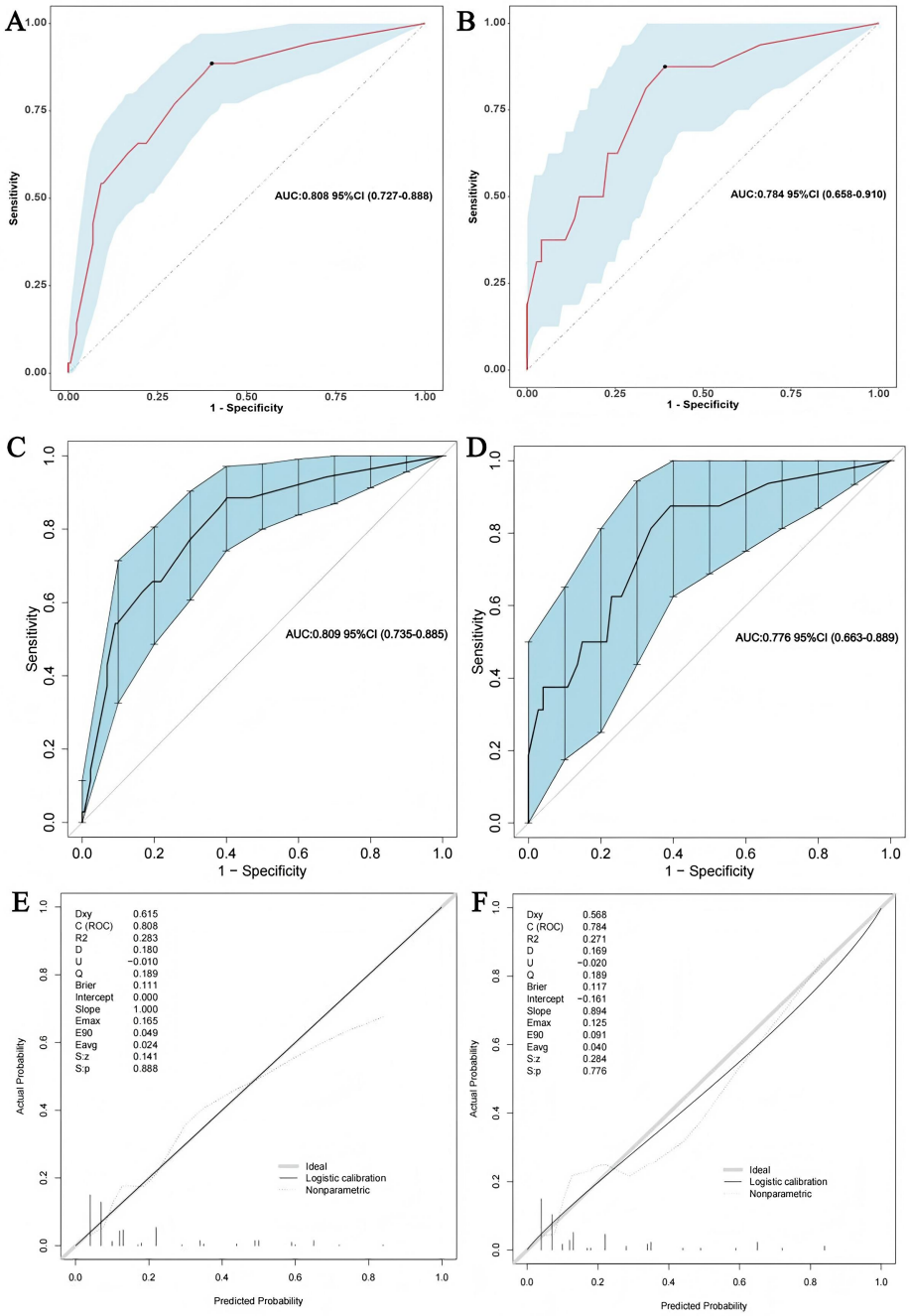

Fig. 3. Model validation diagram. (A) ROC curve for the training set. (B) ROC curve for the validation set. (C) ROC curve for the training set after 500 bootstraps. (D) ROC curve for the validation set after 500 bootstraps. (E) Calibration curve for the training set. (F) Calibration curve for the validation set. The blue shaded area represents the confidence interval. ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval.

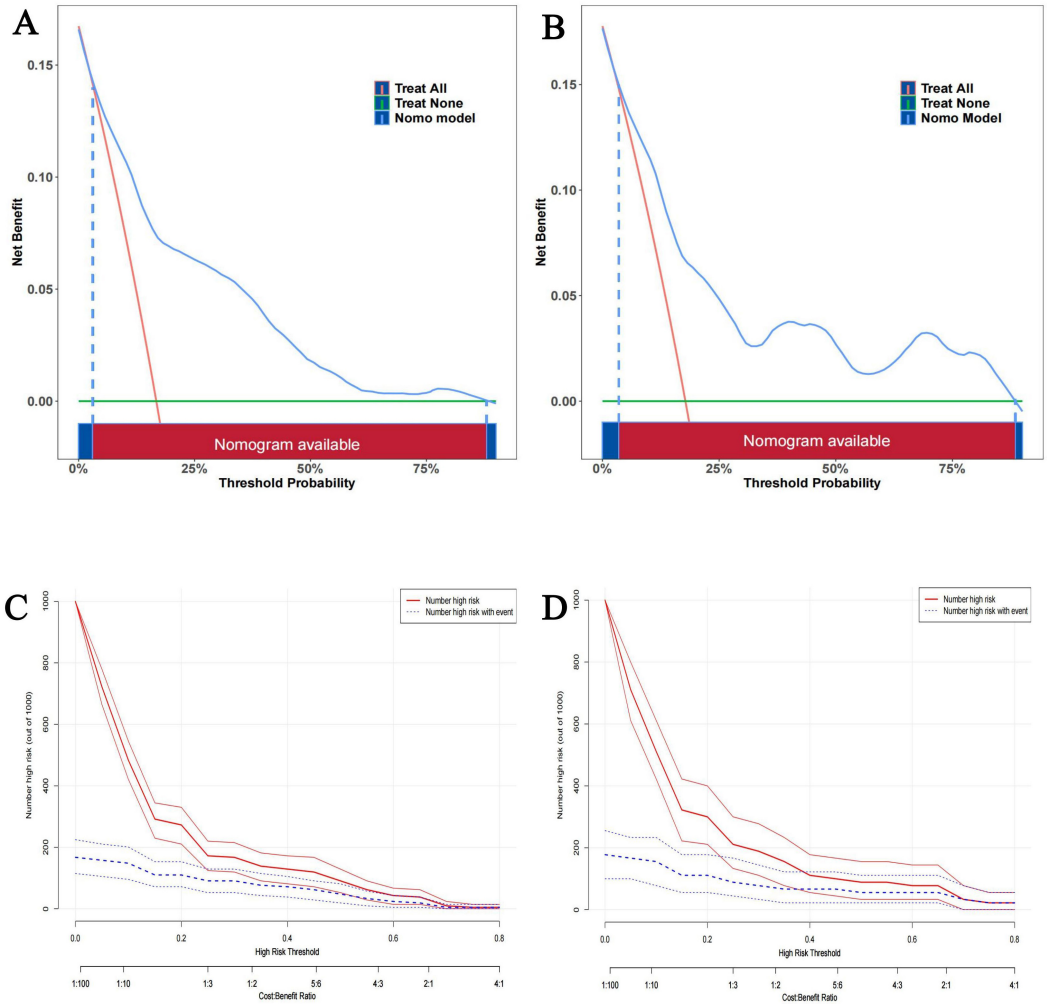

Fig. 4. Clinical benefit curve of the model. (A) DCA for the training set. (B) DCA for the validation set. (C) CIC for the training set. (D) CIC for the validation set. The range of clinical benefit is represented between the two vertical dashed lines; The red curve represents the number of individuals classified by the model as high risk at each threshold probability; the blue curve represents the number of individuals classified as high-risk by the model who subsequently experienced the outcome at each threshold probability. DCA, decision curve analysis; CIC, clinical impact curve.

Discussion

In this study, we developed a predictive model for peritoneal metastasis following curative resection of CRC based on four variables: postoperative ctDNA status, preoperative CA-125 levels, pathological N staging, and perineural invasion. The model was internally validated using bootstrap methods and a validation set, thus demonstrating stability and strong predictive performance. This study, for the first time, combined clinical and pathological factors with ctDNA in predicting peritoneal metastasis after curative surgery for CRC.

Globally, CRC has a high incidence and mortality rate. Several studies have revealed that metastasis is a primary cause of recurrence and a poor prognosis in CRC (Böckelman et al, 2015; Hansdotter et al, 2023). Peritoneal metastasis in CRC is second to liver and lung metastases, and patients with peritoneal metastasis usu-

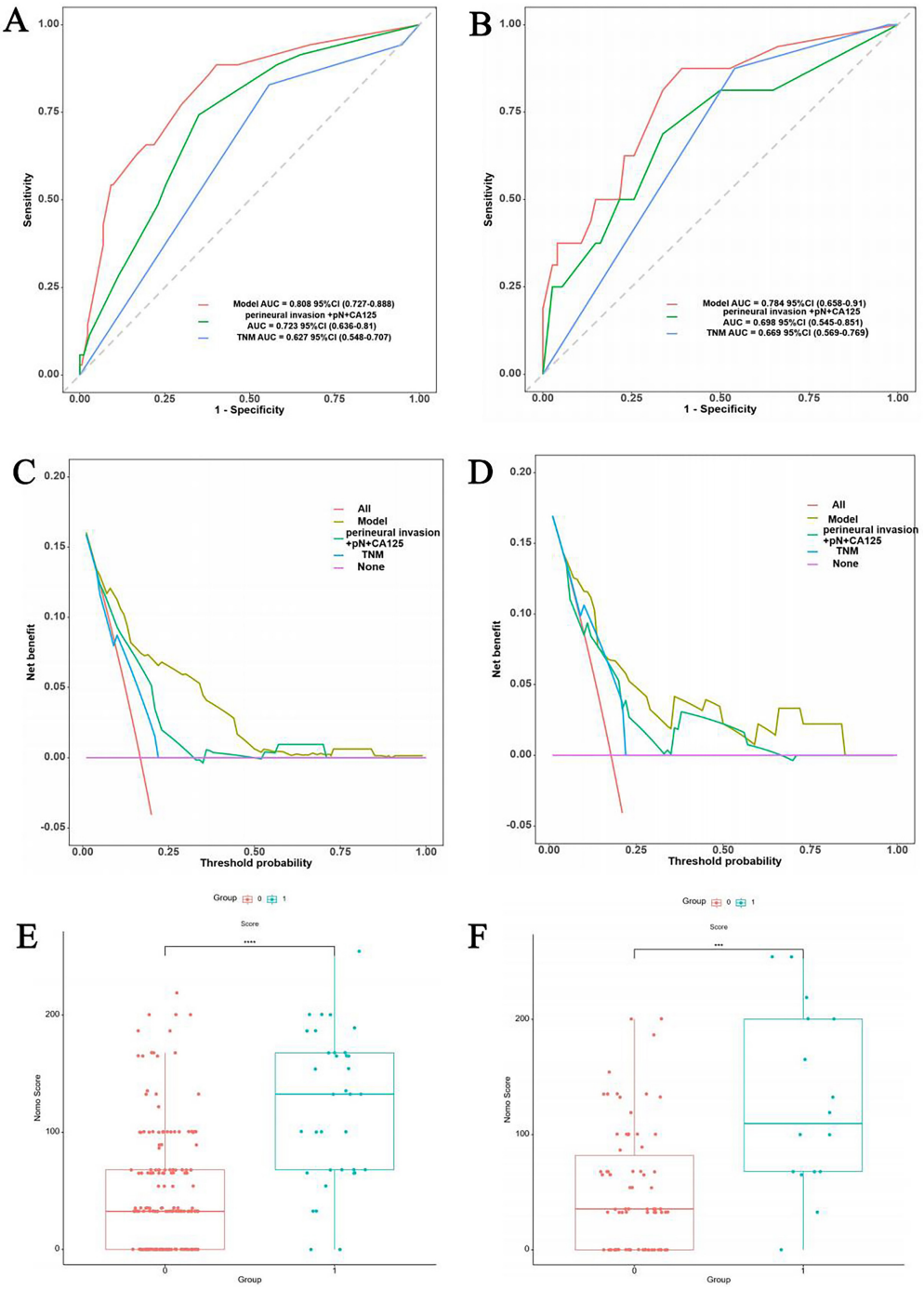

Fig. 5. Rationale analysis. (A) Comparative ROC curves for multiple models in the training set. (B) Comparative ROC curves for multiple models in the validation set. (C) Comparative DCA of multiple models in the training set. (D) Comparative DCA of multiple models in the validation set. (E) Box plot analysis of peritoneal metastasis based on nomoscore in the training set. (F) Box plot analysis of peritoneal metastasis based on nomoscore in the validation set. The Y-axis represents the nomoscore, while the X-axis indicates the peritoneal metastasis group and the non-peritoneal metastasis group. This figure illustrates the distribution of nomoscores across both populations, aiding in assessing the model's predictive efficacy for peritoneal metastasis. "0", non-peritoneal metastasis group; "1", peritoneal metastasis group. "***", $p < 0.001$. "****", $p < 0.0001$.

ally show a poor prognosis. Additionally, these patients are more likely to develop malignant ascites, with a one-year survival rate of less than 10% for those affected by malignant ascites (Mo and Cai, 2016). Therefore, early detection of peritoneal metastasis and timely intervention are particularly crucial.

The diagnosis of peritoneal metastasis in CRC usually relies on imaging studies, such as CT, MRI, and positron emission tomography-computed tomography (PET-CT). Furthermore, diagnostic models for peritoneal metastasis in CRC based on CT characteristics are developed, demonstrating significant diagnostic value (Yuan et al, 2020). However, imaging in early detection is challenging, particularly in nodules sized 5 mm or less, which are often difficult to identify using conventional methods (De Wever et al, 2007). Additionally, imaging diagnoses are usually delayed, and when peritoneal metastasis is confirmed via imaging, a more advanced disease stage has already been reached. Furthermore, traditional tumour markers sometimes yield false positives and false negatives, complicating the diagnosis of peritoneal metastasis (Sørbye and Dahl, 2004). Our model incorporates ctDNA, offering earlier indications of recurrence compared to imaging techniques. Integrating ctDNA with tumour markers for predicting peritoneal metastasis enhances prediction accuracy, reducing the likelihood of both false-positive and false-negative results.

Currently, ctDNA has emerged as a highly promising prognostic biomarker with widespread application in CRC. Besides its potential to predict a high risk, if ctDNA remains continuously positive without systemic treatment, then 95–100% of patients will experience recurrence within two years (Tie et al, 2016). Consistent with prior research (Mo et al, 2023), we observed that ctDNA positivity was significantly associated with reduced recurrence-free survival (RFS) rates and increased mortality. The recurrence risk for patients who were postoperative ctDNA-positive was 32.729 times higher than ctDNA-negative, and the corresponding mortality risk was 11.244 times greater.

Numerous studies have reported that elevated CA-125 levels are significantly associated with poor prognosis in various cancers, such as gastric, ovarian, colorectal, and pancreatic (Huang et al, 2022; Luo et al, 2023b). Clinically, elevated CA-125 levels are routinely interpreted as a potential indicator of peritoneal metastasis. Moreover, CA-125 is closely linked to high-risk clinicopathological characteristics and is routinely used as a serological marker for monitoring treatment efficacy in patients with peritoneal metastasis from advanced gastric cancer (Ando et al, 2023). The increase in CA-125 during peritoneal metastasis may be due to an inflammatory response in the peritoneum, subsequently increasing its levels. Alternatively, it may also result from excessive ascites production, leading to a substantial release of CA-125 from mesothelial cells into the bloodstream (Zhu et al, 2017). Consequently, CA-125 is widely regarded as a crucial biomarker for peritoneal metastasis.

Growing evidence from current studies reported perineural invasion as a key factor in cancer progression, significantly linked to lower survival rates and increased tumour recurrence rates (Alotaibi et al, 2017). In CRC patients, the perineural invasion occurs in 20% to 57% of cases (Chu et al, 2024). Current guidelines consider perineural invasion as a high-risk factor for postoperative recurrence.

Moreover, in patients with stage II CRC exhibiting perineural invasion, the use of adjuvant chemotherapy is recommended (Benson et al, 2017). Gastric cancer research has found that the presence of perineural invasion significantly elevates the risk of both local recurrence and peritoneal dissemination, with the risk of peritoneal dissemination being higher than that of local recurrence (Li et al, 2023; Luo et al, 2023a). The process through which perineural invasion may facilitate cancer cell dissemination is complex and involves intricate interactions. Additionally, the spread of cancer cells causes neuronal damage, which in turn promotes the dissemination of cancer cells, ultimately leading to recurrence and metastasis (Martyn et al, 2019).

The TNM staging system remains the most widely adopted and recognized tumour staging system worldwide. The N staging specifically refers to the involvement of regional lymph nodes, which is highly linked to tumour recurrence and metastasis. Among the various metastatic pathways in CRC, lymph node metastasis is predominant and a crucial factor (Song et al, 2024). In CRC, pathological N staging is an essential determinant of patient survival and plays a vital role in the formulation of personalized treatment strategies (Sun et al, 2024). Moreover, the integration of N staging with other molecular biomarkers to predict prognosis has been shown to offer new avenues for enhancing the predictive power of N staging alone (Li et al, 2024). Pathological N staging has been a crucial predictive factor in our model.

Currently, limited studies have investigated the potential predictive role of ctDNA in determining metastasis sites, and conventional imaging presents significant challenges in accurately diagnosing peritoneal metastases. While postoperative ctDNA has shown significant prognostic value, the role of tumour clinical and pathological characteristics in personalized treatment and monitoring should not be underestimated. These characteristics indicate the inherent features of tumours and play a crucial role in clinical decision-making (Argilés et al, 2020; Grothey et al, 2018). Combining ctDNA with clinical and pathological factors offers the potential for enhanced risk stratification and more informed clinical decisions. Therefore, we constructed predictive models for peritoneal metastasis that combine ctDNA and clinical-pathological risk factors. Previous research revealed that combining ctDNA with clinical and pathological factors offers better predictive accuracy for recurrence than models relying solely on ctDNA or clinical indicators (Gao et al, 2023). Among the multiple models analyzed, our model, which integrates ctDNA and clinical-pathological risk factors, demonstrated superior performance in both the training and validation sets compared to models based solely on clinical-pathological factors. Notably, this model was explicitly established for stage I–III colorectal cancer patients, highlighting its significant role in guiding follow-up surveillance. It is an essential tool for closely monitoring high-risk patients and enabling early intervention for those at risk of peritoneal metastasis.

Despite its promising outcomes, this study has specific limitations. Firstly, it was conducted retrospectively rather than prospectively, and while samples were not deliberately selected, the possibility of selection bias cannot be entirely ruled out. Moreover, the findings of this study align with previous research on ctDNA

concerning adjuvant therapy; the limited size of ctDNA panels restricts their ability to provide comprehensive guidance for subsequent treatment decisions. Furthermore, although pathological biopsy remains the diagnostic gold standard, obtaining biopsies in patients with peritoneal metastasis is challenging, and the associated positive rates are generally low. To mitigate diagnostic bias for peritoneal metastasis, we relied on the response of peritoneal lesions to anti-tumour treatments and assessments by experienced oncologists and radiologists based on imaging studies. While the results of our validation set were promising, external validation using independent datasets was not performed. In the future, we plan to conduct prospective studies to validate and refine our model. This approach will enhance the robustness of our findings and potentially improve the clinical utility of the predictive model across diverse patient populations.

Conclusion

We developed and validated a predictive model for peritoneal metastasis after curative surgery for CRC, providing a valuable tool for guiding clinical decision-making. This model underscores the significance of implementing enhanced follow-up protocols for patients identified as high-risk for peritoneal recurrence, enabling early detection and timely intervention. Notably, integrating clinicopathological factors with ctDNA status significantly enhances the model's predictive accuracy and capacity to anticipate metastasis.

Key Points

- This study reveals the significant role of ctDNA in predicting metastasis sites.
- We developed and validated a predictive model for peritoneal metastasis in stage I–III CRC patients after surgery, providing a valuable diagnostic tool for clinical use.
- Integrating clinicopathological factors with ctDNA status significantly improves model's predictive accuracy.
- Patients identified as high risk for peritoneal metastasis by the model require more rigorous follow-up to enable early intervention.

Availability of Data and Materials

All data included in this study are available upon request by contact with the corresponding author.

Author Contributions

BH designed the research study. BH, XX and YD performed the research. BH, HH and JZ handled data collection and analysis. BH and YD contributed to drafting the manuscript. All authors engaged in the critical revision of the manuscript. All

authors approved the final manuscript and took responsibility for the integrity of the work, ensuring that any concerns about accuracy or completeness were addressed.

Ethics Approval and Consent to Participate

The Ethics Committee of The Sixth Affiliated Hospital, Sun Yat-sen University approved the protocols (Approval ID: 2023ZSLYEC-686), which complied with the principles of the Declaration of Helsinki. Informed consent was waived by the Ethics Committee of The Sixth Affiliated Hospital, Sun Yat-sen University.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://www.magonlinelibrary.com/doi/suppl/10.12968/hmed.2024.0704.

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