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Newest challenges and advances in the treatment of colorectal disorders; from predictive biomarkers to minimally invasive techniques

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Newest challenges and advances in the treatment of colorectal disorders; from predictive biomarkers to minimally invasive techniques

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Table of contents

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Editorial: Newest challenges and advances in the treatment of colorectal disorders; from predictive biomarkers to minimally invasive techniques

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Editorial on the Research Topic
Newest challenges and advances in the treatment of colorectal disorders; from predictive biomarkers to minimally invasive techniques

The latest issue of Frontiers in Surgery highlights significant strides in colorectal disorders research, with an array of studies examining novel prognostic tools, surgical techniques, and treatment strategies. These studies collectively underscore the importance of personalized medicine, offering new insights into predictive markers, surgical innovations, and the nuanced role of adjuvant therapies.

One study by Zheng et al. provides a detailed comparative analysis of laparoscopic-assisted transanal natural orifice specimen extraction (NOSE) vs. conventional laparoscopic surgery (CLS) for sigmoid and rectal cancer. Among 121 patients, NOSE was associated with a shorter total incision length, highlighting its cosmetic advantage. However, this benefit was offset by a longer operation time compared to CLS. Importantly, there were no significant differences in postoperative complications, such as bacterial culture positivity, intra-abdominal infections, or anastomotic leakage, nor were there differences in overall survival (OS) and disease-free survival (DFS) outcomes between the two groups. The findings suggest that while NOSE may be particularly suitable for patients who prioritize cosmetic outcomes, the extended operative duration warrants careful consideration in clinical decision-making.

In another significant contribution, Ying et al. conducted a systematic review and meta-analysis to assess the impact of adjuvant chemotherapy (ACT) on survival outcomes in node-negative CRC patients, with a focus on the presence of perineural invasion (PNI). Their analysis revealed that ACT significantly improved OS and DFS in patients with PNI, with hazard ratios (HR) of 0.52 and 0.53, respectively. However, ACT did not significantly affect DFS in patients without PNI. These findings underscore the potential of ACT as a particularly beneficial intervention for patients

with PNI, while also suggesting that it may confer some survival advantage for those without PNI.

Colorectal cancer (CRC) is characterized by significant genetic, anatomical, and transcriptional diversity. The tumor microenvironment (TME) plays a critical role in CRC prognosis and treatment outcomes. It consists of various cellular components like cancer-associated fibroblasts, tumor-associated macrophages, and regulatory *T* cells, as well as extracellular elements that contribute to therapeutic resistance through mechanisms such as fibrosis and enzymatic degradation. Given its influence on therapy efficacy, the TME presents a promising area for drug discovery, with ongoing research focused on targeting TME components to improve CRC treatment strategies (1).

Colorectal cancer (CRC) with the BRAF V600E mutation is aggressive and resistant to conventional therapies, largely due to the enhanced MAPK pathway activation. Although MAPK inhibitors have shown limited clinical success, combining these inhibitors with immune checkpoint inhibitors (ICIs) offers promise, particularly for patients with microsatellite instability-high (MSI-H) tumors (2).

The study by Jiang et al. investigated the relationship between collagen structure and the Immunoscore in the tumor microenvironment (TME) of CRC Using multiphoton imaging, they developed a collagen signature from 327 stage I-III CRC patients, which was strongly correlated with the Immunoscore. A collagen nomogram was subsequently constructed, integrating the collagen signature with clinicopathological predictors. This nomogram demonstrated high predictive accuracy for prognosis, particularly in high-risk stage II and III patients, and was shown to be a valuable tool in identifying patients who might benefit most from adjuvant chemotherapy. This study highlights the potential of collagen structure as a biomarker for immunological activity within the TME, offering a novel approach to prognosis in CRC.

Zhao et al. focused on patients with pT4M0 colon adenocarcinoma (COAD), analyzing optimal treatment strategies using data from the SEER database. Their study, which included 8,843 patients, revealed that those who received surgery combined with postoperative adjuvant therapy had significantly better 3-year OS and cancer-specific survival (CSS) rates compared to those who underwent surgery alone. A nomogram was developed, incorporating variables such as age, race, N stage, serum CEA levels, tumor differentiation, and the number of resected lymph nodes, demonstrating strong predictive accuracy. This study emphasizes the importance of integrating surgery with adjuvant chemoradiotherapy in improving long-term survival in high-risk COAD patients.

In the realm of imaging, Bai et al. investigated the utility of contrast-enhanced ultrasound (CEUS) in evaluating the response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer (LARC). Their retrospective study of 83 patients found that certain CEUS parameters, such as peak intensity (PI) and area under the curve (AUC), were significantly associated with clinical outcomes. Patients with higher PI and AUC values, along with poorly differentiated tumors, had worse overall survival (OS) and progression-free survival (PFS). This study highlights the potential of CEUS quantitative analysis as a non-invasive tool for predicting treatment response and prognosis in LARC patients.

Zhong et al. examined predictive factors for achieving a pathologic complete response (pCR) in LARC patients treated with neoadjuvant chemoradiation (nCRT). Their analysis identified gross tumor volume (GTV) and tumor differentiation as significant predictors of pCR, with a tumor volume threshold of 21.1 cm^3 showing a high sensitivity for predicting pCR. These findings suggest that GTV and tumor differentiation are crucial in preoperative assessments, helping clinicians in tailoring treatment plans more effectively.

Gallo et al. reported that, minimally invasive techniques such as laparoscopic surgery and SILS have transformed colorectal surgery, significantly improving patient outcomes by reducing recovery times and hospital stays. Emerging technologies, including robotic platforms and AI integration, are further enhancing surgical precision, setting the stage for future advancements in colorectal care (3).

A retrospective multicenter study, analyzing 5,398 rectal cancer surgeries, identified independent risk factors for anastomotic leakage, including sex, BMI, tumor location, and surgical approach (4). The overall leak incidence was 10.2%, with a 2.6% 30-day leak-related mortality. While protective stomas did not reduce leakage rates, they effectively minimized the severity and need for reoperation. The study introduced a clinical prediction model, the RALAR score, to assess individual risk and guide decisions on stoma construction post-resection. These findings may assist in optimizing surgical planning and patient outcomes.

A study by Zhao et al. found that the pan-immune-inflammation value (PIV) is significantly associated with tumor stage and other clinicopathological features in colorectal cancer (CRC) patients, showing potential as a preoperative assessment tool (5). PIV, particularly when combined with markers like CEA and CA19-9, demonstrated better predictive efficacy for CRC staging compared to other immune-inflammatory biomarkers.

Lastly, Verras and Mulita explored the potential of butyrylcholinesterase (BChE) as a predictive biomarker for surgical site infections (SSIs) after colorectal surgery (6). Their prospective study found that low BChE levels on the first and third postoperative days were associated with a significantly higher risk of SSIs. This suggests that BChE could serve as a valuable early marker for identifying patients at increased risk of infection, thereby enabling more timely and targeted interventions.

Benign colorectal diseases include a variety of conditions such as adenomatous polyps, diverticular disease, and inflammatory bowel disease (IBD) (7). These conditions pose significant challenges in clinical management, particularly in accurately identifying which lesions require intervention and which can be monitored safely. Over-diagnosis can lead to unnecessary interventions, increasing patient anxiety, complications and healthcare costs. Additionally, managing complications associated with these diseases, like diverticulitis, adds to the complexity. Future directions should focus on improving screening techniques, incorporating advanced imaging and molecular diagnostics for better characterization of lesions. Research into novel biomarkers for early detection and risk

stratification in patients with IBD may also lead to more personalized treatment approaches.

In daily clinical practice, findings from recent studies can enhance decision-making by improving risk assessments and tailoring treatments. For instance, the pan-immune-inflammation value (PIV) can serve as a useful preoperative marker to assess tumor progression in colorectal cancer. The RALAR score aids in identifying patients at higher risk of anastomotic leakage after rectal surgery, guiding stoma construction decisions. Additionally, early postoperative butyrylcholinesterase (BChE) levels can help predict the risk of surgical site infections, enabling timely interventions and improved patient outcomes.

In conclusion, these studies collectively advance our understanding of CRC management, offering new prognostic tools and insights into the effectiveness of various treatment strategies. As personalized medicine continues to evolve, the integration of novel biomarkers, imaging techniques, and tailored therapeutic approaches will be essential in improving patient outcomes in colorectal disorders.

Author contributions

MD: Investigation, Methodology, Project administration, Resources, Writing – original draft. GV: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Writing – review & editing. FM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Gallo G, Vescio G, De Paola G, Sammarco G. Therapeutic targets and tumor microenvironment in colorectal cancer. *J Clin Med.* (2021) 10(11):2295. doi: 10.3390/jcm10112295

2. Liu M, Liu Q, Hu K, Dong Y, Sun X, Zou Z, et al. Colorectal cancer with BRAF V600E mutation: trends in immune checkpoint inhibitor treatment. *Crit Rev Oncol Hematol.* (2024) 204:104497. doi: 10.1016/j.critrevonc.2024.104497

3. Gallo G, Goglia M. Grand challenges in colorectal and proctological surgery. *Front Surg.* (2023) 10:1331877. doi: 10.3389/fsurg.2023.1331877

4. Degiuli M, Elmore U, De Luca R, De Nardi P, Tomatis M, Biondi A, et al. Risk factors for anastomotic leakage after anterior resection for rectal cancer (RALAR study): a nationwide retrospective study of the Italian society of surgical oncology colorectal cancer network collaborative group. *Colorectal Dis.* (2022) 24(3):264–76. doi: 10.1111/codi.15997

5. Zhao H, Chen X, Zhang W, Cheng D, Lu Y, Wang C, et al. Pan-immune-inflammation value is associated with the clinical stage of colorectal cancer. *Front Surg.* (2022) 9:996844. doi: 10.3389/fsurg.2022.996844

6. Verras G-I, Mulita F. Butyrylcholinesterase levels correlate with surgical site infection risk and severity after colorectal surgery: a prospective single-center study. *Front Surg.* (2024) 11:1379410. doi: 10.3389/fsurg.2024.1379410

7. Sawayama H, Miyamoto Y, Yoshida N, Baba H. Essential updates 2020/2021: colorectal diseases (benign)-current topics in the surgical and medical treatment of benign colorectal diseases. *Ann Gastroenterol Surg.* (2022) 6(3):321–35. doi: 10.1002/ags3.12548

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A nomogram based on collagen signature for predicting the immunoscore in colorectal cancer

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Objectives: The Immunoscore can categorize patients into high- and low-risk groups for prognostication in colorectal cancer (CRC). Collagen plays an important role in immunomodulatory functions in the tumor microenvironment (TME). However, the correlation between collagen and the Immunoscore in the TME is unclear. This study aimed to construct a collagen signature to illuminate the relationship between collagen structure and Immunoscore.

Methods: A total of 327 consecutive patients with stage I-III stage CRC were included in a training cohort. The fully quantitative collagen features were extracted at the tumor center and invasive margin of the specimens using multiphoton imaging. LASSO regression was applied to construct the collagen signature. The association of the collagen signature with Immunoscore was assessed. A collagen nomogram was developed by incorporating the collagen signature and clinicopathological predictors after multivariable logistic regression. The performance of the collagen nomogram was evaluated via calibration, discrimination, and clinical usefulness and then tested in an independent validation cohort. The prognostic values of the collagen nomogram were assessed using Cox regression and the Kaplan–Meier method.

Results: The collagen signature was constructed based on 16 collagen features, which included 6 collagen features from the tumor center and 10 collagen features from the invasive margin. Patients with a high collagen signature were more likely to show a low Immunoscore (Lo IS) in both cohorts (P<0.001). A collagen nomogram integrating the collagen signature and clinicopathological predictors was developed. The collagen nomogram yielded satisfactory discrimination and calibration, with an AUC of 0.925 (95% CI: 0.895-0.956) in the training cohort and 0.911 (95% CI: 0.872-0.949) in the validation cohort. Decision curve analysis confirmed that the collagen nomogram was clinically

useful. Furthermore, the collagen nomogram-predicted subgroup was significantly associated with prognosis. Moreover, patients with a low-probability Lo IS, rather than a high-probability Lo IS, could benefit from chemotherapy in high-risk stage II and stage III CRC patients.

Conclusions: The collagen signature is significantly associated with the Immunoscore in the TME, and the collagen nomogram has the potential to individualize the prediction of the Immunoscore and identify CRC patients who could benefit from adjuvant chemotherapy.

KEYWORDS

immunoscore, colorectal cancer, tumor microenvironment, collagen signature, chemotherapy benefit

1 Introduction

The incidence rate of colorectal cancer (CRC) has gradually increased over the past decades and has become one of the leading causes of cancer burden and cancer deaths worldwide (1). Currently, the tumor-node-metastasis (TNM) staging system is widely utilized in the clinic as the reference standard for prognosis and treatment (2). Nevertheless, there is significant heterogeneity in the clinical outcomes of CRC patients with the same stage who receive a similar treatment regimen. This suggests that the current TNM staging system does not supply adequate prognostic and chemotherapy benefit information (3, 4). Several studies have demonstrated that the tumor microenvironment (TME), including the extracellular matrix (ECM) and immune cells, intensely impacts tumor initiation, proliferation, invasion, and metastasis (5, 6). Among the immune effector cells in the tumor, tumor-infiltrating lymphocytes (TILs) reflect the antitumor immune status of the host and are related to the prognosis and therapeutic response of CRC patients (7, 8). The density of CD3+ and CD8+ T cells at the tumor center (TC) and invasive margin (IM) was quantified and scored, namely, the Immunoscore (9, 10). Recently, several high-quality international studies have validated the prognostic value of the Immunoscore (11–14). Thus, the Immunoscore has been described as a new element for the TNM staging system of CRC and is recommended by the NCCN guidelines (15).

Epithelial-mesenchymal transition (EMT) is known to enhance the migratory and invasive abilities of cancer cells, thereby facilitating tumor formation and metastasis (16). Collagen, as a major component of the extracellular matrix (ECM), is upregulated during the process of EMT under the influence of various transcription factors, such as Twist, Slug, Snail, and Zeb (17–19). Concurrently, the integrins $\alpha1\beta1$ and $\alpha2\beta1$, which interact with collagen and have been shown to mediate the degradation of epithelial cadherin complexes, are also upregulated (20). Previous research indicated that the interaction between cells and the ECM is regulated through ECM-binding proteins, such as SPARC, which promotes the interaction between collagen and $\alpha2\beta1$ (21). SPARC has been demonstrated to induce EMT by regulating SLUG expression and is associated with increased invasiveness (22). Thus, under the influence of various biological signals, the structure of collagen undergoes dynamic changes during the development and progression of tumors (23, 24). Collagen also plays a vital role in the localization, dynamic behavior, and function of TILs in the TME (25, 26). However, the correlation between collagen structure alterations and the Immunoscore remains unclear. Multiphoton imaging, which is a nonlinear optical imaging method, can visualize collagen structure at the supramolecular level and is especially sensitive to collagen structure due to its physical basis (27). This technique has become a powerful tool for investigating the alteration of collagen structure during disease progression (28, 29). Furthermore, our previous studies have established a robust framework that enables automatic high-throughput acquisition of fully quantitative collagen structure features for disease diagnosis and prediction (30–32). Therefore, we hypothesized that we could elucidate the relationship between collagen structure and Immunoscore in the TME of CRC patients using multiphoton imaging and collagen quantification analysis.

Integrating multiple biomarkers into a biomarker panel using a machine learning algorithm can significantly improve the prediction performance compared to individual biomarkers (33, 34). Least absolute shrinkage and selection operator (LASSO) regression is an effective algorithm for analyzing high-throughput data and is widely accepted for model construction (35). Hence, we aimed to construct a fully quantitative collagen biomarker, i.e., a collagen signature, via multiphoton imaging and LASSO regression to comprehensively describe the correlation between collagen structure and the Immunoscore in the TME. Then, we investigated the potential predictive ability of a collagen nomogram that integrated the collagen signature and clinicopathological predictors for individualized prediction of Immunoscore in CRC patients.

2 Materials and methods

2.1 Patients and tissue specimens

Ethics approval was obtained from the institutional review boards of NanFang Hospital and Fujian Provincial Cancer Hospital (NFEC-2023-221). The requirement for informed consent was waived for this study. The study was conducted following the guidelines of the Declaration of Helsinki and the Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) statement criteria.

The flow chart of patient recruitment in this study is shown in Supplementary Figure 1. The inclusion criteria were patients who underwent radical surgery with pathologically diagnosed stage I-III CRC, available follow-up data and clinicopathological characteristics, and hematoxylin and eosin (HE) slides with invasive tumor components. The exclusion criteria were patients with unavailable formalin-fixed paraffin-embedded (FFPE) specimens, a history of cancer, or received neoadjuvant treatment. As a result, a total of 327 consecutive patients were included in the training cohort between January 2011 and December 2013 from Nanfang Hospital. An independent validation cohort contained 327 consecutive patients from Fujian Provincial Cancer Hospital between October 2011 and December 2013. Two independent pathologists reassessed all samples based on the 8th edition AJCC staging criteria.

Clinicopathological characteristics included age, sex, primary tumor location, preoperative carcinoembryonic antigen (CEA) level, preoperative carbohydrate antigen 199 (CA199) level, tumor differentiation, tumor size, pT stage, and pN stage. Adjuvant chemotherapy after radical surgery is recommended for patients with high-risk stage II and stage III CRC according to NCCN guidelines.

A standardized follow-up protocol was implemented, including a serum CEA test every 3 months after surgery and every 6 months after 3 years; CT examination from chest to pelvis every 6 months in the first 5 years after surgery; and colonoscopy at 1 year after surgery.

2.2 Immunohistochemistry and immunoscore construction

FFPE samples were cut into 4-μm sections and stained with antibodies against CD3 and CD8 (Maixin Biotech. Co., Ltd., Fuzhou, China). Immunohistochemical staining was performed as previously described (36, 37). Whole slide images of stained slices were digitized by Aperio ImageScope (Leica Biosystems, CA, USA) at 20× magnification as.svs format files.

The Immunoscore was assessed in the following steps (Figure 1). First, two pathologists who were blinded to the prognostic information selected five representative regions at the TC and five representative regions at the IM. Second, CD3+ and CD8+ stained immune cells were quantified using QuPath software (version 0.2.3). Third, CD3+ and CD8+ density was used to divide the individual cases into "high" or "low" immune groups, and patients with a mean density ≥ 75th percentile were considered a "high" immune group. A high immune group score was set as 1, and a low immune group score was set as 0. The $CD3_{TC}$, $CD3_{IM}$, $CD8_{TC}$, and $CD8_{IM}$ scores were added and converted into an Immunoscore (I0 - I4). Finally, patients were divided into two groups based on their Immunoscore: I0–I1 was classified as low Immunoscore (Lo IS), and I2-I4 was classified as intermediate-high Immunoscore (Int-Hi IS).

2.3 Multiphoton imaging and collagen feature extraction

The regions at the TC and IM, which were used to calculate the density of CD3+ and CD8+, were used for multiphoton imaging.

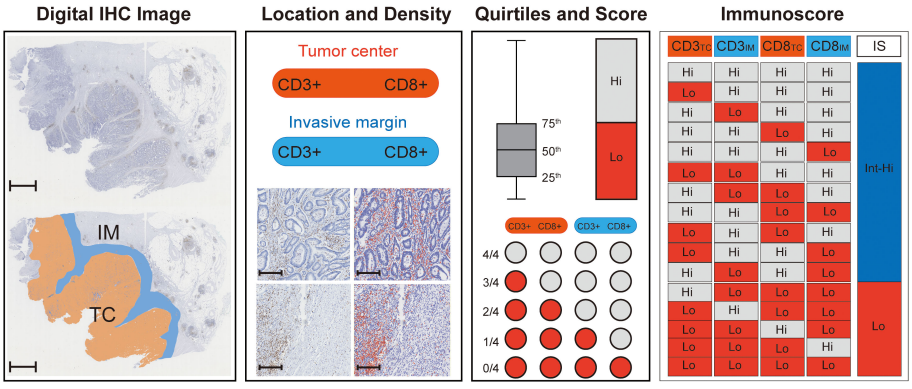

FIGURE 1
Flowchart for calculating Immunoscore. First, digital IHC images (CD3+ for example) were acquired and opened with Qupath software, and 5 representative images were randomly circled in the TC (orange) and IM (blue) regions (scale: 2,000 μm). Then, the densities of CD3+ (brown) in the CT and IM were counted by Qupath software (red), and the number of positive TILs was calculated per mm^2, scale: 250 μm. The mean TIL density was used to divide the individual cases into "high" or "low" immune groups, and patients with a mean density ≥ 75th percentile were regarded as a "high" immune group. A high immune group score was set as 1, and a low immune group score was set as 0. The $CD3_{TC}$, $CD3_{IM}$, $CD8_{TC}$, and $CD8_{IM}$ scores were added and converted into an Immunoscore (I0 - I4), where I0-I1 is a low Immunoscore (Lo IS) and 2-4 is an intermediate-high Immunscore (Int-Hi IS). TC, tumor center; IM, invasive margin; IHC, immunohistochemistry; TILs, tumor-infiltrating lymphocytes; IS, Immunoscore; Lo, low; Int-Hi, intermediate-high.

Image acquisition for multiphoton imaging was performed with a 200× original magnification objective on another unstained serial section and then compared with the HE image for histologic assessment (27). More information about the multiphoton imaging system can be found in the Supplementary Methods.

The framework we constructed for the quantitative extraction of collagen features is shown in the Supplementary Methods. In summary, 142 collagen features (Supplementary Table 1), including morphological features, histogram-based features, gray level concurrence matrix (GLCM) features, and Gabor wavelet transform features, were achieved automatically via MATLAB 2016b (Mathworks, Natick, MA, USA) (30–32). Finally, a total of 284 collagen features were obtained, including 142 from TC and 142 from IM, for further statistical analyses.

2.4 LASSO regression and collagen signature construction

LASSO regression, which is suitable for the regression of high-dimensional data, was used to select the most useful predictive features (33–35). The LASSO regression used an L1 penalty to shrink the coefficients to zero. The penalty parameter λ, also called the tuning constant, controls the number of collagen features to enter the model. In this study, we applied 10-fold cross-validations to select the optimal value of λ via 1-standard error (SE) criteria in the training cohort, and the collage signature was calculated for each patient via a linear combination of selected features that were weighted by their respective coefficients in the training cohort. Then, the collage signature in the validation cohort was calculated by the selected features with their respective coefficients obtained from the training cohort. Details of the LASSO regression are provided in the Supplementary Methods.

2.5 Development and assessment of the collagen nomogram

The collagen signature and clinicopathologic characteristics were included in univariate analysis to investigate their association with Lo IS, and variables with $P < 0.10$ were included in multivariable analysis. A backward stepwise selection method with Akaike's information criterion as the stopping rule was used to select the independent predictors of Lo IS (38). To facilitate clinical application, we developed a collagen nomogram according to the independent predictors in the training cohort (39).

The Hosmer–Lemeshow test was applied to estimate the goodness of fit of the model (40). The multicollinearity of the collagen nomogram was evaluated through the variance inflation factor (VIF) (41). The area under the curve (AUC) and the calibration curve were applied to assess the discrimination and calibration of the collagen nomogram. Then, the collagen nomogram was performed in the validation cohort, and its AUC and calibration curve were acquired. More information on the nomogram is shown in the Supplementary Methods.

2.6 Clinical application value of the collagen nomogram

To assess the clinical application value of the collagen nomogram, a traditional model was developed for comparison with the collagen nomogram. In our study, the traditional model was constructed based on clinicopathological predictors after univariate and multivariable logistic regression in the training cohort. The clinical usefulness of the collagen nomogram was evaluated by decision curve analysis (DCA) and clinical impact curves (CICs) (42). The maximum Youden index value of the ROC curve of the two models was measured to estimate the specificity, sensitivity, accuracy, positive predictive value (PPV), and negative predictive value (NPV). Moreover, the net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were used to show the improvement of the collagen nomogram compared with the traditional model (43, 44). Details of the NRI and IDI are provided in the Supplementary Methods.

2.7 Statistical analysis

Baseline characteristics were compared between the training and validation cohorts by t test, *U* test, Fisher's exact test, and $\chi 2$ test when applicable. The odds ratio (OR) and 95% confidence interval (CI) of the predictors were calculated using multivariable logistic regression. Survival curves were generated by using the Kaplan–Meier method and compared by log-rank tests. Univariate and multivariable analyses with Cox proportional hazards regression determined the hazard ratio (HR) of predictors for disease-free survival (DFS) and overall survival (OS). All statistical analyses were performed with SPSS version 22.0 software (IBM, Armonk, New York USA) and R version 4.0.3 (http://www.r-project.org/). All *P* values were two-sided, and statistical significance was defined as $P < 0.05$.

3 Results

3.1 Patient characteristics and immunoscore

The baseline characteristics of the patients in the training and validation cohorts are summarized in Table 1. A total of 421 (64.3%) patients were < 65 years old, with 405 (61.9%) men. The clinicopathological characteristics of the two cohorts were similar (Supplementary Table 2).

The density of CD3+ and CD8+ TILs in the TC and IM is shown in Supplementary Figure 2, with a higher density of TILs in the IM than in the TC for both CD3+ and CD8+ cells. The cutoff values of CD3+ and CD8+ cells were 593 and 382 cells/mm^2 in the TC and 1382 and 714 cells/mm^2 in the IM, respectively (Supplementary Table 3). Finally, the proportions of patients with Lo IS and Int-Hi IS were 34.3% and 65.7% in the training cohort and 35.8% and 64.2% in the validation cohorts, respectively.

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TABLE 1 Clinicopathological characteristics of the patients in the training and validation cohorts.

Characteristic	Training cohort (*n* = 327)		*P*	Validation cohort (*n* = 327)		P
	Lo IS (*n* = 112)	Int-Hi IS (*n* = 215)		Lo IS (*n* = 117)	Int-Hi IS (*n* = 210)	
Age, years			0.284			0.376
≥ 65	78 (69.6)	137 (63.7)		70 (59.8)	136 (64.8)	
< 65	34 (30.4)	78 (36.3)		47 (40.2)	74 (35.2)	
Sex			0.660			0.310
Male	70 (62.5)	129 (60.0)		64 (54.7)	127 (60.5)	
Female	42 (37.5)	86 (40.0)		53 (45.3)	83 (39.5)	
Primary tumor location			0.823			0.698
Left-sided	65 (58.0)	122 (56.7)		70 (59.8)	121 (57.6)	
Right-sided	47 (42.0)	93 (43.3)		47 (40.2)	89 (42.4)	
Preoperative CEA level			0.127			0.081
Normal	71 (63.4)	154 (71.6)		72 (61.5)	149 (71.0)	
Elevated	41 (36.6)	61 (28.4)		45 (38.5)	61 (29.0)	
Preoperative CA19-9 level			0.225			0.115
Normal	93 (83.0)	189 (87.9)		97 (82.9)	187 (89.0)	
Elevated	19 (17.0)	26 (12.1)		20 (17.1)	23 (11.0)	
Tumor differentiation			<0.001			<0.001
Well or moderately	72 (64.3)	179 (83.3)		73 (62.4)	173 (82.4)	
Poorly or undifferentiated	40 (35.7)	36 (16.7)		44 (37.6)	37 (17.6)	
Tumor size, cm			0.098			0.059
< 4	46 (41.1)	109 (50.7)		48 (41.0)	109 (51.9)	
≥ 4	66 (58.9)	106 (49.3)		69 (59.0)	101 (48.1)	
pT stage			0.013			<0.001
pT1-T3	82 (73.2)	182 (84.3)		78 (66.7)	177 (84.3)	
pT4	30 (26.8)	33 (15.3)		39 (33.3)	33 (15.7)	
pN stage			<0.001			0.002
pN0	44 (39.3)	133 (61.9)		49 (41.9)	125 (59.5)	
pN+	68 (60.7)	82 (38.1)		68 (58.1)	85 (40.5)	
Collagen signature, median (IQR)	3.018 (-0.493, 3.481)	-0.988 (-1.223, -0.800)	<0.001	3.086 (0.867, 3.580)	-1.103 (-1.434, -0.799)	<0.001

Values in parentheses are percentages unless indicated otherwise.
The P value was derived from the univariable association analyses between each of the clinicopathological characteristics and IS.
Lo, low; Int-Hi, intermediate-high; IS, Immunoscore; IQR, interquartile range; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 199.

The median follow-up duration [interquartile range (IQR)] was 72 (42–85) months in the training cohort and 71 (40–83) months in the validation cohort. The 5-year DFS and OS rates were 67.6% and 74.6%, respectively, in the training cohort. Similarly, the DFS and OS rates were 67.0% and 74.3%, respectively, in the validation cohort (Supplementary Figure 3). Patients with Int-Hi IS from the training cohort had a significantly better 5-year DFS (76.7% vs. 49.1%; $P < 0.001$) and 5-year OS (84.2% vs. 55.5%; $P < 0.001$) than patients with Lo IS (Figure 2A). Likewise, patients with Int-Hi and Lo IS had significant differences in 5-year DFS (80.5% vs. 42.7%; $P < 0.001$) and 5-year OS (83.8% vs. 56.4%; $P < 0.001$) in the validation cohort (Figure 2B).

3.2 Collagen signature construction

The framework of the collagen signature is presented in Figure 3. As a result, a collagen signature was constructed based

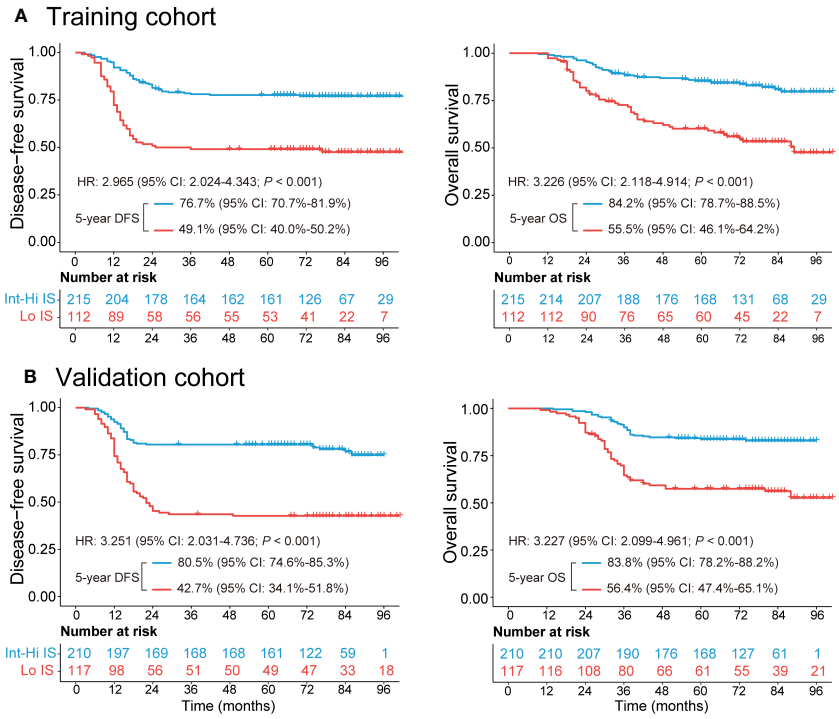

FIGURE 2
Kaplan–Meier survival analysis of the training and validation cohorts grouped by Immunoscore. **(A)** The 5-year DFS and OS comparison between the Lo and Int-Hi IS groups in the training cohort. **(B)** The 5-year DFS and OS comparison between the Lo and Int-Hi IS groups in the validation cohort. Lo, low; Int-Hi, intermediate-high; IS, Immunoscore; DFS, disease-free survival; OS, overall survival; HR, hazard ratio.

on sixteen collagen predictors from 284 collagen features by LASSO regression. (Supplementary Figure 4). The calculation formula for the collagen signature is proposed in the Supplementary Results. The distributions of the 16 collagen predictors and Immunoscore for each patient in the training and validation cohorts are shown in Supplementary Figure 5. The patients with a high collagen signature were more likely to show Lo IS in both cohorts (Figure 4). The collagen signature yielded an AUC of 0.896 (95% CI, 0.854-0.936) in the training cohort and 0.903 (95% CI, 0.863-0.944) in the validation cohort. A significant association between the collagen signature and Lo IS was found when stratified analysis was performed (Supplementary Tables 4, 5).

We also assessed the performance of the collagen signature and the single selected collagen feature in predicting Immunoscore. The results indicated that the collagen signature was more powerful than any individual parameter, demonstrating the added predictive value of the collagen signature (Figure 5).

3.3 Development and validation of the collagen nomogram

Univariate and multivariable logistic regression was performed to identify independent predictors of Lo IS. The results showed that the collagen signature (OR: 4.632, 95% CI: 3.068-6.993; $P < 0.001$), tumor differentiation (OR: 2.537, 95% CI: 1.121-5.741; $P = 0.026$), pT stage (OR: 2.602, 95% CI: 1.106-6.121; $P = 0.028$), and pN stage (OR: 2.550, 95% CI: 1.197-5.433; $P = 0.015$) were independent predictors of Lo IS (Table 2). Then, the collagen nomogram was developed, integrating the above four predictors (Figure 6A). ROC curve analysis indicated that the collagen signature had the most discrimination ability compared with the other predictors (Supplementary Figure 6). Alluvial diagrams were employed to intuitively illustrate the relationship between the four predictors and Immunoscore (Supplementary Figure 7). The variance inflation factor (VIF) values of each predictor were < 10, indicating that there was no multicollinearity among the four predictors (Supplementary Table 6). The Hosmer–Lemeshow test yielded a nonsignificant statistic ($P = 0.299$), demonstrating that there was no departure from a perfect fit.

In the training cohort, the collagen nomogram yielded satisfactory discrimination with an AUC of 0.925 (95% CI: 0.895-0.956). The calibration curve showed good agreement between the predicted and the actual Lo IS probability (Figure 6B). Similar results were observed in the validation cohort (AUC: 0.911, 95% CI: 0.872-0.949) and all patients (AUC: 0.918, 95% CI: 0.893-0.942) (Figure 6C).

3.4 Clinical application value of the collagen nomogram

A traditional model was developed based on tumor differentiation, pT stage, and pN stage in the training cohort (Supplementary Table S7). The traditional model yielded AUCs of 0.683 (95% CI, 0.622-0.744) in the training cohort, 0.680 (95%

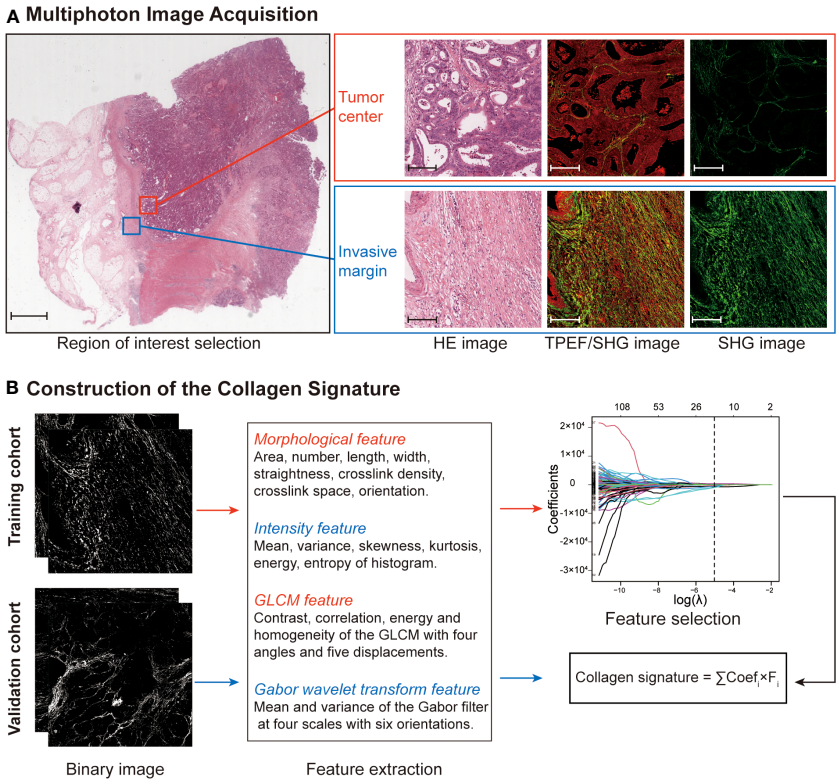

FIGURE 3
Construction framework of the collagen signature. **(A)** Selection of the region of interest in the TC and IM by comparing HE staining and multiphoton imaging. Ten regions (five at the TC and five at the IM) per sample are used for multiphoton imaging. Scale bars: 2,000 μm and 200 μm, respectively. **(B)** Framework for constructing the collagen signature. SHG images were converted to binary images for collagen feature extraction. The collagen signature was constructed using LASSO regression from 284 collagen features (142 from the TC and 142 from the IM). Then, the relationship between the collagen signature and the Immunoscore was evaluated and validated. HE, hematoxylin and eosin; TPEF, two-photon excitation fluorescence; SHG, second harmonic generation; GLCM, gray-level cooccurrence matrix; LASSO, least absolute shrinkage and selection operator; TC, tumor center; IM, invasive margin.

CI, 0.619-0.742) in the validation cohort, and 0.685 (95% CI, 0.642-0.728) in all patients. The collagen nomogram exhibited better discrimination ability than the traditional model (training cohort: 0.925 vs. 0.683; validation cohort: 0.911 vs. 0.680; all patients: 0.918 vs. 0.685; all $P < 0.001$) (Figure 6B). Moreover, the stratified analysis showed that the collagen nomogram was still superior to the traditional model among the subgroups in the training cohort, the validation cohort, and all patients (Supplementary Figures S8–S10). DCA revealed that the collagen nomogram could add more benefits than the traditional model (Figure 7A). CICs were generated to intuitively recognize the application value of the collagen nomogram to more accurately identify patients with Lo IS (Figure 7B).

Furthermore, the collagen nomogram exhibited better sensitivity (97.6% vs. 82.1%), specificity (87.3% vs. 43.7%), accuracy (89.9% vs. 56.6%), PPV (72.3% vs. 43.2%), and NPV (99.1% vs. 82.5%) in the training cohort. Similar results were observed in the validation cohort and all patients (Table 3). The corresponding NRI and IDI both showed significantly improved classification accuracy of the collagen nomogram compared with the traditional model in the training cohort, validation cohort and all patients (Table 4).

3.5 Association of the collagen nomogram with prognosis and chemotherapy benefits

Patients were divided into high- and low-probability Lo IS groups based on the ROC curve of the collagen nomogram. We found that patients with a low-probability Lo IS subgroup showed a better prognosis than patients with a high-probability Lo IS subgroup in the training cohort (Supplementary Figure S11A), the validation cohort (Supplementary Figure S11B) and all patients (Supplementary Figure S11C). This result was also observed in stage I-II (Supplementary Figure 12) and III patients (Supplementary Figure S13). Cox regression analysis demonstrated that the probability of Lo IS was an independent prognostic factor after adjusting for other variables in the training cohort [DFS: HR 2.475 (95% CI, 1.667-3.675), $P < 0.001$; OS: HR 2.179 (95% CI: 1.409-3.370), $P < 0.001$] (Supplementary Table 8), the validation cohort [DFS: HR 2.211 (95% CI, 1.510-3.239), $P < 0.001$; OS: HR 2.111 (95% CI: 1.366-3.262), $P < 0.001$] (Supplementary Table 9), and all patients [DFS: HR 2.350 (95% CI, 1.787-3.091), $P < 0.001$; OS: HR 2.119 (95% CI: 1.559-2.881), $P < 0.001$] (Supplementary Table 10). The collagen signature and clinicopathological predictors with the corresponding DFS and OS status are presented in Figure 8.

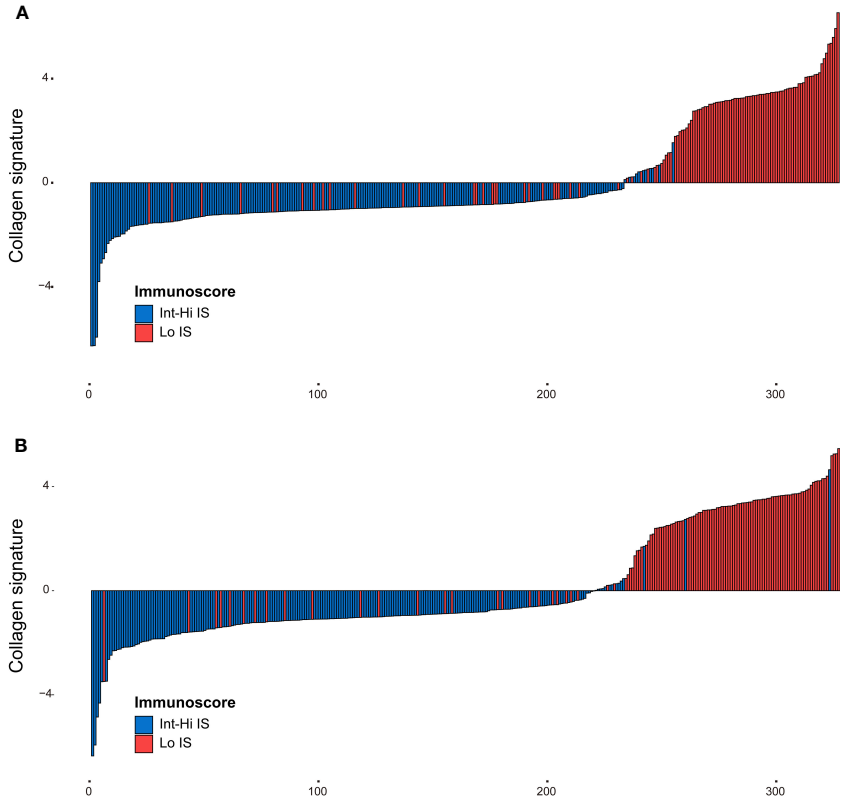

FIGURE 4
Distribution of the collagen signature in the training and validation cohorts. **(A)** Collagen signature for each patient in the training cohort. **(B)** Collagen signature for each patient in the validation cohort. Red represents the Lo Immunoscore, and blue represents the Int-Hi Immunoscore. Lo, low; Int-Hi, intermediate-high; IS, Immunoscore.

In addition, we investigated the chemotherapy benefits of high-risk stage II and stage III CRC patients in the high- and low-probability Lo IS subgroups. The results of the survival analysis showed that chemotherapy was associated with high-risk II and stage III CRC patients (Supplementary Figure S14). A test for an interaction between the probability of Lo IS and chemotherapy demonstrated that in either high-risk stage II or stage III, the benefit observed in the low-probability Lo IS patients [high-risk stage II (Figure 9): DFS, HR: 0.486 (95% CI: 0.280-0.842), P = 0.010; OS, HR: 0.441 (95% CI: 0.229-0.852), P = 0.015; stage III (Figure 10): DFS, HR: 0.464 (95% CI: 0.284-0.758), P = 0.002; OS, HR: 0.452 (95% CI: 0.266-0.770), P = 0.003; all $P < 0.05$ for interaction; Table 5] was superior to that observed in the high-probability Lo IS patients. The results indicated that chemotherapy significantly improved survival outcomes in the low-probability Lo IS group (high-risk stage II: P = 0.010 and P = 0.015; stage III: P = 0.002 and P = 0.003, respectively) but had no significant influence in the high-probability Lo IS group (high-risk stage II: P = 0.459 and P = 0.319; stage III: P = 0.535 and P = 0.449, respectively).

4 Discussion

In the current era of precision medicine, Immunoscore is a standard assay that quantifies the density of TILs, and its prognostic value has been internationally validated. In this study, we found a significant association between the collagen signature and the Immunoscore in the TME, and the collagen nomogram combining the collagen signature, tumor differentiation, pT stage, and pN stage could predict the Immunoscore with satisfactory performance. Moreover, the collagen nomogram was able to classify chemotherapy benefits in high-risk stage II and stage III CRC patients, indicating its potential as a tool to predict prognosis and facilitate treatment decision-making.

During tumor development, collagen in the extracellular matrix (ECM) undergoes notable remodeling, which affects the biological behavior of tumor cells, including infiltration, proliferation, and metastasis (18, 19). Importantly, collagen has also been found to influence various types of tumor-infiltrating immune cells (25, 26). In 3D culture assays, T-cell migration was significantly slower in high-density collagen gels than in low-density collagen gels (45). Increased collagen density also results in increased matrix stiffness, which can further affect T-cell migration (46, 47). In addition, high collagen density can influence immunological synapse formation between T cells and antigen-presenting cells (48), leading to reduced T-cell activity (49, 50). Collagen density has also been found to intensely affect the activity of T cells after the initial activation stage (51). These findings suggest that collagen has important immunomodulatory functions, which lays a foundation

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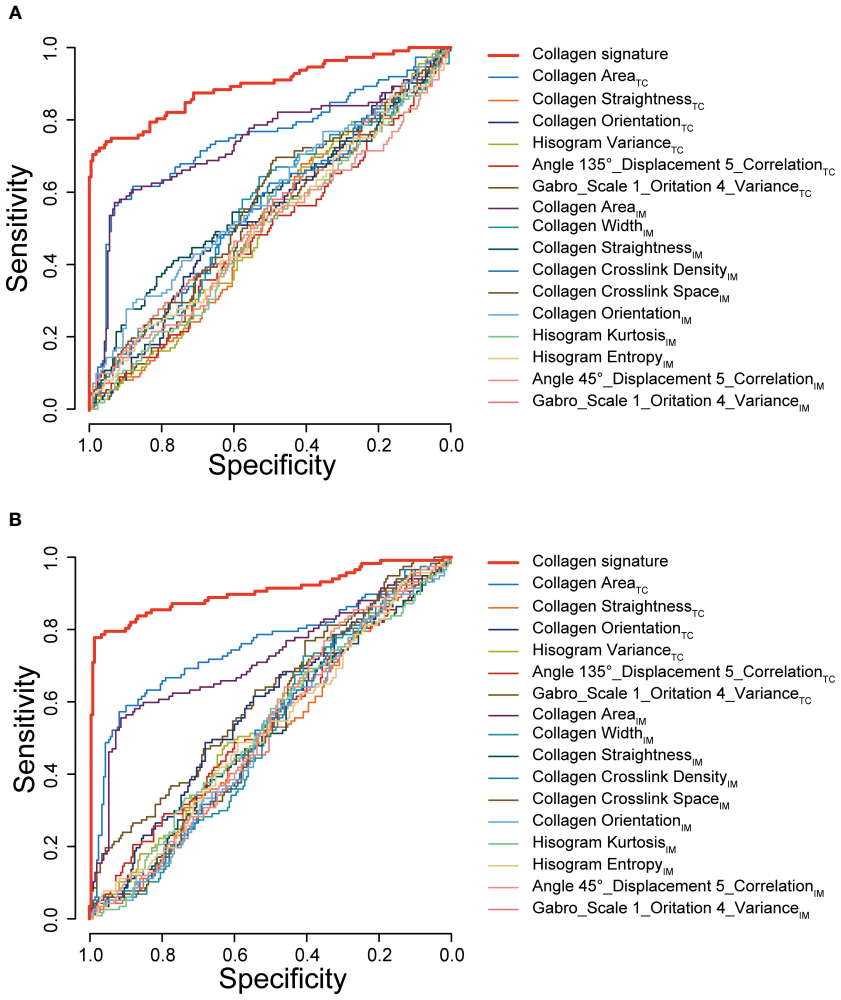

FIGURE 5
ROC curves of the collagen signature and the single selected collagen features. ROC curves of the collagen signature and the 16 selected collagen features in predicting Immunoscore in the training cohort **(A)** and validation cohort **(B)**. TC, tumor center; IM, invasive margin.

TABLE 2 Univariate and multivariable analyses of the predictors of Lo IS in the training cohort.

Variables	Univariate analysis		Multivariable analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age, years				
≥ 65	Ref			
< 65	1.306 (0.801, 2.131)	0.285		
Sex				
Male	Ref			
Female	0.900 (0.563, 1.440)	0.660		
Primary tumor location				
Left-sided	Ref			
Right-sided	0.949 (0.597, 1.506)	0.823		

(Continued)

TABLE 2 Continued

Variables	Univariate analysis		Multivariable analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Preoperative CEA level				
Normal	Ref			
Elevated	1.458 (0.897, 2.369)	0.128		
Preoperative CA19-9 level				
Normal	Ref			
Elevated	1.485 (0.782, 2.821)	0.227		
Tumor differentiation				
Well or moderately	Ref		Ref	
Poorly or undifferentiated	2.762 (1.631, 4.678)	<0.001	2.537 (1.121, 5.741)	0.026
Tumor size, cm				
< 4	Ref			
≥ 4	1.475 (0.930, 2.341)	0.099	NA	NA
pT stage				
pT1-3	Ref		Ref	
pT4	2.018 (1.154, 3.529)	0.014	2.602 (1.106, 6.121)	0.028
pN stage				
pN0	Ref		Ref	
pN+	2.507 (1.569, 4.005)	<0.001	2.550 (1.197, 5.433)	0.015
Collagen signature	4.596 (3.075, 6.870)	<0.001	4.632 (3.068, 6.993)	<0.001

Lo IS, low Immunoscore; OR, odds ratio; CI, confidence interval; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen 199; NA, not available; Ref, reference.

for quantitatively analyzing the relationship between collagen structure and the Immunoscore in the TME.

Collagen is a noncentrosymmetric structure, and multiphoton imaging can provide detailed information about the structure and organization of collagen fibers in tissue (52, 53). In this study, we acquired high-resolution multiphoton images from the TC and IM of the tumor sample. We then extracted quantitative high-throughput collagen features from the images using a robust framework, which could objectively quantify the collagen structural information contained in the TME. LASSO regression, an effective algorithm with variable selection and complexity regularization, was used to shrink and choose the most predictive collagen predictors from the high-throughput features to construct the collagen signature. Variable selection means selectively choosing variables in the model to achieve more satisfactory performance parameters, rather than including all variables in the model, while complexity regularization is retained through the penalty parameter λ to avoid overfitting (35, 54, 55). Using this approach, the collagen signature, based on 6 collagen features from TC and 10 collagen features from IM, was developed and was significantly related to the Immunoscore. Our findings revealed that patients with a high collagen signature exhibited a low T-cell density microenvironment, resulting in Lo IS in CRC patients with poor prognosis, consistent with previous reports (10, 12, 13). Thus, the collagen signature could comprehensively and quantitatively determine the correlation between collagen structure and Immunoscore in the TME. Then, we constructed a collagen nomogram that included the collagen signature, tumor differentiation, pT stage, and pN stage. The collagen nomogram has better discrimination and clinical application value for estimating the Immunoscore than the traditional model. To the best of our knowledge, this is the first study to assess the association between the collagen structure and the Immunoscore in the TME and build an effective prediction model based on the fully quantitative collagen signature using multiphoton imaging.

From a clinical practice standpoint, the clinical translation of the collagen nomogram is feasible. First, the clinicopathological predictors required for the nomogram are routinely supplied in the postoperative pathological report. Second, unlike immunohistochemistry, which requires staining agents and is

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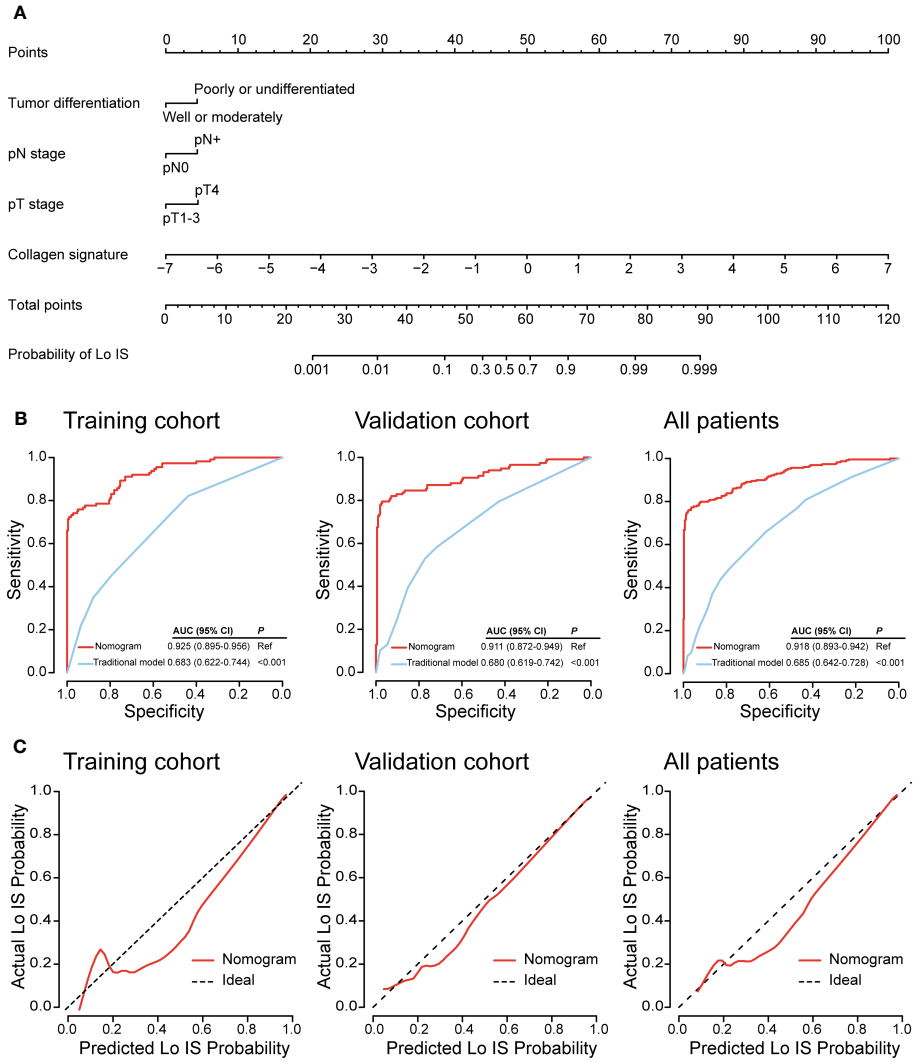

FIGURE 6
Collagen nomogram construction and performance assessment. **(A)** The collagen nomogram was constructed in the training cohort, incorporating the collagen signature, tumor differentiation, pT stage, and pN stage. **(B)** The ROC curves of the nomogram and the traditional model in the training cohort, the validation cohort, and all patients. **(C)** The calibration curves of the nomogram in the training cohort, the validation cohort, and all patients. In the calibration curve, the y-axis represents the actual Lo IS probability, and the x-axis represents the predicted Lo IS probability. The diagonal black dotted line represents a perfect prediction model. The solid red line is a representation of the nomogram; better prediction is indicated when the solid red line has a closer fit to the diagonal black dotted line. AUC, area under the curve; CI, confidence interval; Lo IS, low Immunoscore.

time consuming, multiphoton imaging can quickly image unstained sections in a label-free manner, and collagen feature extraction can be automatically completed using MATLAB software. Third, our study revealed a correlation between collagen structure and Immunoscore, indicating that future treatment might regulate collagen in the TME to potentially tune the antitumor immune status. Taken together, we believe that the collagen nomogram is both time efficient for pathologists and cost contained for patients while also providing a potential therapeutic target for improving the prognosis of CRC patients.

According to the NCCN guidelines, adjuvant chemotherapy is recommended for high-risk stage II and stage III CRC patients. However, not all patients can benefit from chemotherapy. Previous clinical trials have shown that patients with Lo IS could not benefit from chemotherapy, while patients with Hi-Int IS could improve their prognosis from chemotherapy; therefore, the Immunoscore is useful for the selection of individualized chemotherapy (12, 13). Because the collagen nomogram demonstrated satisfactory performance in predicting Lo IS, we further evaluated whether the collagen nomogram can identify patients who could benefit from chemotherapy. Patients were divided into high- and low-probability Lo IS groups according to the collagen nomogram. The results showed that patients with a low-probability Lo IS could benefit from chemotherapy, while patients with a high-probability Lo IS could not. This finding suggests that the collagen nomogram could be a potential tool to assist in individualizing chemotherapy

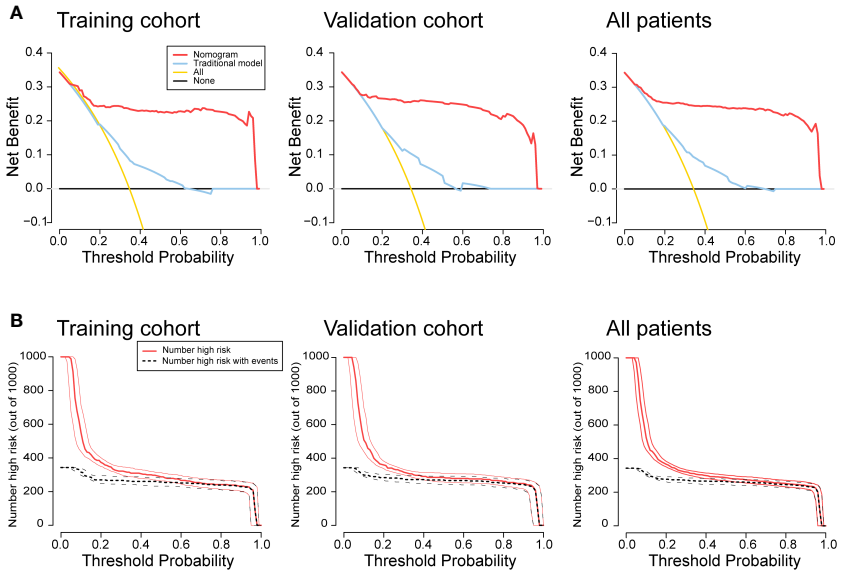

FIGURE 7
Clinical application value of the nomogram. **(A)** Decision curve analysis for the nomogram. The y-axis represents the net benefit, and the x-axis represents the different threshold probabilities. **(B)** Clinical impact curves for the nomogram. Of 1,000 patients, the red line shows the total number of patients who would be deemed to have a low Immunoscore for each threshold probability. The black line shows how many of those would be true positives (cases). The closer the curves are, the higher the probability that the nomogram would identify low Immunoscore patients from the total estimated number of low Immunoscore patients.

selection in high-risk stage II and stage III CRC patients when Immunoscore evaluation is not feasible.

Artificial intelligence (AI) technologies, especially deep learning, have advanced rapidly in medical care, providing powerful methods for constructing accurate prediction models (56, 57). AI has demonstrated comparable performance to pathologists in distinguishing between benign and malignant colorectal diseases (58). Although this approach cannot entirely supplant the role of pathologists, AI can be harnessed as an assistive tool to improve diagnostic efficiency, reduce workload, and improve medical image readability, ultimately reducing the rates of misdiagnosis and missed diagnoses (59). Furthermore, a multistain deep learning model based on AI could also be used to determine the AImmunoscore (AIS) in CRC patients and predict the response to neoadjuvant therapy in rectal cancer patients (60). The potential of AI to revolutionize the clinical landscape of CRC is

TABLE 3 Predictive power of Lo IS between the nomogram and traditional model.

Model	AUC	Sensitivity(%)	Specificity(%)	Accuracy(%)	PPV(%)	NPV(%)
Training cohort						
Nomogram	0.925 (0.895, 0.956)	97.6 (91.6, 99.6)	87.3 (82.5, 90.9)	89.9 (86.2, 92.7)	72.3 (63.4, 79.8)	99.1 (96.7, 99.8)
Traditional model	0.683 (0.622, 0.744)	82.1 (74.0, 88.1)	43.7 (37.3, 50.4)	56.6 (51.2, 61.9)	43.2 (36.7, 49.9)	82.5 (74.4, 88.3)
Validation cohort						
Nomogram	0.911 (0.872, 0.949)	95.7 (89.6, 98.3)	88.4 (83.7, 91.9)	90.5 (86.9, 93.2)	76.9 (68.5, 83.6)	98.1 (95.2, 99.3)
Traditional model	0.680 (0.619, 0.742)	79.5 (71.3, 85.8)	42.9 (36.4, 49.6)	56.0 (50.5, 61.2)	43.7 (37.2, 50.4)	81.8 (73.6, 87.9)
All patients						
Nomogram	0.918 (0.893, 0.942)	96.6 (92.8, 89.4)	87.8 (84.6, 90.5)	90.2 (87.7, 92.3)	74.7 (86.7, 79.9)	98.6 (97.0, 99.4)
Traditional model	0.685 (0.642, 0.728)	80.8 (75.2, 85.4)	43.3 (38.7, 48.0)	56.4 (52.6, 60.2)	43.4 (38.8, 48.2)	80.7 (75.1, 85.3)

Lo IS, low Immunoscore; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

TABLE 4 NRI and IDI test for prediction of Lo IS improvements of the nomogram compared with the traditional model.

Models	NRI (95% CI)	*P*	IDI (95% CI)	*P*
Nomogram vs. Traditional model				
Training cohort	0.551 (0.443, 0.660)	<0.001	0.516 (0.445, 0.587)	<0.001
Validation cohort	0.606 (0.496, 0.717)	<0.001	0.532 (0.467, 0.597)	<0.001
All patients	0.564 (0.484, 0.645)	<0.001	0.523 (0.475, 0.571)	<0.001

Lo IS, low Immunoscore; CI, confidence interval; NRI, net reclassification improvement; IDI, integrated discrimination improvement.

substantial. However, it is important to recognize that AI is still in its early stages of clinical application in CRC. Several challenges that must be addressed include the validation and generalizability of the predictive models, interpretation of the model, and the safe management and use of data. We believe that in the future, AI technologies will assume a considerably more prominent role in the context of screening, diagnosis, surgical treatment, and prognosis prediction.

Our study has some limitations. First, this was a retrospective study. Second, all specimens were obtained from a single medical center in China. Hence, multicenter, international, prospective clinical trials will be necessary to validate the robustness of the collagen nomogram. Third, the probability of Lo IS based on the collagen nomogram was associated with survival; however, additional survival parameters were not added to our nomogram for model accuracy estimation.

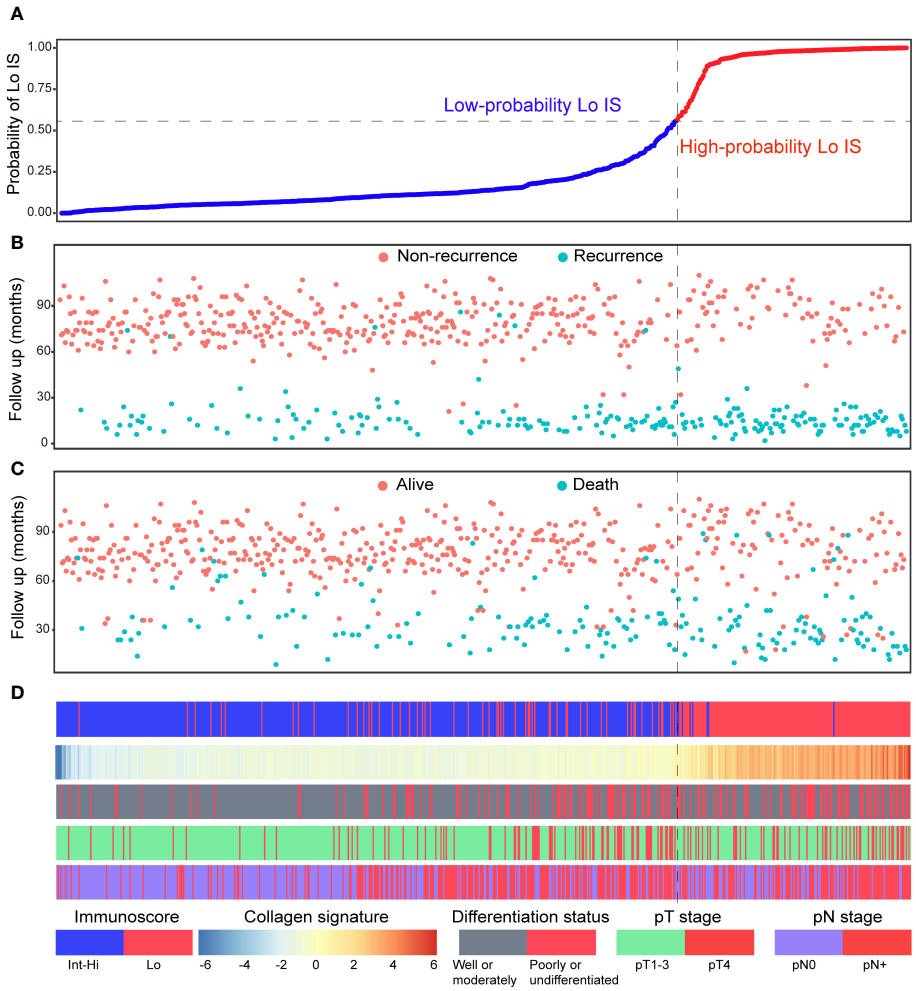

FIGURE 8
Distribution of the nomogram-predicted subgroups with the corresponding survival status in all patients. **(A)** Nomogram-predicted probability of Lo IS distribution; **(B)** Disease-free survival status of all patients; **(C)** Overall survival status of all patients. **(D)** Distribution of the collagen signature and clinicopathological predictors with the corresponding survival status. Lo IS, low Immunoscore; Int-Hi IS, intermediate-high Immunoscore.

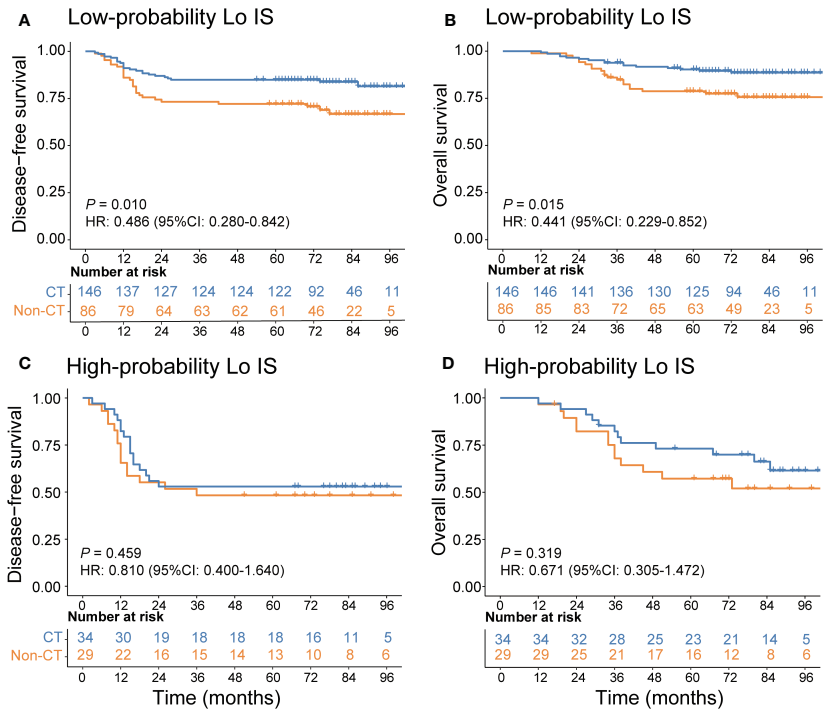

FIGURE 9
Adjuvant chemotherapy benefits in high-risk stage II CRC patients. **(A)** DFS and **(B)** OS comparison of high-risk stage II CRC according to the receipt of adjuvant chemotherapy in patients with a low-probability Lo IS. **(C)** DFS and **(D)** OS comparison of stage high-risk stage II CRC according to the receipt of adjuvant chemotherapy in patients with a high-probability Lo IS. Lo IS, low Immunoscore; CRC, colorectal cancer; DFS, disease-free survival; OS, overall survival; CT, chemotherapy.

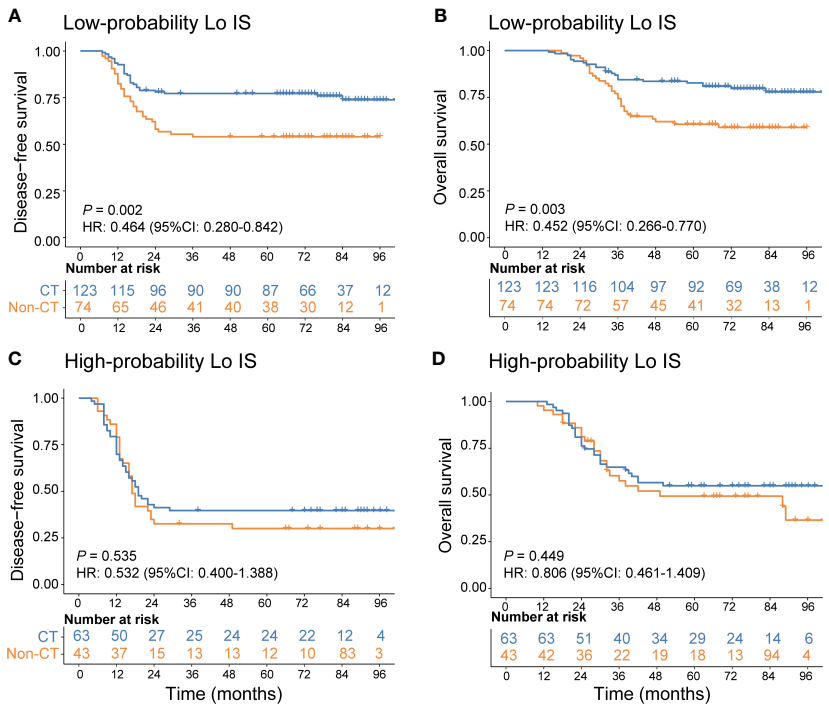

FIGURE 10
Adjuvant chemotherapy benefits in stage III CRC patients. **(A)** DFS and **(B)** OS comparison of stage III CRC according to the receipt of adjuvant chemotherapy in patients with a low-probability Lo IS. **(C)** DFS and **(D)** OS comparison of stage III CRC according to the receipt of adjuvant chemotherapy in patients with a high-probability Lo IS. Lo IS, low Immunoscore; CRC, colorectal cancer; DFS, disease-free survival; OS, overall survival; CT, chemotherapy.

TABLE 5 Adjuvant chemotherapy interaction with the probability of Lo IS for survival in patients with high-risk stage II and stage III disease.

Probability of Lo IS	Chemotherapy		Disease-free survival			Overall survival		
	No CT	CT	HR (95% CI)	*P*	$P_{interaction}$	HR (95% CI)	*P*	$P_{interaction}$
High-risk stage II (n = 295)								
Low	86 (37.1)	146 (62.9)	0.486 (0.280, 0.842)	0.010	0.001	0.441 (0.229, 0.852)	0.015	<0.001
High	29 (46.0)	34 (54.0)	0.810 (0.400, 1.640)	0.459		0.671 (0.305, 1.472)	0.319	
Stage III (n = 303)								
Low	74 (37.6)	123 (62.4)	0.464 (0.284, 0.758)	0.002	<0.001	0.452 (0.266, 0.770)	0.003	<0.001
High	43 (40.6)	63 (59.4)	0.859 (0.532, 1.388)	0.535		0.806 (0.461, 1.409)	0.449	

Lo IS, low Immunoscore; CT, chemotherapy; DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

In conclusion, this study proposed that the collagen signature was significantly associated with the Immunoscore in the TME and that the collagen nomogram is useful for the individualized prediction of the Immunoscore in CRC patients. Moreover, the collagen nomogram could be a potential tool to assist in individualizing chemotherapy selection in high-risk stage II and stage III CRC patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by ethics approval was obtained from the institutional review boards of NanFang Hospital and Fujian Provincial Cancer Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this retrospective study met the following criteria: 1) the study involves no more than minimal risk to subjects, and 2) the waiver or alteration will not adversely affect the rights and welfare of the subjects.

Author contributions

WJ: Conceptualization, Data curation, Formal Analysis, Methodology, Software, Writing – original draft. XD: Conceptualization, Data curation, Methodology, Software, Writing – original draft. XY: Conceptualization, Data curation, Formal Analysis, Resources, Software, Writing – original draft. CL: Data curation, Methodology, Software, Writing – original draft. DC: Data curation, Formal Analysis, Methodology, Software, Writing – original draft. JC: Data curation, Investigation, Software, Writing – review & editing. BY: Data curation, Investigation, Methodology, Software, Writing – original draft. SX: Formal Analysis, Investigation, Methodology, Software, Writing – review & editing. ZL: Formal Analysis, Methodology, Software, Visualization, Writing – review & editing. GC: Conceptualization, Methodology, Supervision, Validation, Visualization, Writing – review & editing. SZ: Conceptualization, Methodology, Software, Supervision, Validation, Visualization, Writing – review & editing. JY: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1269700/full#supplementary-material

References

1. Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Delattre JF, Selcen Oguz Erdogan A, Cohen R, Shi Q, Emile JF, Taieb J, et al. A comprehensive overview of tumour deposits in colorectal cancer: Towards a next TNM classification. *Cancer Treat Rev* (2022) 103:102325. doi: 10.1016/j.ctrv.2021.102325

3. National Cancer Institute's SEER database. Available at: http://seercancergov/ (Accessed 26 August 2020).

4. Dienstmann R, Mason MJ, Sinicrope FA, Phipps AI, Tejpar S, Nesbakken A, et al. Prediction of overall survival in stage II and III colon cancer beyond TNM system: a retrospective, pooled biomarker study. *Ann Oncol* (2017) 28(5):1023–31. doi: 10.1093/annonc/mdx052

5. Kirchhammer N, Trefny MP, Auf der Maur P, Laubli H, Zippelius A. Combination cancer immunotherapies: Emerging treatment strategies adapted to the tumor microenvironment. *Sci Transl Med* (2022) 14(670):eabo3605. doi: 10.1126/scitranslmed.abo3605

6. Xiang X, Niu YR, Wang ZH, Ye LL, Peng WB, Zhou Q. Cancer-associated fibroblasts: Vital suppressors of the immune response in the tumor microenvironment. *Cytokine Growth Factor Rev* (2022) 67:35–48. doi: 10.1016/j.cytogfr.2022.07.006

7. Aristin Revilla S, Kranenburg O, Coffer PJ. Colorectal cancer-infiltrating regulatory T cells: functional heterogeneity, metabolic adaptation, and therapeutic targeting. *Front Immunol* (2022) 13:903564. doi: 10.3389/fimmu.2022.903564

8. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science* (2015) 348(6230):74–80. doi: 10.1126/science.aaa6204

9. Angell HK, Bruni D, Barrett JC, Herbst R, Galon J. The immunoscore: colon cancer and beyond. *Clin Cancer Res* (2020) 26(2):332–9. doi: 10.1158/1078-0432.CCR-18-1851

10. Bruni D, Angell HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer* (2020) 20(11):662–80. doi: 10.1038/s41568-020-0285-7

11. Malka D, Lievre A, Andre T, Taieb J, Ducreux M, Bibeau F. Immune scores in colorectal cancer: Where are we? *Eur J Cancer* (2020) 140:105–18. doi: 10.1016/j.ejca.2020.08.024

12. Mlecnik B, Bifulco C, Bindea G, Marliot F, Lugli A, Lee JJ, et al. Multicenter international society for immunotherapy of cancer study of the consensus immunoscore for the prediction of survival and response to chemotherapy in stage III colon cancer. *J Clin Oncol* (2020) 38(31):3638–51. doi: 10.1200/JCO.19.03205

13. Pages F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, et al. International validation of the consensus immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet* (2018) 391(10135):2128–39. doi: 10.1016/S0140-6736(18)30789-X

14. El Sissy C, Kirilovsky A, Van den Eynde M, Musina AM, Anitei MG, Romero A, et al. A diagnostic biopsy-adapted immunoscore predicts response to neoadjuvant treatment and selects patients with rectal cancer eligible for a watch-and-wait strategy. *Clin Cancer Res* (2020) 26(19):5198–207. doi: 10.1158/1078-0432.CCR-20-0337

15. NCCN. *Clinical practice guidelines in oncology-colon cancer (2022 version 3)* . Available at: http://www.nccn.org.

16. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* (2014) 15(3):178–96. doi: 10.1038/nrm3758

17. Ang HL, Mohan CD, Shanmugam MK, Leong HC, Makvandi P, Rangappa KS, et al. Mechanism of epithelial-mesenchymal transition in cancer and its regulation by natural compounds. *Med Res Rev* (2023) 43(4):1141–200. doi: 10.1002/med.21948

18. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* (2009) 139(5):891–906. doi: 10.1016/j.cell.2009.10.027

19. Mouw JK, Ou G, Weaver VM. Extracellular matrix assembly: a multiscale deconstruction. *Nat Rev Mol Cell Biol* (2014) 15(12):771–85. doi: 10.1038/nrm3902

20. Zhang Y, Cheng K, Xu B, Shi J, Qiang J, Shi S, et al. Epigenetic input dictates the threshold of targeting of the integrin-dependent pathway in non-small cell lung cancer. *Front Cell Dev Biol* (2020) 8:652. doi: 10.3389/fcell.2020.00652

21. Girotti MR, Fernandez M, Lopez JA, Camafeita E, Fernandez EA, Albar JP, et al. SPARC promotes cathepsin B-mediated melanoma invasiveness through a collagen I/alpha2beta1 integrin axis. *J Invest Dermatol* (2011) 131(12):2438–47. doi: 10.1038/jid.2011.239

22. Lopez-Moncada F, Torres MJ, Lavanderos B, Cerda O, Castellon EA, Contreras HR. SPARC Induces E-Cadherin Repression and Enhances Cell Migration through Integrin alphavbeta3 and the Transcription Factor ZEB1 in Prostate Cancer Cells. *Int J Mol Sci* (2022) 23(11):5874. doi: 10.3390/ijms23115874

23. Han W, Chen S, Yuan W, Fan Q, Tian J, Wang X, et al. Oriented collagen fibers direct tumor cell intravasation. *Proc Natl Acad Sci USA* (2016) 113(40):11208–13. doi: 10.1073/pnas.1610347113

24. Martins Cavaco AC, Damaso S, Casimiro S, Costa L. Collagen biology making inroads into prognosis and treatment of cancer progression and metastasis. *Cancer Metastasis Rev* (2020) 39(3):603–23. doi: 10.1007/s10555-020-09888-5

25. Romer AMA, Thorseth ML, Madsen DH. Immune modulatory properties of collagen in cancer. *Front Immunol* (2021) 12:791453. doi: 10.3389/fimmu.2021.791453

26. Salmon H, Franciszkiewicz K, Damotte D, Dieu-Nosjean MC, Validire P, Trautmann A, et al. Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J Clin Invest* (2012) 122(3):899–910. doi: 10.1172/JCI45817

27. Yan J, Zheng X, Liu Z, Liu W, Lin D, Chen D, et al. Multiphoton imaging provides a superior optical biopsy to that of confocal laser endomicroscopy imaging for colorectal lesions. *Endoscopy* (2019) 51(2):174–8. doi: 10.1055/a-0641-5091

28. Skala MC, Riching KM, Gendron-Fitzpatrick A, Eickhoff J, Eliceiri KW, White JG, et al. *In vivo* multiphoton microscopy of NADH and FAD redox states, fluorescence lifetimes, and cellular morphology in precancerous epithelia. *Proc Natl Acad Sci USA* (2007) 104(49):19494–9. doi: 10.1073/pnas.0708425104

29. Zoumi A, Yeh A, Tromberg BJ. Imaging cells and extracellular matrix in *vivo* by using second-harmonic generation and two-photon excited fluorescence. *Proc Natl Acad Sci USA* (2002) 99(17):11014–9. doi: 10.1073/pnas.172368799

30. Chen D, Chen G, Jiang W, Fu M, Liu W, Sui J, et al. Association of the collagen signature in the tumor microenvironment with lymph node metastasis in early gastric cancer. *JAMA Surg* (2019) 154(3):e185249. doi: 10.1001/jamasurg.2018.5249

31. Xu S, Wang Y, Tai DCS, Wang S, Cheng CL, Peng Q, et al. qFibrosis: a fully-quantitative innovative method incorporating histological features to facilitate accurate fibrosis scoring in animal model and chronic hepatitis B patients. *J Hepatol* (2014) 61(2):260–9. doi: 10.1016/j.jhep.2014.02.015

32. Chen W, Dong S, Liu X, Wang G, Xu S, Lei S, et al. Association of the collagen signature in the tumor microenvironment with recurrence and survival of patients with T4N0M0 colon cancer. *Dis Colon Rectum* (2021) 64(5):563–75. doi: 10.1097/DCR.0000000000001907

33. Jiang Y, Zhang Q, Hu Y, Li T, Yu J, Zhao L, et al. ImmunoScore signature: A prognostic and predictive tool in gastric cancer. *Ann Surg* (2018) 267(3):504–13. doi: 10.1097/SLA.0000000000002116

34. Huang Y, Liang C, He L, Tian J, Liang C, Chen X, et al. Development and validation of a radiomics nomogram for preoperative prediction of lymph node

metastasis in colorectal cancer. *J Clin Oncol* (2016) 34(18):2157–64. doi: 10.1200/jco.2015.65.9128

35. Jian H, Ma S, Zhang CH. Adaptive LASSO for sparse high-dimensional regression. *Stat Sin* (2008) 18(4):1603–18. doi: 10.1007/s11135-007-9120-4

36. Kuang DM, Wu Y, Chen N, Cheng J, Zhuang SM, Zheng L. Tumor-derived hyaluronan induces formation of immunosuppressive macrophages through transient early activation of monocytes. *Blood* (2007) 110(2):587–95. doi: 10.1182/blood-2007-01-068031

37. Xu J, Ding T, He Q, Yu XJ, Wu WC, Jia WH, et al. An in *situ* molecular signature to predict early recurrence in hepatitis B virus-related hepatocellular carcinoma. *J Hepatol* (2012) 57(2):313–21. doi: 10.1016/j.jhep.2012.03.027

38. Zhang Z. Variable selection with stepwise and best subset approaches. *Ann Transl Med* (2016) 4(7):136. doi: 10.21037/atm.2016.03.35

39. Balachandran V, Gonen M, Smith J, DeMatteo R. Nomograms in oncology: more than meets the eye. *Lancet Oncol* (2015) 16(4):e173–80. doi: 10.1016/s1470-2045(14)71116-7

40. Rozeboom PD, Henderson WG, Dyas AR, Bronsert MR, Colborn KL, Lambert-Kerzner A, et al. Development and validation of a multivariable prediction model for postoperative intensive care unit stay in a broad surgical population. *JAMA Surg* (2022) 157(4):344–52. doi: 10.1001/jamasurg.2021.7580

41. Cheng J, Sun J, Yao K, Xu M, Cao Y. A variable selection method based on mutual information and variance inflation factor. *Spectrochim Acta A Mol Biomol Spectrosc* (2022) 268:120652. doi: 10.1016/j.saa.2021.120652

42. Fitzgerald M, Saville B, Lewis R. Decision curve analysis. *JAMA* (2015) 313(4):409–10. doi: 10.1001/jama.2015.37

43. Bragg F, Trichia E, Aguilar-Ramirez D, Besevic J, Lewington S, Emberson J. Predictive value of circulating NMR metabolic biomarkers for type 2 diabetes risk in the UK Biobank study. *BMC Med* (2022) 20(1):159. doi: 10.1186/s12916-022-02354-9

44. Jiang Y, Jin C, Yu H, Wu J, Chen C, Yuan Q, et al. Development and validation of a deep learning CT signature to predict survival and chemotherapy benefit in gastric cancer: a multicenter, retrospective study. *Ann Surg* (2020) 274(6):e1153–61. doi: 10.1097/sla.0000000000003778

45. Wolf K, Te Lindert M, Krause M, Alexander S, Te Riet J, Willis AL, et al. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *J Cell Biol* (2013) 201(7):1069–84. doi: 10.1083/jcb.201210152

46. Nicolas-Boluda A, Vaquero J, Vimeux L, Guilbert T, Barrin S, Kantari-Mimoun C, et al. Tumor stiffening reversion through collagen crosslinking inhibition improves T cell migration and anti-PD-1 treatment. *Elife* (2021) 10:e58688. doi: 10.7554/eLife.58688

47. Tabdanov ED, Rodriguez-Merced NJ, Cartagena-Rivera AX, Puram VV, Callaway MK, Ensminger EA, et al. Engineering T cells to enhance 3D migration through structurally and mechanically complex tumor microenvironments. *Nat Commun* (2021) 12(1):2815. doi: 10.1038/s41467-021-22985-5

48. Dustin ML, Choudhuri K. Signaling and polarized communication across the T cell immunological synapse. *Annu Rev Cell Dev Biol* (2016) 32:303–25. doi: 10.1146/annurev-cellbio-100814-125330

49. Dustin ML, de Fougerolles AR. Reprogramming T cells: the role of extracellular matrix in coordination of T cell activation and migration. *Curr Opin Immunol* (2001) 13(3):286–90. doi: 10.1016/s0952-7915(00)00217-x

50. Gunzer M, Schafer A, Borgmann S, Grabbe S, Zanker KS, Brocker EB, et al. Antigen presentation in extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential. *Immunity* (2000) 13(3):323–32. doi: 10.1016/s1074-7613(00)00032-7

51. Kuczek DE, Larsen AMH, Thorseth ML, Carretta M, Kalvisa A, Siersbaek MS, et al. Collagen density regulates the activity of tumor-infiltrating T cells. *J Immunother Cancer* (2019) 7(1):68. doi: 10.1186/s40425-019-0556-6

52. Campagnola PJ, Loew LM. Second-harmonic imaging microscopy for visualizing biomolecular arrays in cells, tissues and organisms. *Nat Biotechnol* (2003) 21(11):1356–60. doi: 10.1038/nbt894

53. Chen X, Nadiarynkh O, Plotnikov S, Campagnola P. Second harmonic generation microscopy for quantitative analysis of collagen fibrillar structure. *Nat Protoc* (2012) 7(4):654–69. doi: 10.1038/nprot.2012.009

54. Meier L, Geer SVD, Bhlmann P, Zrich ETH. The group Lasso for logistic regression. *J R Stat Soc B* (2008) 70(1):53–71. doi: 10.2307/20203811

55. TibshIrani R. The lasso method for variable selection in the Cox model. *Stat Med* (1997) 16(4):385–95. doi: 10.1002/(SICI)1097-0258(19970228)16:43.0.CO;2-3

56. Qiu H, Ding S, Liu J, Wang L, Wang X. Applications of artificial intelligence in screening, diagnosis, treatment, and prognosis of colorectal cancer. *Curr Oncol* (2022) 29(3):1773–95. doi: 10.3390/curroncol29030146

57. Bilal M, Nimir M, Snead D, Taylor GS, Rajpoot N. Role of AI and digital pathology for colorectal immuno-oncology. *Br J Cancer* (2023) 128(1):3–11. doi: 10.1038/s41416-022-01986-1

58. Sharma A, Kumar R, Yadav G, Garg P. Artificial intelligence in intestinal polyp and colorectal cancer prediction. *Cancer Lett* (2023) 565:216238. doi: 10.1016/j.canlet.2023.216238

59. Chlorogiannis DD, Verras G-I, Tzelepi V, Chlorogiannis A, Apostolos A, Kotis K, et al. Tissue classification and diagnosis of colorectal cancer histopathology images using deep learning algorithms. Is the time ripe for clinical practice implementation? *Gastroenterol Review/Przegląd Gastroenterologiczny* (2023). doi: 10.5114/pg.2023.130337

60. Foersch S, Glasner C, Woerl AC, Eckstein M, Wagner DC, Schulz S, et al. Multistain deep learning for prediction of prognosis and therapy response in colorectal cancer. *Nat Med* (2023) 29(2):430–9. doi: 10.1038/s41591-022-02134-1

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Can neoadjuvant chemoradiotherapy combined with immunotherapy benefit patients with microsatellite stable locally advanced rectal cancer? a pooled and integration analysis

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Objective: To assess the clinical efficacy of neoadjuvant chemoradiotherapy combined with immunotherapy for patients with microsatellite stable (MSS) locally advanced rectal cancer and provide evidence to support clinical decision-making.

Methods: A systematic search was conducted on the PubMed, Embase, Cochrane Collaboration databases, conference summaries, and Chinese databases for clinical studies that investigated neoadjuvant chemoradiotherapy combined with immunotherapy for the treatment of locally advanced rectal cancer with MSS status. The search spanned from the inception of each database through July 2023. Data from the identified studies were extracted using a pre-designed table, and efficacy outcomes were analyzed. An integrated analysis was conducted using Stata 12.0 software.

Results: Eight studies were included, comprising 204 patients with locally advanced MSS rectal cancer who received chemoradiotherapy combined with immunotherapy. The integrated analysis revealed a pathologic complete remission rate of 0.33, a sphincter preservation rate of 0.86, an R0 resection rate of 0.83, a major pathologic remission rate of 0.33, and a clinical complete remission rate of 0.30.

Conclusion: Neoadjuvant chemoradiotherapy combined with immunotherapy demonstrates significant short-term efficacy in MSS-type locally advanced rectal cancer, notably enhancing the pathologic complete remission and sphincter preservation rates. This combination is a recommended treatment for patients with MSS-type rectal cancer.

KEYWORDS

neoadjuvant chemoradiotherapy, immunotherapy, microsatellite stable, rectal cancer, pooled and integration analysis

1 Introduction

Neoadjuvant chemoradiotherapy, when combined with surgery and followed by adjuvant chemotherapy, remains a primary treatment strategy for stage II/III locally advanced rectal cancer (LARC). Research indicates that after neoadjuvant radiotherapy, the local recurrence rate for advanced rectal cancer is maintained between 5% and 7% (1–3). A phase II clinical trial verified that six cycles of mFOLFOX6 after chemoradiotherapy and total mesorectal excision significantly elevated the pathologic complete remission (PCR) rate (4). Despite these advances, enhancing the PCR rate and managing distant metastasis remain central challenges. Emphasizing improved clinical outcomes, treatments for LARC now prioritize both survival duration and quality of life. Achieving complete tumor remission, especially for low rectal cancer patients, can lead to anus preservation, which holds immense clinical importance.

Recent discoveries highlight the efficacy of immune checkpoint inhibitors of PD1 in the advanced treatment of various tumors (5). While immunotherapy is particularly effective for colorectal cancer patients with mismatch repair gene expression deficiency or those with high microsatellite instability (MSI-H, which is associated with higher tumor mutation load and tumor-infiltrating lymphocytes [TILs]), Several studies have shown that the microsatellite instability status of tumors is an independent indicator of survival and prognosis of colorectal cancer patients with prognostic significance. Patients with MMR-deficient colorectal cancer generally have a better prognosis than patients with non-MMR-deficient cancer (6, 7). Therefore, the microsatellite instability status of tumors can be an essential influence on the prognosis of colorectal cancer patients, prompting physicians to quantify treatment and long-term follow-up. It has been shown that microsatellite instability can enhance tumor immunogenicity to a certain extent, as well as recognize and kill tumor cells through a variety of immune cells. Therefore, microsatellite instability may be an adjunctive indicator in immunotherapy (8, 9). However, 95% of rectal cancer is of the microsatellite stable (MSS) type. This type typically does not respond well to standalone immunotherapy (10). Many malignant tumors, including rectal cancer, develop immune resistance through diverse mechanisms leading to immune tolerance. PD-1/PD-L1 emerges as the pivotal immune checkpoint pathway in this context (11). PD-1, predominant in activated T-lymphocytes, interacts with PD-L1 on tumor cells, suppressing effector T-cell immune functionality (12). Notably, radiotherapy, especially ionizing radiation, amplifies the anti-tumor immune response of immune checkpoint inhibitors. This amplification is realized by promoting cytotoxic T-cell activity, increasing antigen production, and fostering synergy with immune checkpoint inhibitors (13).

The approach that starts with neoadjuvant chemoradiotherapy followed by sequential combined immunotherapy, primarily as consolidation therapy, was first reported by the investigators of the Japanese VOLTAGE-A study. This study used a conventional long course of radiotherapy with capecitabine and sequential ravulizumab immunotherapy for five courses after the end of radiotherapy. They found that 11 of 37 MSS-type patients achieved a PCR rate of 30%, with three reaching near PCR (8%) and one attaining clinical complete remission (CCR), leading to watchful waiting. Additionally, three of five MSI-H–type patients realized a PCR rate of 60% (14). In another study by Shamseddine et al., a combination of short-term radiotherapy mFOLFOX-6 and avelumab treated locally advanced rectal adenocarcinoma. This study enrolled 13 MSS-type patients, of which three (25%) achieved PCR (tumor regression grade 0), three (25%) approached PCR (tumor regression grade 1), and six (50%) manifested a major pathologic response (15). Recent clinical trials combining neoadjuvant chemoradiotherapy with immunotherapy for LARC have predominantly commenced in the last 2 years. Most are phase II clinical trials focusing on PCR rate as the primary endpoint. Preliminary results from several immunoclinical trials confirm that an improved PCR rate is vital for anus preservation.

Although the efficacy of neoadjuvant radiotherapy combined with immunotherapy is being widely investigated, data on its impact on advanced MSS rectal cancer remain sparse due to limited sample sizes. Thus, this study systematically evaluates the efficacy of neoadjuvant chemoradiotherapy coupled with immunotherapy in MSS/pMMR-type patients with LARC, aiming to offer renewed clinical guidance.

2 Methods

2.1 Literature search strategy

Two researchers independently executed a detailed and systematic exploration of databases, including PubMed, Embase, Cochrane Library, Web of Knowledge, and ClinicalTrials.gov, China National Knowledge Infrastructure (CNKI), along with other sources, such as conference papers (e.g., https://ascopubs.org/doi/10.1200). The following search terms were used: rectal cancer, *nivolumab*, *camrelizumab*, *sintilimab*, *tislelizumab*, *pembrolizumab*, *toripalimab*, *durvalumab*, *avelumab*, *atezolizumab*, *PD1/PD-L1*, *neoadjuvant*, *preoperative avelumab*, and *atezolizumab*. Logical operators (AND/OR) facilitated combining subject terms with free words. The search strategy adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (16).

2.2 Inclusion and exclusion criteria

For inclusion, the articles considered had to meet the following criteria: the study population should have comprised patients with advanced rectal cancer, specifically those with the MSS/pMMR type. The intervention under investigation must have been neoadjuvant chemoradiotherapy combined with immunotherapy for LARC. Additionally, eligible articles reported on randomized controlled studies, prospective or retrospective studies, or single-arm clinical studies. On the other hand, the exclusion criteria encompassed reviews, commentaries, and other literature types. Studies that were

published multiple times, had incomplete data, or from which data could not be extracted were also excluded.

2.3 Outcomes

The primary endpoints were PCR (pathologic complete remission), sphincter preservation rate, major pathologic remission (MPR), R0 resection rate (R0 resection represents complete resection of the tumor and negative microscopic margins), and CCR (clinical complete remission).

2.4 Data extraction and literature quality evaluation

Two investigators independently reviewed the complete texts, extracted relevant data, and verified the extracted information. Disagreements between them were resolved by a third investigator. Both researchers also assessed the bias risk of the included studies and evaluated the literature quality. If consensus could not be reached, a third researcher intervened to assess the quality of the literature in question. The Newcastle/Ottawa Scale (NOS) was employed to gauge the quality of controlled and single-arm trials (17).

2.5 Statistical analysis

Stata 12.0 (StataCorp, College Station, TX, USA) software was used for data processing, and there was significant heterogeneity in the included literature, so the heterogeneity analysis was integrated using a random effects model. Additionally, publication bias was detected using Begg's test, Egger's test, and funnel plot analysis.

3 Results

3.1 Literature screening and quality evaluation

A thorough search of the literature database yielded 507 articles. After duplicates were removed using Endnote, 153 articles remained. Subsequent screening of titles and abstracts narrowed the pool down to 28 articles. Of these, 20 were excluded due to them being review or commentary articles or not being relevant to the predefined study indicators. Ultimately, eight articles were selected for inclusion, encompassing 204 MSS-type LARC patients who underwent neoadjuvant chemoradiotherapy coupled with immunotherapy (14, 15, 18–23) (Figure 1). Out of the included studies, five case-control studies achieved an NOS score of 8, while the other three single-arm studies each scored 6.

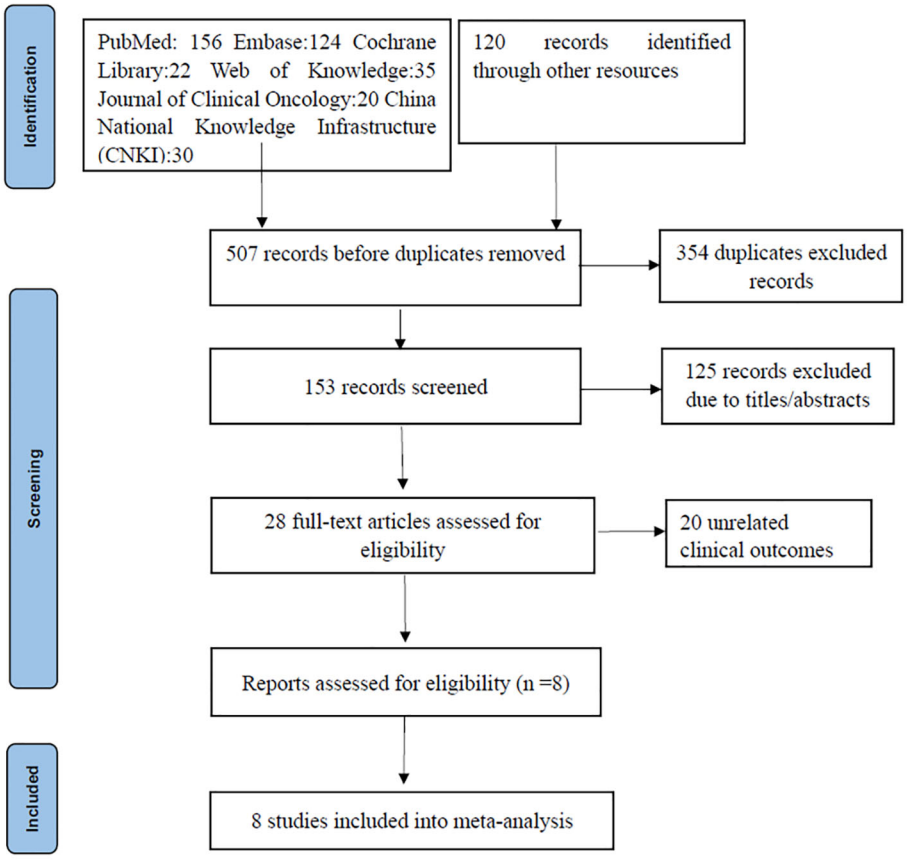

FIGURE 1
Flow chart of study selection PRISMA.

3.2 Baseline information of the included studies

Key details from the incorporated studies, such as the research program, country, sample size, patients' pathologic characteristics, study design, study outcomes, and NOS scores, are summarized in Tables 1, 2.

3.3 Study outcomes

3.3.1 PCR rate

Eight studies (14, 15, 18–23), with a total of 204 patients, reported PCR rates in a range of 0.2-0.56, and the result of the integrated analysis of PCR rates in this study was 0.33 (95% CI: 0.24-0.43, I^2 = 50.9%; Figure 2).

3.3.2 Sphincter preservation rate

Six studies (14, 18–20, 22, 23), totaling 185 patients, reported sphincter retention rates in a range of 0.71-1, and the integrated analysis of sphincter retention in this study was 0.86 (95% CI: 0.77-0.94, I^2 = 59.4%; Figure 3).

3.3.3 MPR rate

Two studies (15, 22), with a total of 32 patients, reported MPR rates of 0.2 and 0.5, respectively, and the result of the integrated analysis of MPR rates in this study was 0.33 (95% CI: 0.04-0.62, I^2 = 68.0%; Figure 4).

3.3.4 R0 resection rate

Three studies (14, 19, 22), with a total of 84 patients, reported R0 resection rates of 0.71, 1, and 0.95, respectively, and the

TABLE 1 Baseline information of included studies.

Study	country	Sample Size	Patient characteristics	Clinical Trial Registration Number	Treatment Programs	NOS score
George 2019 (18)	American	45	Stage II/III; MSS	NCT03102047	CRT[Capeox + 50.4 Gy] +Durvalumab × 4 - TME	6
Shamseddine 2020 (15)	Belgium	44	stage II/III;MSS	NCT03503630	5×5Gy+mFOLFOX × 6 + Ave × 6 -TME	8
Lin 2021 (19)	China	30	T3-4N0M0 or T1-4N+M0;MSS	NCT04231552	5 × 5Gy + Capeox × 2 + Camrelizumab× 2 - TME	8
Li 2021 (22)	China	24	pMMR/MSS	NCT02864849	(sintilimab+Capeox) ×3+IMRT+ Capeox×2-TME	8
Bando 2022 (14)	Japan	39	cT3-4N0-2M0;III; MSS	NCT02948348	CRT [Capeox + 50.4 Gy] +Nivolumab × 5- TME	8
Zhou 2022 (20)	China	23	T1-3aN0-1M0; pMMR/MSS	NCT05215379	CRT [Capeox + 50.4 Gy] + Sintilimab × 2 - Cape/Capeox × 6 + Sintilimab × 2 - TME	6
WU 2022 (21)	China	25	pMMR/MSS	NCT04340401	Capeox×3+ Camrelizumab × 3 - CRT [Capeox + 50.4 Gy] - Capeox × 2 - TME	6
Wang 2023 (23)	China	32*	pMMR/MSS	NCT045182820	consolidation group: 25 Gy/5 Fx+ (CAPOX+ toripalimab) ×6-TME Induction group: (CAPOX+ toripalimab) ×2 + 25 Gy/5 Fx+(CAPOX+PD-1) ×4-TME	8

CRT, chemoradiotherapy; TME, total mesorectal excision; mFOLFOX, modify oxaliplatin + leucovorin + 5-fluorouracil; Capeox, capecitabine + oxaliplatin; mFOLFIRINOX, modify oxaliplatin + irinotecan + calcium folinate + 5-fluorouracil; Cape, capecitabin; 5FU, 5-fluorouracil; AF, LV, leucovorin; OXA, oxaliplatin; FOLFOX, oxaliplatin + calcium folinate + 5-fluorouracil; cCR, clinical complete response; pCR, pathologic complete response; MPR, major pathological response; NAR, neoadjuvant rectal score; HR, Hazard Ratio.*: The subjects included in this study were patients who underwent TME after neoadjuvant chemoradiotherapy combined with immunotherapy, so the sample size included in this article was patients who underwent surgery.

TABLE 2 Outcome indicators for inclusion in the study.

Study	PCR rate	Sphincter preservation rate	MPR rate	R0 resection rate	CCR rate
George 2019 (18)	22.2%	71.1%			31.1%
Shamseddine 2020 (15)	25%	–	50%		
Lin 2021 (19)	48.1%	88.9%		100%	
Li 2021 (22)	30%	80%	20%	95%	13.6%
Bando 2022 (14)	29.7%	87.1%		70.3%	
Zhou 2022 (20)	20%	95.5			43.4%
WU 2022 (21)	33.3%	–			
Wang 2023 (23)	56.3%	100%			34.4%

pCR, pathologic complete response; MPR, major pathological response;"-", not reported.

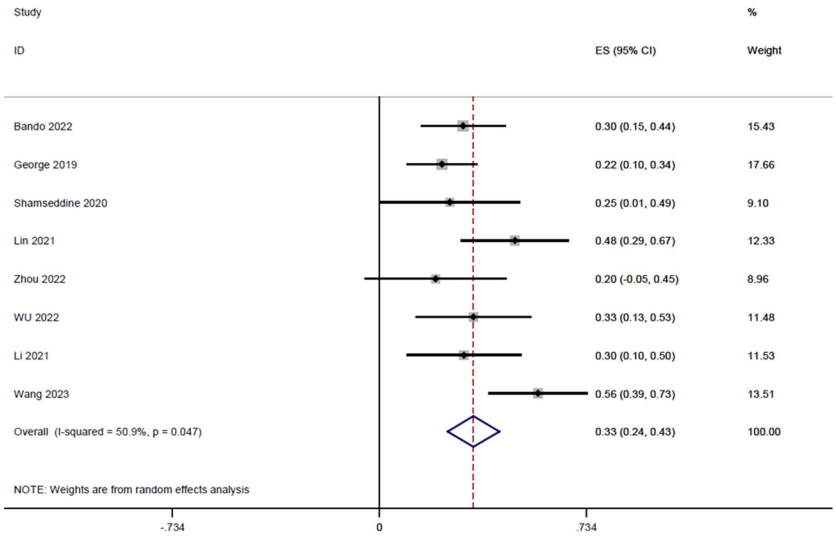

FIGURE 2
Forest plot of single arm integrated analysis of PCR rate in rectal cancer patients with MSS type.

integrated analysis of R0 resection rates in this study resulted in 0.83 (95% CI: 0.59-1.07, I^2 = 86.9%; Figure 5).

3.3.5 CCR rate

Four studies (18, 20, 22, 23), with a total of 122 patients, reported CCR rates of 0.31, 0.43, 0.13, and 0.34 respectively, and the integrated analysis of CCR rates in this study resulted in 0.30 (95% CI: 0.18-0.42, I^2 = 56.3%; Figure 6).

4 Publication bias

Begg's test for the PCR rate yielded Pr>|z| = 0.276 (Figure 7). Meanwhile, Egger's test showed Pr>|t| = 0.183 (95% CI: -0.25-0.75; Figure 8), indicating no observed publication bias.

5 Discussion

Innovations in neoadjuvant therapy for LARC have steadily progressed. According to the NCCN guidelines, long-range radiotherapy (50 Gy/25 Fx) combined with 5-FU or capecitabine concurrently and short-range radiotherapy (25 Gy/5 Fx), which markedly reduces local recurrence rates, are now considered standard-of-care recommendations (24, 25). Nonetheless, the PCR rate of neoadjuvant therapy stands at a mere 10-20%, leaving much to be desired regarding long-term prognosis.

However, immunotherapy has emerged as a revolutionary treatment for various malignant tumors. Preclinical studies have shown that radiotherapy can bolster anti-tumor immune responses. It can also cause an upsurge in PD-L1 expression in tumor tissues,

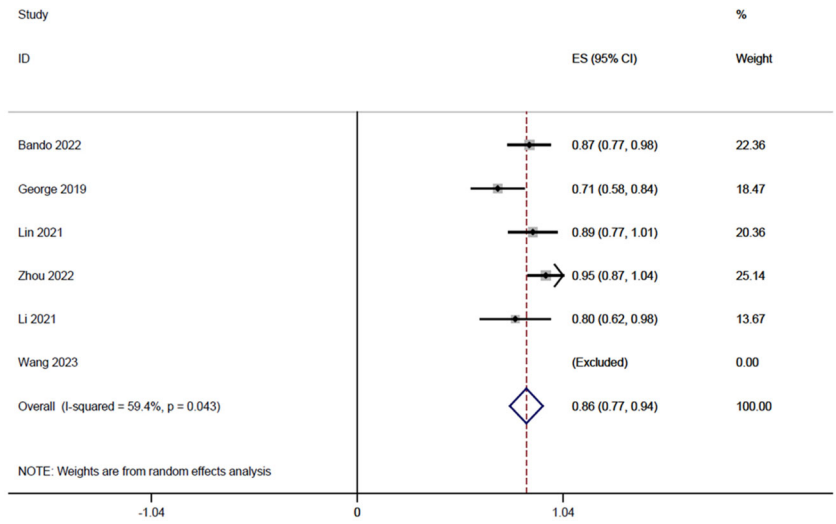

FIGURE 3
Forest plot of single-arm integrated analysis of mean sphincter preservation rate in patients with MSS-type rectal cancer.

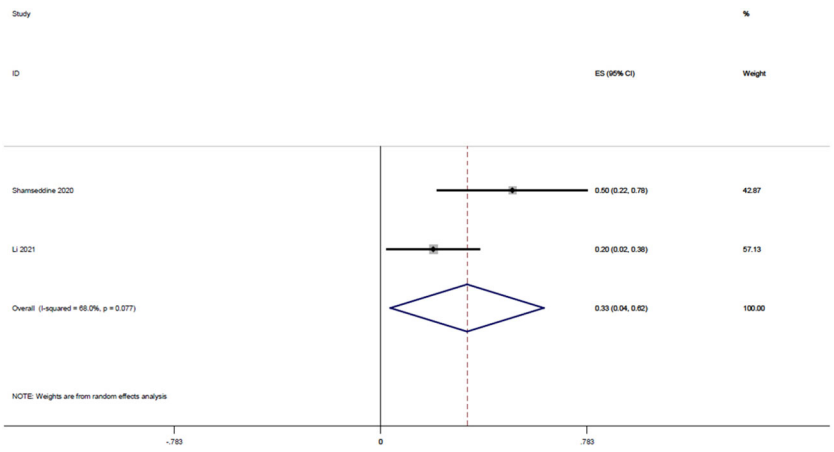

FIGURE 4
Forest plot of single arm integrated analysis of MPR rate in rectal cancer patients with MSS type.

heightening their susceptibility to immunotherapy. Additionally, when combined with immune checkpoint inhibitors, radiotherapy can modulate the tumor microenvironment, mitigating its immunosuppressive effects (26, 27). Furthermore, clinical research has unveiled a "distant effect" wherein chemoradiotherapy paired with immunotherapy leads to significant regression in both the irradiated tumor component and distant tumor tissue, a phenomenon attributed to the systemic immune response triggered by radiotherapy (28, 29).

This synergy offers promising avenues in the neoadjuvant treatment of LARC. Combining neoadjuvant radiotherapy and immunotherapy could potentially break through the bottleneck slowing efforts to improve outcome data related to PCR and CCR in MSS rectal cancer responses. Clinical trials exploring this combination have been steadily progressing, yielding positive results in terms of enhanced tumor regression and improved PCR rates in both MSI-H and MSS rectal cancer patients. This therapeutic approach also increases the probability of anal preservation, bolstering the feasibility of a "wait-and-see" strategy.

In our investigation, we evaluated the efficacy of neoadjuvant chemoradiotherapy in tandem with immunotherapy for patients diagnosed with MSS-type rectal cancer. We incorporated seven clinical trials, encompassing a total of 172 MSS-type rectal cancer patients. An aggregated analysis revealed a PCR rate of 29% after treatment, a substantial improvement compared with using neoadjuvant chemoradiotherapy in isolation. At the 2020 ASCO meeting, findings from the Japanese VOLTAGE -A study were presented. They applied a standard long course of radiotherapy combined with capecitabine, followed by five rounds of sequential ravulizumab immunotherapy after radiotherapy. Out of 37 MSS-type patients, 11 achieved PCR (30%), three achieved near PCR (8%), and one reached CCR, opting for a watchful waiting approach. Given that traditional radiotherapy combined with capecitabine typically reaches a PCR rate of 15-20%, these results

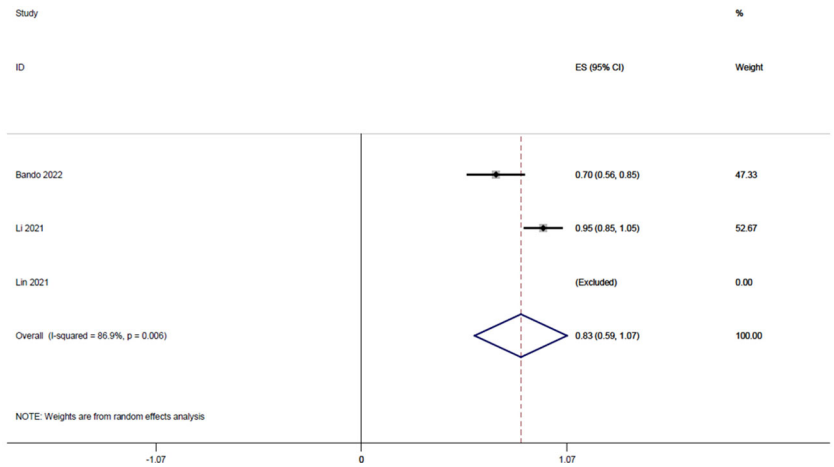

FIGURE 5
Forest plot of single-arm integrated analysis of R0 resection rate in patients with MSS type of rectal cancer.

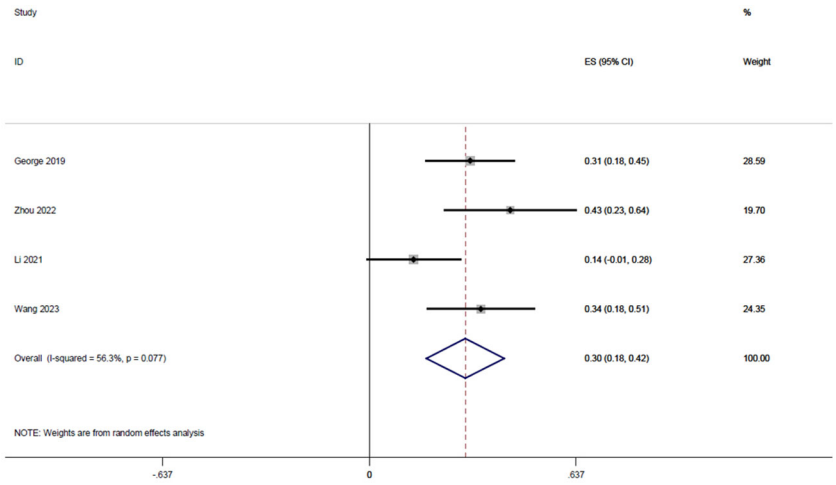

FIGURE 6
Forest plot of single arm integrated analysis of CCR rate in rectal cancer patients with MSS type.

(30% PCR) indicate superior efficacy when paired with immunotherapy (14). The recently released NRG-GI002 study showcased the pembrolizumab cohort study results. All cohorts followed the TNT (total neoadjuvant therapy) model, involving eight cycles of FOLFOX (oxaliplatin + folinic acid + 5-fluorouracil) chemotherapy, with the control group receiving sequential long-term radiotherapy (alongside capecitabine). In contrast, the experimental group had the same but combined with pembrolizumab. The PCR rate was 29.4% for the control group and 31.9% for the experimental group, underlining the effectiveness of integrating neoadjuvant chemoradiotherapy with immunotherapy (30). A phase II clinical trial, known as the averectalstudy, was conducted in Lebanon and Jordan. It combined short-course radiotherapy with mFOLFOX6 (oxaliplatin + folinic acid + 5-fluorouracil adjusted regimen 6) chemotherapy and avelumab immunotherapy. Of the 44 patients enrolled, four were excluded for various reasons. Among the remaining 40, 15 achieved PCR (37.5%), and 12 (30%) achieved near PCR with a tumor regression grade of 1. This means that 67.5% of the patients demonstrated significant tumor regression (15).

In 2004, Prof. Habr-Gama from Brazil introduced the "wait-and-see" strategy for patients achieving CCR after neoadjuvant therapy for rectal cancer (nCRT). This approach has shown marked improvements in patient quality of life without impacting long-term survival (31). However, after conventional nCRT, the CCR rates remain less than optimal. A study by Martens et al. showed that out of 141 rectal cancer patients treated with nCRT, only 24 (17%) achieved CCR (32).

Furthermore, a recent evaluation at the Cancer Hospital of Peking University in China assessed the outcomes of the PD1

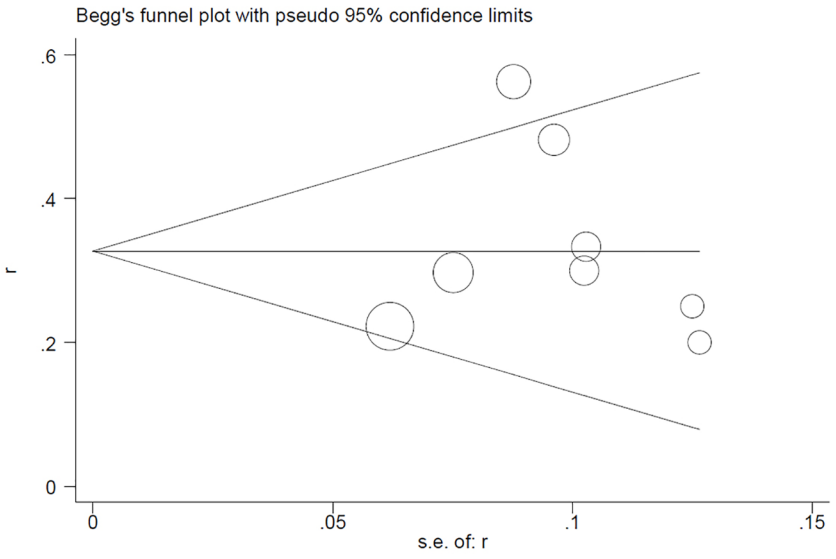

FIGURE 7
Begg's funnel plot for PCR rate.

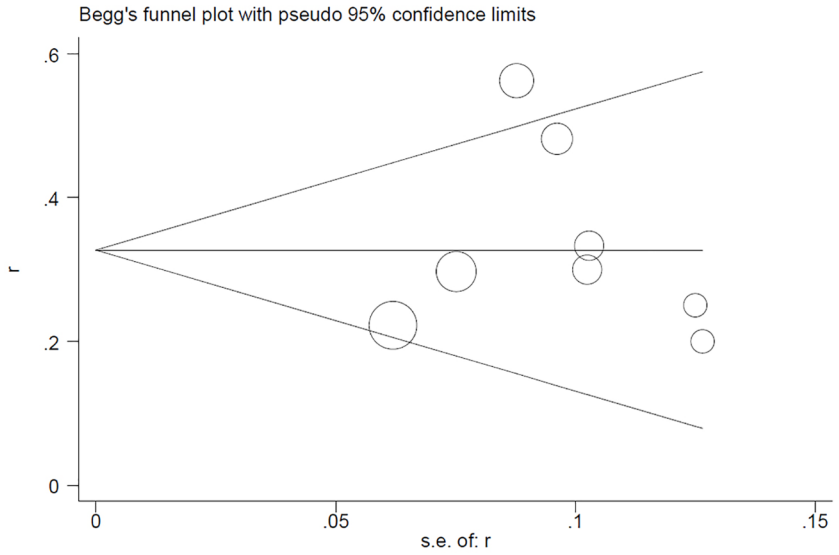

FIGURE 8
Egger's funnel plot for PCR rate.

antibody combined with full neoadjuvant chemoradiotherapy for high-risk, locally progressive, low- and intermediate-stage rectal cancer patients. Among the 24 MSS-type rectal cancer patients, 19 had R0 resection, 16 underwent anal sphincter preservation surgery, and the PCR rate stood at 30.0% (6 out of 20). An additional 20.0% (4 out of 20) had major pathologic responses. The study showed that the combination of PD-1 antibody with full neoadjuvant chemoradiotherapy yielded positive safety and histopathologic regression outcomes. Combining histologic and genetic testing can further assist in identifying individuals who may benefit most from this approach (22).

Our comprehensive analysis of neoadjuvant chemoradiotherapy combined with immunotherapy yielded the following findings associated with this strategy: a CCR rate of 28%, an R0 resection rate of 83%, and an anal preservation rate of 86%. These findings underscore the potential of this combination to enhance the regression of MSS-type rectal tumors, elevate the PCR rate, and offer a safe and tolerable treatment option. It emerges as a viable choice for those patients keen on organ preservation and achieving CCR. This combination serves as a promising option for LARC patients opting for the "wait-and-see" approach and desiring CCR.

Our study has integrated and analyzed the most recent clinical efficacy data regarding neoadjuvant chemoradiotherapy paired with immunotherapy for MSS-type advanced colorectal cancer. The findings solidify the potential clinical significance of this combined approach for MSS-type advanced colorectal cancer patients. The synergy of nCRT and immunotherapy for treating LARC leads to favorable PCR/CCR rates and increases the probability of preserving the anus. It is crucial to note that our study had a limited sample size. Additionally, differences in treatment regimens and the sequence of applying radiotherapy and immunotherapy across studies led to heterogeneity in outcomes. Nonetheless, in the evolving landscape of immunotherapy, while radiotherapy remains pivotal in treating rectal cancer, we are optimistic that immunotherapy combined with neoadjuvant chemoradiotherapy heralds a brighter future for patients with the MSS type.

6 Conclusion

Our comprehensive analysis, encompassing eight single-arm clinical studies, underscores the promising efficacy of neoadjuvant chemoradiotherapy in tandem with immunotherapy for patients with MSS-type LARC. This combined approach has demonstrated notable enhancements in outcomes, including the PCR rate, MPR rate, R0 resection rate, major sphincter preservation rate, and CCR rate. Given these promising preliminary findings, the combination of neoadjuvant chemoradiotherapy with immunotherapy heralds vast potential in the therapeutic landscape. Nonetheless, for a definitive endorsement of its efficacy, there is a pressing need for large-scale, randomized controlled trials focusing on MSS-type LARC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

YY: Data curation, Writing – original draft. MC: Conceptualization, Data curation, Formal Analysis, Writing – review & editing. XX: Conceptualization, Investigation, Software, Writing – review & editing. MW: Conceptualization, Investigation, Supervision, Validation, Writing

– review & editing. QW: Writing – review & editing. BZ: Formal Analysis, Methodology, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1280995/full#supplementary-material

References

1. Hofheinz RD, Wenz F, Post S, Matzdorff A, Laechelt S, Hartmann JT, et al. Chemoradiotherapy with capecitabine versus fluorouracil for locally advanced rectal cancer: a randomised, multicentre, non-inferiority, phase 3 trial. *Lancet Oncol* (2012) 13(6):579–88. doi: 10.1016/S1470-2045(12)70116-X

2. Body A, Prenen H, Lam M, Davies A, Tipping-Smith S, Lum C, et al. Neoadjuvant therapy for locally advanced rectal cancer: recent advances and ongoing challenges. *Clin Colorectal Cancer* (2021) 20(1):29–41. doi: 10.1016/j.clcc.2020.12.005

3. Xiong K, Bao T, Cao Y, Hu W, Deng J, Chen J, et al. Efficacy and safety of total neoadjuvant therapy in locally advanced rectal cancer: a meta-analysis. *Int J Colorectal Dis* (2023) 38(1):89. doi: 10.1007/s00384-023-04376-y

4. Marco MR, Zhou L, Patil S, Marcet JE, Varma MG, Oommen S, et al. Consolidation mFOLFOX6 chemotherapy after chemoradiotherapy improves survival in patients with locally advanced rectal cancer: final results of a multicenter phase II trial. *Dis Colon Rectum* (2018) 61(10):1146–55. doi: 10.1097/DCR.0000000000001207

5. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Five-year survival and correlates among patients with advanced melanoma, renal cell carcinoma, or non-small cell lung cancer treated with nivolumab. *JAMA Oncol* (2019) 5 (10):1411–20. doi: 10.1001/jamaoncol.2019.2187

6. Gelsomino F, Barbolini M, Spallanzani A, Pugliese G, Cascinu S. The evolving role of microsatellite instability in colorectal cancer: A review. *Cancer Treat Rev* (2016) 51:19–26. doi: 10.1016/j.ctrv.2016.10.005

7. Cohen R, Rousseau B, Vidal J, Colle R, Diaz LA Jr, André T. Immune checkpoint inhibition in colorectal cancer: microsatellite instability and beyond. *Target Oncol* (2020) 15(1):11–24. doi: 10.1007/s11523-019-00690-0

8. Zhang X, Wu T, Cai X, Dong J, Xia C, Zhou Y, et al. Neoadjuvant immunotherapy for MSI-H/dMMR locally advanced colorectal cancer: new strategies and unveiled opportunities. *Front Immunol* (2022) 13:795972. doi: 10.3389/fimmu.2022.795972

9. Ding PR. [Immunotherapy for microsatellite-instability-high advanced colorectal cancer]. *Zhonghua Wei Chang Wai Ke Za Zhi* (2022) 25(3):199–204. doi: 10.3760/cma.j.cn441530-20220118-00025

10. Wang Y, Shen L, Wan J, Zhang H, Wu R, Wang J, et al. Neoadjuvant chemoradiotherapy combined with immunotherapy for locally advanced rectal cancer: A new era for anal preservation. *Front Immunol* (2022) 13:1067036. doi: 10.3389/fimmu.2022.1067036

11. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* (2014) 515(7528):568–71. doi: 10.1038/nature13954

12. Blank C, Kuball J, Voelkl S, Wiendl H, Becker B, Walter B, et al. Blockade of PD-L1 (B7-H1) augments human tumor-specific T cell responses in *vitro*. *Int J Cancer* (2006) 119(2):317–27. doi: 10.1002/ijc.21775

13. Deng L, Liang H, Burnette B, Beckett M, Darga T, Weichselbaum RR, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest* (2014) 124(2):687–95. doi: 10.1172/JCI67313

14. Bando H, Tsukada Y, Inamori K, Togashi Y, Koyama S, Kotani D, et al. Preoperative Chemoradiotherapy plus Nivolumab before Surgery in Patients with Microsatellite Stable and Microsatellite Instability-High Locally Advanced Rectal Cancer. *Clin Cancer Res* (2022) 28(6):1136–46. doi: 10.1158/1078-0432.CCR-21-3213

15. Shamseddine A, Zeidan YH, El Husseini Z, Kreidieh M, Al Darazi M, Turfa R, et al. Efficacy and safety-in analysis of short-course radiation followed by mFOLFOX-6 plus avelumab for locally advanced rectal adenocarcinoma. *Radiat Oncol* (2020) 15 (1):233. doi: 10.1186/s13014-020-01673-6

16. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* (2015) 4(1):1. doi: 10.1186/2046-4053-4-1

17. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* (2010) 25 (9):603–5. doi: 10.1007/s10654-010-9491-z

18. George TJ, Yothers G, Lee JJ, Jacobs SA, Deutsch M, Allegra CJ, et al. NSABPFR-2: Phase II study of durvalumab following neoadjuvant chemoRT in stage II-IV rectal cancer. *JCO* (2019) 37:TPS727–7. doi: 10.1200/jco.2019.37.4_suppl.tps727

19. Lin Z, Cai M, Zhang P, Li G, Liu T, Li X, et al. single-arm trial of preoperative short-course radiotherapy followed by chemotherapy and camrelizumab in locally advanced rectal cancer. *J Immunother Cancer* (2021) 9(11):e003554. doi: 10.1136/jitc-2021-003554

20. Zhou L, Yu G, Shen Y, Ding H, Zheng K, Wen R, et al. The clinical efficacy and safety of neoadjuvant chemoradiation therapy with immunotherapy for the organ preservation of ultra low rectal cancer: A single arm and open label exploratory study. *JCO* (2022) 40:e15603–3. doi: 10.1200/JCO.2022.40.16_suppl.e15603

21. Wu A, Li Y, Ji D, Zhang L, Zhang X, Cai Y, et al. PKUCH 04 trial: Total neoadjuvant chemoradiation combined with neoadjuvant PD-1 blockade for pMMR/MSS locally advanced middle to low rectal cancer. *JCO* (2022) 40:3609–9. doi: 10.1200/JCO.2022.40.16_suppl.3609

22. Li YJ, Zhang L, Dong QS, Cai Y, Zhang YZ, Wang L, et al. Short-term outcome of programmed cell death protein1 (PD-1) antibody combined with total neoadjuvant chemoradiotherapy in the treatment of locally advanced middle-low rectal cancer with high risk factors. *Zhonghua Wei Chang Wai Ke Za Zhi* (2021) 24(11):998–1007. doi: 10.3760/cma.j.cn441530-20210927-00386

23. Wang YQ, Shen LJ, Wan JF, Zhang H, Wang Y, Wu X, et al. [Short-course radiotherapy combined with CAPOX and PD-1 inhibitor for the total neoadjuvant therapy of locally advanced rectal cancer: the preliminary single-center findings of a prospective, multicentre, randomized phase II trial (TORCH)]. *Zhonghua Wei Chang Wai Ke Za Zhi* (2023) 26(5):448–58. doi: 10.3760/cma.j.cn441530-20230107-00010

24. Bachet JB, Benoist S, Mas L, Huguet F. Traitement néoadjuvant des cancers du rectum [Neoadjuvant treatment for rectal cancer]. *Bull Cancer* (2021) 108(9):855–67. doi: 10.1016/j.bulcan.2021.03.018IF

25. Liu S, Jiang T, Xiao L, Yang S, Liu Q, Gao Y, et al. Total neoadjuvant therapy (TNT) versus standard neoadjuvant chemoradiotherapy for locally advanced rectal cancer: A systematic review and meta-analysis. *Oncologist* (2021) 26(9):e1555–66. doi: 10.1002/onco.13824

26. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* (2017) 357(6349):409–13. doi: 10.1126/science.aan6733IF

27. Michael-Robinson JM, Biemer-Hüttmann A, Purdie DM, Walsh MD, Simms LA, Biden KG, et al. Tumour infiltrating lymphocytes and apoptosis are independent features in colorectal cancer stratified according to microsatellite instability status. *Gut* (2001) 48(3):360–6. doi: 10.1136/gut.48.3.360

28. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. *N Engl J Med* (2012) 366(10):925–31. doi: 10.1056/NEJMoa1112824

29. Brix N, Tiefenthaller A, Anders H, Belka C, Lauber K. Abscopal, immunological effects of radiotherapy: Narrowing the gap between clinical and preclinical experiences. *Immunol Rev* (2017) 280(1):249–79. doi: 10.1111/imr.12573

30. Rahma OE, Y others G, Hong TS, Russell MM, You YN, Parker W, et al. Use of total neoadjuvant therapy for locally advanced rectal cancer: initial results from the pembrolizumab arm of a phase 2 randomized clinical trial. *JAMA Oncol* (2021) 7 (8):1225–30. doi: 10.1001/jamaoncol.2021.1683

31. Habr-Gama A, Perez RO, Nadalin W, Sabbaga J, Ribeiro UJr, Silva e Sousa AHJr, et al. Operative versus nonoperative treatment for stage 0 distal rectal cancer following chemoradiation therapy: long-term results. *Ann Surg* (2004) 240(4):711–7; discussion 717-8. doi: 10.1097/01.sla.0000141194.27992.32

32. Martens MH, Maas M, Heijnen LA, Lambregts DM, Leijtens JW, Stassen LP, et al. Long-term outcome of an organ preservation program after neoadjuvant treatment for rectal cancer. *J Natl Cancer Inst* (2016) 108(12):djw171. doi: 10.1093/jnci/djw171

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Case Report: A management strategy and clinical analysis of primary squamous cell carcinoma of the colon

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Primary colorectal squamous cell carcinoma (CSCC) is a rare pathological subtype. Currently, clinical data with regards to its prognosis and treatment is limited, and there is no optimal treatment method. The case presented involves a proficient mismatch repair (pMMR) and microsatellite-stable (MSS) Colorectal cancer (CRC) patient with squamous cell carcinoma (SCC) located transversely in the colon. Based on the imaging assessment, the tumor infiltration depth is classified as T4. After receiving 4 cycles of neoadjuvant treatment with oxaliplatin and capecitabine (XELOX), the patients were evaluated for partial response (PR) in 2 cycles and stable disease (SD) in 4 cycles. The patient underwent a right hemicolectomy and received postoperative paclitaxel/cisplatin (TC) adjuvant chemotherapy. After 23 months, a systemic examination revealed abdominal metastasis. A needle biopsy was conducted on the detected abdominal metastases, with the resulting pathology indicating the presence of metastatic SCC. The individual exhibited expression of programmed cell death ligand 1 (PD-L1) and a mutation in the TP53 gene. Considering the patient's disease recurrence based on medical history, a treatment plan was formulated. This involved Sintilimab plus Cetuximab and the combination of leucovorin, fluorouracil, and irinotecan (FOLFIRI) regimen. The patient received four cycles of treatment with an efficacy evaluation of SD- and seven cycles of treatment with an efficacy evaluation of SD+, which resulted in a progression-free survival (PFS) duration of 7 months. This case study presents the conventional XELOX chemotherapy protocol, which has shown limited effectiveness, and highlights the favorable results achieved by implementing the TC adjuvant chemotherapy regimen in individuals diagnosed with primary colonic SCC. Furthermore, combining immune checkpoint blockade (ICB) with other therapies for patients with advanced disease is anticipated to provide an extended duration of survival.

KEYWORDS

colorectal cancer, squamous cell carcinoma, adjuvant chemotherapy, immune checkpoint inhibitors, microsatellite instability, case report

1 Introduction

Primary CSCC is an infrequent form of tumor, representing a mere 0.01-0.025% of the total cases of colorectal cancer (1). The mean age of the patients was 63.5 ± 15.3, with no significant difference in incidence between men and women (2). The most common site of occurrence was the rectum, followed by the right colon (3). The majority of cases were found to be complicated by lymph node and liver metastases (4). The patient's clinical presentation resembled that of colorectal adenocarcinoma, with nearly a half of patients displaying symptoms of gastrointestinal bleeding or abdominal distress (3, 5). The initial diagnosis is mostly advanced, with a poor prognosis (6). Usually, patients with metastasis in distant organs have a median survival rate of about 8 months (7). The five-year relative survival rate is notably inferior compared to that of colorectal adenocarcinoma (2).

Patients with primary rectal SCC are mainly treated with a combination of surgical intervention and radio chemotherapy (4, 7). Significantly, primary rectal SCC at stage II manifests a high sensitivity to chemoradiotherapy, and the administration of neoadjuvant chemoradiotherapy in patients prior to surgery has demonstrated a positive correlation with improved survival rates (8). Patients diagnosed with stage III or IV rectal SCC are typically treated through a combination of radiotherapy and chemotherapy; adding surgical interventions concurrently does not improve the overall survival (OS) of these patients (1). However, for patients experiencing recurrence or an ineffective response to radiotherapy and chemotherapy, the option of surgical intervention is available (9).

Divergent from rectal SCC, the most important treatment for patients with primary colonic SCC is surgery, and the efficacy of chemotherapy or radiotherapy is still unclear (10). Surgical intervention is the primary therapeutic modality employed for patients diagnosed with colonic SCC in Stage II (11). In cases of stage III colonic SCC, the treatment regimen involves a combination of surgical intervention and chemotherapy. In general, patients diagnosed with this condition commonly receive fluorouracil with or without cisplatin adjuvant chemotherapy (12, 13). Palliative treatment is the main approach for patients with metastatic primary colonic SCC. Hence, it is imperative to engage in further discourse regarding the management of patients diagnosed with colonic SCC.

2 Case presentation

The 41-year-old female patient presented to the clinic with abdominal pain and was diagnosed with colon cancer on January 16, 2020 (Figure 1A). Colonoscopy, the pathological results of the biopsy, and immunohistochemistry (IHC) indicated poorly differentiated SCC located in the transverse colon. The patient received a comprehensive examination that ruled out the presence

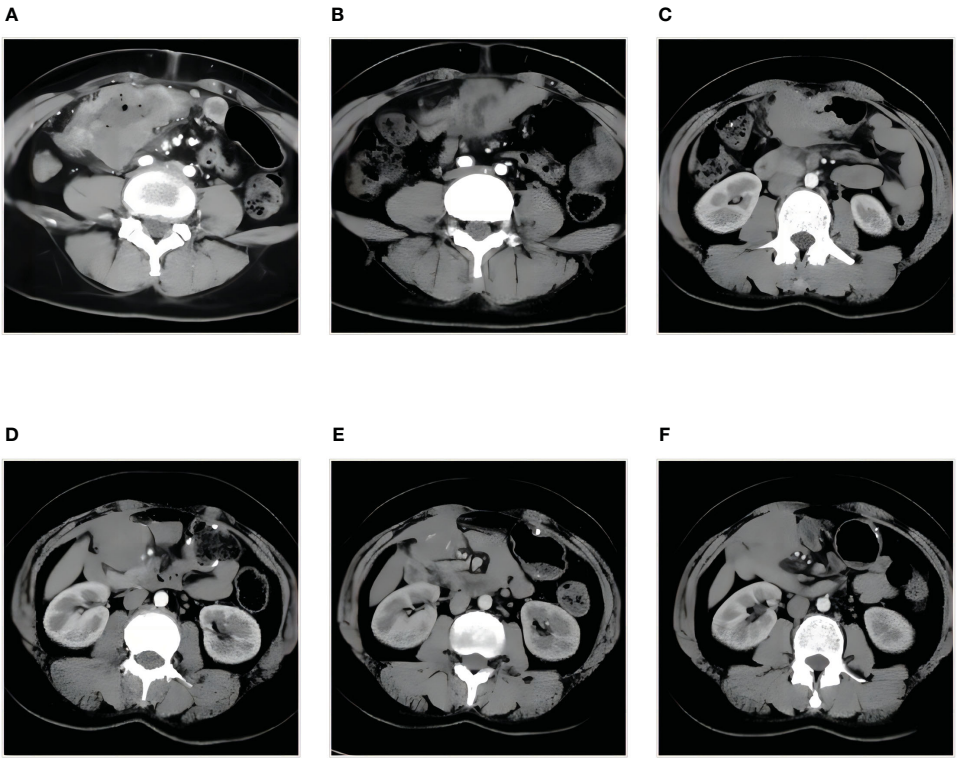

FIGURE 1
Treatment assessment by abdominal enhanced CT **(A-F)**. **(A)** Diagnosis, the tumor infiltration depth is classified as T4. **(B)** PR, after two cycles of XELOX therapy. **(C)** SD, after four cycles of XELOX therapy. **(D)** PD, abdominal metastasis. **(E)** SD-, after four cycles of combination therapy. **(F)** SD+, after seven cycles of combination therapy. PR, partial response; PD, progressive disease; SD, stable disease.

of distant metastases and primary tumors. The imaging assessment results indicate that the tumor infiltration depth was classified as T4, suggesting local progression of the disease. As a result, neoadjuvant treatment was administered. The XELOX regimen was utilized for therapy, which was then followed by 2 cycles of treatment with a PR efficacy evaluation (Figure 1B). Subsequently, 4 cycles of treatment were given with an efficacy evaluation of SD (Figure 1C). The MDT (Multidisciplinary Team) that deliberated on the case after the neoadjuvant treatment concluded that surgical resection of the neoplasia had become feasible. The patient underwent a right hemicolectomy procedure on May 22, 2020. The postoperative pathological examination yielded a poorly differentiated SCC located in the colon (Figure 2A). The tumor's largest diameter measured 6.5cm. The tumor infiltrated the muscular layer and reached the subserosal fibrous adipose tissue. The malignancy was visible at the circumferential cutting edge, and no clear vascular tumor thrombus or nerve infiltration was found, which was in line with the chemotherapy response (AJCC/TRG grade 3). No metastatic cancer was found in lymph nodes (0/43). The IHC indicated BRAFV600E (-), PMS 2 (+), MLH 1 (+), MSH 2 (+), MSH 6 (+), CD56 (-), Syn (focal weak +), CgA (+), CK20 (partial +), CDX 2 (+), P40 (+), P63 (+), and Ki-67 (70%). Between June 25th, 2020, and November 30th, 2020, the patient commenced treatment with the TC adjuvant chemotherapy protocol. The patients were then regularly monitored, and the medical condition remained stable.

On December 9, 2021, a mass located in the right upper quadrant of the anterior abdominal wall was observed during the reexamination of an abdominal enhanced CT (Figure 1D). On December 23, 2021, the abdominal mass was subjected to a CT-guided percutaneous needle biopsy. The pathology report suggested the possibility of metastatic SCC. Genetic testing revealed a mutation in the TP53 gene, but RAS and BRAF were wild-type, and IHC indicated that the patient had PD-L1: CPS (Combined Positive Score) = 20 (Figure 2B). On January 14, 2022, she was treated with Sintilimab plus Cetuximab and the FOLFIRI regimen. The patient received four cycles of treatment with an efficacy evaluation of SD- (Figure 1E) and seven cycles of treatment with an efficacy evaluation of SD+ (Figure 1F). The patient's review on July 19, 2022, revealed PD in the condition. The patient's PFS was approximately 7 months. Considering that the patient is progressing with oligolesions, there is presently a lack of a standardized second-line treatment plan with limited effectiveness. Considering that the patient was progressing by developing oligolesions, there did not, at the time, exist a standardized second-line treatment plan with a certain degree of effectiveness. In September of the same year, the patient underwent a surgical procedure to remove the abdominal mass. Postoperative pathological consideration revealed metastatic SCC. Subsequently, the patient did not receive any further anti-tumor treatment. Regrettably, the patient passed away on June 9, 2023. (Figure 3).

3 Discussion

As a rare pathological type, primary CSCC currently has no established standard treatment available. Most of the studies were focused on rectal SCC, with limited research available on colonic SCC. There was no comprehensive and systematic evidence-based study on its treatment regimen and survival prognosis. Most of the information comes from individual case reports. The present article presents the case of a patient who initially received a diagnosis of locally advanced SCC of the transverse colon. However, the surgical evaluation did not result in an R0 resection. Consequently, the patient underwent four cycles of neoadjuvant chemotherapy, followed by a right hemicolectomy procedure. Postoperative pathology suggested an AJCC/TRG grading of 3. The patient was administered adjuvant chemotherapy with TC with a PFS of 18 months. According to reports, the TC systemic chemotherapy regimen has shown superior treatment outcomes when compared to the 5-fluorouracil and cisplatin (FP) regimen for the management of esophageal squamous cell carcinoma (ESCC) in

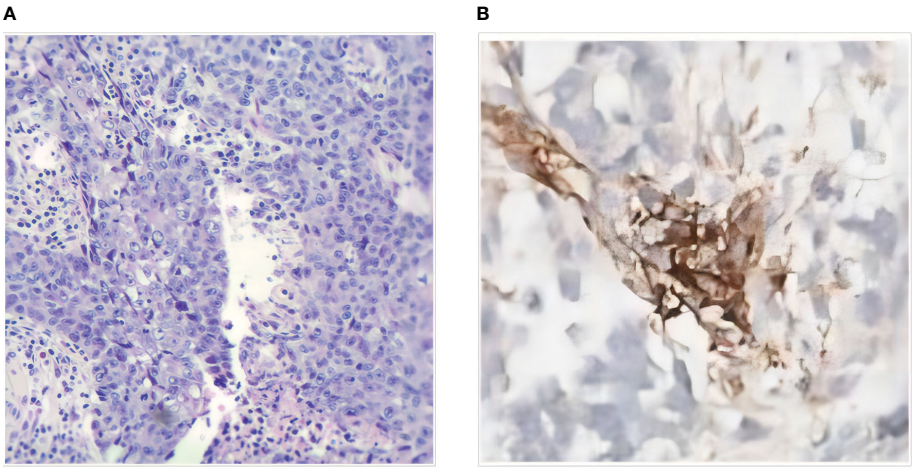

FIGURE 2
(A) Histopathology of SCC of transverse colon (HE×100). **(B)** Immunohistochemistry (IHC) of the Abdominal nodule biopsies.

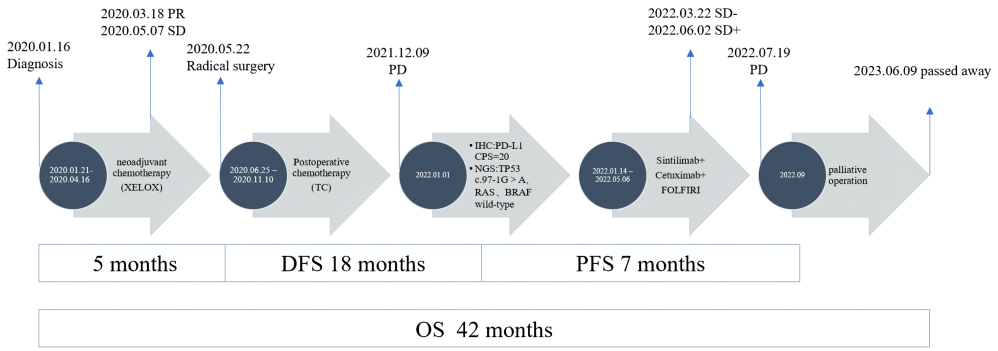

FIGURE 3
Treatment summary of the patient from diagnosis to last follow-up. PR, partial response; PD, progressive disease; SD, stable disease.

patients who receive adjuvant chemotherapy after surgery (14). In the postoperative treatment of head and neck squamous cell carcinoma (HNSCC), TC combined with radiotherapy improves the disease control rate (DCR) (15). Meanwhile, the utilization of TC in postoperative settings has been found to extend the period of disease-free survival (DFS) among patients diagnosed with cervical SCC (16). The TC regimen for individuals diagnosed with SCC has some clinical benefits. In the case of colonic SCC, it may be worth comparing the effectiveness of TC and FP.

Hence, given its unique pathological characteristics, CSCC appears to require a distinct approach to treatment when compared to colon adenocarcinoma. Currently, the accepted adjuvant therapy protocol for colon adenocarcinoma consists of the XELOX, mFOLFOX6 (oxaliplatin, fluorouracil, and leucovorin), etc. As per the standard treatment protocol, patients diagnosed with colon adenocarcinoma have shown a 3-year PFS rate of 76% (17). The primary approach for treating patients with advanced colon adenocarcinoma continues to be chemotherapy, plus Bevacizumab or Cetuximab is a feasible treatment option (18, 19). Immunotherapy has shown limited efficacy in treating gastrointestinal tumors, particularly in patients with colon cancer. However, it has been observed that Pembrolizumab is effective as a first-line treatment for metastatic colon cancer patients who have MSI-H or dMMR (20, 21). In recent times, it has been put forth the notion that the fusion of ICB and other treatment methods could emerge as a novel therapeutic choice for pMMR or MSS CRC (22). Research has demonstrated that Avelumab and Cetuximab possess complementary modes of operation that can effectively collaborate to counter the negative feedback of immunosuppression through synergistic action (23). The joint utilization of Pembrolizumab and Cetuximab results in a synergistic antitumor outcome by promoting a more advantageous anti-tumor microenvironment via the amplification of intracellular cytotoxic T lymphocytes and NK cells (24). The phase II CAVE clinical trial findings indicate that the combination of Cetuximab and Avelumab effectively targets patients with MSS metastatic CRC, exhibiting significant rechallenge therapy (25). Incorporating Avelumab into the treatment regimen consisting of Cetuximab and chemotherapy resulted in a noteworthy increase in the objective response rate (ORR) to 83% among patients with MSS CRC (26).

The current case report showcases a patient diagnosed with colonic SCC and exhibiting PD-L1 expression. After experiencing disease relapse, the patient underwent treatment with a regimen consisting of Sintelimab, Cetuximab, and chemotherapy. As a result, the patient achieved a PFS of 7 months. In the palliative treatment of colonic SCC, there are individual case reports indicating that immunotherapy can be used for these patients with PD-L1 expression. A case of a patient diagnosed with pMMR/MSS primary rectosigmoid-junction SCC and presented with high PD-L1 (CPS = 60) expression and tumor mutational burden (TMB-High, 18.99 mutations/mb), received Sintilimab combined with chemotherapy, and obtained a disease-stabilizing period of one year (27). A case of a patient suffering from pMMR/MSS primary colonic SCC with high PD-L1 (CPS = 20) expression underwent treatment involving Sintilimab combined with mFOLFOX6 and achieved a PFS of 8.5 months (28). According to previous data, the median OS for patients with advanced colonic SCC who only received chemotherapy was approximately 8 months (7). It is evident that immunotherapy treatment for colon SCC offers a survival advantage, possibly linked to the expression of PD-L1.

Based on preclinical experiments, it has been demonstrated that SCC with PD-L1 expression can be effectively suppressed through the use of ICB (29). The expression of PD-L1 on tumors reflects an immunocompetent microenvironment and is considered a major factor in anti-PD-1 therapy (30). And research has shown that the expression of PD-L1 on tumor cells is directly proportional to the response to ICB (31, 32). According to previous reports, the expression of PD-L1 is found in diverse solid tumors, encompassing lung, esophageal, and head and neck squamous cell carcinomas (33). However, there is limited available data on the expression of PD-L1 in colonic SCC.

In addition, immune response as a potential target for the treatment of SCC is associated with distinct gene expression profiles (34). The different mRNA expression patterns suggest that each SCC possesses unique immune signatures (35). Song et al. analyzed the proteome of SCC cancers from 17 organs and identified six distinct immune subtypes of pan SCC, each exhibiting unique tumor microenvironment (TME) characteristics and varying prognostic outcomes. However, it is worth noting that these

samples contain common and rare sites of SCC, but do not involve the colon (36). Therefore, there is still no good description of the molecular mechanism of CSCC.

According to this report, it is suggested that the use of TC as an adjuvant chemotherapy regimen may exhibit favorable anti-tumor effects when treating colonic SCC. Additionally, the report recommends exploring the potential of ICB when used in conjunction with other therapies for treating patients with progressive colonic SCC. And the expression level of PD-L1 could be used as a biomarker for the application of ICB therapy in patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Affiliated Hospital of Hebei University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XW: Writing – original draft. SS: Writing – review & editing. YW: Writing – review & editing. DH: Writing – review & editing. ZW: Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Dyson T, Draganov PV. Squamous cell cancer of the rectum. *World J Gastroenterol* (2009) 15:4380–6. doi: 10.3748/wjg.15.4380

2. Kang H, O'Connell JB, Leonardi MJ, Maggard MA, McGory ML, Ko CY. Rare tumors of the colon and rectum: a national review. *Int J Colorectal Dis* (2007) 22:183–9. doi: 10.1007/s00384-006-0145-2

3. Schizas D, Katsaros I, Mastoraki A, Karela NR, Zampetaki D, Lazaridis II, et al. Primary squamous cell carcinoma of colon and rectum: A systematic review of the literature. *J Invest Surg* (2022) 35:151–6. doi: 10.1080/08941939.2020.1824044

4. Frizelle FA, Hobday KS, Batts KP, Nelson H. Adenosquamous and squamous carcinoma of the colon and upper rectum: a clinical and histopathologic study. *Dis Colon Rectum* (2001) 44:341–6. doi: 10.1007/BF02234730

5. Linardoutsos D, Frountzas M, Feakins RM, Patel NH, Simanskaite V, Patel H. Primary colonic squamous cell carcinoma: a case report and review of the literature. *Ann R Coll Surg Engl* (2020) 102:e1–7. doi: 10.1308/rcsann.2020.0149

6. Ozuner G, Aytac E, Gorgun E, Bennett A. Colorectal squamous cell carcinoma: a rare tumor with poor prognosis. *Int J Colorectal Dis* (2015) 30:127–30. doi: 10.1007/s00384-014-2058-9

7. Yang Y, Yu J, Hu J, Zhou C, Niu J, Ma H, et al. A systematic and comprehensive analysis of colorectal squamous cell carcinoma: Implication for diagnosis and treatment. *Cancer Med* (2022) 11:2492–502. doi: 10.1002/cam4.4616

8. Wang ML, Heriot A, Leong T, Ngan SY. Chemoradiotherapy in the management of primary squamous-cell carcinoma of the rectum. *Colorectal Dis* (2011) 13:296–301. doi: 10.1111/j.1463-1318.2009.02154

9. Clark J, Cleator S, Goldin R, Lowdell C, Darzi A, Ziprin P. Treatment of primary rectal squamous cell carcinoma by primary chemoradiotherapy: should surgery still be considered a standard of care? *Eur J Cancer* (2008) 44:2340–3. doi: 10.1016/j.ejca.2008.07.004

10. Nassar H, Ataya K, Hafez B, El Bsat A, Geagea L, Faraj W. Primary squamous cell carcinoma of the colon: A rare case report. *Int J Surg Case Rep* (2022) 9:107383. doi: 10.1016/j.ijscr.2022.107383

11. Abdelqader A, Jabaji R, Albugeaey M, Palese C. Squamous cell carcinoma of the ascending colon: two cases. *J Community Hosp Intern Med Perspect* (2017) 7:53–5. doi: 10.1080/20009666.2017.1309339

12. Mondal SK. Primary squamous cell carcinoma of the cecum. *J Cancer Res Ther* (2009) 5:328–30. doi: 10.4103/0973-1482.59900

13. Yokoyama Y, Wada R, Yamada T, Uchida E, Naito Z. A case of ulcerative colitis with squamous cell carcinomas and multiple foci of squamous dysplasia. *Pathol Int* (2017) 67:414–8. doi: 10.1111/pin.12539

14. Zhang L, Li W, Lyu X, Song Y, Mao Y, Wang S, et al. Adjuvant chemotherapy with paclitaxel and cisplatin in lymph node-positive thoracic esophageal squamous cell carcinoma. *Chin J Cancer Res* (2017) 29:149–55. doi: 10.21147/j.issn.1000-9604.2017.02.08

15. Rosenthal DI, Harris J, Forastiere AA, Weber RS, Ridge JA, Myers JN, et al. Early postoperative paclitaxel followed by concurrent paclitaxel and cisplatin with radiation therapy for patients with resected high-risk head and neck squamous cell carcinoma: report of the phase II trial RTOG 0024. *J Clin Oncol* (2009) 27:4727–32. doi: 10.1200/JCO.2008.21.4197

16. Qin T, Zhen J, Zhou M, Wu H, Ren R, Qu B, et al. Efficacy of neoadjuvant chemotherapy plus radical surgery in patients with bulky stage II cervical squamous cell carcinoma: A retrospective cohort study. *Int J Surg* (2016) 30:121–5. doi: 10.1016/j.ijsu.2016.04.038

17. André T, Vernerey D, Mineur L, Bennouna J, Desrame J, Faroux R, et al. Three versus 6 months of oxaliplatin-based adjuvant chemotherapy for patients with stage III colon cancer: disease-free survival results from a randomized, open-label, international duration evaluation of adjuvant (IDEA) France, phase III trial. *J Clin Oncol* (2018) 36(15):1469–77. doi: 10.1200/JCO.2017.76.0355

18. Cervantes A, Prager GW. FOLFOXIRI plus bevacizumab as standard of care for first-line treatment in patients with advanced colon cancer. *ESMO Open* (2023) 8(2):100883. doi: 10.1016/j.esmoop.2023.100883

19. Qin S, Li J, Wang L, Xu J, Cheng Y, Bai Y, et al. Efficacy and tolerability of first-line cetuximab plus leucovorin, fluorouracil, and oxaliplatin (FOLFOX-4) versus FOLFOX-4 in patients with RAS wild-type metastatic colorectal cancer: the open-label, randomized, phase III TAILOR trial. *J Clin Oncol* (2018) 36(30):3031–9. doi: 10.1200/JCO.2018.78.3183

20. Casak SJ, Marcus L, Fashoyin-Aje L, Mushti SL, Cheng J, Shen YL, et al. FDA approval summary: pembrolizumab for the first-line treatment of patients with MSI-H/dMMR advanced unresectable or metastatic colorectal carcinoma. *Clin Cancer Res* (2021) 27(17):4680–4. doi: 10.1158/1078-0432.CCR-21-0557

21. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* (2015) 372(26):2509–20. doi: 10.1056/NEJMoa1500596

22. Wang H, Zhou Y, Zhang Y, Fang S, Zhang M, Li H, et al. Subtyping of microsatellite stability colorectal cancer reveals guanylate binding protein 2 (GBP2) as a potential immunotherapeutic target. *J Immunother Cancer* (2022) 10:e004302. doi: 10.1136/jitc-2021-004302

23. Bourhis J, Stein A, Paul de Boer J, Van Den Eynde M, Gold KA, Stintzing S, et al. Avelumab and cetuximab as a therapeutic combination: An overview of scientific rationale and current clinical trials in cancer. *Cancer Treat Rev* (2021) 97:102172. doi: 10.1016/j.ctrv.2021.102172

24. Fountzilas C, Bajor DL, Mukherjee S, Saltzman J, Witkiewicz AK, Maguire O, et al. Phase ib/II study of cetuximab plus pembrolizumab in patients with advanced RAS wild-type colorectal cancer. *Clin Cancer Res* (2021) 27:6726–36. doi: 10.1158/1078-0432.CCR-21-1650

25. Martinelli E, Martini G, Famiglietti V, Troiani T, Napolitano S, Pietrantonio F, et al. Cetuximab rechallenge plus avelumab in pretreated patients with RAS wild-type metastatic colorectal cancer: the phase 2 single-arm clinical CAVE trial. *JAMA Oncol* (2021) 7:1529–35. doi: 10.1001/jamaoncol.2021.2915

26. Stein A, Simnica D, Schultheiß C, Scholz R, Tintelnot J, Gökkurt E, et al. PD-L1 targeting and subclonal immune escape mediated by PD-L1 mutations in metastatic colorectal cancer. *J Immunother Cancer* (2021) 9:e002844. doi: 10.1136/jitc-2021-002844

27. He Y, Wang L, Li X, Zhang T, Song T, Zhang J, et al. Rectosigmoid-junction squamous cell carcinoma with pMMR/MSS achieved a partial response following PD-1 blockade combined with chemotherapy: A case report. *Front Oncol* (2021) 11:596342. doi: 10.3389/fonc.2021.596342

28. Liu Y, Du J, Zhang P, Xiao H. Squamous cell carcinoma of ascending colon with pMMR/MSS showed a partial response to PD-1 blockade combined with chemotherapy: A case report. *Front Oncol* (2023) 13:1051786. doi: 10.3389/fonc.2023.1051786

29. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clin Cancer Res* (2014) 20:5064–74. doi: 10.1158/1078-0432.CCR-13-3271

30. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer* (2016) 16:275–87. doi: 10.1038/nrc.2016.36

31. Sun JM, Shen L, Shah MA, Enzinger P, Adenis A, Doi T, et al. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3 study. *Lancet.* (2021) 398:759–71. doi: 10.1016/S0140-6736(21)01234-4

32. Lu Z, Wang J, Shu Y, Liu L, Kong L, Yang L, et al. Sintilimab versus placebo in combination with chemotherapy as first line treatment for locally advanced or metastatic oesophageal squamous cell carcinoma (ORIENT-15): multicentre, randomised, double blind, phase 3 trial. *BMJ.* (2022) 377:e068714. doi: 10.1136/bmj-2021-068714

33. Ritprajak P, Azuma M. Intrinsic and extrinsic control of expression of the immunoregulatory molecule PD-L1 in epithelial cells and squamous cell carcinoma. *Oral Oncol* (2015) 51:221–8. doi: 10.1016/j.oraloncology.2014.11.014

34. Campbell JD, Yau C, Bowlby R, Liu Y, Brennan K, Fan H, et al. Genomic, pathway network, and immunologic features distinguishing squamous carcinomas. *Cell Rep* (2018) 23(1):194–212.e6. doi: 10.1016/j.celrep.2018.03.063

35. Li B, Cui Y, Nambiar DK, Sunwoo JB, Li R. The immune subtypes and landscape of squamous cell carcinoma. *Clin Cancer Res* (2019) 25(12):3528–37. doi: 10.1158/1078-0432.CCR-18-4085

36. Song Q, Yang Y, Jiang D, Qin Z, Xu C, Wang H, et al. Proteomic analysis reveals key differences between squamous cell carcinomas and adenocarcinomas across multiple tissues. *Nat Commun* (2022) 13(1):4167. doi: 10.1038/s41467-022-31719-0

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The effect of adjuvant chemotherapy on survival in node negative colorectal cancer with or without perineural invasion: a systematic review and meta-analysis

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Purpose: It was aimed at assessing the benefits of adjuvant chemotherapy (ACT) for patients with node-negative colorectal cancer (CRC) either with or without perineural invasion (PNI).

Methods: We systematically searched PubMed, Cochrane Library, Embase, and Web of Science from database inception through October 1, 2023. Survival outcomes were analyzed using hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). The methodological quality of included studies was assessed using the Newcastle-Ottawa Scale (NOS). Heterogeneity for the descriptive meta-analyses was quantified using the I^2 statistic.

Results: Ten studies included in this review. ACT improved overall survival (OS) (HR 0.52, 95% CI 0.40–0.69) and disease-free survival (DFS) (HR 0.53, 95% CI 0.35–0.82) in PNI + patients but did not affect DFS (HR 1.13, 95% CI 0.72–1.77) in PNI- patients. A disease-specific survival (DSS) benefit with chemotherapy was observed in PNI + (HR 0.76, 95% CI 0.58–0.99) and PNI- patients (HR 0.76, 95% CI 0.57–1.00). And PNI decreased DFS (HR 1.94, 95% CI 1.52–2.47) and OS (HR 1.75, 95% CI 0.96–3.17) in node-negative CRC.

Conclusions: In conclusion, chemotherapy appears most beneficial for survival outcomes in node-negative patients with PNI, but may also confer some advantage in those without PNI.

Systematic Review Registration: Identifier INPLASY2021120103.

KEYWORDS

perineural invasion, adjuvant chemotherapy, node negative, colorectal cancer, retrospective cohort

Introduction

Colorectal cancer is the third most common type of cancer in both men and women. Globally, almost 1.5 million new cases of CRC are diagnosed every year, of which more than a third are fatal (1). The most common cause of death is complications arising from metastasis (2). The primary treatment for stage I–II CRC is radical surgery (3). However, undetected micrometastases that persist after curative surgery may cause cancer recurrence (4). This micrometastasis is eradicated with ACT to enhance cure rates (5).

It is unfortunate that few reliable prognostic and predictive markers exist to identify patients at a high risk for disease progression during the early stages of CRC (6). Stage II CRC recurrence rates range from 7.9%–22%, whereas only 2%–5% of patients benefit from ACT (7–10). Due to these reasons, the National Comprehensive Cancer Network (NCCN) does not recommend conventional ACT for stage II CRC unless certain risk factors exist. There were pT4 lesions, intestinal perforation, obstruction, 12-sample lymph nodes (LNs), lymph vascular invasion, PNI, poorly differentiated histology and margins that are positive, indeterminate, or close (11). Patients with these risk factors have a relatively poor prognosis (12). According to Lin et al., ACT was beneficial to patients with CRC and certain risk factors (13). In contrast, O'Connor et al. reported that ACT had no effect on any of these risk factors (14). Kumar et al. found that ACT was most effective for patients with pT4 in high-risk patients (15). Recent studies suggest, however, that ACT can benefit patients with PNI (16–18). PNI refers to tumor cells spreading through nerves. It was Bataskis who first described the prognostic value of PNI, which he defined as "tumor invasion around and through nerves (19)." PNI has been recognized as an unfavorable prognostic factor in CRC since it is associated with poor survival rates (20).

ACT, however, remains controversial because it is unclear whether these patients will benefit patients with PNI (21). This study was conducted to determine whether node-negative CRC patients with and without PNI receive different benefits from ACT.

Materials and methods

Search strategy

Our search focused on academic papers published in English between inception through October 1, 2023 in PubMed, Cochrane Library, Web of Science, and Embase databases. The following keywords were used: "perineural invasion", "PNI", "colon cancer", "rectal neoplasms", "Corectal cancer", "colorectal neoplasms", "adjuvant chemotherapy", "cohort", "randomized controlled trial", and "randomized trial". Additionally, we searched the references of relevant articles.

Selection criteria and exclusion criteria

Studies were included if they met the following criteria: (1) Enrolled patients with stage II CRC who underwent radical resection, confirmed by postoperative histopathology. (2) Assessed the association between PNI and survival among patients receiving ACT. (3) Published in English. (4) Reported sufficient data to calculate HRs and 95% CIs. Studies were excluded if they: (1) Were not published in English. (2) Included node-positive or mixed stage CRC patients. (3) Were case reports or case series with <50 patients. (4) Did not report outcomes of interest including OS, DFS.

Data extraction and quality assessment

The researchers (W. Yu and H. Ying) independently assessed the eligibility of all the studies and extracted the following information: The first author's name, the country in which the study was conducted, the sample size, the year of the study, the ages of the participants, the stage of their cancer, the chemotherapy regimen, and the period of follow-up. As well as OS, DFS, DSS, recurrence-free survival (RFS), and NOS. We consulted with a third reviewer (W. Hong) to resolve any discrepancies between the reviewers. In order to rate the quality of the articles, we used the NOS score. Articles that have an NOS score >6 (on a scale of 0–9) were considered to be of high quality (22).

Risk of bias analysis

Using non-parametric correlation tests, we examined the association between quality of reporting and HR. Begg and Egger tests were also conducted to determine whether publication bias was present (23).

Statistical analysis

Our analysis used HRs and 95% CIs to compare PNI with survival. When HRs and 95% CIs were not included, data were derived from survival curves according to Parmar et al. and Tierney et al. (24, 25). Study heterogeneity was examined using I^2 statistics. Whenever there was obvious heterogeneity, as indicated by a p-value < 0.10 or I^2 exceeding 50%, a random effect model was used. In other cases, a fixed effect model was used. Our findings were further enhanced by performing meta-regressions and subgroup analyses in order to identify the sources of heterogeneity. We conducted sensitivity analysis to determine the stability of our combined results, and we assessed publication bias using the Begg and Egger test (26, 27). Statistical significance was set at $p < 0.05$ using STATA 16.0 (Stata Corporation, College Station, TX, USA).

Results

Search results and quality assessment

We conducted electronic searches of MEDLINE, Embase, Cochrane Library, and Web of Science, which yielded 743 studies. An additional 21 studies were identified from reference lists. After removing 442 duplicate records, 322 studies underwent title and abstract screening, of which 199 were excluded as Records excluded. The full texts of the remaining 123 studies were assessed; 17 studies could not be retrieved and 52 further studies were excluded based on predefined criteria. Ultimately, 10 studies met the inclusion criteria and

were included in the systematic review and meta-analysis, comprising data on 118,529 patients in total. The study selection process is outlined in the PRISMA flow diagram (Figure 1). All patients underwent curative-intent resection of their CRC prior to ACT. Some studies also analyzed the high-risk factors after colon cancer surgery. Table 1 summarizes the 10 retrospective cohort studies included in the systematic review. These studies involved 118,529 patients with stage II CRC who underwent surgery. The studies compared ACT vs. no chemotherapy and reported on outcomes including OS, DFS, and recurrence. Follow-up times ranged from 5 to 10 years. We assessed the quality of ten articles by using the NOS score since they were retrospective cohort studies. A total of seven articles scored 7 points and six articles scored 8 points, with the main loss being the study controls for confounding factors (Table 2).

Effect of adjuvant chemotherapy on perineural invasion

We evaluated the survival of node-negative CRC patients who received ACT compared to no chemotherapy. Across the 10 included studies, 6,196 patients had PNI, with 1,467 receiving ACT. The prevalence of PNI ranged from 5.2% to 11.3% based on tumor location. OS was analyzed in 6 studies comprising 3,794 PNI + patients, of whom 786 underwent ACT, as well as 54,177 PNI- patients, including 6,535 who received ACT (12, 15, 28–31). DFS was examined in 4 studies including 344 PNI + patients (with 62 receiving ACT) and 3,285 PNI- patients (with 1,191 receiving ACT) (29, 32–34). Two studies with 2,461 PNI + (262 ACT) and 55,257 PNI- (4,675 ACT) patients analyzed DSS (15, 35). Recurrence-free survival (RFS) was assessed in 2 studies: one with 108 PNI + patients (43 ACT) and another with 2,498

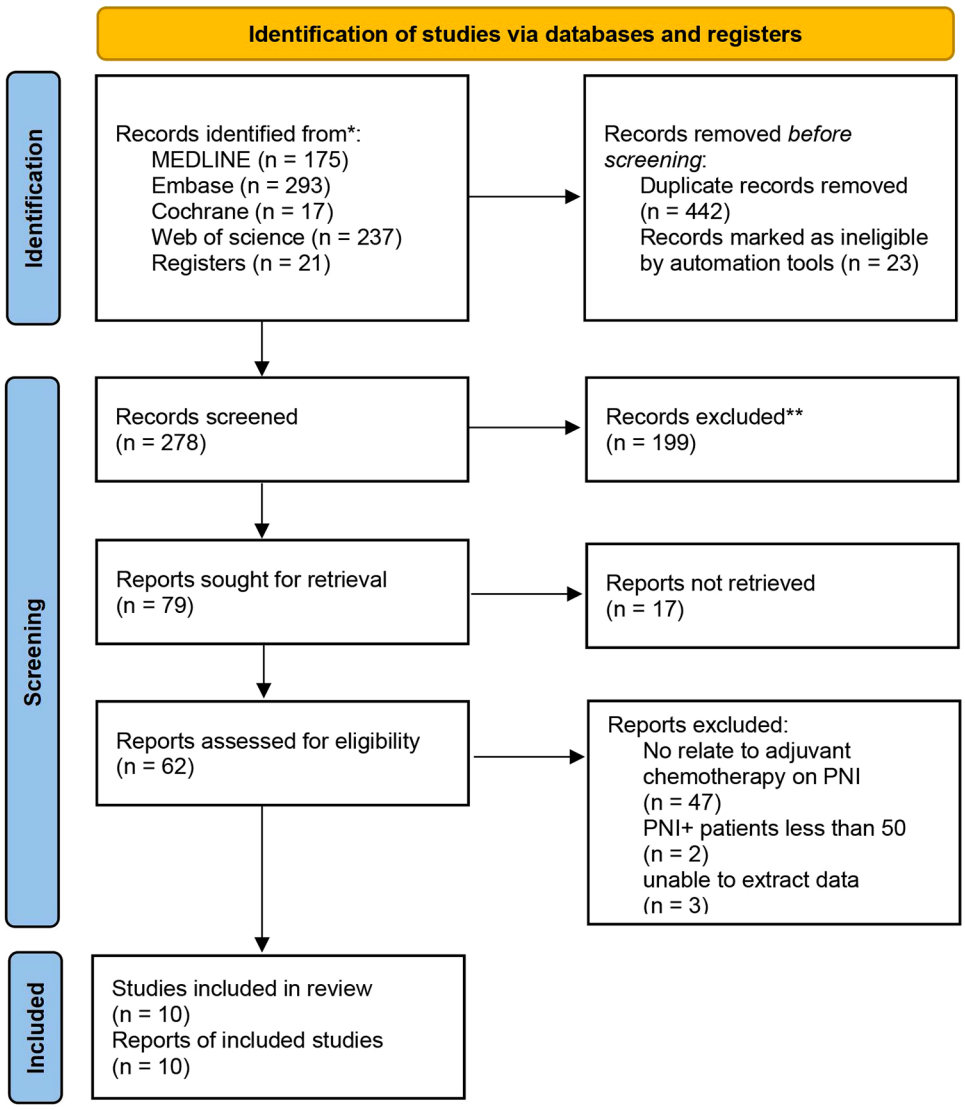

FIGURE 1
Flow chart of patient search strategy.

TABLE 1 Characteristics of included studies.

References	Country	Age (mean)	Timeframe	Stage	Tumor location	Patients	PNI + patients (ACT)	Study design	Follow-up	Outcome	NOS score
Skancke	USA	70	2010–2014	II	Colon cancer	32,493	2,404 (480)	RSC	5.0	OS	8
Mirkin	USA	72	2004–2012	I, II	Colon cancer	21,488	987 (202)	RSC	5.0	OS	8
Huh	Korea	63	2001–2006	II	Colorectal	341	57 (46)	RSC	5.0	DFS	7
Cienfuegos	Spain	64	2000–2012	I, II	Colon cancer	507	57 (36)	RSC	10	DFS	7
Tu	China	65	2010–2015	II	Colon cancer	57,255	2,372 (486)	RSC	7.0	DSS	8
Kumar	Canada	67	1999–2008	II	Colon cancer	1,697	89 (37)	RSC	5.3	OS,DSS,RFS	8
Loree	British	69	1999–2009	II	Rectal cancer	851	51 (18)	RSC	8.5	DFS, RFS	7
Song	Korea	62	2005–2014	II	Rectal cancer	1,232	185 (150)	RSC	5.0	RFS, DFS	8
Babcock	USA	NR	2010–2013	II	Colon cancer	2,374	248 (52)	RSC	5.0	OS	8
Morris	Germany	65	1993–2003	II	Colon cancer	812	47 (7)	RSC	5.0	OS	7

ACT, adjuvant chemotherapy; PNI+, perineural invasion positive; RSC, retrospective cohort; OS, overall survival; DFS, disease-free survival; NOS, Newcastle-Ottawa scale; NR, not reported.

TABLE 2 Results of quality assessment using the Newcastle-Ottawa scale.

				Selection		Comparability		Outcome			
References	Years	REC	SNEC	AE	DO	SC	AF	AO	FU	AFU	Total score
Skancke	2019	1	1	1	1	1	0	1	1	1	8
Mirkin	2017	1	1	1	1	1	0	1	1	1	8
Huh	2010	1	1	1	0	1	0	1	1	1	7
Cienfuegos	2017	1	1	1	1	1	0	1	1	1	7
Tu	2021	1	1	1	1	1	0	1	1	1	8
Kumar	2015	1	1	1	1	1	0	1	1	1	8
Jonathan	2016	1	1	1	0	1	0	1	1	1	7
Song	2019	1	1	1	1	1	0	1	1	1	8
Babcock	2018	1	1	1	1	1	0	1	1	1	8
Morris	2007	1	1	1	0	1	0	1	1	1	7

AE, indicates ascertainment of exposure; AF, study controls for any additional factors; AFU, adequacy of follow-up of cohorts; "1" means that the study is satisfied the item and "0" means the opposite situation. AO, assessment of outcome; DO, demonstration that outcome of interest was not present at start of study; FU, follow-up long enough for outcomes to occur; REC, representativeness of the exposed cohort; SC, study controls for age; SNEC, selection of the nonexposed cohort.

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PNI- patients (471 ACT) (15, 29). RFS was improved with ACT in PNI + patients (HR 0.79, 95% CI 0.42–1.46, $I^2 = 0\%$), but RFS data were unavailable for PNI- patients (Table 1).

We compared OS, DFS and DSS between patients who received ACT and those who observation only, stratified by PNI status. For patients with node-negative CRC and PNI+, ACT was associated with significantly improved OS compared to observation (HR 0.52, 95% CI 0.40–0.69). There was moderate heterogeneity between the 3 included studies ($I^2 = 41.3\%$, $p = 0.130$). In the PNI- subgroup, ACT also conferred an OS benefit over observation (HR 0.52, 95% CI 0.27–0.78). However, there was substantial heterogeneity between the 2 studies in this analysis ($I^2 = 77.1\%$, $p = 0.013$). ACT appeared to improve OS regardless of PNI status. The OS benefit with ACT was similar between PNI + and PNI- patients (Figure 2).

Among PNI + patients, ACT significantly improved DFS compared to observation alone (HR 0.53, 95% CI 0.35–0.82). There was no heterogeneity between the 4 studies in this subgroup ($I^2 = 0\%$, $p = 0.797$). In the PNI- subgroup, ACT did not provide a DFS benefit over observation (HR 1.13, 95% CI 0.72–1.77). No significant heterogeneity was found between the 2 PNI- studies ($I^2 = 0\%$, $p = 0.328$). ACT appeared to improve DFS in node-negative CRC patients with PNI, but not in those without PNI (Figure 3).

In the PNI + subgroup, ACT was associated with improved DSS compared to observation (HR 0.76, 95% CI 0.58–0.99). There was no heterogeneity between the 2 studies ($I^2 = 0\%$, $p = 0.980$). For PNI- patients, ACT also showed a trend towards improved DSS over observation that did not reach statistical significance (HR 0.76, 95% CI 0.57–1.00). Only 1 study was available for this subgroup analysis (Figure 4).

Effect of perineural invasion on survival

Five studies involving 91,828 patients provided data on the impact of PNI on survival (28, 32–35). In three studies, PNI was found to decrease DFS (HR = 1.94, 95% CI = 1.52–2.47,

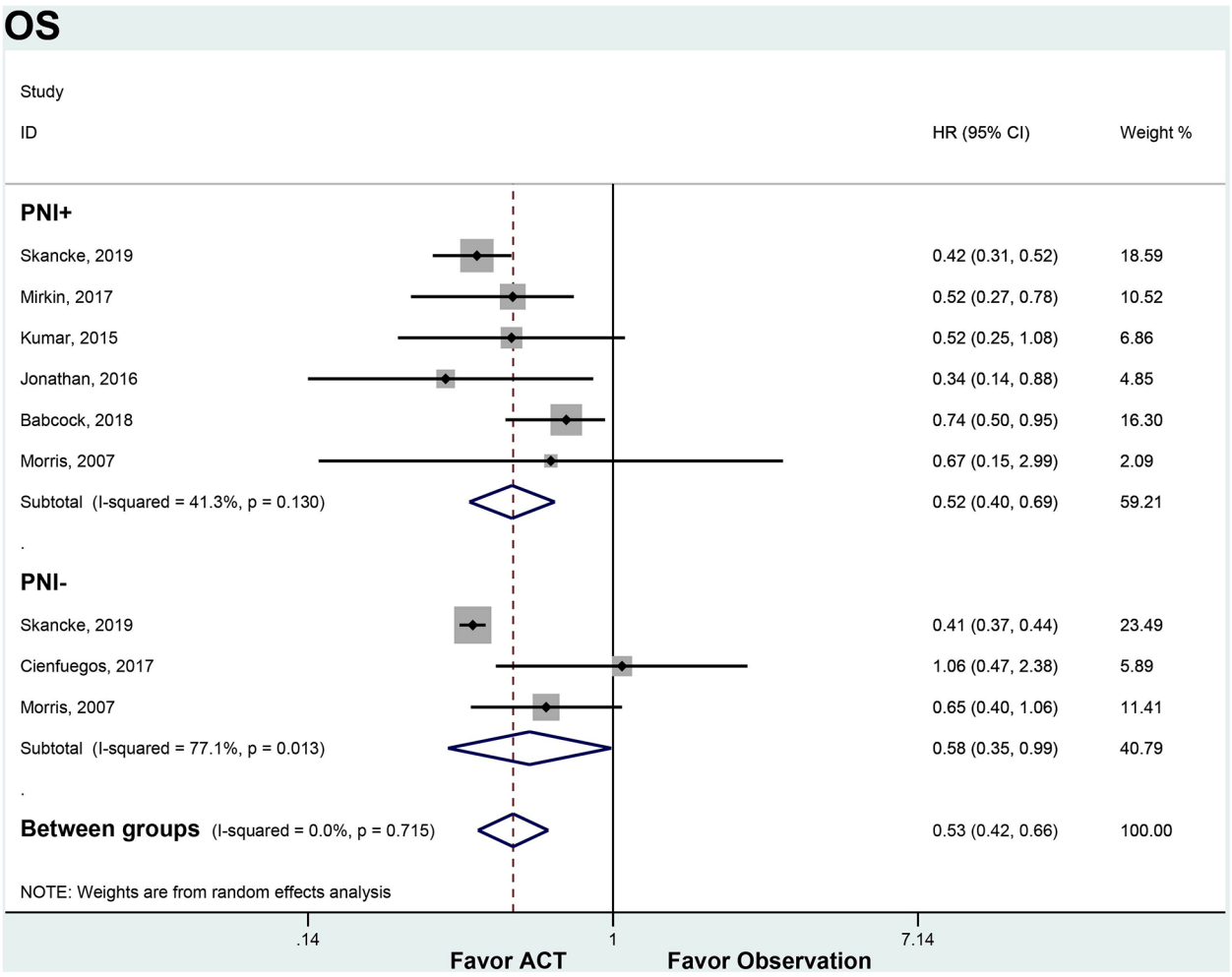

FIGURE 2
ACT versus observation-only patients stratified by PNI, OS. Diamond represents the pooled effect estimate of the overall analysis. Data are represented as HRs with 95% CIs. Inter-study heterogeneity quantified by I^2 with significance $p < 0.10$. HR, hazard ratio; OS, overall survival; ACT, adjuvant chemotherapy; PNI, perineural invasion.

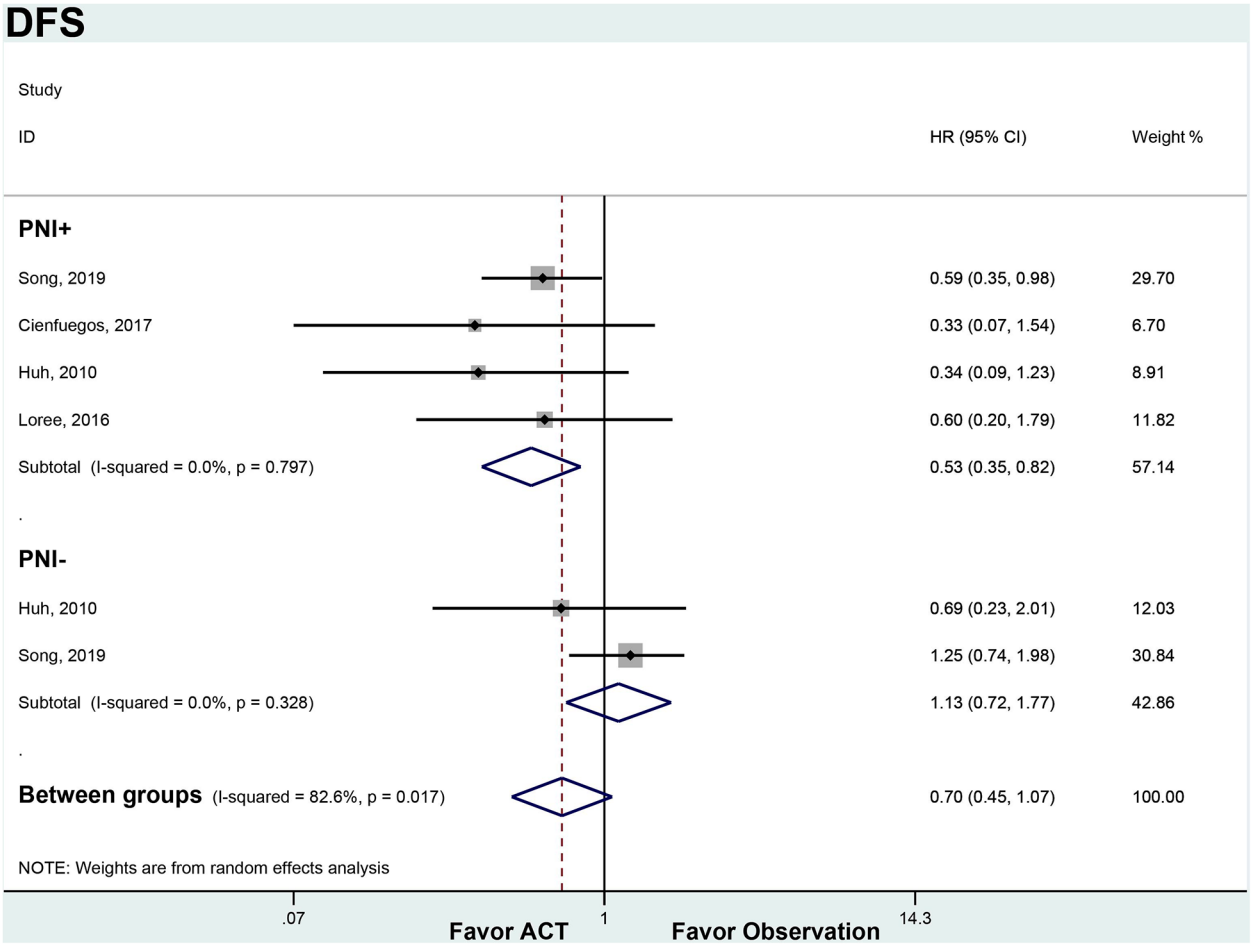

FIGURE 3
ACT versus observation-only patients stratified by PNI, DFS. Diamond represents the pooled effect estimate of the overall analysis. Data are represented as HRs with 95% CIs. Inter-study heterogeneity quantified by I^2 with significance $p < 0.10$. HR, hazard ratio; DFS, disease-free survival; ACT, adjuvant chemotherapy; PNI, perineural invasion.

$p < 0.001$). There was no significant heterogeneity between studies ($I^2 = 0.00\%$, $p < 0.001$). There were two studies analyzing the OS (28, 34). The OS decreased in the presence of PNI (HR = 1.75, 95% CI = 0.96–3.17). Significant heterogeneity was observed between studies ($I^2 = 89.8\%$, $p = 0.002$) (Figure 5).

Sensitivity analysis

Fixed effects and random effects models were compared to analyze prognosis (OS) in patients with PNI who were treated with ACT.

We analyzed the prognosis (OS) of patients with PNI who received ACT by comparing fixed effect and random effect models. OS did not differ significantly between the two models (fixed effect model: HR = 0.51, 95% CI = 0.43–0.61, random effect model: HR = 0.52, 95% CI = 0.40–0.69). In the sensitivity analysis, we arbitrarily deleted the OS and DFS literature, which did not affect the results of this study (Figure 6).

Publication bias

Our analysis included ten studies, but the subgroup studies were relatively few because they assessed different outcomes. There is an inherent risk of public bias in all reviews. According to Egger and Begg tests (Egger test: $p = 0.189$; Begg test: $p = 0.308$), DFS analysis did not detect a significant publication bias. In addition, the DFS analysis found no evidence of publication bias (Egger test: $p = 0.925$; Begg's test: $p = 1.00$).

Discussion

This systematic review and meta-analysis examined the efficacy of ACT for node-negative CRC stratified by PNI status. Our results suggest that chemotherapy improves overall and DFS in patients with PNI, but may not affect DFS in those without PNI.

OS was significantly improved with ACT vs. observation in the PNI + subgroup (HR 0.52, 95% CI 0.40–0.69), consistent with prior

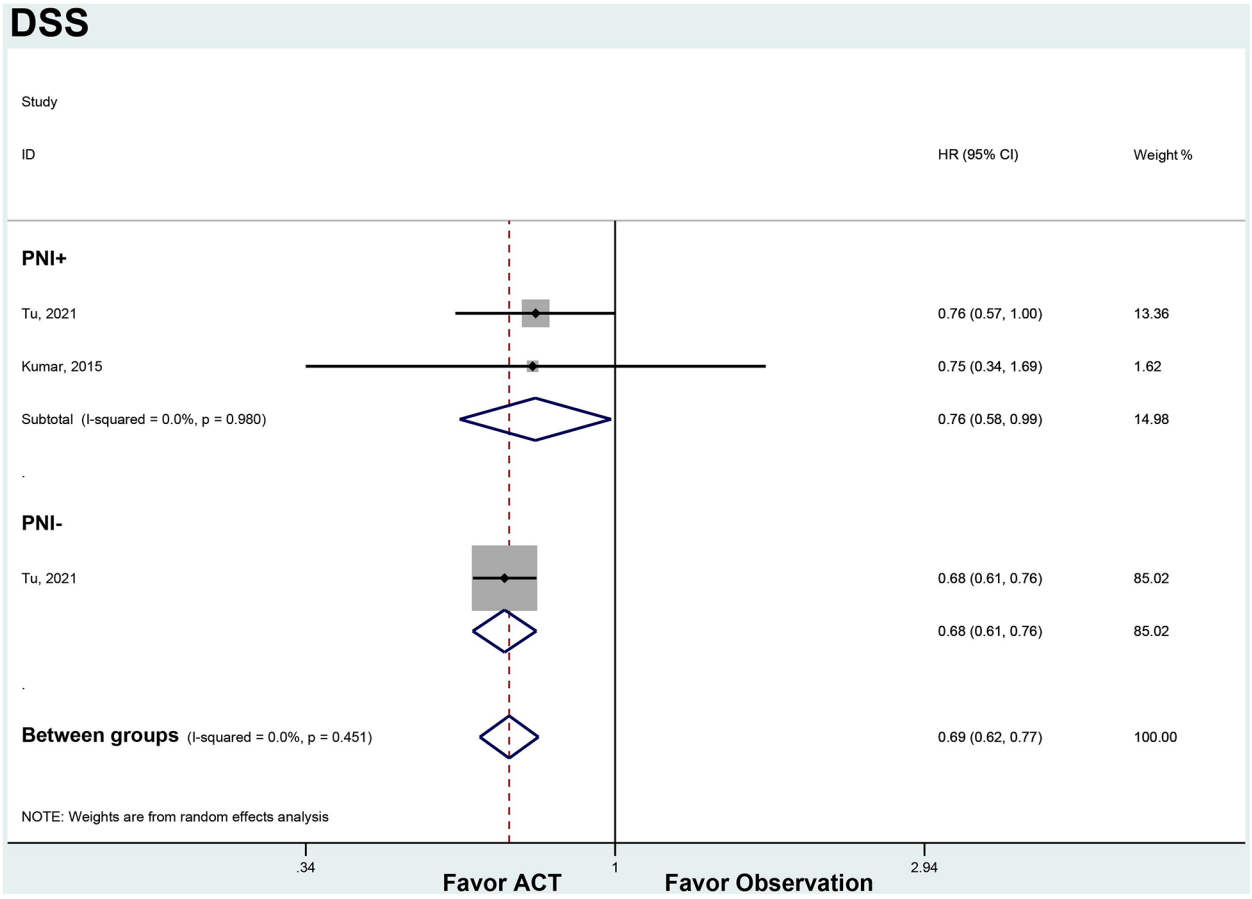

FIGURE 4
ACT versus observation-only patients stratified by PNI, DFS. Diamond represents the pooled effect estimate of the overall analysis. Data are represented as HRs with 95% CIs. Inter-study heterogeneity quantified by I^2 with significance $p < 0.10$. HR, hazard ratio; DSS, disease-specific survival; ACT, adjuvant chemotherapy; PNI, perineural invasion.

studies showing a survival benefit for high-risk stage II patients receiving chemotherapy (36, 37). A recent cohort study of 500 colon cancer patients also found the addition of oxaliplatin to standard 5-FU chemotherapy prolonged OS and DFS selectively in the subgroup with PNI (33). The survival gain seen with chemotherapy in PNI + patients may be due to eradication of occult micrometastases not detectable on standard pathology (38). Interestingly, we also observed an OS benefit with chemotherapy in the PNI- subgroup (HR 0.52, 95% CI 0.27–0.78), although prior analyses have been conflicting (10, 39). The reason for improved OS with chemotherapy even for lower risk PNI- patients is unclear and warrants investigation.

DFS was significantly improved by chemotherapy in the PNI + subgroup (HR 0.53, 95% CI 0.35–0.82) but not in the PNI- subgroup (HR 1.13, 95% CI 0.72–1.77). These findings align with other studies demonstrating PNI is an independent prognostic factor for DFS (40). A potential explanation is that PNI + tumors are more aggressive and prone to early micrometastases or local recurrence after surgery that is eradicated by chemotherapy (41). The lack of DFS benefit with chemotherapy in PNI- patients highlights the need for risk-stratified treatment approaches to avoid over-treatment (42). Recent data suggest molecular profiling may help further stratify risk in node negative CRC (43).

This study has several limitations. The pooled sample size was relatively small for PNI subgroup analyses, particularly for secondary outcomes like DFS and DSS, warranting cautious interpretation. Publication bias remains a concern given the limited number of studies. There was heterogeneity between studies that may relate to differences in chemotherapy regimens, follow-up times, and underlying study populations. The retrospective observational nature of the included studies also has inherent biases compared to prospective trials. And this systematic review included studies published over a long timespan, ranging from 1993 to 2015. The inclusion of literature covering many decades could introduce bias, as changes in cancer treatments, staging modalities, and other factors over time may impact outcomes. Despite these limitations, this systematic review provides a comprehensive synthesis of current evidence regarding efficacy of ACT in early stage CRC with vs. without PNI.

ACT appears to improve survival outcomes primarily in node-negative CRC patients with PNI. PNI may be an important factor to guide chemotherapy decisions in this population. Additional

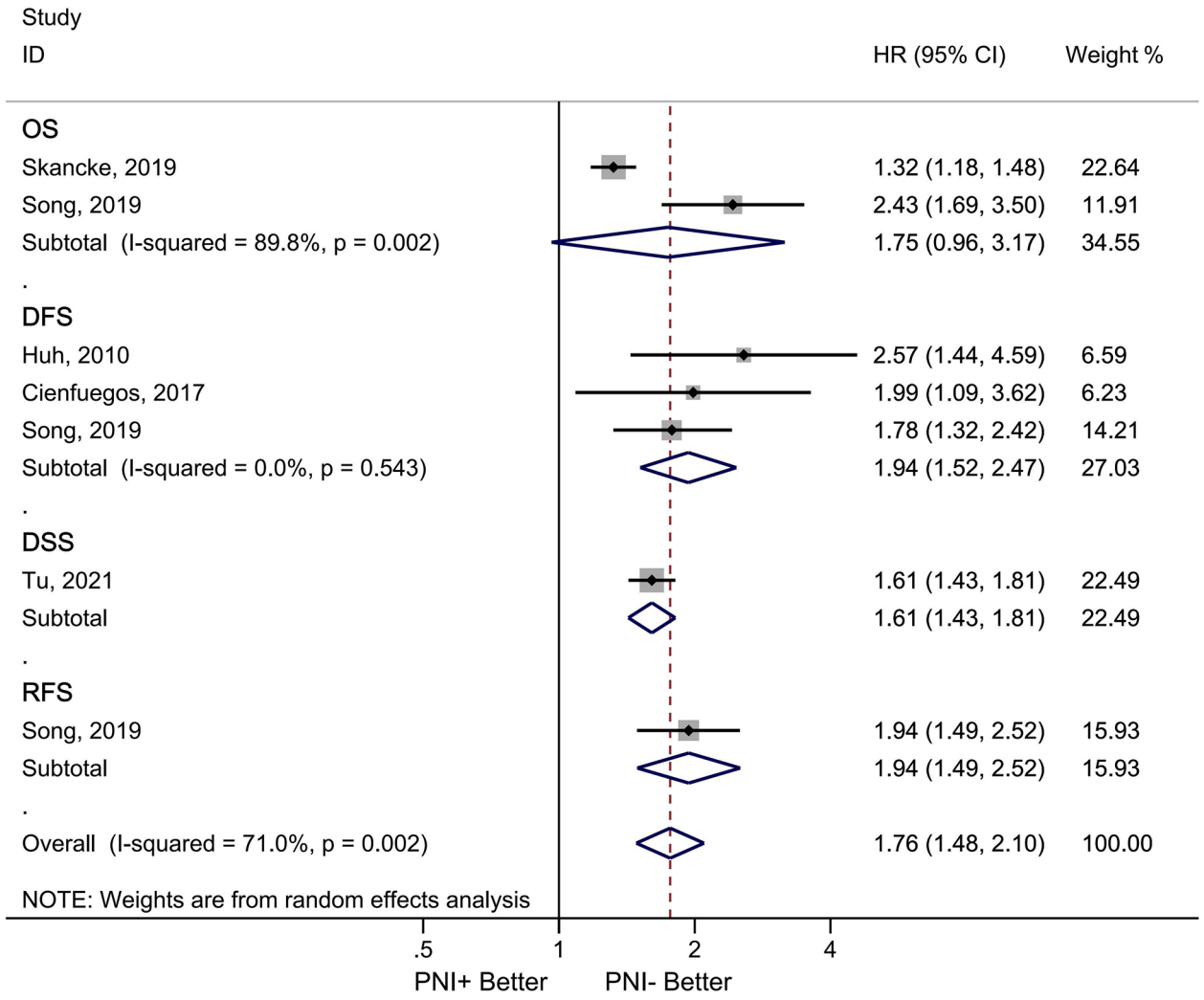

FIGURE 5
Association between PNI and survival in node negative colorectal cancer patients. Diamond represents the pooled effect estimate of the overall analysis. Data are represented as HRs with 95% CIs. Inter-study heterogeneity quantified by I^2 with significance $p < 0.10$, HR, hazard ratio; ACT, adjuvant chemotherapy; PNI, perineural invasion; OS, overall survival; DFS, disease free survival overal; DSS, disease-specific survival; RFS, recurrence-free survival; PNI, perineural invasion.

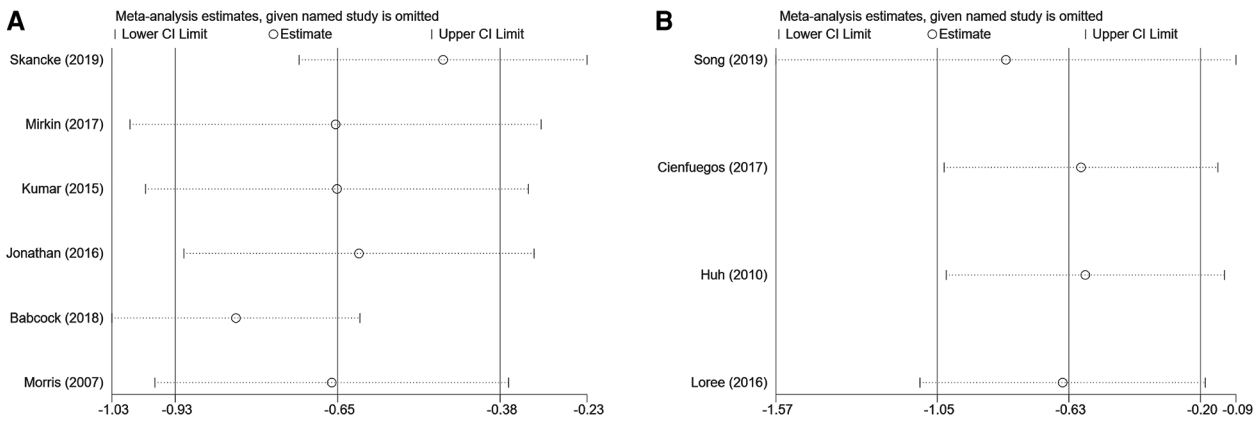

FIGURE 6
Sensitivity analysis of overall high-risk factors receiving adjuvant hemotherapy on OS (A) and disease-free survival (B).

well-designed prospective studies are needed to clarify the risk-benefit ratio of adjuvant treatment based on PNI status. Future research should also examine how emerging prognostic factors and individualized risk prediction models can optimize personalized adjuvant therapy for early stage CRC.

Conclusion

ACT improved OS and DSS in node-negative CRC patients regardless of PNI status. But DFS benefit with chemotherapy was observed only in patients with PNI. Overall, chemotherapy appears most beneficial for survival outcomes in node-negative patients with PNI, but may also confer some advantage in those without invasion.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

Author contributions

HY: Conceptualization, Investigation, Writing – original draft. JS: Conceptualization, Data curation, Writing – original draft. NL: Formal Analysis, Validation, Writing – original draft. XX: Software, Visualization, Writing – review & editing. WY: Conceptualization, Data curation, Writing – original draft. WH: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J Clin*. (2023) 73(3):233–54. doi: 10.3322/caac.21772

2. Lebeck Lee CM, Ziogas IA, Agarwal R, Alexopoulos SP, Ciombor KK, Matsuoka LK, et al. A contemporary systematic review on liver transplantation for unresectable liver metastases of colorectal cancer. *Cancer*. (2022) 128(12):2243–57. doi: 10.1002/cncr.34170

3. Mothes H, Bauschke A, Schuele S, Eigendorff E, Altendorf-Hofmann A, Settmacher U. Surgery for colorectal cancer in elderly patients: how can we improve outcome. *J Cancer Res Clin Oncol*. (2017) 143(9):1879–89. doi: 10.1007/s00432-017-2438-y

4. Miyamoto Y, Hiyoshi Y, Sawayama H, Tokunaga R, Baba H. Precision medicine for adjuvant chemotherapy of resected colorectal cancer. *Ann Gastroenterol Surg*. (2020) 4(6):635–45. doi: 10.1002/ags3.12397

5. Shibutani M, Nagahara H, Fukuoka T, Iseki Y, Hirakawa K, Ohira M. Efficacy of adjuvant chemotherapy according to the classification of recurrence risk based on systemic inflammatory markers in patients with liver metastases of colorectal cancer. *Anticancer Res*. (2019) 39(9):5039–45. doi: 10.21873/anticanres.13695

6. Chung SS, Ali SI, Cash BD. The present and future of colorectal cancer screening. *Gastroenterol Hepatol (N Y)*. (2022) 18(11):646–53.

7. Schrag D, Rifas-Shiman S, Saltz L, Bach PB, Begg CB. Adjuvant chemotherapy use for medicare beneficiaries with stage II colon cancer. *J Clin Oncol*. (2002) 20 (19):3999–4005. doi: 10.1200/JCO.2002.11.084

8. Efficacy of adjuvant fluorouracil and folinic acid in B2 colon cancer. International multicentre pooled analysis of B2 colon cancer trials (IMPACT B2) investigators. *J Clin Oncol*. 1999 17(5): 1356–63. doi: 10.1200/JCO.1999.17.5.1356

9. Gertler R, Rosenberg R, Schuster T, Friess H. Defining a high-risk subgroup with colon cancer stages I and II for possible adjuvant therapy. *Eur J Cancer*. (2009) 45 (17):2992–9. doi: 10.1016/j.ejca.2009.07.008

10. Gray R, Barnwell J, McConkey C, Hills RK, Williams NS, Kerr DJ. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. *Lancet*. (2007) 370(9604):2020–9. doi: 10.1016/S0140-6736(07)61866-2

11. Weiss JM, Gupta S, Burke CA, Axell L, Chen LM, Chung DC, et al. NCCN guidelines® insights: genetic/familial high-risk assessment: colorectal, version 1.2021. *J Natl Compr Canc Netw*. (2021) 19(10):1122–32.

12. Mirkin KA, Hollenbeak CS, Mohamed A, Jia Y, El-Deiry WS, Messaris E. Impact of perineural invasion on survival in node negative colon cancer. *Cancer Biol Ther*. (2017) 18(9):740–5. doi: 10.1080/15384047.2017.1323602

13. Liu LL, Xiang ZL. Adjuvant chemotherapy improves survival in high-risk stage II colon cancer: a retrospective cohort study. *Therap Adv Gastroenterol*. (2022) 15:17562848221137758. doi: 10.1177/17562848221137758

14. O'Connor ES, Greenblatt DY, LoConte NK, Gangnon RE, Liou JI, Heise CP, et al. Adjuvant chemotherapy for stage II colon cancer with poor prognostic features. *J Clin Oncol*. (2011) 29(25):3381–8. doi: 10.1200/JCO.2010.34.3426

15. Kumar A, Kennecke HF, Renouf DJ, Lim HJ, Gill S, Woods R, et al. Adjuvant chemotherapy use and outcomes of patients with high-risk versus low-risk stage II colon cancer. *Cancer*. (2015) 121(4):527–34. doi: 10.1002/cncr.29072

16. Cho JR, Lee KW, Oh HK, Kim JW, Kim JW, Kim DW, et al. Effectiveness of oral fluoropyrimidine monotherapy as adjuvant chemotherapy for high-risk stage II colon cancer. *Ann Surg Treat Res*. (2022) 102(5):271–80. doi: 10.4174/astr.2022.102.5.271

17. Paolo M, Cruz J, George C, Pales C, Min Kim K, Wan Kim Y. Adjuvant chemotherapy for high-risk stage II and stage III colon cancer: timing of initiation and optimal duration. *J BUON*. (2018) 23(3):568–73.

18. Sinicrope FA, Chakrabarti S, Laurent-Puig P, Huebner L, Smyrk TC, Tabernero J, et al. Prognostic variables in low and high risk stage III colon cancers treated in two adjuvant chemotherapy trials. *Eur J Cancer*. (2021) 144:101–12. doi: 10.1016/j.ejca.2020.11.016

19. Batsakis JG. Nerves and neurotropic carcinomas. *Ann Otol Rhinol Laryngol*. (1985) 94(4 Pt 1):426–7. doi: 10.1177/000348948509400420

20. Knijn N, Mogk SC, Teerenstra S, Simmer F, Nagtegaal ID. Perineural invasion is a strong prognostic factor in colorectal cancer: a systematic review. *Am J Surg Pathol*. (2016) 40(1):103–12. doi: 10.1097/PAS.0000000000000518

21. Rebuzzi SE, Pesola G, Martelli V, Sobrero AF. Adjuvant chemotherapy for stage II colon cancer. *Cancers (Basel)*. (2020) 12(9):2584. doi: 10.3390/cancers12092584

22. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol*. (2010) 25 (9):603–5. doi: 10.1007/s10654-010-9491-z

23. Lin L, Chu H, Murad MH, Hong C, Qu Z, Cole SR, et al. Empirical comparison of publication bias tests in meta-analysis. *J Gen Intern Med*. (2018) 33(8):1260–7. doi: 10.1007/s11606-018-4425-7

24. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med*. (1998) 17 (24):2815–34. doi: 10.1002/(SICI)1097-0258(19981230)17:24<2815::AID-SIM110>3.0.CO;2-8

25. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials*. (2007) 8:16. doi: 10.1186/1745-6215-8-16

26. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics*. (1994) 50(4):1088–101. doi: 10.2307/2533446

27. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. (1997) 315(7109):629–34. doi: 10.1136/bmj.315.7109.629

28. Skancke M, Arnott SM, Amdur RL, Siegel RS, Obias VJ, Umapathi BA. Lymphovascular invasion and perineural invasion negatively impact overall survival for stage II adenocarcinoma of the colon. *Dis Colon Rectum*. (2019) 62(2):181–8. doi: 10.1097/DCR.0000000000001258

29. Loree JM, Kennecke HF, Renouf DJ, Lim HJ, Vickers MM, Speers CH, et al. Effect of adjuvant chemotherapy on stage II rectal cancer outcomes after preoperative short-course radiotherapy. *Clin Colorectal Cancer*. (2016) 15 (4):352–9.e1. doi: 10.1016/j.clcc.2016.04.003

30. Babcock BD, Aljehani MA, Jabo B, Choi AH, Morgan JW, Selleck MJ, et al. High-risk stage II colon cancer: not all risks are created equal. *Ann Surg Oncol*. (2018) 25(7):1980–5. doi: 10.1245/s10434-018-6484-8

31. Morris M, Platell C, McCaul K, Millward M, van Hazel G, Bayliss E, et al. Survival rates for stage II colon cancer patients treated with or without chemotherapy in a population-based setting. *Int J Colorectal Dis*. (2007) 22 (8):887–95. doi: 10.1007/s00384-006-0262-y

32. Cienfuegos JA, Martínez P, Baixauli J, Beorlegui C, Rosenstone S, Sola JJ, et al. Perineural invasion is a major prognostic and predictive factor of response to adjuvant chemotherapy in stage I–II colon cancer. *Ann Surg Oncol*. (2017) 24(4):1077–84. doi: 10.1245/s10434-016-5561-0

33. Huh JW, Kim HR, Kim YJ. Prognostic value of perineural invasion in patients with stage II colorectal cancer. *Ann Surg Oncol*. (2010) 17(8):2066–72. doi: 10.1245/s10434-010-0982-7

34. Song JH, Yu M, Kang KM, Lee JH, Kim SH, Nam TK, et al. Significance of perineural and lymphovascular invasion in locally advanced rectal cancer treated by preoperative chemoradiotherapy and radical surgery: can perineural invasion be an indication of adjuvant chemotherapy. *Radiother Oncol*. (2019) 133:125–31. doi: 10.1016/j.radonc.2019.01.002

35. Tu J, Yao Z, Wu W, Ju J, Xu Y, Liu Y. Perineural invasion is a strong prognostic factor but not a predictive factor of response to adjuvant chemotherapy in node-negative colon cancer. *Front Oncol*. (2021) 11:663154. doi: 10.3389/fonc.2021.663154

36. Yang Y, Lu Y, Tan H, Bai M, Wang X, Ge S, et al. The optimal time of starting adjuvant chemotherapy after curative surgery in patients with colorectal cancer. *BMC Cancer*. (2023) 23(1):422. doi: 10.1186/s12885-023-10863-w

37. Sargent D, Sobrero A, Grothey A, O'Connell MJ, Buyse M, Andre T, et al. Evidence for cure by adjuvant therapy in colon cancer: observations based on individual patient data from 20,898 patients on 18 randomized trials. *J Clin Oncol*. (2009) 27(6):872–7. doi: 10.1200/JCO.2008.19.5362

38. Delattre JF, Selcen Oguz Erdogan A, Cohen R, Shi Q, Emile JF, Taieb J, et al. A comprehensive overview of tumour deposits in colorectal cancer: towards a next TNM classification. *Cancer Treat Rev*. (2022) 103:102325. doi: 10.1016/j.ctrv.2021.102325

39. Morris EJ, Maughan NJ, Forman D, Quirke P. Who to treat with adjuvant therapy in dukes B/stage II colorectal cancer? The need for high quality pathology. *Gut*. (2007) 56(10):1419–25. doi: 10.1136/gut.2006.116830

40. Kim YI, Kim CW, Kim JH, Kim J, Ro JS, Lee JL, et al. Clinical implication of perineural and lymphovascular invasion in rectal cancer patients who underwent surgery after preoperative chemoradiotherapy. *Dis Colon Rectum*. (2022) 65 (11):1325–34. doi: 10.1097/DCR.0000000000002219

41. Yang Y, Huang X, Sun J, Gao P, Song Y, Chen X, et al. Prognostic value of perineural invasion in colorectal cancer: a meta-analysis. *J Gastrointest Surg*. (2015) 19(6):1113–22. doi: 10.1007/s11605-015-2761-z

42. Dotan E, Cohen SJ. Challenges in the management of stage II colon cancer. *Semin Oncol*. (2011) 38(4):511–20. doi: 10.1053/j.seminoncol.2011.05.005

43. Sinicrope FA, Shi Q, Smyrk TC, Thibodeau SN, Dienstmann R, Guinney J, et al. Molecular markers identify subtypes of stage III colon cancer associated with patient outcomes. *Gastroenterology*. (2015) 148(1):88–99. doi: 10.1053/j.gastro.2014.09.041

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Preoperative radiotherapy does not improve and may even be detrimental to the long-term prognosis of patients diagnosed with stage III colon adenocarcinoma: a propensity score-matched SEER database analysis

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Background: Currently, for patients with colon adenocarcinoma who are diagnosed with local lymph node metastasis, it is typically recommended to undergo neoadjuvant treatment before undergoing curative surgical intervention. Nowadays, the focus of preoperative adjuvant therapy for colon adenocarcinoma patients mainly revolves around chemotherapy, and the impact of preoperative radiotherapy on long-term prognosis remains uncertain.

Methods: We extracted data from the Surveillance, Epidemiology, and End Results database for patients with stage III colon adenocarcinoma between 2004 and 2019. Using propensity score matching (PSM), the patients were divided into a preoperative radiotherapy group and a non-preoperative radiotherapy group, and the differences in Kaplan-Meier (KM) survival curves between the two groups were compared. Cox regression analysis was employed to identify clinical factors that influence survival in stage III colon adenocarcinoma, and the prognostic differences between the two groups were compared within specific subgroups of these clinical factors.

Results: After PSM, a total of 242 patients were included in the study, divided into the preoperative radiotherapy group and the non-preoperative radiotherapy group. There were no statistically significant differences in important clinical characteristics between the two groups. KM analysis revealed no statistically significant difference in overall survival (OS) between the two groups. Furthermore, age, chemotherapy, T staging, N staging, race, tumor grade, gender, tumor location, and tumor diameter were identified as important factors influencing the prognosis of patients. Within each level of the aforementioned subgroups, there were no differences in OS between the two groups. In fact, in specific subgroups, the non-preoperative radiotherapy group exhibited better OS than the preoperative radiotherapy group.

Conclusion: Preoperative radiotherapy does not improve the long-term prognosis of patients with stage III colon adenocarcinoma. In certain patient populations with specific clinical characteristics, preoperative radiotherapy may even lead to a decrease in OS.

KEYWORDS

preoperative radiotherapy, colon adenocarcinoma, SEER, overall survival, propensity score matching

1 Introduction

Colorectal cancer is one of the most prevalent malignant tumors worldwide. Recent global cancer statistics have shown that the incidence of colorectal cancer has risen to the third highest, with the mortality rate ranking second, and the number of newly diagnosed cases ranking fifth (1). Currently, adjuvant chemotherapy following curative surgery remains the preferred curative treatment for colorectal cancer (2). However, due to population aging and urban industrialization, the incidence and mortality rates of colorectal cancer have significantly increased (3). Moreover, an increasing number of colorectal cancer patients are being diagnosed with regional lymph node metastasis (stage III according to AJCC staging), which further complicates effective treatment.

In recent years, more researchers believe that patients with lymph node metastasis at the time of preoperative diagnosis should consider receiving neoadjuvant therapy in order to reduce tumor staging, improve R0 resection rate, decrease local recurrence rate, and achieve clinical complete response (cCR) or even pathological complete response (pCR) for some patients (4–6).

However, current research on preoperative neoadjuvant therapy for colorectal cancer mainly focuses on chemotherapy, while the safety of adjuvant radiotherapy and its impact on long-term prognosis still remain controversial (4, 7, 8). In this study, we selected stage III colon adenocarcinoma (CA) patients diagnosed between 2004 and 2019 from the Surveillance, Epidemiology, and End Results (SEER) database to determine the long-term survival benefits of preoperative radiotherapy. We also conducted comparative analyses within different subgroups to explore characteristics of populations that may benefit from preoperative radiotherapy.

2 Materials and methods

2.1 Data source

The dataset of CA patients in this study is derived from the SEER database. Patients were selected based on the World Health Organization's International Classification of Diseases, Third Edition (ICD-3) codes (8140-8389) for pathologically diagnosed primary colon adenocarcinoma from 2004 to 2019. Data including age, sex, race, tumor size, tumor differentiation, tumor location, tumor staging, surgery, preoperative radiotherapy (RBS), chemotherapy, and survival period (survival time and status) were extracted from the SEER database.

2.2 Patient selection criteria

This study included patients who met the following criteria: (1) underwent curative surgery, (2) were classified as stage III according to AJCC staging, and (3) were pathologically diagnosed with CA. Patients were excluded from this study if they met any of the following criteria: (1) diagnosed through autopsy or based on death certificates, (2) had unknown clinical data, or (3) had a survival time of less than one month. Based on whether patients received radiotherapy before surgery (RBS), they were divided into two groups: the surgery group (None-RBS) and the radiotherapy before surgery group (RBS).

2.3 Outcome variable and covariates

The primary outcome variable in our study is overall survival (OS) of patients. OS is defined as the time from the date of diagnosis to the date of patient's death or last follow-up. Additionally, we selected several clinical covariates that are closely associated with OS in colorectal cancer patients, including age, sex, race, tumor size, tumor differentiation, tumor location, tumor staging, surgery, and chemotherapy. We stratified patients based on each covariate and constructed Cox models within each subgroup to assess the impact of preoperative radiotherapy on OS among different subgroups of patients.

2.4 Propensity score matching

The propensity score is defined as the likelihood of receiving RBS (within the range of 0 to 1) based on individual characteristics. It is derived from a logistic regression model that considers the independent associations of all available variables (i-x) with the RBS status (xi). In summary, a 1:1 nearest neighbor matching method was used to match baseline characteristics between the two groups, with a caliper width of 0.02 standard deviations. By comparing the survival outcomes of matched RBS and None-RBS patient groups, we aim to mitigate selection bias for specific patients receiving RBS (9, 10). The

validation of PSM is achieved by comparing various observed variables between the RBS and None-RBS groups before and after PSM.

2.5 Statistical analyses

All statistical analyses in this study were performed using R software (version 4.3.1). All tests conducted were two-sided, and a p-value of <0.05 was considered statistically significant. The chi-square ($\chi 2$) test or Fisher's exact test was used for comparing baseline data between the two patient groups. Overall survival (OS) analysis comparing the two groups was performed using Kaplan-Meier (K-M) method with log-rank test. Cox proportional hazards models were applied to analyze all predictor variables (i-xi) using the procedure in the MuMIn package, with Breslow approximation for handling ties. This procedure generated a set of Cox models with different combinations of variables. Within this set, we utilized an information-theoretic framework to identify the best-fitting models (11, 12). Specifically, the adjusted Akaike information criterion (AICc) was calculated, which measures the amount of information provided by a model while penalizing for overfitting. The AICc values were used to select a 95% confidence set, representing the best-approximating models that may include the true model. Hazard ratio estimates for RBS and other predictive factors within the 95% confidence set were averaged (weighted by AICc) to infer prognostic indicators for survival. Subsequently, patients were stratified within each subgroup of the identified risk factors in the best model to explore differences in OS between the two cohorts within specific stratified patient populations.

3 Results

3.1 Baseline characteristics of the study population

A total of 72,365 eligible patients were included in this study, with 121 patients in the RBS group and 72,144 patients in the None-RBS group. As shown in Table 1, significant differences in baseline characteristics were observed between the two groups. Compared to the None-RBS group, the RBS group had a higher proportion of young patients, male patients, Grade I-II patients, left-sided colon cancer patients, and patients receiving chemotherapy (all p<0.05). However, after performing 1:1 propensity score matching (PSM) (Figure 1A), the baseline characteristics between the two groups became comparable (all p>0.05, Table 1).

TABLE 1 The baseline characteristics before and after propensity score matching reveal the statistical comparison between the RBS group (highlighted as the reference group) and the None-RBS group (chi-square test).

	Pre-PSM		RBS (N=121)	Post-PSM	
	None-RBS (N=72144)	Comparison		Comparison	None-RBS (N=121)
Age		$\chi 2$ = 22.15		$\chi 2$ = 0.09	
<60	21105 (29.3%)	p<0.001	55 (45.5%)	p=0.955	57 (47.1%)
60-70	19515 (27.1%)		37 (30.6%)		35 (28.9%)
>70	31524 (43.7%)		29 (24.0%)		29 (24.0%)
Sex		$\chi 2$ = 8.88		$\chi 2$ = 0.00	
Female	36905 (51.2%)	p=0.003	45 (37.2%)	p=1.000	44 (36.4%)
Male	35239 (48.8%)		76 (62.8%)		77 (63.6%)
Race		$\chi 2$ = 3.10		$\chi 2$ = 0.00	
White	56221 (77.9%)	p=0.213	90 (74.4%)	p=1.000	90 (74.4%)
Black	8648 (12.0%)		13 (10.7%)		13 (10.7%)
Other	7275 (10.1%)		18 (14.9%)		18 (14.9%)
Grade		$\chi 2$ = 9.94		$\chi 2$ = 0.07	
Grade I	3989 (5.5%)	p=0.019	11 (9.1%)	p=0.995	12 (9.9%)
Grade II	49408 (68.5%)		92 (76.0%)		92 (76.0%)
Grade III	16462 (22.8%)		17 (14.0%)		16 (13.2%)
Grade IV	2285 (3.2%)		1 (0.8%)		1 (0.8%)

(Continued)

TABLE 1 Continued

	Pre-PSM		RBS (N=121)	Post-PSM	
	None-RBS (N=72144)	Comparison		Comparison	None-RBS (N=121)
T stage		χ2 = 4.10		χ2 = 0.77	
T1	2662 (3.7%)	p=0.251	4 (3.3%)	p=0.856	2 (1.7%)
T2	6507 (9.0%)		14 (11.6%)		13 (10.7%)
T3	48125 (66.7%)		71 (58.7%)		72 (59.5%)
T4	14850 (20.6%)		32 (26.4%)		34 (28.1%)
N stage		χ2 = 2.27		χ2 = 0.00	
N1	48731 (67.5%)	p=0.132	90 (74.4%)	p=1.000	91 (75.2%)
N2	23413 (32.5%)		31 (25.6%)		30 (24.8%)
Tumor size		χ2 = 5.68		χ2 = 0.00	
<30	13109 (18.2%)	p=0.058	27 (22.3%)	p=1.000	27 (22.3%)
30-50	34034 (47.2%)		44 (36.4%)		44 (36.4%)
>50	25001 (34.7%)		50 (41.3%)		50 (41.3%)
Tumor site		χ2 = 132.83		χ2 = 0.27	
Left-side	28636 (39.7%)	p<0.001	110(90.9%)	p=0.966	110 (90.9%)
Right-side	35664 (49.4%)		7 (5.8%)		8 (6.6%)
Transverse colon	6873 (9.5%)		3 (2.5%)		2 (1.7%)
Overlapping lesion	971 (1.3%)		1 (0.8%)		1 (0.8%)
Chemotherapy		χ2 = 53.96		χ2 = 0.00	
Yes	44187 (61.2%)	p<0.001	114(94.2%)	p=1.000	113 (93.4%)
No/Unknown	27957 (38.8%)		7 (5.8%)		8 (6.6%)

3.2 Effect of preoperative radiotherapy on OS in stage III CA patients

Prior to PSM, patients receiving RBS exhibited slightly better OS rates at various time points compared to the None-RBS group, but the difference was not statistically significant (p=0.13, Figure 1B). After PSM, non-RBS patients showed a trend of better early OS rates compared to RBS patients. However, as the follow-up time increased and the number of censoring events grew, the OS rates between the two groups became more consistent. Nonetheless, there was still no statistically significant difference in OS between the two groups (p=0.16, Figure 1C).

3.3 Effect of levels of various factors on OS in stage III CA patients

The IT-AIC method was employed to estimate the effect of RBS in a multivariable context and identify additional prognostic factors that contribute to the selection of RBS patients. According to AICc, there was no single model that clearly best explained overall survival (Table 2). The top-ranking models included 10 variables, with a likelihood of being the best-approximating model at 53.6%. To improve the expected predictive accuracy while maintaining low overfitting, we considered a "confidence set" consisting of two models, which together accounted for 100% likelihood of including the best model. These models indicated that the following factors were informative for predicting survival rates: (1) age, (2) chemotherapy, (3) T staging, (4) N staging, (5) race, (6) tumor grade, (7) sex, (8) tumor location, (9) tumor diameter, and (10) RBS. Based on the variables included in the models, corresponding Cox forest plots were constructed (Figure 2). It can be observed that advanced age, later T and N staging, and larger tumor diameter were unfavorable for the prognosis of stage III CA patients. On the other hand, receiving chemotherapy, specific tumor locations, and certain racial backgrounds were associated with improved survival time for CA patients.

3.4 Effect of preoperative radiotherapy on OS in various subgroups

To investigate the impact of RBS on the prognosis of CA patients with specific clinical characteristics more precisely, we stratified

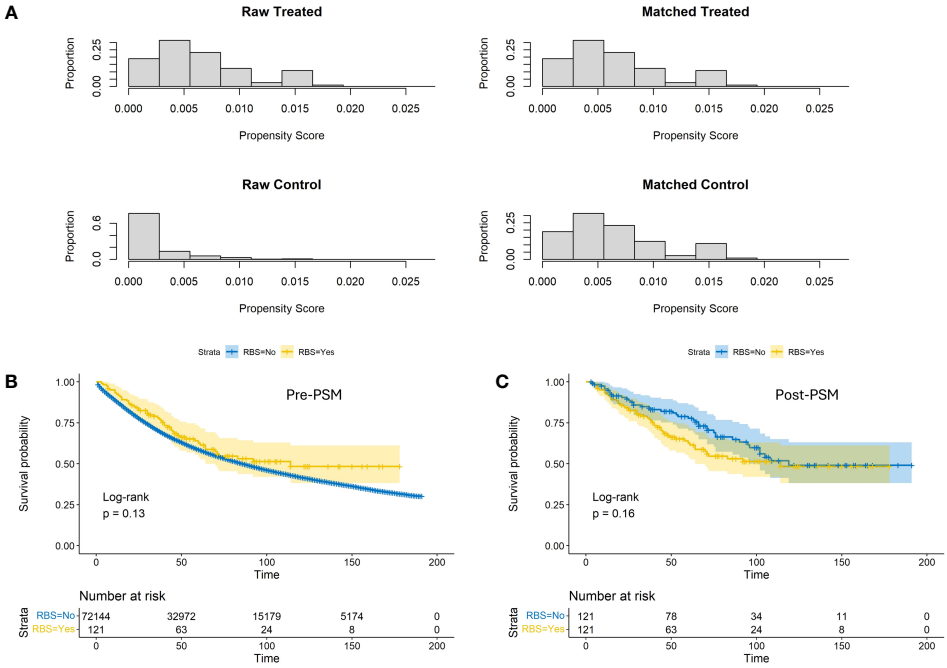

FIGURE 1
Propensity score distributions **(A)** and Kaplan-Meier estimates of OS with corresponding 95% confidence intervals before **(B)** and after **(C)** conducting propensity score matching between the RBS group and the none-RBS group.

patients within each factor's subgroup in the aforementioned models and compared the OS between RBS and No-RBS groups before and after PSM. The results showed that in the subgroups with tumor diameter <30mm (Figure 3D) and T staging of T1-T1 (Figure 3G), patients who received RBS had significantly better prognosis than those without RBS (p=0.018; p=0.013). Meanwhile, in the subgroups of age <60 years (Figure 3A) and Grade I-II (Figure 4A), non-RBS patients exhibited a better prognosis for a significant duration of time, although the differences were not statistically significant (p=0.098; p=0.069). In the remaining subgroup analyses, although there was no statistically significant difference in OS between the two groups, some subgroups still showed a trend of better OS in the RBS group compared to the No-RBS group (Figures 3, 4).

4 Discussion

Currently, there is limited research focusing on whether preoperative radiotherapy provides benefits for patients with stage III CA, and there are no clear guidelines to provide guidance in this regard. Consequently, clinicians face challenges in making appropriate decisions during clinical management, and some physicians tend to lean towards the use of preoperative radiotherapy in patients with locally advanced disease (13–17). In this study, we created a maximally balanced cohort of baseline covariates using propensity score matching and investigated the impact of preoperative radiotherapy on survival. Based on the statistical analysis results, we found that although the incidence rates were higher in the elderly population, females, and

TABLE 2 Set of models created with cox stepwise regression, ranked by corrected AIC.

Age	Chm	T	N	Race	Grd	Sex	Site	Size	RBS	K	LL	AICc	ΔAIC	AICcW
										10	-345485.5	691001.1	0.00	0.536
										9	-345486.7	691001.4	0.29	0.464
										9	-345501.5	691029.0	27.92	0.000
										8	-345502.6	691029.3	28.18	0.000
										8	-345525.7	691075.3	74.25	0.000
										9	-345524.9	691075.9	74.77	0.000
										7	-345544.1	691108.2	107.08	0.000

*The shaded boxes indicate the variables included in the model. Models with darker shading represent the confidence set, which has a likelihood of more than 95% to encompass the variables of the best-approximating model (based on AICcWt). K refers to the number of parameters. LL represents the log-likelihood. ΔAICc indicates the difference in corrected AIC compared to the top-ranked model (values < 2 suggest informational equivalence). AICcWt denotes the relative weight of the AICc for a specific model within the entire set of models (values approximate the likelihood that a given model is the best among those considered).

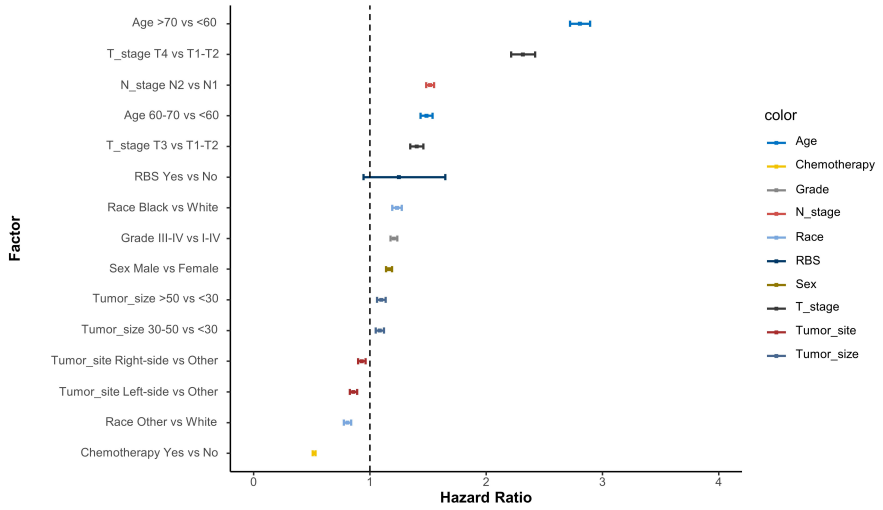

FIGURE 2
Cox proportional hazard ratios with 95% confidence intervals averaged across the model. A dashed line represents the reference hazard ratio (HR=1).

those with the primary site located in the right colon, clinicians tend to preferentially administer preoperative radiotherapy to younger individuals, males, and those with the primary site in the left colon. This preference may be due to considerations of patient tolerance to radiation and the operability of the site (18). However, our results show that preoperative radiotherapy does not improve the overall survival of patients with stage III CA. Cox models further confirm that preoperative radiotherapy is not a significant prognostic factor for patients with stage III CA. Regardless of differences in T staging, N staging, differentiation grade, age, sex, tumor location, and tumor size, preoperative radiotherapy does not confer a survival benefit. In fact, in certain specific subgroups, the OS of the RBS group was significantly lower than that of the No-RBS group. This discrepancy may be attributed to the small sample size and high rate of missing data, but

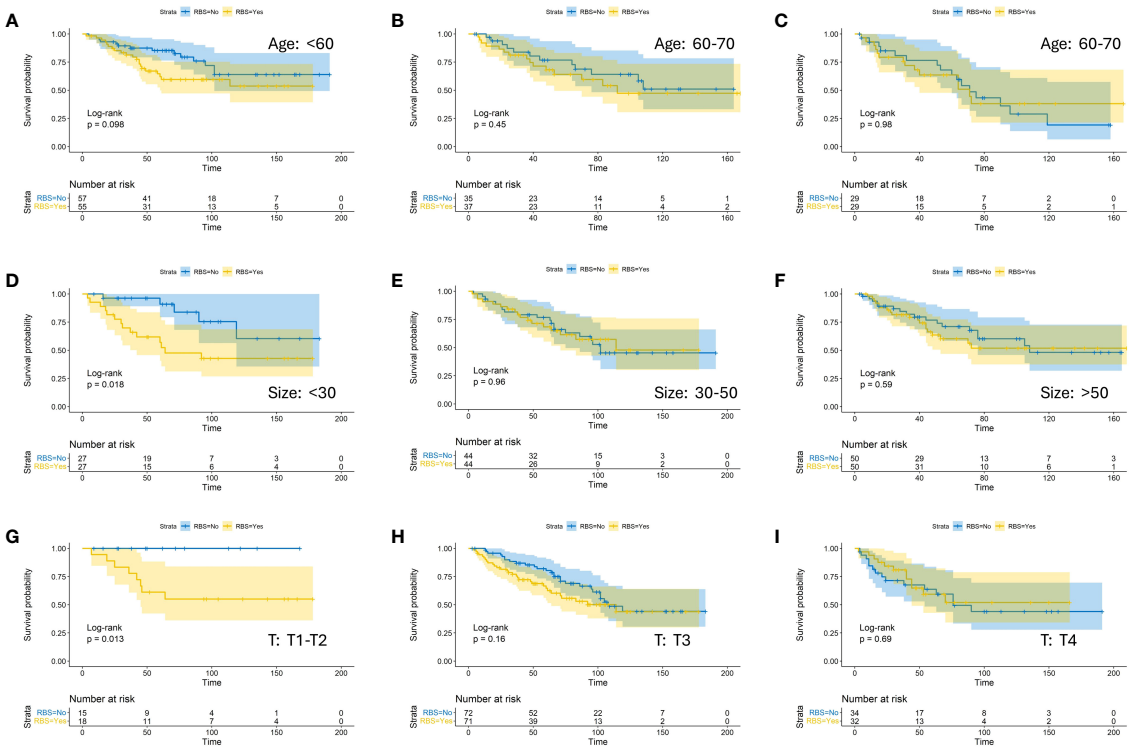

FIGURE 3
Kaplan-Meier estimates of OS with corresponding 95% confidence intervals for patients in the RBS and none-RBS groups after PSM: Stratified by Age **(A–C)**, Tumor Size **(D–F)**, and T-Stage **(G–I)**.

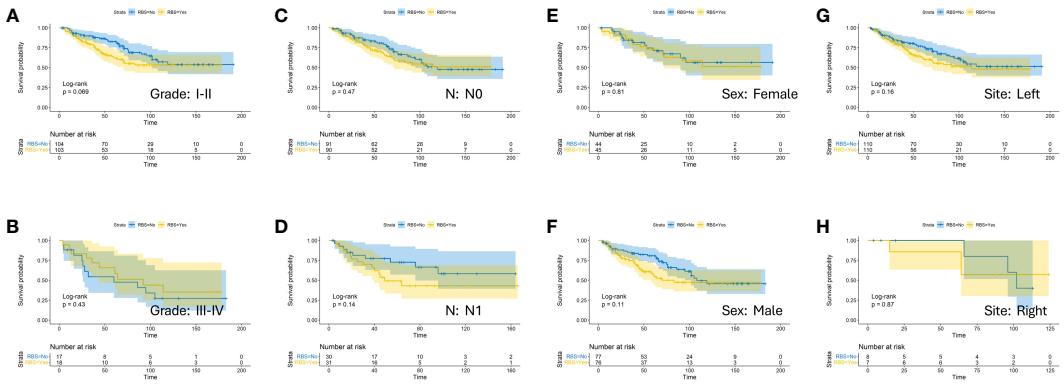

FIGURE 4
Kaplan-Meier estimates of OS with corresponding 95% confidence intervals for patients in the RBS and none-RBS groups after PSM: Stratified by Grade **(A, B)**, N-Stage **(C, D)**, Sex **(E, F)** and Tumor Site **(G, H)**.

it raises questions about whether preoperative radiotherapy not only fails to improve the prognosis of patients with stage III CA but also leads to a decrease in their OS. Furthermore, while many case reports suggest that preoperative radiotherapy may be an effective treatment option for locally advanced colorectal cancer (19), it is important to note that radiotherapy can have negative impacts. For example, radiotherapy can increase the proliferation of residual cells, induce vascular remodeling, and alter cell motility, thereby promoting the regrowth of tumor cells (20). Additionally, preoperative radiotherapy increases the risk of developing subsequent secondary primary tumors in patients (21) and is associated with an increased incidence of anastomotic leakage after surgery (22). The Intergroup 0130 study also indicated that patients who received combined chemoradiotherapy were more likely to experience toxic reactions such as leukopenia and nausea compared to those receiving chemotherapy alone (23).

We have observed that previous studies have indicated that preoperative neoadjuvant radiotherapy can contribute to an increased rate of pathological complete response (pCR) and overall survival (OS) in locally advanced colon cancer (24–27). However, these findings seem to differ from the conclusions drawn in our study. We speculate that this discrepancy may be attributed to several factors. Firstly, Huang et al.'s study focused on patients with T4N2M0 colon cancer, and their study endpoint was 5-year OS, which differs from our study in terms of patient population and research objectives. Additionally, Wang et al.'s study considered chemotherapy regimens concurrently, but they did not conduct a controlled study comparing two cohorts, and their sample size was relatively small. It is worth noting that some scholars argue that adjuvant radiotherapy is not commonly used as definitive treatment for colon cancer (28), although their study primarily focused on postoperative adjuvant radiotherapy (29).

In general, this study incorporated the latest data from a multicenter study with a large sample size. Propensity score matching (PSM) was employed to mitigate potential biases caused by confounding factors, and long-term overall survival (OS) served as the study endpoint, providing robust evidence for clinical decision-making in treatment selection. However, like any SEER-based study, there are limitations to consider. Firstly, the SEER database does not include information on patients' physical fitness or reasons for not receiving adjuvant radiotherapy. Secondly, the SEER database lacks data on preoperative radiotherapy, including clinical target volume and radiation protocols, which weakens the conclusions of the current study. Additionally, whether patients experienced toxic reactions after radiotherapy remains unknown. Thirdly, due to the non-routine inclusion of adjuvant radiotherapy in the preoperative treatment of colon cancer patients, even though we included data from all patients over a 15-year period, the sample size of the study may still be insufficient. Lastly, there may be variations in the acceptance rate of preoperative radiotherapy among different healthcare regions. Therefore, we hope that future randomized multicenter clinical trials on a global scale can provide further validation in this regard.

5 Conclusion

Based on our study findings, we conclude that preoperative radiotherapy does not improve the long-term prognosis of patients with stage III CA. In fact, in certain patient populations with specific clinical characteristics, preoperative radiotherapy may even lead to a decrease in OS.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://seer.cancer.gov/.

Ethics statement

The requirement of ethical approval was waived by Surveillance, Epidemiology, and End Results database for the studies involving humans because Surveillance, Epidemiology, and End Results database. Since the data from SEER are publicly available and deidentified, this study was exempt from local institutional review board review. The studies were conducted in accordance with the

local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JX: Conceptualization, Methodology, Software, Visualization, Writing – original draft. XN: Data curation, Investigation, Supervision, Validation, Writing – review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Lin Y, Wang L, Shao L, Zhang X, Lin H, Wang Y, et al. Prognostic analysis and beneficiary identification of adjuvant external beam radiotherapy for stage pT4b sigmoid colon cancer. *Sci Rep* (2021) 11(1):11782. doi: 10.1038/s41598-021-91172-9

3. Zhang X, Wu T, Cai X, Dong J, Xia C, Zhou Y, et al. Neoadjuvant immunotherapy for MSI-H/dMMR locally advanced colorectal cancer: new strategies and unveiled opportunities. *Front Immunol* (2022) 13:795972. doi: 10.3389/fimmu.2022.795972

4. Sauer R, Liersch T, Merkel S, Fietkau R, Hohenberger W, Hess C, et al. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: results of the German CAO/ARO/AIO-94 randomized phase III trial after a median follow-up of 11 years. *J Clin Oncol* (2012) 30(16):1926–33. doi: 10.1200/JCO.2011.40.1836

5. Huang CM, Huang CW, Ma CJ, Tsai HL, Su WC, Chang TK, et al. Outcomes of neoadjuvant chemoradiotherapy followed by radical resection for T4 colorectal cancer. *World J Gastrointest Oncol* (2020) 12(12):1428–42. doi: 10.4251/wjgo.v12.i12.1428

6. Martorana E, Castorina P, Ferini G, Forte S, Mare M. Forecasting Individual Patients' Best Time for Surgery in Colon-Rectal Cancer by Tumor Regression during and after Neoadjuvant Radiochemotherapy. *J Pers Med* (2023) 13(5):851. doi: 10.3390/jpm13050851

7. Zhou J, Guo Z, Yu W, Li S, Qiao W. Clinical evaluation of preoperative radiotherapy combined with FOLFOX chemotherapy on patients with locally advanced colon cancer. *Am Surg* (2019) 85(4):313–20. doi: 10.1177/000313481908500418

8. Wegner RE, Abel S, Monga D, Raj M, Finley G, Nosik S, et al. Utilization of adjuvant radiotherapy for resected colon cancer and its effect on outcome. *Ann Surg Oncol* (2020) 27(3):825–32. doi: 10.1245/s10434-019-08042-y

9. Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. *Stat Med* (2009) 28(25):3083–107. doi: 10.1002/sim.3697

10. Austin PC. Statistical criteria for selecting the optimal number of untreated subjects matched to each treated subject when using many-to-one matching on the propensity score. *Am J Epidemiol* (2010) 172(9):1092–7. doi: 10.1093/aje/kwq224

11. Burnham KP, Anderson DR. Multimodel inference:Understanding AIC and BIC in model selection. *Sociological Methods Res* (2004) 33(2):261–304. doi: 10.1177/0049124104268644

12. Symonds MRE, Moussalli A. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behav Ecol Sociobiology* (2011) 65(1):13–21. doi: 10.1007/s00265-010-1037-6

13. Wong AC, Stock S, Schrag D, Kahn KL, Salz T, Charlton ME, et al. Physicians' beliefs about the benefits and risks of adjuvant therapies for stage II and stage III colorectal cancer. *J Oncol Pract* (2014) 10(5):e360–7. doi: 10.1200/JOP.2013.001309

14. Chuang JP, Tsai HL, Chen PJ, Chang TK, Su WC, Yeh YS, et al. Comprehensive review of biomarkers for the treatment of locally advanced colon cancer. *Cells* (2022) 11(23):3744. doi: 10.3390/cells11233744

15. Rega D, Granata V, Petrillo A, Pace U, Di Marzo M, Fusco R, et al. Electrochemotherapy of primary colon rectum cancer and local recurrence: case report and prospective analysis. *J Clin Med* (2022) 11(10):2745. doi: 10.3390/jcm11102745

16. Yang J, Xing P, Kong Y, Xu M, Zhang L. PD-1 inhibitor combined with radiotherapy and GM-CSF in MSS/pMMR metastatic colon cancer: a case report. *Front Oncol* (2023) 13:1078915. doi: 10.3389/fonc.2023.1078915

17. Chiang CH, Chao TY, Huang MY. Adaptive radiotherapy of locally advanced sigmoid colon cancer with intra−fractional motion using the MRIdian system: A case report. *Oncol Lett* (2023) 26(5):487. doi: 10.3892/ol.2023.14074

18. Sarasqueta C, Perales A, Escobar A, Baré M, Redondo M, Fernández de Larrea N, et al. Impact of age on the use of adjuvant treatments in patients undergoing surgery for colorectal cancer: patients with stage III colon or stage II/III rectal cancer. *BMC Canc* (2019) 19(1):735. doi: 10.1186/s12885-019-5910-z

19. Sekiya S, Imamura K, Takeuchi S, Teramura K, Watanabe Y, Tamoto E, et al. Pathological complete response of locally advanced colon cancer after preoperative radiotherapy: a case report and narrative review of the literature. *Surg Case Rep* (2018) 4(1):58. doi: 10.1186/s40792-018-0466-8

20. Gladstone M, Su TT. Radiation responses and resistance. *Int Rev Cell Mol Biol* (2012) 299:235–53. doi: 10.1016/B978-0-12-394310-1.00006-0

21. Smith-Gagen J, Goodwin GA3rd, Tay J. Multiple primary tumors following stage II and III rectal cancer in patients receiving radiotherapy, 1998-2010. *J Cancer Res Clin Oncol* (2014) 140(6):949–55. doi: 10.1007/s00432-014-1647-x

22. Zarnescu EC, Zarnescu NO, Costea R. Updates of risk factors for anastomotic leakage after colorectal surgery. *Diagnostics (Basel)* (2021) 11(12):2382. doi: 10.3390/diagnostics11122382

23. Martenson JA Jr., Willett CG, Sargent DJ, Mailliard JA, Donohue JH, Gunderson LL, et al. Phase III study of adjuvant chemotherapy and radiation therapy compared with chemotherapy alone in the surgical adjuvant treatment of colon cancer: results of intergroup protocol 0130. *J Clin Oncol* (2004) 22(16):3277–83. doi: 10.1200/JCO.2004.01.029

24. Huang CM, Huang MY, Ma CJ, Yeh Y, Tsai HL, Huang CW, et al. Neoadjuvant FOLFOX chemotherapy combined with radiotherapy followed by radical resection in patients with locally advanced colon cancer. *Radiat Oncol* (2017) 12(1):48. doi: 10.1186/s13014-017-0790-3

25. Huang Y, Gu X, Ge K, Fu G, Chu J, Wei W. The survival benefit of adjuvant radiotherapy for pathological T4N2M0 colon cancer in the Modern Chemotherapy Era: evidence from the SEER database 2004-2015. *Artif Cells Nanomed Biotechnol* (2020) 48(1):834–40. doi: 10.1080/21691401.2020.1770270

26. Niu SQ, Li RZ, Yuan Y, Xie WH, Wang QX, Chang H, et al. Neoadjuvant chemoradiotherapy in patients with unresectable locally advanced sigmoid colon cancer: clinical feasibility and outcome. *Radiat Oncol* (2021) 16(1):93. doi: 10.1186/s13014-021-01823-4

27. Yang Z, Wu G, Zhang X, Gao J, Meng C, Liu Y, et al. Current progress and future perspectives of neoadjuvant anti-PD-1/PD-L1 therapy for colorectal cancer. *Front Immunol* (2022) 13:1001444. doi: 10.3389/fimmu.2022.1001444

28. McLaughlin C, Kim NK, Bandyopadhyay D, Deng X, Kaplan B, Matin K, et al. Adjuvant radiation therapy for T4 non-rectal colon adenocarcinoma provides a cause-specific survival advantage: A SEER database analysis. *Radiother Oncol* (2019) 133:50–3. doi: 10.1016/j.radonc.2018.11.026

29. Luo D, Zhang R, Yang Y, Li Q, Li X. Pretreatment carcinoembryonic antigen level serves as a potential biomarker to guide adjuvant radiotherapy in pT4N+ Colon cancer patients. *J Oncol* (2023) 2023:4815996. doi: 10.1155/2023/4815996

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Case report: The application of neoadjuvant chemoradiotherapy in anal adenocarcinoma combined with perianal Paget disease involving vulvar skin

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Anal adenocarcinoma combined with perianal Paget's disease (PPD) involving the vulva is rare, and there is no established standard treatment. We present the case of a 69-year-old woman with symptoms of intermittent hematochezia and perianal discomfort for 7 months. Upon examination, we discovered a plaque-like hard mass on the right posterior wall of the anal canal, which extended to encompass the anus and dentate line. The lesion skin also extended forward from the gluteal groove, involving the bilateral labial area. Colonoscopy revealed an extensive protruding lesion on the dentate line, which was confirmed as anal adenocarcinoma (mrT4N0M0). The presence of Paget's cells in perianal and vulvar skins led to the diagnosis of PPD. The strategy of neoadjuvant chemoradiotherapy (nCRT) followed by radical surgery was then made after multi-disciplinary discuss. The scope and extent of perianal and vulvar disease were significantly diminished after nCRT. The patient underwent laparoscopic abdominoperineal resection and vulvar lesion resection, confirming the diagnosis of anal adenocarcinoma (ypT2N0). No evidence of tumor cells was found in perianal and vulvar skin, indicating a complete response. The patient is regularly monitored without recurrence or metastasis.

KEYWORDS

anus neoplasms, Paget disease, extramammary, neoadjuvant therapy, efficacy, case report

Introduction

Paget disease is characterized by adenocarcinoma localized within the epidermis of the nipple or areola of the breast (1). Extramammary Paget disease (EMPD) is a relatively rare malignancy developed on apocrine-rich skin, such as the vulva, scrotum, and penis, with a reported incidence of 0.1-2.4 patients per 1,000,000 person-years (2, 3). EMPD predominantly presents as a slowly enlarging asymmetrical erythematous plaque, and pruritus is a common symptom (4). The mechanism underlying EMPD develop remain unclear; however, a popular theory posits that primary EMPD arises as an intraepidermal neoplasm originating from the cells of the apocrine gland ducts (5). Owing to its nonspecific clinical presentation and insidious onset, EMPD is often misdiagnosed, resulting in delayed treatment (6).

Perianal Paget disease (PPD) develops near the anus, but in rare cases (~20% of EMPD lesions) can spread across the perineum, genitalia, gluteal canal, and anal canal (7). The perianal region is the second most common site, with 5% of all EMPD lesions originating from the anorectal region (1, 7, 8). The standard-of-care management strategy for PPD is comprehensive, including radiotherapy, local excision, and radical surgery; however, no consensus exists regarding the superiority of local excision and radical surgery, especially in cases of accompanied anal adenocarcinoma (9). Here, we report a rare case of an anal tumor combined with PPD involving the vulvar skin with extensive and massive lesions. Based on our experience with the management of rectal cancer, we decided to apply neoadjuvant chemoradiotherapy (nCRT) followed by radical surgical resection, achieving a satisfactory outcome.

Case presentation

Complaints and colonoscopy

The overall treatment procedure is illustrated in Figure 1. A 69-year woman was admitted to our hospital complaining of intermittent hematochezia and perianal discomfort for 7 months. The patient had initially developed intermittent hematochezia, characterized by dark bloody stool, accompanied by perianal pruritus and pain 7 months prior. Upon examination, pigmentation of the local skin and chapped epidermal hyperplasia, as well as a plaque-like hard mass, were found on the right posterior wall of the anal canal, involving the anus and dentate line. The patient's primary manifestations were changes in defecation habits, including diarrhea and a feeling of urgency during defecation (Figure 2A). The aforementioned painful symptoms led the patient to visit the hospital for further examination. A digital colonoscopy (July 14, 2022) revealed an extensive protrudent lesion on the dentate line, and the biopsy results revealed moderately differentiated adenocarcinoma in the anal canal, while papillomatous hyperplasia was characterized by scattered glandular distributed heteromorphic cells with abundant cytoplasm at the lesions around the perianal and vulvar skins, meeting the features of Paget's cells (Figures 3A–C).

Rectal MRI examination

The patient's typical pathologic features led us to consider the diagnosis of PPD (Figure 3D). There were no metastatic lesions in

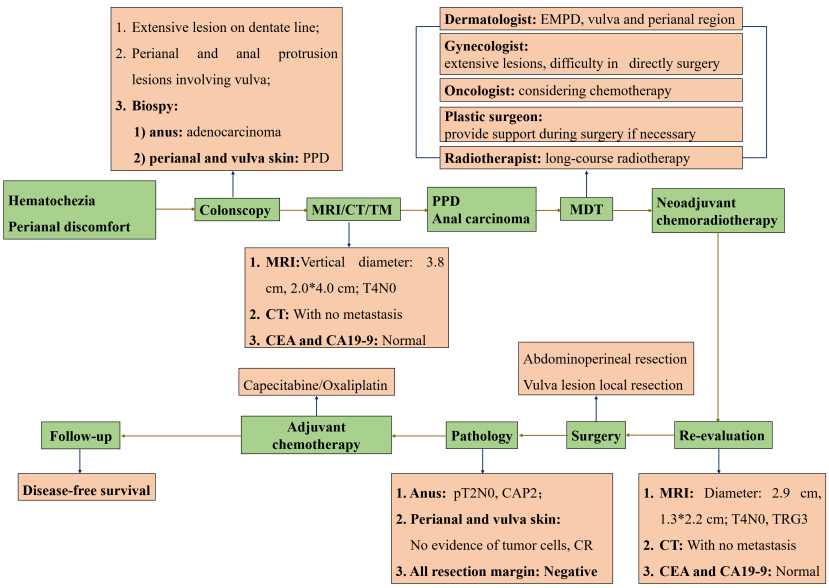

FIGURE 1
The overview of procedures during the treatment. All procedures marked in green were presented in the order of timelines, all contents marked in orange were supplementary explanation of each procedure. PPD, Perianal Paget disease; EMPD, Extramammary Paget disease; MRI, Magnetic resonance imaging; MDT, Multi-disciplinary treatment; CR, Complete response.

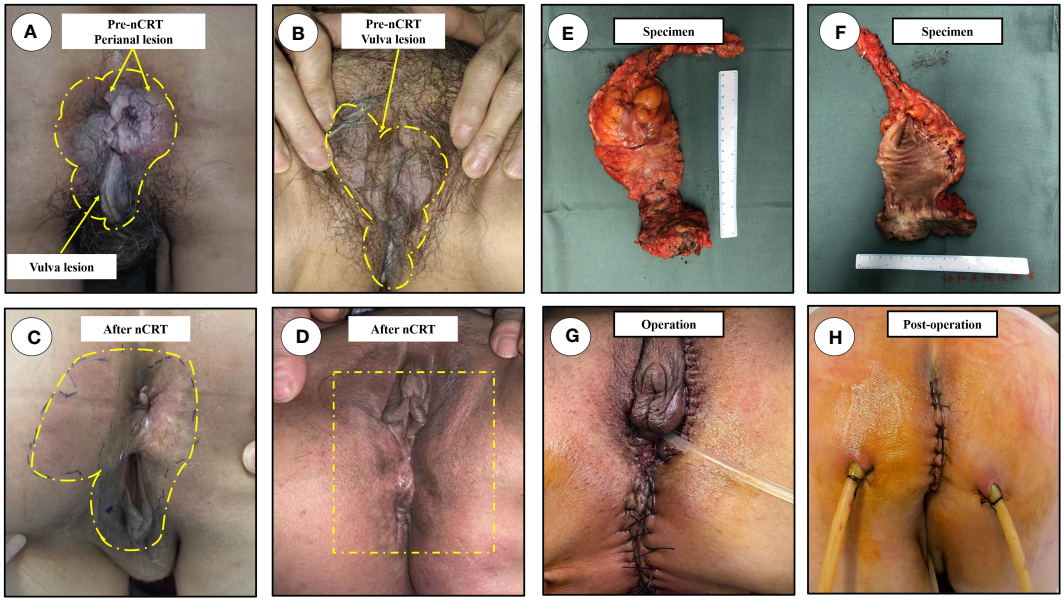

FIGURE 2
Physical examination. The scope of lesions were marked in yellow dotted line, the most typical lesion was marked in yellow arrow. **(A)** The overall outlook of perianal disease, an obvious mass protruding out of anal canal before nCRT; **(B)** The massive scope of vulva lesions, and the lesion skin before nCRT; **(C)** The overall outlook of perianal region after nCRT; **(D)** the lesions of vulvar region with no macroscopic lesions after nCRT; **(E)** Anterior view of the excised specimen; **(F)** The image of the dissected specimen; **(G)** Images after surgery; **(H)** Perineal incision on postoperative day 9. nCRT, neoadjuvant chemoradiotherapy.

the liver or lungs, and tumor markers were normal. An irregular mass protruding into the intestinal cavity was observed from the 6 to 12 o' clock positions in the entire section of the anal canal, detected by rectal MRI. The maximum section was 4.0 cm * 2.0 cm. The lesion protruded from the external anal margin, the involved anal canal became stenotic. The anterior edge of the lesion involved the posterior wall of the vagina and was staged as mrT4N0M0 (Figures 4A–C).

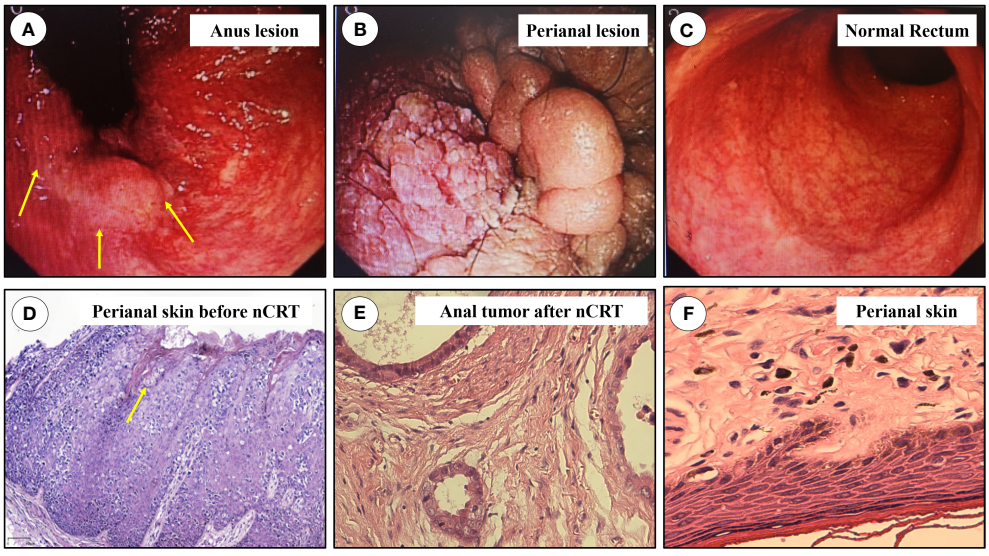

FIGURE 3
The results of colonoscopy and pathology. **(A)** Anus lesion, protrusion and nodular lesions at anal canal and perianal mucosa, the most typical lesion was marked in yellow arrow; **(B)** The lesions at perianal region; **(C)** The proximal mucosa was normal; **(D)** Pathology of biopsied perianal skin before nCRT, Paget's like tumor cells could be found, marked in yellow arrow. **(E)** Pathologic result of anal adenocarcinoma; **(F)** Pathologic result of resected perianal and vulva lesions. nCRT, neoadjuvant chemoradiotherapy.

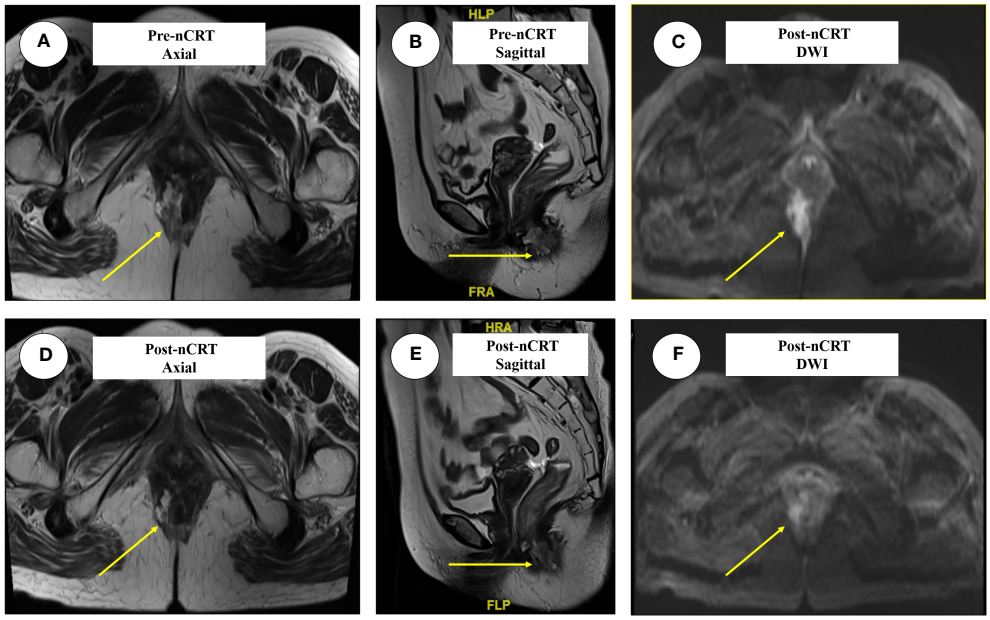

FIGURE 4
The typical images of anal adenocarcinoma before and after nCRT in three dimensions. The lesions were marked in yellow arrow. **(A)** The axial images before nCRT, the irregular mass protruding into the intestinal cavity; **(B)** The sagittal images before nCRT; **(C)** Lesions presented by DWI before nCRT; **(D)** The axial images after nCRT; **(E, F)** represent the sagittal and DWI lesions after nCRT. nCRT, neoadjuvant chemoradiotherapy; DWI, Diffusion weighted imaging; TRG, Tumor regression grade.

Physical examination revealed that local skin pigmentation and chapped epidermic hyperplasia could be found on the right side of the anus with a range 2.0×3.0 cm in scope, while a plaque-like hard mass (~1.5 cm in diameter) was observed on the right posterior wall of the anal canal, involving the anus and dentate line. The lesion skin developed from the gluteal groove forward involving the bilateral area of the labia (Figures 2A, B).

Multi-disciplinary discussion

Considering that diagnosis of anal adenocarcinoma combined with PPD involving the vulvar skin, we organized a multidisciplinary panel to determine the optimal strategies. The dermatologist consulted the pathological sections of the perianal skin again, which showed heterotypic epithelial cells visible in the basal layer and epidermis, accompanied by a slightly stained cytoplasm. PPD was considered firstly when combined with immunohistochemical results (positive for CK7/CK20/CEA/PAS). In addition, the scope of the vulvar lesions was determined using fluorescence diagnostic technology and biopsy, which revealed that the epidermal spinous layer was hypertrophic, with a large number of Paget-like cells with obvious atypia. The gynecologist held the view that the chapped and depigmented skin lesions developed from the perianal area to the bilateral regions of the labia, radical surgery was difficult. Although the diagnosis was clear, the oncologist believed that the lesions involved a large area and that direct surgery might be difficult, and neoadjuvant radiotherapy concurrent with capecitabine might therefore be the optimal choice. The plastic surgeon was recruited to assist in the removal and repair of soft tissue lesions, if necessary.

NCRT and subsequent re-evaluation

A strategy was chosen to administer nCRT followed by radical surgery. The patient received 25 fractions of radiotherapy, followed by 2 cycles of oral capecitabine between October 4, 2022, and November 8, 2022. The treatment course was well-tolerated without any notable discomfort (Figure 1).

Re-evaluation by MRI revealed an irregular mass protruding into the cavity of the anal canal. The maximum cross section was about 1.3 cm × 2.2 cm, the solid component of tumor mass was significantly reduced, and mucus signals were visible in the anal canal. The application of nCRT effectively reduced both the volume and diameter of the tumor mass to an extremely diminished status, with MRI indicating a tumor regression grade reaching TRG 3 (Figures 4D–F).

Physical examination revealed scattered erythematous changes on the perianal skin, although no eminent lesions observed on the perianal skin. Digital rectal examination (thoraco-knee position) revealed a hard mass on the right posterior wall of the anal canal under the dentate line, which was significantly decreased compared with the pre-neoadjuvant period; lesions on the bilateral labia were also significantly improved, with no obvious pigmentation or chapped skin (Figures 2C, D).

Surgery and follow-up

The patient was subsequently scheduled for laparoscopic abdominoperineal resection combined with vulvar lesion resection on February 3, 2023, the resected specimens of anal tumor along with perianal lesions were displayed in Figures 2E, F. The surgery was successful, and the patient was discharged from the hospital on postoperative day 10 (Figures 2G, H). Pathological review of the resected specimens revealed that the anal mass was a moderately differentiated adenocarcinoma staged as ypT2N0, and the tumor regression grade was reaching CAP 2 (Figure 3E), there was no evidence of tumor cells in the resected perianal and vulvar lesions (Figure 3F), Immunohistochemistry indicated positivity for CK20 (+), CDX-2 (+), MUC2 (+), GCDFP-15 (+), and negative CK-7. Follow-up is currently ongoing, and no evidence of recurrence or metastasis has been observed.

Discussion

EMPD is a malignancy originating from epithelial cells, which is predominantly distributed in the perineum, external genitalia, and other apocrine-rich sites. These lesions are generally confined to the epidermis, dermis, or subcutaneous soft tissue, later transforming into invasive lesions through recurrence and metastasis (8). EMPD is mostly a single-organ disease, and only 4% of cases are complicated by multiple lesions (10).

The incidence of EMPD is low, and its clinical manifestations lack specificity, mainly presenting as erythema, erosion, ulcers and hyperpigmentation (8). Multi-point full-layer puncture is an important diagnostic method for EMPD. In EMPD, single cells or clusters of Paget cells can be arranged in the epidermis, dermis, or subcutaneous tissues. Paget cells are larger than keratinocytes, and their cytoplasm is more pale or granular (11). Immunohistochemistry also contributes to EMPD diagnosis. Positivity for keratin CK7/CK20 and CEA and negativity for SOX10 are suggestive of EMPD, whereas positivity for CK20 and CDX2 suggests the possibility of secondary EMPD (12, 13). In addition, site-specific tumor markers are also helpful for differential diagnosis, such as positivity for GCDFP15, indicating the origin of the genital system, and positivity for CDX2 and CK20, which may refer to perianal lesions, as presented in this case (14). Ultrasonography, colonoscopy, magnetic resonance imaging (MRI), computed tomography (CT), and other examinations can clarify and differentiate between the diagnosis (1, 8, 11).

Radical surgery is a vital treatment for primary EMPD, and the essential principle is to ensure that all surgical margins are negative. Local or extended local resection or Mohs minimally invasive surgery can also be performed; however, the postoperative recurrence rate can reach as high as 30% (13). Conservative treatment or local radiotherapy can also be used as alternative treatments. Adjuvant chemotherapy, targeted therapy, and immunotherapy may also be used to manage metastatic EMPD (15). For secondary EMPD, a multidisciplinary collaboration is required to develop precise treatment plans.

Whether nCRT can be applied in the treatment of invasive PPD, particularly when accompanied by simultaneous anal canal adenocarcinoma, remains controversial (2, 16). In this case, anal adenocarcinoma was combined with PPD that invaded the vulva, and the lesions were diffuse and involved a large area between the perianus and labia. If surgical resection is chosen for the first time, combined abdominoperineal resection and extended resection of the vulva may be mandatory, which is extensive and traumatic; further, flap transplantation and vulvar reconstruction may also be required for radical purpose (17). After a multidisciplinary consultation, we decided to gain experience with the comprehensive treatment of mid-to-low locally advanced rectal cancer using neoadjuvant long-course radiotherapy, followed by concurrent single-agent oral capecitabine for a total of two cycles.

This study has certain limitations. Firstly, in the process of assessing the treatment efficacy after neoadjuvant chemoradiotherapy, there was no subsequent biopsy of the perianal skin and the lesion site in the perineum. Confirming complete remission of perianal and perineal lesions before surgery could have further reduced the extent of the surgical procedure. Additionally, this study only reports one-year postoperative survival outcomes, which represents a relatively short follow-up period. Long-term survival prognosis for patients should be tracked more extensively in the future.

The application of nCRT has two purposes: first, the sizes and scope of lesions can be reduced, in some cases even achieving complete clinical response; second, it can achieve a reduction in tumor size and stage through chemoradiotherapy, as well as increasing tumor local control and eradication of micrometastasis to reduce the possibility of local recurrence and distant metastasis (16, 18). In the present patient, after nCRT, the lesions in the perianal region were significantly reduced, no clear swelling lesions were found around the anus after treatment, and the scope of vulvar lesions was also diminished compared to that before treatment (18). Postoperative pathological results confirmed that after nCRT, no tumor cells could be found around the anus and vulva, and the overall treatment effect was promising. During surgery, radical resection was ensured by repeated inspection of the incisal margin. The resection scope of vulvar lesions was relatively limited, and a one-stage suture was feasible, avoiding flap transplantation and vulvar reconstruction.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Peking Union Medical College Hospital (No. JS-1296). The studies were conducted in accordance with the local

legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

GL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft. XQ: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – review & editing. XZ: Conceptualization, Data curation, Formal analysis, Methodology, Resources, Writing – review & editing. NZ: Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing – review & editing. GL: Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Morris CR, Hurst EA. Extramammary paget disease: A review of the literature: history, epidemiology, pathogenesis, presentation, histopathology, and diagnostic work-up. *Dermatol Surg* (2020) 46(2):151–8. doi: 10.1097/DSS.0000000000002064

2. Herrel LA, Weiss AD, Goodman M, Johnson TV, Osunkoya AO, Delman KA, et al. Extramammary Paget's disease in males: survival outcomes in 495 patients. *Ann Surg Oncol* (2015) 22(5):1625–30. doi: 10.1245/s10434-014-4139-y

3. Siesling S, Elferink MA, van Dijck JA, Pierie JP, Blokx WA. Epidemiology and treatment of extramammary Paget disease in the Netherlands. *Eur J Surg Oncol* (2007) 33(8):951–5. doi: 10.1016/j.ejso.2006.11.028

4. Hatta N, Yamada M, Hirano T, Fujimoto A, Morita R. Extramammary Paget's disease: treatment, prognostic factors and outcome in 76 patients. *Br J Dermatol* (2008) 158(2):313–8. doi: 10.1111/j.1365-2133.2007.08314.x

5. Schaapveld M, Visser O, Louwman MJ, de Vries EG, Willemse PH, Otter R, et al. Risk of new primary nonbreast cancers after breast cancer treatment: a Dutch population-based study. *J Clin Oncol* (2008) 26(8):1239–46. doi: 10.1200/JCO.2007.11.9081

6. Funaro D, Krasny M, Lam C, Desy D, Sauthier P, Bouffard D. Extramammary Paget disease: epidemiology and association to cancer in a Quebec-based population. *J Low Genit Tract Dis* (2013) 17(2):167–74. doi: 10.1097/LGT.0b013e31825f4b4f

7. Williams SL, Rogers LW, Quan SH. Perianal Paget's disease: report of seven cases. *Dis Colon Rectum* (1976) . 19(1):30–40. doi: 10.1007/BF02590848

8. Kibbi N, Owen JL, Worley B, Wang JX, Harikumar V, Downing MB, et al. Evidence-based clinical practice guidelines for extramammary Paget disease. *JAMA Oncol* (2022) 8(4):618–28. doi: 10.1001/jamaoncol.2021.7148

9. Luyten A, Sörgel P, Clad A, Gieseking F, Maass-Poppenhusen K, Lellé RJ, et al. Treatment of Extramammary Paget disease of the vulva with imiquimod: a retrospective, multicenter study by the German Colposcopy Network. *J Am Acad Dermatol* (2014) 70(4):644–50. doi: 10.1016/j.jaad.2013.12.008

10. Yélamos O, Hibler BP, Cordova M, Hollmann TJ, Kose K, Marchetti MA, et al. Handheld reflectance confocal microscopy for the detection of recurrent Extramammary Paget disease. *JAMA Dermatol* (2017) 153(7):689–93. doi: 10.1001/jamadermatol.2017.0619

11. Navarrete-Dechent C, Aleissa S, Cordova M, Hibler BP, Erlendsson AM, Polansky M, et al. Treatment of extramammary Paget disease and the role of reflectance confocal microscopy: A prospective study. *Dermatol Surg* (2021) 47(4):473–9. doi: 10.1097/DSS.0000000000002934

12. Pan ZY, Liang J, Zhang QA, Lin JR, Zheng ZZ. *In vivo* reflectance confocal microscopy of extramammary Paget disease: diagnostic evaluation and surgical management. *J Am Acad Dermatol* (2012) 66(2):e47–53. doi: 10.1016/j.jaad.2010.09.722

13. Bayan CY, Khanna T, Rotemberg V, Samie FH, Zeitouni NC. A review of non-invasive imaging in extramammary Paget's disease. *J Eur Acad Dermatol Venereol* (2018) 32(11):1862–73. doi: 10.1111/jdv.15072

14. Kawaguchi M, Kato H, Noda Y, Kobayashi K, Miyazaki T, Hyodo F, et al. Imaging findings of Malignant skin tumors: radiological-pathological correlation. *Insights Imaging* (2022) 13(1):52. doi: 10.1186/s13244-022-01205-8

15. Appert DL, Otley CC, Phillips PK, Roenigk RK. Role of multiple scouting biopsies before Mohs micrographic surgery for extramammary Paget's disease. *Dermatol Surg* (2005) 31(11):1417–22. doi: 10.2310/6350.2005.31207

16. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, et al. NCCN guidelines insights: rectal cancer, version 6.2020. *J Natl Compr Canc Netw* (2020) 8(7):806–15. doi: 10.6004/jnccn.2020.0032

17. Karam A, Dorigo O. Treatment outcomes in a large cohort of patients with invasive Extramammary Paget's disease. *Gynecol Oncol* (2012) 125(2):346–51. doi: 10.1016/j.ygyno.2012.01.032

18. Helbling D, Bodoky G, Gautschi O, Sun H, Bosman F, Gloor B, et al. Neoadjuvant chemoradiotherapy with or without panitumumab in patients with wild-type KRAS, locally advanced rectal cancer (LARC): a randomized, multicenter, phase II trial SAKK 41/07. *Ann Oncol* (2013) 24(3):718–25. doi: 10.1093/annonc/mds519

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Short-term clinical outcomes and five-year survival analysis of laparoscopic-assisted transanal natural orifice specimen extraction versus conventional laparoscopic surgery for sigmoid and rectal cancer: a single-center retrospective study

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Background: The cosmetic benefits of natural orifice specimen extraction (NOSE) are easily noticeable, but its principles of aseptic and tumor-free procedure have caused controversy.

Methods: We conducted a retrospective analysis of the clinical data of patients who underwent laparoscopic-assisted transanal NOSE or conventional laparoscopic surgery (CLS) for sigmoid and rectal cancer at our hospital between January 2018 and December 2018. The study aimed to compare the general characteristics, perioperative indicators, postoperative complications, and five-year follow-up results between the two groups.

Results: A total of 121 eligible patients were enrolled, with 52 underwent laparoscopic-assisted transanal NOSE and 69 underwent CLS. There were no significant differences observed between the two groups in terms of gender, age, body mass index (BMI), TNM stage, etc. ($P > 0.05$). However, the NOSE group exhibited significantly shorter total incision length and longer operation time compared to the CLS group ($P < 0.05$). There were no statistically significant differences observed between the two groups in terms of positive rate of bacterial culture, incidence rates of intraabdominal infections or anastomotic leakage ($P > 0.05$). Furthermore, during follow-up period there was no statistically significant difference observed between these two groups concerning overall survival rate and disease-free survival outcomes ($P > 0.05$).

Conclusions: The management of surgical complications in CLS is exemplary, with NOSE presenting a sole advantage in terms of incision length albeit at the cost of prolonged operative time. Therefore, NOSE may be deemed appropriate for patients who place high emphasis on postoperative cosmetic outcomes.

KEYWORDS

laparoscopy, survival analysis, intraabdominal infections, colorectal surgery, sigmoid

1. Introduction

Despite the gradual acceptance of early screening for colorectal tumors, the global incidence and mortality rates of colorectal cancer remain alarmingly high, currently ranking third in terms of incidence and second in terms of mortality worldwide (1), this persistent trend poses significant challenges to colorectal surgeons. Surgical intervention represents the foremost and efficacious modality for managing colorectal neoplasms (2, 3). The transition from open surgery to laparoscopic surgery represents a groundbreaking milestone in the management of colorectal cancer. Modern surgeons strive not only for radical tumor cure but also for minimizing surgical trauma. Moreover, extensive evidence supports the safety and efficacy of laparoscopic colorectal surgery, which is associated with smaller incisions, faster postoperative recovery, and even improved tumor prognosis compared to open surgery (4–6). Consequently, it has gained widespread utilization in clinical practice. However, laparoscopic surgery inevitably necessitates a lengthy abdominal incision for specimen extraction and digestive tract reconstruction. This lengthy incision has led to numerous complications associated with wounds, including infection and hernia formation, which contradicts the fundamental principle of minimally invasive surgery. In the pursuit of achieving a more minimally invasive approach, the emergence of NOSE has revolutionized surgeons' perspectives. By utilizing the natural cavity passage for specimen retrieval, it eliminates the need for lengthy abdominal incisions, resulting in reduced trauma and enhanced aesthetic outcomes. Since its introduction by Franklin et al. (7) in 1993, who reported a series of patients undergoing laparoscopic sigmoid colon resection with transanal specimen removal, this technique has gained widespread recognition and adoption in China (8).

Despite the numerous advantages associated with NOSE, its principle of aseptic and tumor-free procedure remains a subject of controversy (9). The intraperitoneal opening of the intestinal cavity poses an increased risk for intraperitoneal infection and tumor dissemination, whereas extraction of specimens through the natural duct may potentially lead to rectal stump implantation and metastasis. Some studies pertaining to the NOSE have indeed substantiated its safety; however, there exists a dearth of outcomes derived from bacterial culture analysis of postoperative abdominal drainage fluid. Furthermore, the majority of these investigations suffer from limited availability of data. Meanwhile, the limited duration of NOSE surgery and the lack of long-term survival analysis preclude a comprehensive assessment of the efficacy of NOSE cancer treatment (10). This retrospective study aimed to investigate the short-term clinical outcomes and five-year follow-up of laparoscopic-assisted transanal NOSE compared to CLS for the treatment of sigmoid and rectal cancer.

2. Materials and methods

This retrospective study (Registration No. 2020LWB035) was conducted at Zhangzhou Affiliated Hospital of Fujian Medical University, with approval from the ethics committee and informed consent obtained from all patients involved. The inclusion criteria encompassed: (1) patients who underwent laparoscopic-assisted transanal NOSE or CLS for sigmoid and rectal cancer between January 2018 and December 2018 at our institution; (2) patients with confirmed diagnoses of sigmoid or rectal cancer through preoperative colonoscopy and pathology assessments; (3) patients classified as $T_{0-3}N_{0-2}M_0$ stage based on CT or MRI evaluations prior to surgery; (4) patients without evidence of distant metastasis or invasion into adjacent organs; and finally; (5) patients without any concurrent malignant tumors or significant systemic diseases such as cardiac, hepatic, renal conditions, among others. The exclusion criteria were as follows: (1) patients who had to undergo open surgery due to the discovery of distant metastasis or invasion of adjacent organs during the operation; (2) patients who were unable to provide complete follow-up data after surgery. Based on the different surgical methods, the included patients were divided into two groups: NOSE group and CLS group. The general characteristics, perioperative indicators, postoperative complications, and five-year follow-up results of these two groups were compared (**Figure 1**).

2.1. Preoperative preparation and anesthesia

All patients were given a prescription for metronidazole tablets two days prior to their surgery and received oral laxatives the evening before the procedure to prepare their bowels. Before the surgery, a single infusion of cefmetazole was routinely administered intravenously 30 min beforehand. If the surgical procedure lasted longer than 3 h, the same dosage was repeated. General anesthesia was uniformly administered to all patients during anesthetic induction.

2.2. Surgical intervention

The patient underwent a modified lithotomy position and achieved pneumoperitoneum at 13 mmHg. The abdominal wall was punctured with five trocars: one 10-mm camera port above the navel, one 12-mm surgeon's operation port in the lower right quadrant, two 5-mm ports on the left side aligned with the spina iliaca anterior superior in both middle and lower abdomen, and one 5-mm port on the right side in the middle abdomen. Tumor and lymphoid tissue dissection were conducted following total mesorectal excision (TME). Sigmoidectomy was performed for tumors located in the sigmoid colon, anterior resection was performed for tumors located in the upper rectum, and low anterior resection was performed for tumors located in the lower rectum.

The CLS group underwent a conventional laparoscopic-assisted procedure for radical sigmoidectomy or proctectomy, involving the creation of a hypogastric incision measuring 4–6 cm in length. After separating the mesocolon, they proceeded to divide the proximal colon and remove tumor tissue. Subsequently, an anvil was introduced into the distal colon to facilitate bowel anastomosis.

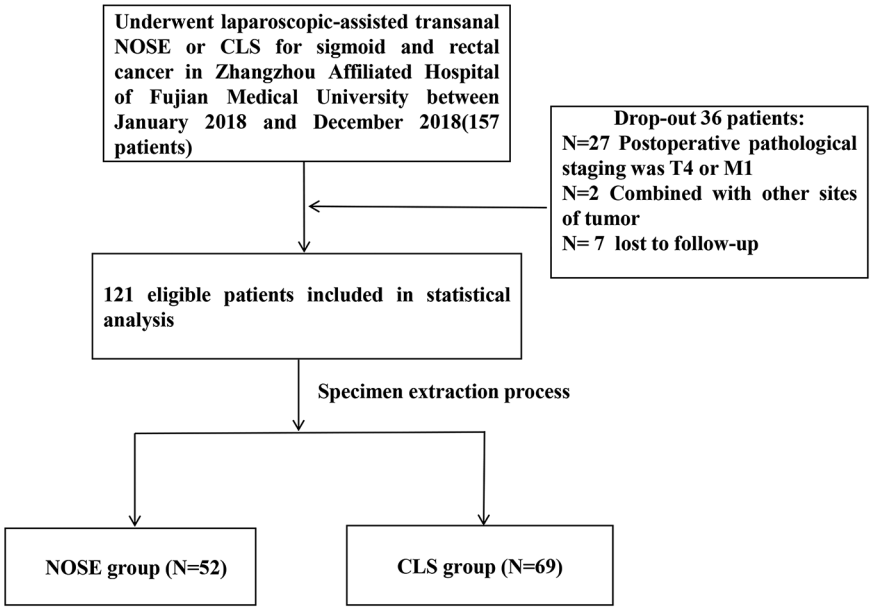

FIGURE 1
Patient recruitment process. NOSE, natural orifice specimen extraction; CLS, conventional laparoscopic surgery.

The NOSE group received treatment using CRC-NOSES VI (11). A linear cutter stapler was utilized to divide the proximal 10 cm of the colon tumor and the lower edge of the tumor (up to 2–3 cm for rectal cancer and 10 cm for sigmoid colon cancer). Following that, a thorough disinfection of the rectal lumen was conducted using diluted povidone-iodine, followed by an incision in the rectum. Subsequently, a sterile protective sleeve was inserted into the abdominal cavity through which excised diseased tissue could be safely extracted along with the protective sleeve. Next, the circular stapling device's anvil was inserted into the abdominal cavity through the rectal stump. Sterile gauze was carefully placed around the proximal colon. A precise longitudinal incision of approximately 2 cm was made on the wall of the proximal colon to allow for insertion of the anvil in this area. Finally, using an endoscopic linear stapler, both the exposed proximal colon and rectal stump were expertly closed.

In both study groups, the circular stapling device was meticulously inserted into the rectum, followed by a laparoscopic-guided end-to-end anastomosis with the anvil junction positioned in the proximal colon. Subsequently, thorough irrigation of the abdomen and pelvis was performed using a substantial volume of normal saline solution, while concurrently placing a pelvic drainage tube. On postoperative day one, peritoneal drainage fluid samples were collected for bacterial culture analysis.

2.3. Follow-up

According to the guidelines provided by the NCCN, adjuvant chemotherapy was administered to all patients who had undergone surgery for T3/T4 or postoperative node-positive tumors. Follow-up appointments were scheduled every 3–6 months within the first three years, which included physical examinations and laboratory tests incorporating tumor biomarkers such as CEA and CA-199. Biannual CT scans of the chest, abdomen, and pelvis were performed, while a complete colonoscopy was planned on an annual basis. The patients were observed at intervals of 6–12 months after the surgery, either through outpatient visits or telephone communication, until the occurrence of CRC recurrence and metastasis or October 01, 2023. The main goals of this study were to assess the long-term outcomes of overall survival (OS) and disease-free survival (DFS) over a period of five years. This approach is in line with the stringent standards expected by Nature journal for scholarly writing.

2.4. Statistical analysis

The statistical data were processed using SPSS software version 27.0 for Windows (IBM Corp., Armonk, NY, United States). Quantitative variables were analyzed utilizing the Student's *t*-test and expressed as mean ± standard deviation (SD). Categorical variables were presented as a percentage (%) and compared employing Pearson's Chi-Square (χ^2) test or Fisher's exact test when appropriate. The Kaplan–Meier method was employed to calculate survival outcomes of patients in both groups, and differences in survival curves (OS and DFS) were compared through the log-rank test. A significance level of $P < 0.05$ was considered statistically significant according to established conventions.

3. Results

3.1. The clinical characteristics of the participants

The CLS group comprised a total of 39 males and 30 females, with an average age of 60.7 ± 11.4 years. Similarly, the NOSE group consisted of 28 males and 24 females, with an average age of 62.2 ± 10.0 years. There were no statistically significant differences in clinical characteristics between the NOSE and CLS groups, including age, gender, BMI, history of abdominal operations, and metastasis (TNM) stages ($P > 0.05$; Table 1).

3.2. Perioperative outcomes

No conversions to open surgery were observed, and there were no incidences of incision infection. When comparing NOSE with CLS group, significant differences were noted in the effect on operation time (213.9 ± 20.0 min vs. 194.1 ± 20.6 min, $t = 5.292$, $p < 0.01$) and total incision length (7.0 ± 0.0 cm vs. 11.7 ± 0.8 cm, $t = -12.435$, $p < 0.01$). However, the differences between the groups regarding positive rate of bacterial culture (15.4% vs. 8.7%, $\chi^2 = 1.297$, $p = 0.255$) and intraabdominal infections (9.6% vs. 2.9%, $\chi^2 = 2.455$, $P = 0.117$) did not reach statistical significance. Eight patients in the NOSE group tested positive for bacterial culture; among them, five patients had escherichia coli cultured from drainage fluid. Six patients in the CLS group tested positive for bacterial culture and five patients had escherichia coli cultured from drainage fluid as shown in Table 2.

3.3. Survival analysis

The median follow-up period was 64.0 months (range, 14–68). Throughout the entire follow-up duration, a total of 14 out of the initial cohort of 121 patients succumbed to mortality, while an additional 17 patients experienced either local recurrence or distant metastasis. Notably, there was no statistically significant disparity observed in terms of tumor recurrence between the NOSE group and the CLS group. Within the NOSE group specifically, one patient exhibited local recurrence and five patients encountered distant recurrence following a median follow-up period of 64 months (range, 23–68). Conversely, within the CLS group, one patient demonstrated local recurrence and ten patients manifested distant recurrence after being monitored for a median follow-up duration of 63 months (range, 14–68). Only one patient in the NOSE group experienced recurrence at the anastomotic site. The Kaplan curves revealed that the overall survival ($p = 0.531$) and disease-free survival ($p = 0.460$) of the NOSE group were comparable to those of the CLS group. In the NOSS group, the 5-year overall survival rate was 90.4% and disease-free survival rate was 88.5%, while in the CLS group, these rates were slightly lower at 87.0% and 84.1%, respectively (Figures 2, 3).

4. Discussion

In recent years, technological advancements and innovations in surgical instruments have facilitated the performance of surgeries with reduced incisions (12, 13). However, conventional laparoscopic colorectal cancer surgery inevitably entails a longer auxiliary incision for specimen extraction and reconstruction of the digestive tract. Compared to CLS, the key distinguishing feature of NOSE in colorectal surgery lies in its ability to extract specimens through natural orifices, perform complete intraperitoneal anastomosis, and avoid lengthy abdominal incisions (14–16). Patients undergoing NOSE experience enhanced pain management and reduced incidence of incision infections, among other notable benefits.

TABLE 1 Patient characteristics.

Clinical characteristics	NOSE group ($n = 52$)	CLS group ($n = 69$)	t/χ^2	P
Age (years)	62.2 ± 10.0	60.7 ± 11.4	0.747	0.457
Gender			0.086	0.769
Male	28	39		
Female	24	30		
BMI (kg/m^2)	22.7 ± 3.2	21.9 ± 3.4	1.468	0.145
Abdominal operation history			0.268	0.605
Presence	6	6		
Absence	46	63		
TNM stages			5.651	0.130
0	7	2		
I	11	13		
II	17	23		
III	17	31		

Data are shown as mean ± SD or *n*.

TABLE 2 Operative and postoperative outcomes.

Perioperative outcomes	NOSE group ($n = 52$)	CLS group ($n = 69$)	t/χ^2	P
Operation time (min)	213.9 ± 20.0	194.1 ± 20.6	5.292	<0.01
Intraoperative blood loss (ml)	32.1 ± 13.3	38.6 ± 15.4	−2.412	0.051
Total incision length (cm)	7.0 ± 0.0	11.7 ± 0.8	−12.435	<0.01
No. of lymph nodes retrieved	18.0 ± 9.5	23.3 ± 8.9	−3.139	0.345
Duration for analgesic (days)	3.3 ± 0.5	3.42 ± 0.6	−0.592	0.558
Duration for the first postoperative exhaust (days)	2.8 ± 2.1	2.9 ± 2.3	−0.292	0.771
Duration for the first postoperative defecation (days)	4.1 ± 2.9	4.0 ± 2.4	0.130	0.991
Length of postoperative stay in hospital (days)	8.6 ± 6.7	7.8 ± 2.7	0.883	0.256
Postoperative complications (%)	17.4	10.1	1.326	0.250
Positive rate of bacterial culture (%)	15.4	8.7	1.297	0.255
Intraabdominal infection (%)	9.6	2.9	2.455	0.117
Anastomotic leakage (%)	3.8	1.4	0.705	0.401
Reoperation (%)	9.6	8.7	0.030	0.862

Data are shown as mean ± SD or %.

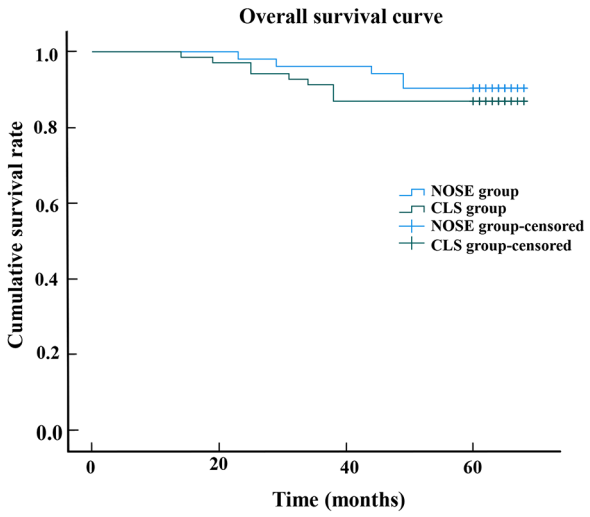

FIGURE 2
The overall survival curve shows that 5-year overall survival rate in the NOSE group and CLS group were 90.4% and 87.0%, respectively. There was no significant difference between the NOSE and CLS groups (p = 09.531).

Numerous studies had conducted comparisons between NOSE and CLS, yielding invaluable insights for the clinical application in colorectal oncology. Studies had demonstrated that NOSE are predominantly conducted using laparoscopic techniques, which offer enhanced precision and obviate the need for lengthy surgical incisions. Consequently, this approach minimized surgical bleeding and did not prolong the duration of the operation (17). Our study demonstrated that the NOSE group exhibited significantly longer surgery times. Clearly, the laparoscopic procedure for NOSE entailed greater complexity, and certain patients undergoing this technique may encounter challenges in extracting specimens due to the narrow rectal cavity and pelvis, consequently leading to prolonged operative duration. Therefore, we propose conducting preoperative assessments for rhinoplasty patients to not only assess tumor dimensions but also evaluate pelvic measurements. As demonstrated by several studies, patients undergoing NOSE exhibit reduced reliance on postoperative analgesia and report lower pain scores (18–20). Additionally, NOSE has been shown to positively impact postoperative intestinal function recovery and lead to a decreased length of hospital stay (19, 21, 22). There may be multiple factors contributing to this outcome: (Ⅰ) The implementation of complete laparoscopic dissection and reconstruction of the digestive tract effectively minimizes excessive traction on the intestinal tract; (Ⅱ) The utilization of smaller incisions resulting in reduced postoperative pain enables patients to regain mobility earlier after surgery. Our study demonstrates comparable postoperative recovery outcomes among patients undergoing NOSE with CLS.

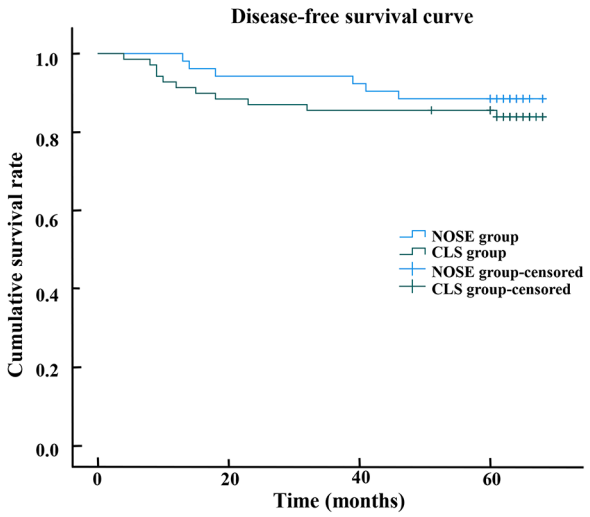

FIGURE 3
The disease-free survival curve shows that 5-year disease-free survival rate in the NOSE group and CLS group were 88.5% and 84.1%, respectively. There was no significant difference between the NOSE and CLS groups (p = 0.460).

The postoperative abdominal or pelvic infection resulting from the dissemination of intestinal bacteria during bowel opening and anvil passage through the anorectum had garnered significant attention. Previous studies had substantiated this potential bacterial contamination following NOSE by assessing the prevalence of positive bacterial culture in intraoperative pelvic fluid (23, 24). Our research findings indicate that the predominant bacterial cultures in abdominal drainage were primarily escherichia coli, as a consequence of the dissemination of bacteria due to intestinal cavity opening. Numerous preventive measures had been implemented to impede the ingress of bacteria into the abdominal cavity, including ensuring meticulous bowel preparation, employing a linear cutter stapler for closure of both the proximal and lower edge of the tumor, irrigating with diluted 1% povidone-iodine prior to opening the rectal stump, and utilizing a sterile protective sleeve. Nevertheless, there remained an increased likelihood for bacterial dissemination through the aperture of the proximal bowel and rectal stump (25). However, it should be noted that not all instances of bacterial spread result in intraabdominal infections, and there were cases where patients with intraabdominal infections did not yield positive results in bacterial culture. Furthermore, our study revealed no significant disparity in celiac infections between the NOSE and CLS groups. Consequently, it is plausible to suggest that intracorporeal bowel opening did not augment the likelihood of abdominal or pelvic contamination. Additionally, patients did not encounter an extension in their hospital stay duration subsequent to receiving appropriate anti-infection treatment.

Another concern of the NOSE pertains to whether intraperitoneal dissection of the tumor bowel, opening of the rectal stump and proximal colon, and transrectal removal of the specimen result in exfoliation of cancer cells, potentially leading to recurrence in the abdominal and rectal stump. However, conclusive evidence regarding this matter is still lacking as only a

limited number of studies have conducted comprehensive five-year survival analyses. The fundamental principle of tumor surgery is to achieve maximal resection, and the adoption of NOSE does not pose additional challenges in achieving complete tumor removal, particularly during lymph node dissection and mesangial separation. Notably, studies have demonstrated that both NOSE and CLS approaches yield comparable oncological outcomes over follow-up periods (20, 26, 27). Our study findings indicated that patients in the NOSE group demonstrated improved disease-free survival and overall survival outcomes compared to those in the CLS group; however, these differences did not reach statistical significance. We propose that this result may be attributed to limitations associated with CLS. Specifically, the vertical pull-out technique employed for excising diseased tissue through an abdominal incision, along with the compression of the incision protector, potentially increased the risk of tumor cells falling outside the protected area due to gravitational forces. Moreover, it is noteworthy that immediate removal of the incision protector following extraction of diseased tissue from the abdominal cavity was not performed. It was imperative to employ the incision protector for safeguarding the wound during the resection of the proximal colon and placement of the anvil of circular stapling device. However, this inadvertently facilitated tumor cell infiltration into the abdominal cavity, potentially leading to metastasis. In contrast, within the NOSE group, we utilized sterile protective sleeves to facilitate smooth specimen extraction and prevent tumor deposition at the open rectal stump. Subsequently, these sleeves were meticulously removed along with the specimen.

The study has certain limitations, as it did not employ a prospective design. Moreover, due to the restricted sample size, obtaining valid data on specific key findings was unattainable. For instance, no statistically significant difference was detected in bacterial culture results. Peritoneal drainage was only cultured for bacteria on the first day after surgery and was not continuously sampled to prevent potential false-negative results. Regrettably, further investigation into the correlation between bacterial culture in abdominal drainage fluid and intraperitoneal metastasis could not be conducted.

5. Conclusions

The management of surgical complications in CLS is exemplary, with NOSE presenting a sole advantage in terms of incision length albeit at the cost of prolonged operative time. Therefore, NOSE may be deemed appropriate for patients who place high emphasis on postoperative cosmetic outcomes.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Zhangzhou Affiliated Hospital of Fujian Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

ZZ: Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. FK: Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. YY: Data curation, Investigation, Writing – review & editing. YF: Data curation, Investigation, Writing – original draft. KY: Investigation, Writing – original draft. QZ: Investigation, Writing – original draft. MF: Formal Analysis, Writing – original draft. LL: Formal Analysis, Writing – original draft. XX: Formal Analysis, Writing – original draft. SL: Writing – original draft. XS: Writing – original draft. XF: Writing – original draft. BZ: Writing – original draft. YG: Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram L. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Hospital Authority of National Health Commission of the People's Republic of China, Chinese Society of Oncology, Chinese Medical Association. National health commission of China colorectal cancer diagnosis and treatment standards (2023 edition). *Chin J Pract Surg*. (2023) 43(06):602–30. doi: 10.19538/j.cjps.issn1005-2208.2023.06.02

3. National Comprehensive Cancer Network. *Colon cancer (version 2.2023)*. Available at: http://www.nccn.org/patients (Accessed April 25, 2023).

4. Yamaguchi S, Tashiro J, Araki R, Okuda J, Sugihara K. Laparoscopic versus open resection for transverse and descending colon cancer: short-term and long-term outcomes of a multicenter retrospective study of 1,830 patients. *Asian J Endosc Surg*. (2017) 10(3):268–75. doi: 10.1111/ases.12373

5. Kai C, Zhuqing Z, Yunfei Z, Shuangyi R. Comparison of the clinical outcomes of laparoscopic-assisted versus open surgery for colorectal cancer. *Oncol Lett*. (2014) 7 (4):1213–8. doi: 10.3892/ol.2014.1859

6. Seshadri RA, Swaminathan R, Srinivasan A. Laparoscopic versus open surgery for rectal cancer after neoadjuvant chemoradiation: long-term outcomes of a propensity score matched study. *J Surg Oncol*. (2018) 3:506–13. doi: 10.1002/jso.24868

7. Franklin ME. Laparoscopic colonic procedures. *World J Surg*. (1993) 17(1):51–6. doi: 10.1007/BF01655705

8. Guan X, Wang G, Zhou Z, Zhou H, Chen Y, Tang Q, et al. Retrospective study of 718 colorectal neoplasms treated by natural orifice specimen extraction surgery in 79 hospitals. *Chin J Colorectal Dis*. (2017) 6(06):469–77. doi: 10.3877/CMA.J.ISSN.2095-3224.2017.06.006

9. Hu Q, Bai JG, Liu Z, Wang XS. Key points and clinical evidence of standardized "aseptic tumor free" specimen extraction through natural cavity passage. *Colorectal Anal Surg*. (2022) 28(5):432–7. doi: 10.19668/j.cnki,issn1674-0491.2022.05.002

10. Liu RJ, Zhang CD, Fan YC, Pei JP, Zhang C, Dai DQ, et al. Safety and oncological outcomes of laparoscopic NOSE surgery compared with conventional laparoscopic surgery for colorectal diseases: a meta-analysis. *Front Oncol*. (2019) 9:597. doi: 10.3389/fonc.2019.00597

11. Guan X, Liu Z, Parvaiz A, Longo A, Saklani AA, Cai JC, et al. International consensus on natural orifice specimen extraction surgery (NOSES) for gastric cancer (2019). *Gastroenterol Rep*. (2020) 8(1):5–10. doi: 10.1093/gastro/goz067

12. Athanasiou CD, Robinson J, Yiasemidou M, Lockwood S, Markides GA. Laparoscopic vs open approach for transverse colon cancer. A systematic review and meta-analysis of short and long term outcomes. *Int J Surg*. (2017) 41:78–85. doi: 10.1016/j.ijsu.2017.03.050

13. Reza MM, Blasco JA, Andradas E, Cantero R, Mayol J. Systematic review of laparoscopic versus open surgery for colorectal cancer. *Br J Surg*. (2006) 93 (8):921–28. doi: 10.1002/bjs.5430

14. Franklin ME, Liang S, Russek K. Natural orifice specimen extraction in laparoscopic colorectal surgery: transanal and transvaginal approaches. *Tech Coloproctol*. (2013) 17(S1):S63–7. doi: 0.1007/s10151-012-0938-y

15. Akamatsu H, Omori T, Oyama T, Tori M, Ueshima S, Nakahara M, et al. Totally laparoscopic sigmoid colectomy: a simple and safe technique for intracorporeal anastomosis. *Surg Endosc*. (2009) 23(11):2605–9. doi: 10.1007/s00464-009-0406-6

16. Awad ZT, Qureshi I, Seibel B, Sharma S, Dobbertien MA. Laparoscopic right hemicolectomy with transvaginal colon extraction using a laparoscopic posterior colpotomy: a 2-year series from a single institution. *Surg Laparosc Endosc Percutan Tech*. (2011) 21(6):403–8. doi: 10.1097/SLE.0b013e31823945ac

17. Ng HI, Sun WQ, Zhao XM, Jin L, Shen XX, Zhang ZT, et al. Outcomes of trans-anal natural orifice specimen extraction combined with laparoscopic anterior resection for sigmoid and rectal carcinoma: an observational study. *Medicine (Baltimore)*. (2018) 97(38):e12347. doi: 10.1097/MD.0000000000012347

18. Mingguang Z, Xiyue H, Xu G, Wei Z, Zheng L, Zheng J, et al. Surgical outcomes and sexual function after laparoscopic colon cancer surgery with transvaginal versus conventional specimen extraction: a retrospective propensity score matched cohort study. *Int J Surg*. (2022) 104:106787. doi: 10.1016/J.IJSU.2022.106787

19. Fuqiang Z, Wei Z, Tixian X, Zhijie W, Fei H, Wei X, et al. Evaluating short-term and survival outcomes of natural orifice specimen extraction surgery for colorectal cancer: a single-centre retrospective study. *Front Surg*. (2023) 10:1078316. doi: 10.3389/FSURG.2023.1078316

20. Zhou S, Wang X, Zhao C, Pei W, Zhou H, Liu Q, et al. Comparison of short-term and survival outcomes for transanal natural orifice specimen extraction with conventional mini-laparotomy after laparoscopic anterior resection for colorectal cancer. *Cancer Manag Res*. (2019) 11:5939–48. doi: 10.2147/CMAR.S209194

21. Awad ZT, Griffin R. Laparoscopic right hemicolectomy: a comparison of natural orifice vs. transabdominal specimen extraction. *Surg Endosc*. (2014) 28(10):2871–6. doi: 10.1007/s00464-014-3540-8

22. Weiwei W, Feipeng X, Feng G, Meixia L, Junjun L, Qidong L, et al. Short-term clinical effects and inflammatory response of natural orifice specimen extraction surgery versus conventional laparoscopic-assisted surgery for the treatment of sigmoid and rectal cancer. *J Gastrointest Oncol*. (2023) 14(2):815–23. doi: 10.21037/JGO-23-144

23. Costantino FA, Diana M, Wall J, Leroy J, Mutter D, Marescaux J. Prospective evaluation of peritoneal fluid contamination following transabdominal vs. transanal specimen extraction in laparoscopic left-sided colorectal resections. *Surg Endosc*. (2012) 26(6):1495–500. doi: 10.1007/s00464-011-2066-6

24. Ngu J, Wong ASY. Transanal natural orifice specimen extraction in colorectal surgery: bacteriological and oncological concerns. *Aust N Z J Surg*. (2016) 86 (4):299–302. doi: 10.1111/ans.13383

25. Shan M, YiBo G, Xu G, Tang QC, Shao F, Guiyu W, et al. Laparoscopic natural orifice specimen extraction, a minimally invasive surgical technique for mid-rectal cancers: retrospective single-center analysis and single-surgeon experience of selected patients. *J IntMed Res*. (2022) 50(11):1–14. doi: 10.1177/03000605221134472

26. Wang S, Tang J, Sun W, Yao H, Li Z. The natural orifice specimen extraction surgery compared with conventional laparoscopy for colorectal cancer: a meta-analysis of efficacy and long-term oncological outcomes. *Int J Surg*. (2022) 97:106196. doi: 10.1016/j.ijsu.2021.106196

27. Seok PJ, Hyun K, Yeun PS, Jin KH, Taek LI, Choi GS, et al. Long-term outcomes after natural orifice specimen extraction versus conventional laparoscopy-assisted surgery for rectal cancer: a matched case-control study. *Ann Surg Treat Res*. (2018) 94(1):26–35. doi: 10.4174/astr.2018.94.1.26

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Analysis of definitive chemo-radiation outcomes in anal cancer: insights from a tertiary cancer center in the MENA Region

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Background: Outcomes of chemo-radiation (CRT) for anal cancer in Middle East and North Africa (MENA) are scarce. We aim to report treatment outcomes for anal cancer treated at tertiary cancer center, with a particular focus on patients managed with non-oncological surgery prior definitive CRT.

Methods: We conducted a retrospective review of patients diagnosed with locally advanced anal carcinoma, who underwent definitive CRT King Hussein Cancer Center, from January 2007 till January 2020. Patient demographics and disease characteristics were extracted, and a univariate chi-squared test was employed to assess the impact of chemotherapy type, HPV status, and pre-treatment non-oncological surgery on outcomes, including complete remission (CR), disease-free survival (DFS), and overall survival (OS). Kaplan–Meier tests were employed to analyze the obtained survival data.

Results: Among the 34 initially identified patients, 30 were eligible, 24 (80%) achieved CR. Notably, 20 out of 21 HPV positive patients achieved CR, versus 1 out 4 HPV-negative achieved CR, p=0.006The 5-years OS for HPV-positive patients was 89% compared with 25% for HPV-negative, p=0001. There was no statistical significant difference in patients outcomes as regard type of chemotherapy, radiation technique and non-oncologic resection prior to CRT.

Conclusion: Herein, we reported the first series of anal cancer from our region. CRT had yielded an oncologic outcome comparable with series in the literature. HPV-positive patients demonstrated better results. Moreover, we found non-oncologic resection prior to CRT did not seem to impact the outcomes. Further studies are warranted to overcome the limitations of our study.

KEYWORDS

anal cancer, chemoradiation, hemorrhoidectomy, MENA (Middle East and North Africa), overall survival

Introduction

Anal cancer is a rare malignancy of the gastrointestinal (GI) tract, comprising approximately 1-2% of all GI cancers. The predominant type is squamous cell carcinoma (SCC), with a higher incidence observed in individuals aged 55-64 years. Additionally, it is more prevalent in female gender (1).

The incidence of SCC has remained steady in certain Asian countries such as India, Japan, and Singapore. An analysis, covering the years 1989 to 2007, demonstrated a relatively stable number of new cases over that period (2). On the other hand, the incidence of anal carcinoma in Middle East and North Africa (MENA) remains lower than other regions in the world (3).

In Jordan, anal cancer constitutes 0.2% of all newly diagnosed malignancies, as per the national cancer registry 2016 (4), which is lower than the global incidence of 0.5%, according to Surveillance, Epidemiology, and End Results (SEER) data (5).

Human papillomavirus (HPV) is the primary risk factor for anal cancer. Other contributing factors include immune deficiency which is linked to Human Immunodeficiency Virus (HIV) infection and immunosuppressiv medications, as well as sexually transmitted diseases, chronic inflammation, fistulas, and tobacco use (6).

A growing body of evidence suggests that oncogenic strains of HPV, particularly subtypes 16 and 18, play a role in the development of SCC in various anatomical sites beyond the anal canal, including cervical and head and neck cancers. In a study conducted by Frisch and colleagues, HPV DNA was identified in 88% of patients with anal cancer, HPV subtype 16 was implicated in 73% of the cases of anal cancer (7).

HPV infection has been linked to sexual practice. Which includes having first intercourse at earlier age, a greater number of male sexual partners, and engaging in receptive anal intercourse (8). These practices have increased in recent years, especially in high-income countries (9). This may explain why the incidence in Asia and MENA region is lower (10).

Hemorrhoids are not widely acknowledged as a risk factor for anal cancer. Nevertheless, persistent irritation associated with long-term hemorrhoids could potentially lead to the development of squamous cell carcinomas. Consequently, it is advisable to conduct histopathologic examinations of hemorrhoidectomies specimens (11). Moreover, anal carcinom usually presents with rectal bleeding and sensation of a mass, which mimic symptoms associated with benign anal conditions like perianal hemorrhoids and fissures. This fact may delay the diagnosis and treatment in many patients (12, 13).

Anal cancer treatment has evolvedsignificantly over the past three decades. Organ-preserving chemoradiation therapy (CRT) has been established asa standard of care for locally advanced diseaseinstead of abdominoperineal resection (14). This approach is formed of concurrent 5-fluorouracil (5-FU) or capecitabine and mitomycin C (MMC) with definitive radiation therapy. Chemoradiation offers a high level of disease control and successfully preserves the anal sphincter (15, 16).

Data on the outcomes of anal cancer from our region remains scarce. Herein, we report a series of patients treated at a tertiary cancer center, under the umbrella of a multidisciplinary clinic. We investigated patients and disease characteristics, and explored those who underwent non-oncologic resection before treatment.

Materials and methods

Patients' population

We conducted a retrospective review of medical records for patients diagnosed with anal carcinoma and treated with definitive CRT at our institution from January 2007 to January 2020. Patients with metastatic disease, and those who received part of their CRT outside our center were excluded. Diagnosis was established through physical examinations, CT scan for the chest, abdomen, and pelvis, pelvic MRI, and colonoscopy with tumor biopsy. Pathology specimens from anal mass biopsies or hemorrhoidectomies were reviewed by a dedicated gastrointestinal pathologis.

Chemoradiation

Management of all patients with locally advanced anal carcinoma were discussed at the institutional multidisciplinary board. Before 2013, radiation therapy utilized a conventional 3D conformal planning based on RTOG 9811 protocol, involving pelvic radiotherapy at 36 Gray (Gy) with a sequential boost to the primary disease and involved nodes up to 59 Gy (17). The introduction of Intensity Modulated Radiation Therapy (IMRT) marked a shift, with anal carcinoma patients being offered IMRT. The dose and target volume definition adhered to the RTOG 0529 protocol, utilizing dose-painted IMRT to mitigate grade 2 or more acute gastrointestinal and genitourinary toxicities. As per RTOG 0529, the dose to the primary tumor and lymph nodes was stage-dependent, and delivery was achieved using the simultaneous boost (SIB) dose painting technique. For T2N0 tumors, the primary PTV received a dose of 50.4 Gy over 28 fractions, with an elective nodal dose of 42 Gy. In the case of T3-T4 N0 tumors, the primary PTV was prescribed a dose of 54 Gy, while the nodal PTV was given 45 Gy over 30 fractions SIB (18).

In cases of node-positive disease, irrespective of the T stage, the administered dose wascontingent upon the size of the affected lymph nodes. Lymph nodes measuring 3 cm or less are prescribed 54 Gy for the tumor and 50.4 Gy for the nodal planning target volume (PTV) over 30 fractions using the simultaneous boost (SIB) technique. Conversely, when dealing with lymph nodes larger than 3 cm, a dose of 54 Gy was delivered to both the primary tumor and nodal PTVs over 30 fractions using the SIB approach (19).

Chemotherapy was administered using a regimen based on 5-FU/Capecitabine in combination with MMC or Cisplatin. The first regimen consisted of either 5-FU (1000 mg/m2/day) delivered continuously on days 1 to 4 and days 29 to 32, or Capecitabine (825 mg/m2 BID) on the days of radiotherapy, along with MMC (10 mg/m2) on Day 1 and Day 29. On the other hand, the second regimen involved the combination of 5-FU/Capecitabine with Cisplatin (75 mg/m2) on day 1 and day 29, maintaining the same

doses for 5-FU or Capecitabine as in the previous protocol with MMC.

Assessment of response involved digital rectal examination, endoscopy, and pelvic MRI. Complete remission (CR) was characterized by the clinical and radiographic evidence confirming the complete disappearance of the tumor.

Statistics

Statistical analyses were conducted using SPSS. The Chi-square test was employed to compare and identify differences between groups in terms of CR and recurrence rates. The primary endpoint, disease-free survival (DFS), was calculated from the date of the initial treatment to the date of death or recurrence (metastasis or local recurrence), while overall survival (OS) was measured from the date of the first treatment to the date of death due to anal cancer, with deaths from other causes considered as censored data. The Kaplan-Meier method was utilized to estimate 5-year DFS and 5-year OS for various groups, and differences in survival outcomes were evaluated using the log-rank test. A significance level of p <0.05 was applied to all analyses.

Results

We identified records of 34 patients, 4 did not meet the inclusion criteria, leaving 30 patients for analysis. The mean age was 57 years (ranging from 32 to 80), 15 (50%) were males and 24 (80%) patients were above the age of 50 years. 21 (70%) were HPV positive, and the test failed in5 samples. Table 1 illustrates patients and disease characteristics.

24 (80%) patients attained CR following definitive CRT. The overall 5-year OS for the entire group was 78%. Notably, 20 out of 21 HPV-positive patients achieved CR, versus 1 out of 4 HPV-negative patients, p=0.006 3 out of 4 HPV negative patients experienced disease recurrence, compared to 3 out of 21 patients in the HPV-positive group, p= 0.03.

The DFS for HPV-negative patients was 10 months,and not reached for HPV-positiv, p= 0.02. As regardOS, HPV-negative patients had a median OS of 17 months, whereas it was not reached for HPV-positive patients. The 5-year OS was 25% for HPV-negative patients compared to 89.6% for HPV-positive patients, p=0.001, as depicted in Figure 1.

10 patients underwent non-oncologic surgery upon diagnosis, with 9 (90%) of them achieving CR compared to 15 (75%) in the remaining 20 cases, p=0.51. The5-year OS for patients who underwent non-oncologic surgery was 73.6%, while for the other group was 88.9%, p=0.32.

For chemotherapy, group 1 comprised 23 patients who received 5-FU/Capecitabine with MMC, 17 of them (73.9%) achieved CR. On the other hand, all the 7 patients in group 2, who received 5-FU/capecitabine with cisplatin, achieved CR, p=0.3. The 5-year DFS was 69% in group 1, compared to 85.7% in group 2, p=0.38. While 5-year OS was 71.6% in group 1 versus 100% in Group 2, p=0.14. as illustrated in Figure 2.

TABLE 1 Patients and disease characteristics.

Characteristics		Number (N)	Percentage (%)
Age (years)	≥50	24	80 %
	<50	6	20 %
Gender	Male	15	50 %
	Female	15	50 %
Smoking	Yes	18	60 %
	No	12	40 %
Stage	I	2	6.7 %
	II	13	43.3 %
	III	15	50 %
Chemotherapy	5 FU/Xeloda + MMC	23	76.7 %
	5 FU/Xeloda + Cisplatin	7	23.3 %
HPV	Positive	21	70 %
	Negative	4	13.3 %
	N/A	5	16.7 %
Radiotherapy Technique	3D	9	30 %
	IMRT	21	70 %
Non-oncologic resection	Yes	10	33.3 %
	No	20	66.7 %

Regarding other patients and disease factors, Table 2 demonstrated the potential impact on CR rate in addition to DFS and OS.

With respect to treatment-related toxicity, skin reaction was the most common in 27 cases (90%) with grade 2 or higher, 5 patients (16.6%) experienced grade 2 diarrhea, and 1 patient (3.3%) developed a rectovaginal fistula.

Discussion

In this retrospective analysis, we explored the clinical outcomes of patients with squamous cell carcinoma of the anal canal. To the best of our knowledge, this is the first report on anal cancer from the MENA region. We reported a 5-years OS of 78%, which aligns with survival rates reportedin the literature (5).

Although CRT is considered a standard treatment for locally advanced anal carcinoma surgery is typically reserved as a salvage option Nevertheless, our cohort demonstrated a distinctive situation, as one third of patients underwent non-oncologic surgery before being referred for chemoradiation. This fact may be attributed to the rarity of the disease and incidental detection of cancer in hemorrhoid specimens.

In large clinical trials, median age at diagnosis was typically above the age 50 years, 80% of patients in our cohort were diagnosed at age more than 50 years (20). Previous reports have indicated that

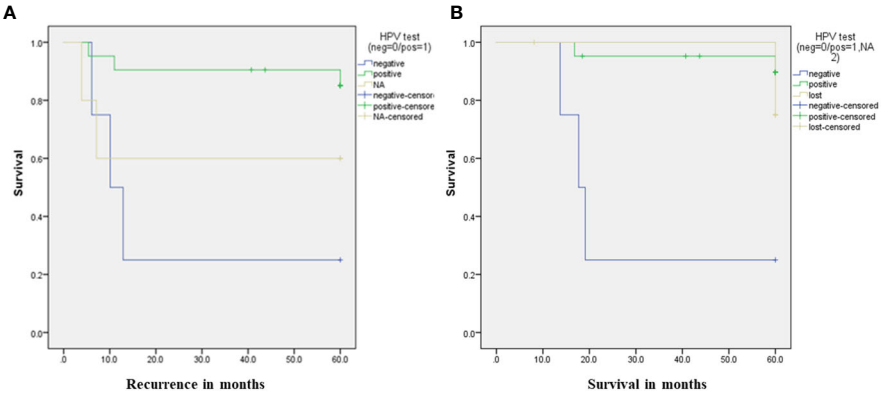

FIGURE 1
(A) Recurrence rate according to HPV status. **(B)** Survival rate according to HPV status.

diagnosis of anal SCC on top of hemorrhoids is not an isolated rare event, it was estimated that up to 4% of anal surgical specimens would uncover malignancy (11). Another trial had explored the risk of anal squamous cell cancer in patients with anal fistulas and fissures. They demonstrated increased risk of cancer in those patients even without presence of irritable bowel disease (21). However, these series did not explore the influence of non-oncologic resection on disease outcomes. In our study, we did not observe difference in oncologic outcomes in patient who underwent surgery before CRT. But we acknowledge that our study might be under-powered to detect the impact of non-oncologic resection.

Some data suggested that patients with HPV-negative tumors exhibited a reduced responsiveness to concurrent CRTand had a higher tendency for relapse (22). This finding came in concordance with our results, but this might be hampered withour small sample size and the fact that the majority of our patients tested positive for HPV.

In a systematic review, that tested the correlation between HPV status and treatment response (23), They found patients with HPV-positive status exhibited superior disease-free survival and overall survival compared to those with HPV-negative disease. This difference is attributed, at least in part, to the more favorable response to CRT in HPV-positive cases. Similarly, another study conducted in Japan, revealed a more favorable response in patients with HPV-positive anal cancer (24). The favorable response in HPV-positive may be related to distinct gene profiles in HPV-positive cancer cells. These differences are frequently identified among genes responsible for DNA regulation and repair, and cellular immune response. These distinctions enhance treatment sensitivity in these patients, especially the radiation effect (25). In our study, we demonstratedsuperior rates of CR, DFS, and OS among patients with HPV-positive status compared with HPV-negative status. Aforementioned, the small number of HPV-negative cases might affect the robustness of our results.

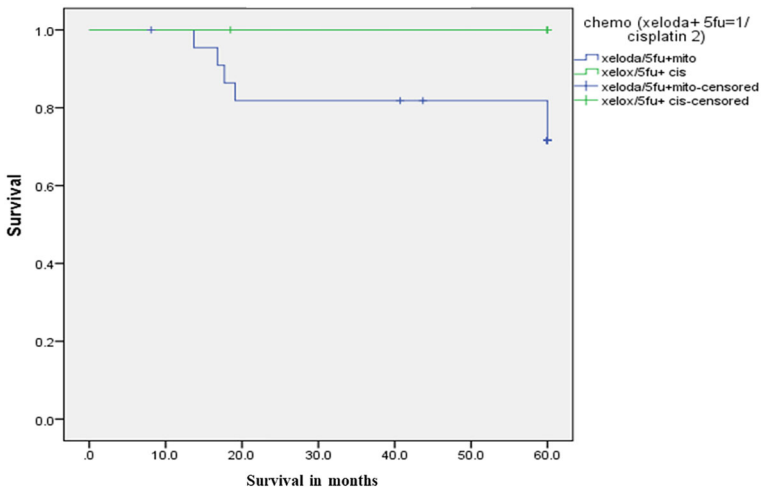

FIGURE 2
Survival curves according to the type of chemotherapy.

TABLE 2 Patient and disease factors in relation to disease outcomes.

Characteristics		pCR	*p* value	DFS	*p* value	OS	*p* value
Age (years)	≥50	79.2 %	0.34	75.0%	0.73	76.5 %	0.79
	<50	83.3 %		66.7%		83.3 %	
Gender	Male	86.7 %	0.54	80.0 %	0.38	85.7 %	0.33
	Female	73.3 %		66.7 %		70.6 %	
Smoking	Yes	73.7 %	0.43	68.0 %	0.66	77.4 %	0.067
	No	90.9 %		81.8 %		78.8 %	
Stage	I	100 %	0.83	100 %	0.26	100 %	0.76
	II	84.6%		84.6%		76.2%	
	III	73.3%		59.3%		77.1%	
Chemotherapy	5 FU/Xeloda + MMC	73.9%	0.3	69.0%	0.38	71.6%	0.14
	5 FU/Xeloda + Cisplatin	100.0%		85.7%		100.0%	
HPV	Positive	95.0%	0.006	85.0%	0.02	89.6%	0.001
	Negative	25.0%		25.0%		25.0%	
Radiotherapy Technique	3D	66.7 %	0.08	66.7 %	0.57	75.0 %	0.83
	IMRT	85.7 %		75.6 %		79.8 %	
Non- oncologic resection	Yes	90.0%	0.51	90.0%	0.15	89.9%	0.32
	No	75.0%		64.2%		73.6%	

In our study, we observed no statistical difference in outcomes based on the type of chemotherapy. A randomized controlled trial by Ajani et al, compared recurrence rates between MMC-5FU and cisplatin5FU, the recurrence rate with MMC was 25% and 33% in the cisplatin group, but the difference between the groups was not statistically significant, (16). In our series, we found comparable results, with a 30.4% local disease recurrence in the MMC group compared to 14.3% in the cisplatin group.

In the ACT-II trial, (26) which assessed the complete response rate between the MMC and cisplatin groups with radiation therapy, including or excluding maintenance treatment, there was no significant difference between the two groups. The complete response rates were 89.6% in the cisplatin arm and 90.5% in the MMC arm, p=0.64. Noteworthy, there was nomaintenance chemotherapy after CRT in our cohort.

A study by O'Brien and colleagues compared patients with hemorrhoidal SCC to those with non-hemorrhoidal SCC. They reported a higher proportion of stage I/II in the hemorrhoidal SCC arm, but no significant difference in OSbetween the two groups (27). These findings aligned with our data, indicating that hemorrhoidal surgery did not impact the oncological outcomes of the underlying anal SCC. Furthermore, It is important to recognize that local excision alone is acceptable treatment for early-stage anal cancer (28).

The emergence of immunotherapy in the treatment of anal cancers is promising. Recent studies have demonstrated encouraging results, and further trials in this context are anticipated (29).

We acknowledge that our cohort has multiple limitations, first is the retrospective nature of the study and potential selection bias. Second is the number of patients in our series is relatively small, which restricts the generalizability of the results and reduces statistical power. Third, is the differences in the treatment approach among the patient's groups, which might have influence the outcomes. Nevertheless, this investigation offers the initial set of oncological outcomes for anal cancer in our region. It aims to enhance comprehension of this underreported disease.

Conclusion

This report evaluated the oncological outcomes of patients diagnosed with locally advanced anal carcinoma undergoing CRT. Our findings reveal comparable oncological outcomes to those reported in the literature for patients with anal cancer. Notably, we observed significantly higher rates of CR, DFS, and OS in HPV-positive patients compared to their HPV-negative counterparts. Noteworthy, non-oncologic resection before CRT did not seem to impact oncologic outcomes. Future prospective trials are essential to validate our results and further enhance our understanding of anal cancer.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

TA: Conceptualization, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. RA: Supervision, Writing – original draft, Writing – review & editing, Methodology. IM: Methodology, Writing – review & editing. SA (4th author): Investigation, Writing – original draft. HA: Data curation, Formal analysis, Writing – original draft. RT: Data curation, Investigation, Writing – review & editing. SA (7th author): Conceptualization, Methodology, Writing – original draft. YK: Methodology, Writing – review & editing. FA: Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Daling JR, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, et al. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. *Cancer* (2004) 101(2):270–80. doi: 10.1002/cncr.20365

2. Islami F, Ferlay J, Lortet-Tieulent J, Bray F, Jemal A. International trends in anal cancer incidence rates. *Int J Epidemiol* (2017) 46(3):924–38. doi: 10.1093/ije/dyw276

3. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer.* (2019) 144(8):1941–53. doi: 10.1002/ijc.31937

4. Statistical digest Jordan cancer registry (JCR) cancer incidence in Jordan-2016 non-communicable diseases directorate-MOH. Available at: https://www.moh.gov.jo/ebv4.0/root_storage/ar/eb_list_page/%D8%A7%D9%84%D8%AA%D9%82%D8%B1%D9%8A%D8%B1_%D8%A7%D9%84%D8%B3%D9%86%D9%88%D9%8A_%D9%84%D9%88%D8%A8%D8%A7%D8%A6%D9%8A%D8%A9_%D8%A7%D9%84%D8%B3%D8%B1%D8%B7%D8%A7%D9%86_%D9%84%D9%84%D8%B9%D8%A7%D9%85_2016.pdf.

5. Johnson LG, Madeleine MM, Newcomer LM, Schwartz SM, Daling JR. Anal cancer incidence and survival: The Surveillance, Epidemiology, and End Results experience, 1973–2000. *Cancer* (2004) 101(2):281–8. doi: 10.1002/cncr.20364

6. Eng C, Messick C, Glynne-Jones R. The management and prevention of anal squamous cell carcinoma. *Am Soc Clin Oncol Educ book Am Soc Clin Oncol Annu Meet* (2019) 39(39):216–25. doi: 10.1200/EDBK_237433

7. Nelson VM, Benson AB. Epidemiology of anal canal cancer. *Surg Oncol Clin N Am* (2017) 26(1):9–15. doi: 10.1016/j.soc.2016.07.001

8. Tseng HF, Morgenstern H, Mack TM, Peters RK. Risk factors for anal cancer: results of a population-based case–control study. *Cancer Causes Control* (2003) 14 (9):837–46. doi: 10.1023/B:CACO.0000003837.10664.7f

9. Herbenick D, Reece M, Schick V, Sanders SA, Dodge B, Fortenberry JD. Sexual behavior in the United States: results from a national probability sample of men and women ages 14-94. *J Sex Med* (2010) 7 Suppl 5(SUPPL. 5):255–65. doi: 10.1111/j.1743-6109.2010.02012.x

10. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, De Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis* (2010) 202(12):1789–99. doi: 10.1086/657321

11. Luca N, Valentina A, Federico S, Renato P. Unexpected anal squamous cells carcinoma after open hemorrhoidectomy. *Case Rep Surg* (2015) 2015:1–2. doi: 10.1155/2015/616274

12. Chiu S, Joseph K, Ghosh S, Cornand RM, Schiller D. Reasons for delays in diagnosis of anal cancer and the effect on patient satisfaction. *Can Fam Physician* (2015) 61(11):e509.

13. Barbeiro S, Atalaia-Martins C, Marcos P, Goncalves C, Cotrim I, Vasconcelos H. A case series of anal carcinoma misdiagnosed as idiopathic chronic anal fissure. *GE Port J Gastroenterol* (2017) 24(5):227–31. doi: 10.1159/000452869

14. Glynne-Jones R, Saleem W, Harrison M, Mawdsley S, Hall M. Background and current treatment of squamous cell carcinoma of the anus. *Oncol Ther* (2016) 4(2):135. doi: 10.1007/s40487-016-0024-0

15. Souza KT, Pereira AAL, Araujo RL, Oliveira SCR, Hoff PM, Riechelmann RP. Replacing 5-fluorouracil by capecitabine in localised squamous cell carcinoma of the anal canal: systematic review and meta-analysis. *Ecancermedicalscience* (2016) 10:699. doi: 10.3332/ecancer.2016.699

16. Ajani JA, Winter KA, Gunderson LL, Pedersen J, Benson AB, Thomas CR, et al. Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. *JAMA* (2008) 299 (16):1914–21. doi: 10.1001/jama.299.16.1914

17. Ajani JA, Winter KA, Gunderson LL, Pedersen J, Benson AB, Thomas C, et al. Intergroup RTOG 98–11: A phase III randomized study of 5-fluorouracil (5-FU), mitomycin, and radiotherapy versus 5-fluorouracil, cisplatin and radiotherapy in carcinoma of the anal canal. *J Clin Oncol* (2006) 24(18_suppl):4009–9. doi: 10.1200/jco.2006.24.18_suppl.4009

18. Kachnic LA, Winter K, Myerson RJ, Goodyear MD, Willins J, Esthappan J, et al. RTOG 0529: a phase 2 evaluation of dose-painted intensity modulated radiation therapy in combination with 5-fluorouracil and mitomycin-C for the reduction of acute morbidity in carcinoma of the anal canal. *Int J Radiat Oncol Biol Phys* (2013) 86 (1):27–33. doi: 10.1016/j.ijrobp.2012.09.023

19. Gay HA, Barthold HJ, O'Meara E, Bosch WR, El Naqa I, Al-Lozi R, et al. Pelvic normal tissue contouring guidelines for radiation therapy: a Radiation Therapy Oncology Group consensus panel atlas. *Int J Radiat Oncol Biol Phys* (2012) 83(3): e353-62. doi: 10.1016/j.ijrobp.2012.01.023

20. Foo M, Link E, Leong T, Chu J, Lee MT, Chander S, et al. Impact of advancing age on treatment and outcomes in anal cancer. *Acta Oncol (Madr)* (2014) 53(7):909–16. doi: 10.3109/0284186X.2013.876513

21. Chae W, Kang SY, Jang SI, Han YD. Risk of anorectal cancer associated with benign anal inflammatory diseases: A retrospective matched cohort study. *Int J Environ Res Public Health* (2022) 19(12):7467. doi: 10.3390/ijerph19127467

22. Soares PC, Abdelhay ES, Thuler LCS, Soares BM, Demachki S, Ferro GVR, et al. HPV positive, wild type TP53, and p16 overexpression correlate with the absence of residual tumors after chemoradiotherapy in anal squamous cell carcinoma. *BMC Gastroenterol* (2018) 18(1):30. doi: 10.1186/s12876-018-0758-2

23. Yao JN, Zhang XX, Zhou HN, Le L, Xu HR, CF W, et al. Human papillomavirus related anal squamous cell carcinoma survival: a systematic review and meta-analysis. *Transl Cancer Res* (2017) 6(3):463–73. doi: 10.21037/tcr.2017.06.13

24. Yamada K, Shiraishi K, Takashima A, Takayanagi D, Saiki Y, Takano S, et al. Characteristics of anal canal squamous cell carcinoma as an HPV-associated cancer in Japan. *Int J Clin Oncol* (2023) 28(8):990–8. doi: 10.1007/s10147-023-02339-5

25. Liu C, Mann D, Sinha UK, Kokot NC. The molecular mechanisms of increased radiosensitivity of HPV-positive oropharyngeal squamous cell carcinoma (OPSCC): an extensive review. *J Otolaryngol Head Neck Surg* (2018) 47(1):59. doi: 10.1186/s40463-018-0302-y

26. James RD, Glynne-Jones R, Meadows HM, Cunningham D, Myint AS, Saunders MP, et al. Mitomycin or cisplatin chemoradiation with or without maintenance chemotherapy for treatment of squamous-cell carcinoma of the anus (ACT II): a randomised, phase 3, open-label, 2 × 2 factorial trial. *Lancet Oncol* (2013) 14(6):516–24. doi: 10.1016/S1470-2045(13)70086-X

27. O'Brien SJ, Ellis CT, McDowell J, Galandiuk S, Polk HC. Anal squamous cell carcinoma incidentally found at hemorrhoidectomy. *Surgery* (2021) 169(3):610–6. doi: 10.1016/j.surg.2020.08.026

28. Chai CY, Cao HT, Awad S, Massarweh NN. Management of stage I squamous cell carcinoma of the anal canal. *JAMA Surg* (2018) 153(3):209. doi: 10.1001/jamasurg.2017.3151

29. Ciardiello D, Pio Guerrera L, Anna Maiorano B, Parente P, Pia Latiano T, Di Maio M, et al. Anti-tumour Treatment Immunotherapy in advanced anal cancer: Is the beginning of a new era? *Cancer Treat Rev* (2022) 105:102373. doi: 10.1016/j.ctrv.2022.102373

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Optimal treatment strategy and prognostic analysis for patients with non-metastatic pT4 colon adenocarcinoma

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Objective: This study endeavored to explore the optimal treatment strategy and conduct a prognostic analysis for patients diagnosed with pT4M0 (pathologic stage T4) colon adenocarcinoma (COAD).

Methods and materials: A total of 8,843 patients diagnosed with pT4M0 COAD between January 2010 and December 2015 were included in this study from the Surveillance, Epidemiology, and End Results (SEER) database. These patients were randomly divided into a training set and an internal validation set using a 7:3 ratio. Variables that demonstrated statistical significance (P<0.05) in univariate COX regression analysis or held clinical significance were incorporated into the multivariate COX regression model. Subsequently, this model was utilized to formulate a nomogram. The predictive accuracy and discriminability of the nomogram were assessed using the C-index, area under the curve (AUC), and calibration curves. Decision curve analysis (DCA) was conducted to confirm the clinical validity of the model.

Results: In the entire SEER cohort, the 3-year overall survival (OS) rate (74.22% vs. 63.20%, P<0.001) and the 3-year cancer-specific survival (CSS) rate (76.25% vs. 66.98%, P<0.001) in the surgery combined with postoperative adjuvant therapy (S+ADT) group surpassed those in the surgery (S) group. Multivariate COX regression analysis of the training set unveiled correlations between age, race, N stage, serum CEA (carcinoembryonic antigen), differentiation, number of resected lymph nodes, and treatment modalities with OS and CSS. Nomograms for OS and CSS were meticulously crafted based on these variables, achieving C-indexes of 0.692 and 0.690 in the training set, respectively. The robust predictive ability of the nomogram was further affirmed through receiver operating characteristic (ROC) and calibration curves in both the training and validation sets.

Conclusion: In individuals diagnosed with pT4M0 COAD, the integration of surgery with adjuvant chemoradiotherapy demonstrated a substantial extension of long-term survival. The nomogram, which incorporated key factors such as age, race, differentiation, N stage, serum CEA level, tumor

size, and the number of resected lymph nodes, stood as a dependable tool for predicting OS and CSS rates. This predictive model held promise in aiding clinicians by identifying high-risk patients and facilitating the development of personalized treatment plans.

KEYWORDS

pT4M0 colon cancer, adenocarcinoma, prognosis, model, treatment modality

1 Introduction

Colon adenocarcinoma (COAD) ranks among the most prevalent malignant tumors of the digestive system. Global statistics from 2018 reveal an alarming incidence, with over 1.8 million new cases of colon cancer reported, constituting 10.2% of all cancer cases. This malignancy, standing as the second most common cancer worldwide, follows closely behind lung and breast cancer. Furthermore, the associated mortality figures are equally concerning, with over 840,000 deaths attributed to colon cancer, accounting for 9.2% of all cancer-related deaths (1).

In the secondary analysis of global cancer statistics for 2020, colon cancer maintains its significant impact, ranking second in incidence and fifth in mortality within China (2). Despite advancements in systemic therapy that have contributed to a decline in the incidence of distant metastasis in colon cancer, postoperative local recurrence rates remain notable, ranging between 10% and 40% (3). This underscores the imperative for effective postoperative local treatments, such as radiotherapy (4, 5). Traditionally, adjuvant radiotherapy is not routinely recommended for COAD and is typically reserved for specific clinical scenarios, including locally advanced disease (pT4) and/or positive margins (6). However, due to the limited utilization of radiotherapy in clinical practice and the scarcity of comprehensive clinical trials, the therapeutic efficacy of radiotherapy in COAD remains uncertain (7).

Recent updates in the 2020 NCCN guidelines mark a subtle expansion in the indications for adjuvant radiotherapy. Remarkably, individuals with a confirmed postoperative T4 stage with fixation are currently being contemplated for radiotherapy, albeit with a class II recommendation. Nevertheless, the influence of adjuvant radiotherapy on the overall prognosis of patients with COAD remains elusive.

In light of this, our retrospective study utilized the Surveillance, Epidemiology, and End Results (SEER) database to analyze the survival outcomes of patients with pT4M0 COAD who underwent various treatment modalities. The study aimed to elucidate prognostic factors influencing the outcomes of these patients and, subsequently, construct and validate nomograms to predict overall survival (OS) and cancer-specific survival (CSS).

2 Materials and methods

2.1 Study population

The inclusion criteria for patients in the SEER database search were as follows (1): patients diagnosed with COAD as their initial malignancy between January 2010 and December 2015 (2); T4 and M0 staging according to the American Joint Committee on Cancer 7th Edition Staging System (3); definitive cause of death and treatment details, including initial surgery or external radiotherapy, with or without chemotherapy; and (4) survival time of at least 1 month. Ultimately, this study included 8,843 patients with stage pT4M0 COAD. All treatments, including surgery, radiotherapy, and chemotherapy, were administered as the initial treatment upon diagnosis.

2.2 Treatment groups

All patients underwent surgery. Patients exclusively undergoing surgery were included in the S group. Patients receiving postoperative adjuvant radiotherapy were included in the S+R group. Those receiving postoperative adjuvant chemotherapy were included in the S+C group. Patients undergoing postoperative adjuvant chemoradiotherapy were included in the S +R+C group. For subsequent analysis convenience, the S+R group, S+C group, and S+R+C group were combined into the surgery with the postoperative adjuvant therapy (S+ADT) group.

2.3 Statistical analysis

The study delineated OS as the duration from randomization to death from any cause. CSS was specifically defined as the duration from randomization to death caused by COAD. OS was designated as the primary endpoint, with CSS serving as the secondary endpoint.

The statistical analysis was performed using SPSS 26.0 and R-Software 4.1.2, and graphs were generated through R packages, including 'survival,' 'timeROC,' 'ggDCA,' 'dplyr,' and 'rms.'

Pearson's chi-square test was employed to compare the characteristics of different treatment groups. Univariate and multivariate analyses utilized COX proportional hazards models to assess and compare the prognostic significance of clinicopathologic variables on OS and CSS rates.

The Kaplan-Meier method, followed by a log-rank test, was employed to analyze survival curves. Variables found significant in the univariate analysis were included in the multivariate analysis to construct the nomogram. Statistical significance was set at $P < 0.05$.

The accuracy of the nomogram was evaluated using the C-index, receiver operating characteristic (ROC) curve, and calibration curve. Clinical usefulness and benefits were estimated using decision curve analysis (DCA) plots. Additionally, using risk score and X-tile software version 3.6.1 (Yale University, New Haven, CT), patients were stratified into low-, intermediate-, and high-risk groups.

3 Results

3.1 Patient characteristics

We identified 10,041 patients diagnosed with pT4M0 colon cancer between January 2010 and December 2015 from the SEER database. Exclusions were applied to 649 patients with a pathological type other than adenocarcinoma, 491 based on treatment modality, and 59 with a survival period of less than 1 month. This resulted in a final cohort of 8,843 patients for the current analysis. The entire SEER cohort was randomly divided into training and validation sets in a 7:3 ratio, and summarized characteristics are provided in Table 1.

In the total SEER cohort, the median survival for the overall population was 49 (1–119) months, with 1-, 3-, and 5-year OS rates of 86.63%, 68.97%, and 61.19%, respectively. Corresponding CSS rates were 87.89%, 71.83%, and 65.01%. Within the S+C and S+R+C groups, 1-, 3-, and 5-year OS rates were 92.47% vs. 94.56%, 74.41% vs. 73.11%, and 65.16% vs. 64.95%, respectively. For CSS, the rates were 93.08% vs. 95.17%, 76.39% vs. 75.83%, and 61.89% vs. 70.09%. Survival curves for the S+C and S+R+C groups (Figures 1A, C) were similar and superior to those in the S group. Due to limited cases in the S+R and S+R+C groups, they were combined into the S+ADT group. The 1-year OS rate for the S+ADT group was 92.48%, compared to 80.21% in the S group. At 3 years, the rates were 74.22% vs. 63.20%, and at 5 years, 65.06% vs. 56.94%, all significantly better in the S+ADT group ($P<0.01$). The 1-, 3-, and 5-year CSS rates (93.09%, 76.25%, and 68.13%) in the S+ADT group surpassed those in the S group (Figure 1). These findings strongly supported the recommendation of surgery with postoperative adjuvant therapy for pT4M0 COAD.

3.2 Independent prognostic predictors of OS and CSS

Univariate COX regression analysis during training revealed significant influences on OS, including age, race, primary site, degree of differentiation, N stage, serum carcinoembryonic antigen (CEA), tumor size, and number of lymph nodes resected. Similarly, age, race, primary site, gender, degree of differentiation, N stage, serum CEA, tumor size, and the number of lymph nodes resected were associated with CSS (Table 2). In a multifactorial COX regression analysis adjusting for covariates, independent predictors of both OS and CSS included age, race, differentiation grade, N stage, serum CEA, tumor size, and the number of resected lymph nodes (Table 3).

3.3 Construction and validation of the nomogram

Nomograms for OS and CSS were developed using independent predictors identified through multifactorial COX regression analysis in the training set (Figure 2). The nomograms highlighted race as the most significant factor impacting OS, followed by N stage, treatment modality, number of resected lymph nodes, age, tumor size, serum CEA levels, and degree of differentiation. Similarly, for CSS, race emerged as the foremost influential factor, succeeded by N stage, number of resected lymph nodes, age, tumor size, treatment modality, degree of differentiation, and serum CEA levels. The R2 values for the OS and CSS models were 0.148 and 0.134, respectively (Supplementary Table 4).

The C-index values for predicting OS and CSS in the training set were 0.692 and 0.690, respectively. In the internal validation set, these values improved to 0.703 and 0.708, respectively (Figure 3), indicating commendable accuracy. The area under the curve (AUC) for 1-, 3-, and 5-year OS in the training set was 0.78, 0.74, and 0.72, respectively. In the validation set, these figures were 0.80, 0.75, and 0.73, respectively. As for CSS, the AUC for 1-, 3-, and 5-year OS in the training set was 0.78, 0.73, and 0.72, respectively. In the validation set, these AUCs were 0.80, 0.76, and 0.74, respectively.

Calibration curves for predicting 1-, 3-, and 5-year OS and CSS exhibited no deviation from the 45-degree diagonal lines in both the training set and the validation set (Figures 4, 5), signifying a high level of agreement between predicted and observed outcomes. Clinical DCA confirmed the robust clinical applicability of the nomograms in predicting 1-, 3-, and 5-year OS and CSS in both the training set (Figure 6) and the validation set (Figure 7).

In conclusion, risk scores, computed through the nomogram, facilitated effective risk stratification. Patients were stratified into three risk subgroups based on cutoff values determined by X-tile software. For the OS nomogram, patients fell into low risk (points ≤ 176.28), intermediate risk (176.28 < points ≤ 236.60), and high risk (points > 236.60) categories. Similarly, the CSS nomogram classified patients into three risk categories: low risk (points ≤ 178.67), intermediate risk (178.67 < points ≤ 245.69), and high risk (points > 245.69). Kaplan-Meier survival curves depicted distinct differentiation among the various risk subgroups (Figure 8).

3.4 Subgroup analysis

As depicted in Figure 9, Kaplan-Meier analysis of the treatment group within the pT4aM0 subgroup revealed a more favorable

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TABLE 1 Clinical data of the study subjects.

	Overall	Training set	Validation set	P
	N=8843 NO.(%)	N=6190 NO.(%)	N=2653 NO.(%)	
Age (%)				
<50	1049 (11.86)	729 (11.78)	320 (12.06)	0.577
50~75	3119 (35.27)	2166 (34.99)	953 (35.92)	
≥75	4675 (52.87)	3295 (53.23)	1380 (52.02)	
Sex (%)				
Female	4500 (50.89)	3156 (50.99)	1344 (50.66)	0.797
Male	4343 (49.11)	3034 (49.01)	1309 (49.34)	
Race (%)				
White	6937 (78.45)	4878 (78.80)	2059 (77.61)	0.619
Black	909 (10.28)	622 (10.05)	287 (10.82)	
Other	973 (11.00)	674 (10.89)	299 (11.27)	
Unknown	24 (0.27)	16 (0.26)	8 (0.30)	
Primary site (%)				
Ascending Colon	2130 (24.09)	1495 (24.15)	635 (23.94)	0.987
Hepatic Flexure	547 (6.19)	377 (6.09)	170 (6.41)	
Transverse Colon	1294 (14.63)	901 (14.56)	393 (14.81)	
Splenic Flexure	492 (5.56)	347 (5.61)	145 (5.47)	
Descending Colon	790 (8.93)	546 (8.82)	244 (9.20)	
Descending Colon	3191 (36.09)	2245 (36.27)	946 (35.66)	
Large Intestine	399 (4.51)	279 (4.51)	120 (4.52)	
Grade (%)				
I	417 (4.72)	321 (5.19)	96 (3.62)	0.021
II	5621 (63.56)	3936 (63.59)	1685 (63.51)	
III	2083 (23.56)	1430 (23.10)	653 (24.61)	
IV	458 (5.18)	318 (5.14)	140 (5.28)	
unknow	264 (2.99)	185 (2.99)	79 (2.98)	
NStage (%)				
N0	3766 (42.59)	2628 (42.46)	1138 (42.89)	0.855
N1	2978 (33.68)	2102 (33.96)	876 (33.02)	
N2	2042 (23.09)	1420 (22.94)	622 (23.45)	
NX	57 (0.64)	40 (0.65)	17 (0.64)	
TStage (%)				
T4	38 (0.43)	22 (0.36)	16 (0.60)	0.259
T4a	5526 (62.49)	3867 (62.47)	1659 (62.53)	
T4b	3279 (37.08)	2301 (37.17)	978 (36.86)	
Treatment (%)				
S	4215 (47.66)	2980 (48.14)	1235 (46.55)	0.575

(Continued)

TABLE 1 Continued

	Overall	Training set	Validation set	P
	N=8843 NO.(%)	N=6190 NO.(%)	N=2653 NO.(%)	
S+C	4248 (48.04)	2945 (47.58)	1303 (49.11)	
S+R	49 (0.55)	35 (0.57)	14 (0.53)	
S+R+C	331 (3.74)	230 (3.72)	101 (3.81)	
CEA (%)				
	2579 (29.16)	1808 (29.21)	771 (29.06)	0.974
abnormal	2838 (32.09)	1982 (32.02)	856 (32.27)	
unknown	3426 (38.74)	2400 (38.77)	1026 (38.67)	
Node removed (%)				
normal	1435 (16.23)	1017 (16.43)	418 (15.76)	0.515
abnormal	7363 (83.26)	5139 (83.02)	2224 (83.83)	
unknown	45 (0.51)	34 (0.55)	11 (0.41)	
Size (%)				
<5	3355 (37.94)	2347 (37.92)	1008 (37.99)	0.679
≥5	5154 (58.28)	3602 (58.19)	1552 (58.50)	
unknown	334 (3.78)	241 (3.89)	93 (3.51)	

S, Surgery; S+R, Surgery+Radiotherapy; S+C, Surgery+Chemotherapy; S+R+C, Surgery+Radiotherapy+Chemotherapy.

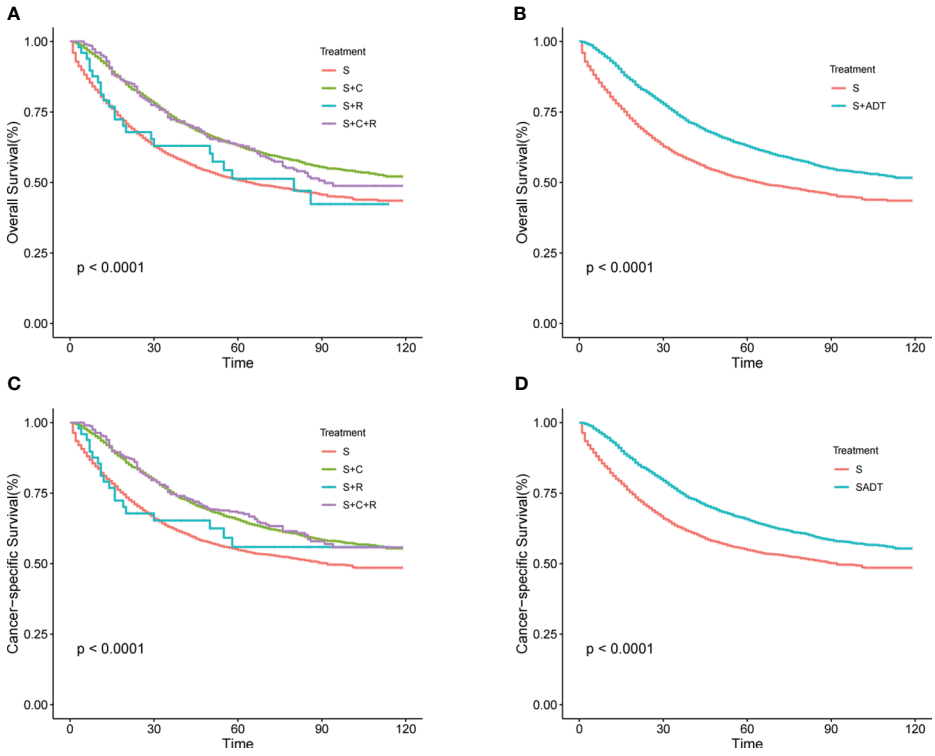

FIGURE 1
(A–D) Survival rates for T4M0 COAD patients grouped by treatment throughout the cohort. **(A)** OS rates between four groups **(B)** OS rates between S and S+ ADT groups **(C)** CSS rates between four groups **(D)** CSS rates between S and S+ADT groups.

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TABLE 2 Univariate COX regression analysis of OS and CSS.

Variable	Univariate COX regression analysis					
	OS			CSS		
	HR	95%CI	P	HR	95%CI	P
Age						
<50						
50~75	1.279	1.114-1.468	0.000	1.253	1.084-1.448	0.002
≥75	2.199	1.911-2.531	0.000	2.176	1.879-2.521	0.000
Race						
White						
Black	1.134	0.945-1.211	0.288	0.798	0.670-0.951	0.012
Other	0.786	0.735-0.951	0.006	0.255	0.064-1.026	0.054
Unknown	0.243	0.062-0.992	0.049	0.921	0.808-1.049	0.215
Sex						
Female						
Male	0.926	0.857-1.000	0.050	0.921	0.849-0.999	0.047
Primary site						
Ascending Colon						
Hepatic Flexure	1.118	0.942-1.325	0.202	1.139	0.952-1.361	0.154
Transverse Colon	0.965	0.847-1.100	0.595	0.970	0.846-1.112	0.662
Splenic Flexure	0.985	0.823-1.180	0.872	0.974	0.805-1.179	0.789
Descending Colon	0.965	0.829-1.122	0.641	0.978	0.835-1.146	0.786
Sigmoid Colon	0.937	0.847-1.037	0.21	0.924	0.83-1.029	0.150
Large Intestine	1.258	1.041-1.519	0.017	1.301	1.069-1.582	0.009
Grade						
I						
II	0.985	0.821-1.181	0.871	1.031	0.849-1.253	0.757
III	1.440	1.191-1.741	0.000	1.49	1.215-1.826	0.000
IV	1.650	1.308-2.083	0.000	1.719	1.342-2.203	0.000
unknown	2.022	1.559-2.621	0.000	2.065	1.564-2.726	0.000
N Stage						
N0						
N1	1.355	1.235-1.488	0.000	1.380	1.250-1.523	0.000
N2	2.059	1.871-2.267	0.000	2.082	1.881-2.305	0.000
NX	5.578	3.938-7.901	0.000	5.677	3.940-8.181	0.000
Treatment						
S						
S+C	0.683	0.631-0.74	0.000	0.709	0.652-0.770	0.000
S+R	1.085	0.672-1.75	0.739	1.081	0.650-1.799	0.764
S+R+C	0.745	0.608-0.913	0.005	0.733	0.589-0.911	0.005

(Continued)

TABLE 2 Continued

Variable	Univariate COX regression analysis					
	OS			CSS		
	HR	95%CI	P	HR	95%CI	P
CEA						
normal						
abnormal	1.591	1.438-1.761	0.000	1.587	1.426-1.767	0.000
unknown	1.431	1.296-1.580	0.000	1.440	1.297-1.599	0.001
Node removed						
<12						
≥12	0.518	0.472-0.568	0.000	0.517	0.468-0.570	0.000
unknown	0.982	0.635-1.518	0.934	0.984	0.622-1.555	0.984
Size						
<5						
≥5	1.068	0.986-1.156	0.012	1.094	1.004-1.191	0.041
unknown	1.914	1.611-2.274	0.000	2.181	1.825-2.606	0.000

TABLE 3 Multivariate COX regression analysis of OS and CSS.

Variable	Multivariate COX regression analysis					
	OS			CSS		
	HR	95%CI	P	HR	95%CI	P
Age						
<50						
50~75	1.189	1.035-1.368	0.015	1.172	1.012-1.356	0.034
≥75	1.932	1.665-2.241	0.000	1.943	1.662-2.272	0.000
Race						
White						
Black	1.108	0.976-1.258	0.114	1.125	0.984-1.286	0.084
Other	0.775	0.681-0.883	0.000	0.805	0.703-0.921	0.002
Unknown	0.216	0.054-0.866	0.031	0.245	0.061-0.985	0.047
Grade						
I						
II	1.003	0.836-1.204	0.973	1.046	0.86-1.272	0.655
III	1.266	1.044-1.535	0.017	1.299	1.056-1.597	0.013
IV	1.507	1.191-1.906	0.001	1.555	1.21-1.997	0.001
unknown	1.361	1.036-1.789	0.027	1.374	1.026-1.839	0.033
N Stage						
N0						
N1	1.600	1.452-1.763	0.000	1.611	1.455-1.875	0.000

(Continued)

TABLE 3 Continued

Variable	Multivariate COX regression analysis					
	OS			CSS		
	HR	95%CI	P	HR	95%CI	P
N2	2.671	2.410-2.960	0.000	2.670	2.394-2.977	0.000
NX	2.311	1.582-3.377	0.000	2.291	1.539-3.41	0.000
Treatment						
S						
S+C	0.666	0.61-0.727	0.000	0.696	0.635-0.764	0.000
S+R	1.246	0.770-2.015	0.370	1.269	0.760-2.117	0.362
S+R+C	0.842	0.684-1.038	0.107	0.844	0.675-1.055	0.136
CEA						
normal						
abnormal	1.526	1.378-1.690	0.000	1.520	1.364-1.693	0.000
unknown	1.310	1.185-1.448	0.000	1.321	1.189-1.469	0.000
node removed						
<12						
≥12	0.505	0.458-0.558	0.000	0.505	0.455-0.56	0.000
unknown	0.720	0.461-1.124	0.148	0.703	0.44-1.123	0.140
Size						
<5						
≥5	1.158	1.065-1.258	0.001	1.145	1.048-1.25	0.003
unknown	1.839	1.515-2.233	0.000	1.907	1.559-2.333	0.000

prognosis for the S+R group. Conversely, in the T4bM0 subgroup, Kaplan-Meier analysis demonstrated that the S+R group exhibited similar OS and CSS rates compared to the S group. This suggested that postoperative radiotherapy might confer greater benefits to patients with pT4aM0 COAD. Single and multiple COX regression analyses for OS and CSS were performed for various variables in the pT4aM0 and pT4bM0 subgroups, with results presented in Supplementary Tables 2A, B and Supplementary Tables 3A, B, respectively. Forest plots were generated to visualize the findings (Supplementary Figures 1, 2). The outcomes indicated that the S+C group derived benefits across all subgroups. However, a significant benefit was observed in the S+R+C group within the pT4bM0 subgroup, while no such benefit was evident in the pT4aM0 subgroup.

4 Discussion

The primary recommended treatment for pT4M0 COAD currently involves surgery followed by adjuvant chemotherapy, with adjuvant radiotherapy not considered a standard approach. Early studies (8, 9) indicating improved local control and disease-free survival (DFS) through radiotherapy in locally advanced COAD date back to the 1980s and 1990s. While many studies suggest enhanced survival with postoperative radiation therapy, the absence of supportive phase III trials has limited its acceptance for COAD. Notably, the only randomized controlled phase III trial has failed to establish a role for adjuvant radiotherapy (10).

The prognostic impact of radiotherapy on COAD remains unclear, and its application is generally discouraged in clinical practice. Owing to the limited availability of clinical data, prior studies in this domain often resort to analyzing information from large public databases such as the National Cancer Database (NCDB) and the SEER databases (11–14).

In this study, we conducted a Kaplan-Meier survival a0alysis for the S group, S+C group, S+R group, and S+R+C group, revealing a significant difference (P<0.05). Notably, the S+R+C group exhibited the highest OS rate and CSS rate. The S+C group demonstrated the second-highest rates, while the S group had the lowest rates. However, survival analysis between the S+C and S+R+C groups did not reveal statistically significant differences. A phase III trial has indicated similar OS and DFS for patients receiving chemotherapy, with higher toxicity observed in those undergoing single chemoradiotherapy (10).

Several studies, have investigated the progression of COAD, suggesting that the critical period for progression often occurs

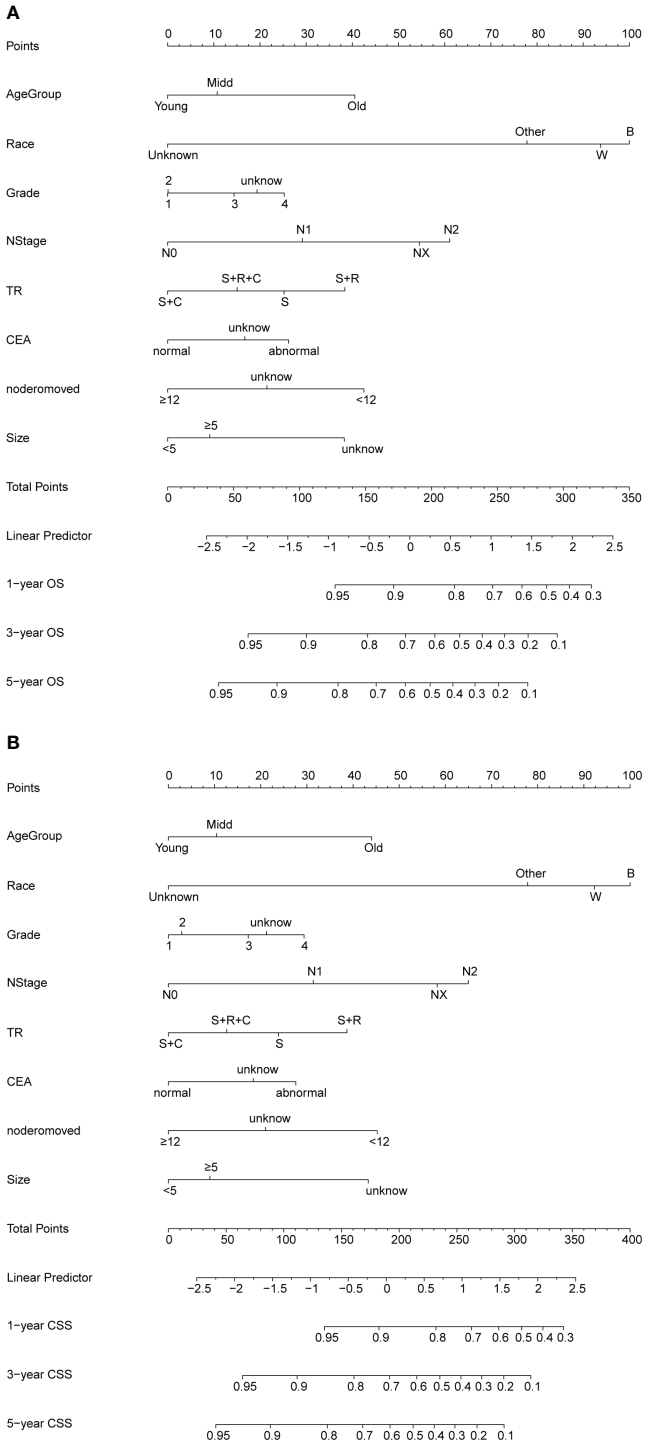

FIGURE 2
(A, B) Nomogram for predicting OS and CSS of patients with PT4M0 COAD. **(A)** Nomogram for predicting 1-, 3-, and 5-year OS; **(B)** Nomogram for predicting 1-, 3-, and 5-year CSS.

within 3 years post-surgery (15, 16). Our study aligned with these findings, revealing a 3-year OS of 68.97% and a 1-year OS of 86.63% for patients with pT4M0 COAD. The 3-year CSS was 71.83%, and the 1-year CSS was 87.89%.

The determinants of prognosis for COAD remain inconclusive, with varied results across studies. Wang et al. (17) have emphasized the significance of the tumor primary site, T stage, and serum CEA level, while Vergara-Fernandez et al. (18) have underscored the importance of the number of resected lymph nodes and nerve invasion. This complexity suggests that the recurrence of COAD metastasis is likely influenced by multiple and intricate factors.

To predict OS and CSS, we constructed nomograms based on multifactorial COX regression analysis, incorporating factors such as age, race, degree of differentiation, N stage, serum CEA levels,

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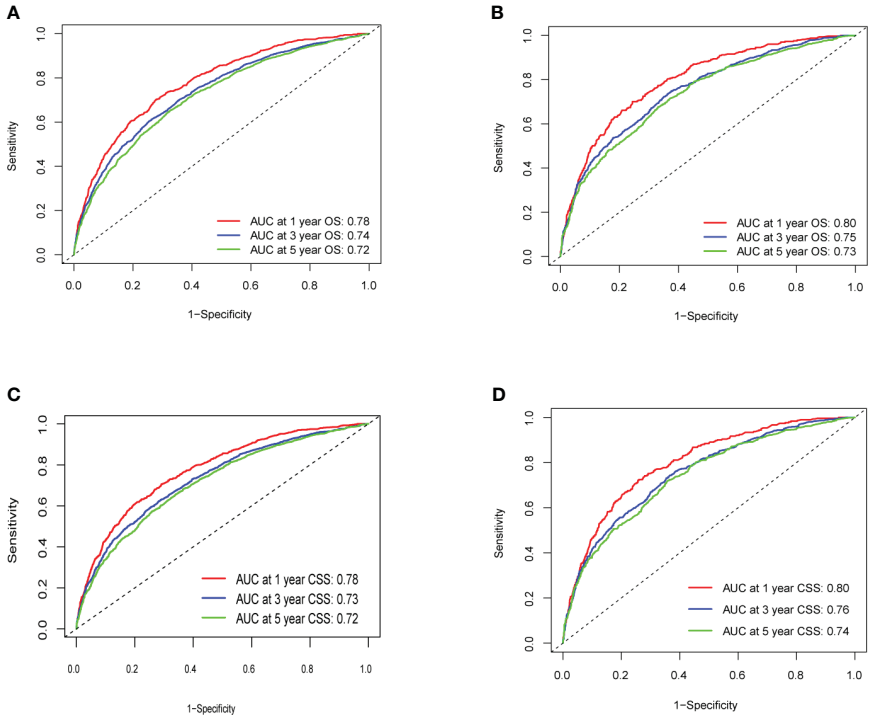

FIGURE 3
(A–D) ROC Curve of OS and CSS of Training Group and Validation Group. **(A)** Training Group OS; **(B)** Validation Group OS; **(C)** Training Group CSS; **(D)** Validation Group CSS.

tumor size, and the number of resected lymph nodes. Validation was performed using calibration curves, ROC curves, and DCA, with further confirmation from an independent validation group.

Our findings revealed that age independently impacted prognosis, with 52.87% of patients aged ≥75 years in the entire SEER cohort. Patients in this age group faced a more than twofold higher risk of death compared to those aged <50 years (HR = 1.943, 95% CI: 1.662-2.272, P < 0.001), likely associated with poorer health status and a higher prevalence of comorbidities, consistent with previous studies (5, 6, 11, 19).

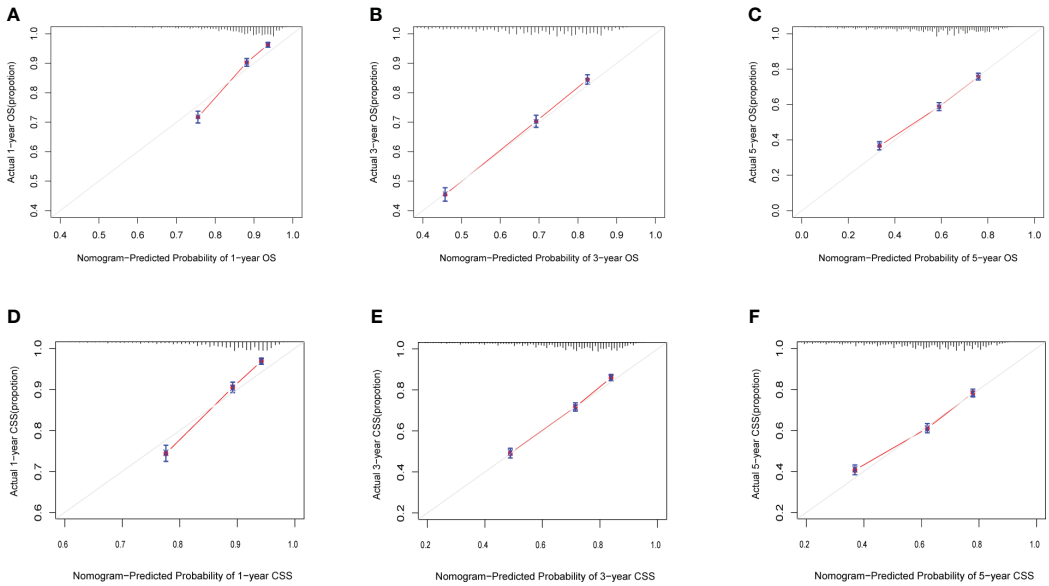

FIGURE 4
(A–F) Calibration curves of training group 1-, 3-, and 5-year OS and CSS. **(A)** Training group 1-year OS; **(B)** Training group 3-year OS; **(C)** Training group 5-year OS; **(D)** Training group 1-year CSS; **(E)** Training group 3-year CSS; **(F)** Training group 5-year CSS.

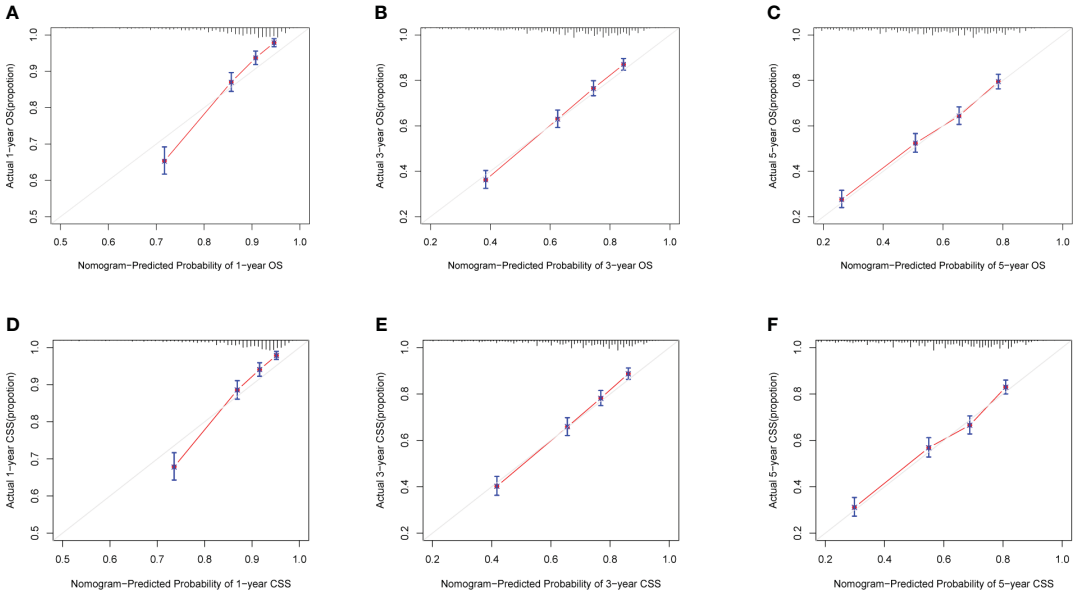

FIGURE 5
(A-F) Calibration curves of validation group 1-, 3-, and 5-year OS and CSS. **(A)** Validation group 1-year OS; **(B)** Training group 3-year OS; **(C)** Validation group 5-year OS; **(D)** Validation group 1-year CSS; **(E)** Validation group 3-year CSS; **(F)** Validation group 5-year CSS.

Serum CEA level was routinely used as an indicator for diagnosing and monitoring COAD (20–22). In our study, elevated serum CEA emerged as an independent risk factor for prognosis (HR = 1.319, 95% CI: 1.186-1.466, P = 0.000). Although serum CEA levels can rise in various malignant tumors and inflammatory or degenerative diseases, our study supported its role as an independent prognostic factor.

N stage, reflecting the extent of local advancement, was a significant prognostic factor. Patients with stage N1 faced a 1.611 times higher risk of death than those with stage N0 (95% CI: 1.455-1.875, P = 0.000), while stage N2 patients had a 2.67 times higher risk (95% CI: 2.394-2.977, P = 0.000). The number of resected lymph nodes, with a threshold of 12, influenced prognosis, with better outcomes for patients with ≥12 lymph nodes resected

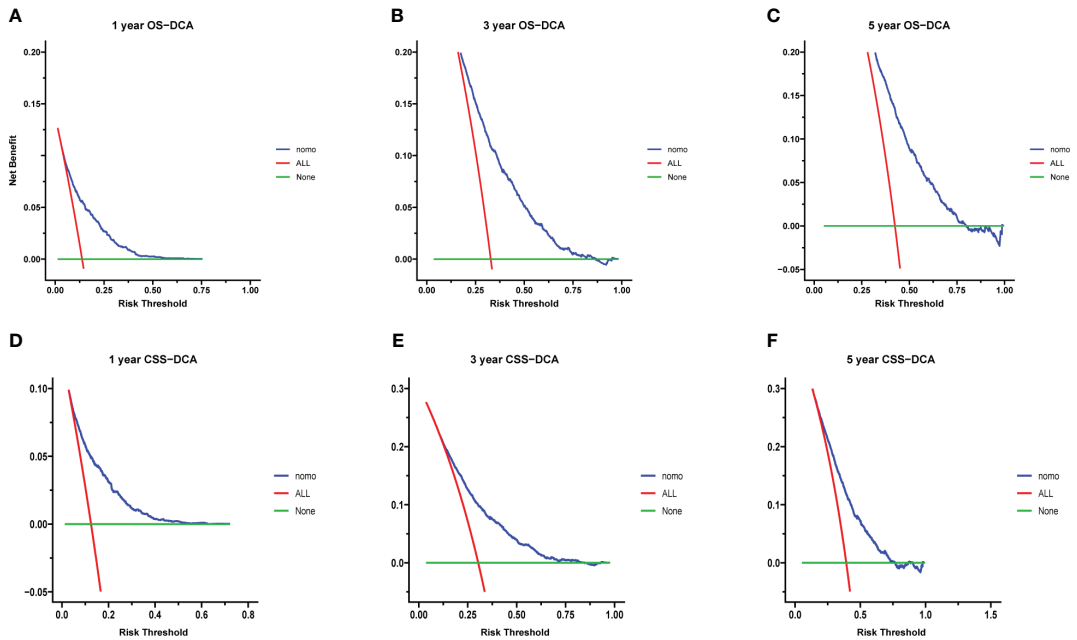

FIGURE 6
(A–F) Decision curves of training group 1-, 3-, and 5-year OS and CSS. **(A)** 1-year OS; **(B)** 3-year OS; **(C)** 5-year OS; **(D)** 1-year CSS; **(E)** 3-year CSS; **(F)** 5-year CSS.

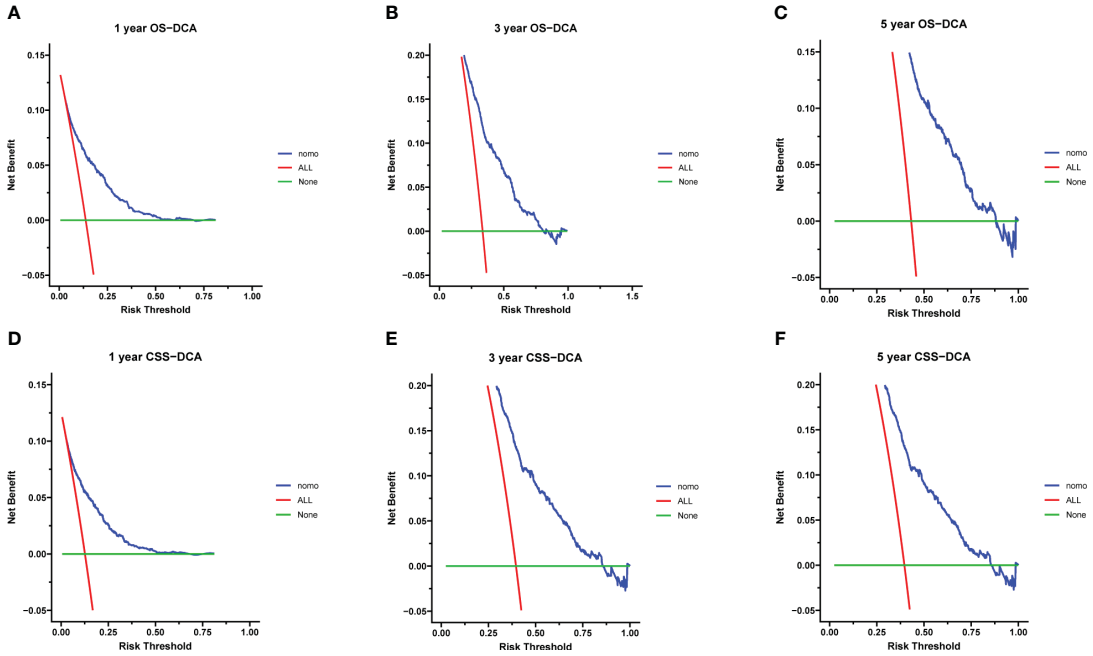

FIGURE 7
(A–F) Decision curves of validation group 1-, 3-, and 5-year OS and CSS **(A)** 1-year OS; **(B)** 3-year OS; **(C)** 5-year OS; **(D)** 1-year CSS; **(E)** 3-year CSS; **(F)** 5-year CSS.

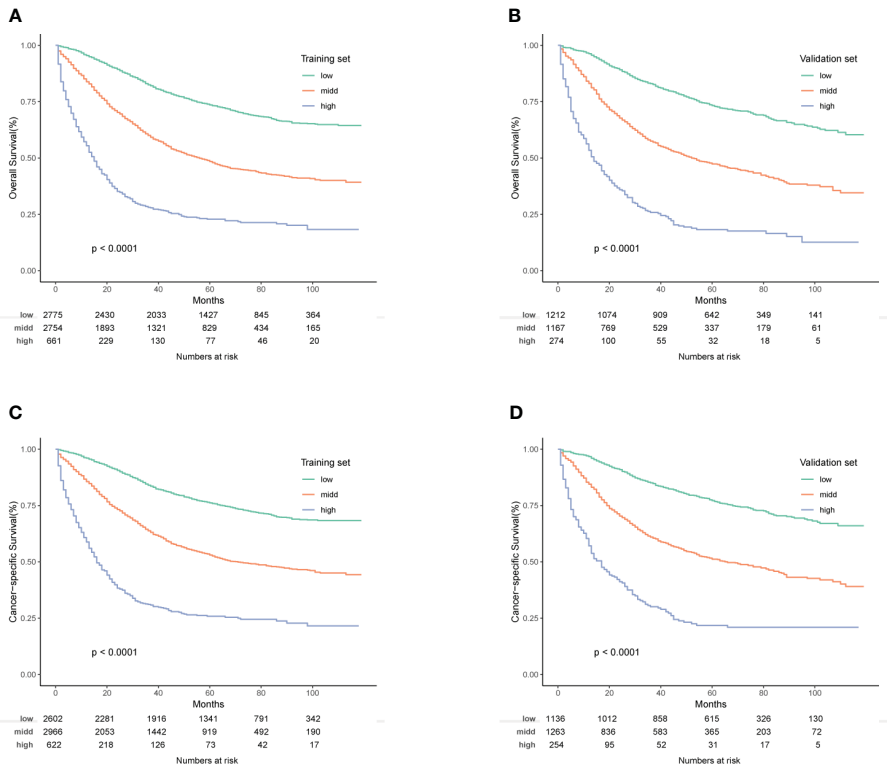

FIGURE 8
(A–D) Kaplan-Meier OS and CSS survival curves for different risk groups in the training and validation groups. **(A)** Training group of OS; **(B)** Validation group of OS; **(C)** Training group of CSS; **(D)** Validation group of CSS.

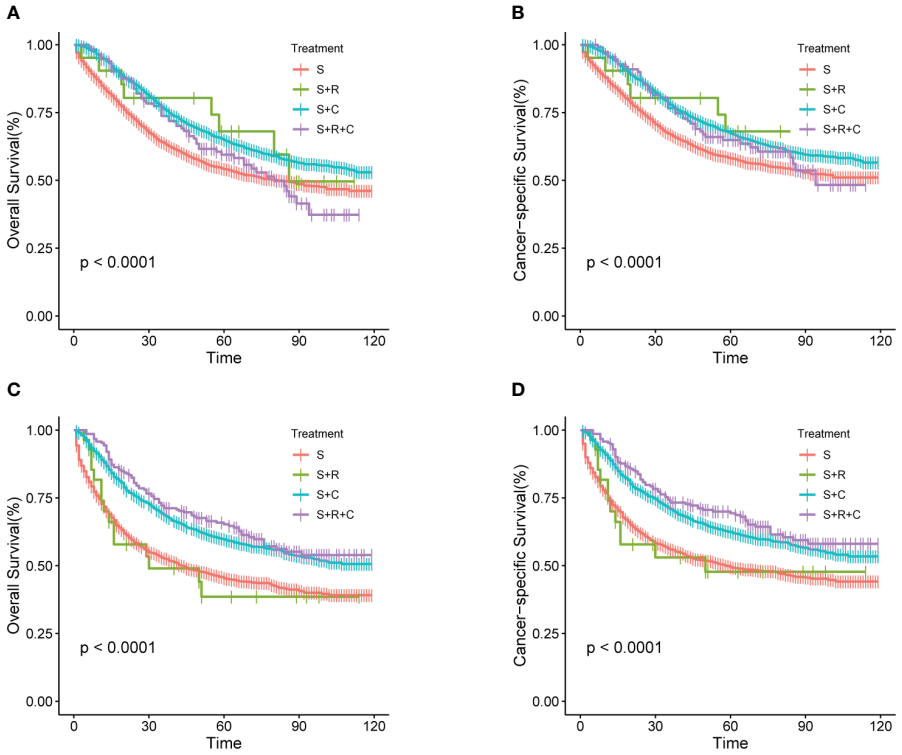

FIGURE 9
(A–D) Kaplan-Meier Survival curves of OS and CSS for stage pT4aM0 and pT4bM0 COAD comparing different treatments. **(A)** pT4aM0 group of OS; **(B)** pT4aM0 group of CSS; **(C)** pT4bM0 group of OS; **(D)** pT4bM0 group of CSS.

compared to those with <12 (P < 0.001, 95% CI: 0.455-0.560), consistent with prior studies (4, 18, 23, 24).

Tumor size ≥5 cm was associated with a 1.145 times higher risk of death than sizes <5 cm (95% CI: 1.048-1.25, P = 0.003). Pathopathological grades III and IV carried a higher risk of death compared to grade I (HRs: 1.299 vs. 1.555, P < 0.05), while the risk in grade II, though higher than grade I, did not reach statistical significance (P = 0.655).

In contrast to previous analyses of COAD prognosis, this study focused on a survival analysis of the treatment modality. While the radiotherapy group had a relatively small number of cases in the survival analysis, there was only a slight difference in the distribution of baseline clinical characteristics of the data (P>0.05), and therefore, propensity score matching (PSM) was not performed.

Acknowledging certain limitations in our study is essential. Being a retrospective study, it is susceptible to selection bias between groups. First, the information in the SEER database, collected by a single center, did not provide insight into whether patients received subsequent treatment at other facilities, potentially impacting their survival time. Second, the database lacked detailed information on factors such as physical status, CEA expression level, radiotherapy dose, chemotherapy regimen, and infiltration depth, which could enhance the accuracy of diagnostic and prognostic models. Third, the SEER database did not furnish comprehensive details about patients' underlying diseases, such as severe coronary heart disease, liver and kidney diseases, or diabetes, which play a pivotal role in treatment decisions. Lastly, the patients included in the SEER database are predominantly from the United States, raising the question of the generalizability of the results to the Chinese population. Our study lacked Chinese patients for external validation. Notably, according to the modeling in this study, race independently influenced OS and CSS. Consequently, large-scale randomized controlled trials (RCTs) conducted in China are imperative to validate the potential benefits of postoperative adjuvant chemoradiotherapy.

5 Conclusion

For patients with COAD at the pT4M0 stage, the combination of surgery and adjuvant chemoradiotherapy demonstrated a significant extension of long-term survival. The nomogram, incorporating variables such as age, race, degree of differentiation, N stage, serum CEA level, tumor size, and the number of resected lymph nodes, stood as a reliable tool for predicting OS and CSS rates in this specific cohort. The utilization of this nomogram can prove instrumental for clinicians in identifying high-risk patients and formulating personalized treatment plans tailored to the unique characteristics of individuals with pT4M0 COAD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

XZ: Data curation, Software, Writing – original draft. LH: Methodology, Supervision, Writing – review & editing. QM: Data curation, Writing – review & editing. MZ: Data curation, Writing – review & editing. JL: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1342289/full#supplementary-material

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(2):209–49. doi: 10.3322/caac.21492

2. Cao W, Chen Hd Fau - Yu Y-W, Yu Yw Fau - Li N, Li N Fau - Chen W-Q, Chen WQ. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. *Chin Med J (Engl)* (2021) 134(7):783–91. doi: 10.1097/CM9.0000000000001474

3. Deijen CL, Vasmel JE, de Lange-de Klerk ESM, Cuesta MA, Coene PLO, Lange JF, et al. Ten-year outcomes of a randomised trial of laparoscopic versus open surgery for colon cancer. *Surg Endosc* (2017) 31(6):2607–15. doi: 10.1007/s00464-016-5270-6

4. Hosseini S, Bananzadeh AM, Mohammadianpanah M, Salek R, Taghizadeh-Kermani A. Prognostic significance of adjuvant radiation therapy in adenocarcinoma of the cecum. *Radiat Oncol J(2018)* (2018) 36(1):45–53. doi: 10.3857/roj.2017.00332

5. Krishnamurty DM, Hawkins AT, Wells KO, Mutch MG, Silviera ML, Glasgow SC, et al. Neoadjuvant radiation therapy in locally advanced colon cancer: a cohort analysis. *J Gastrointest Surg* (2018) 22(5):906–12. doi: 10.1007/s11605-018-3676-2

6. Wegner RE, Abel S, Monga D, Raj M, Finley G, Nosik S, et al. Utilization of adjuvant radiotherapy for resected colon cancer and its effect on outcome. *Ann Surg Oncol* (2020) 27(2):825–32. doi: 10.1245/s10434-019-08042-y

7. Haddock MG. Intraoperative radiation therapy for colon and rectal cancers: a clinical review. *Radiat Oncol* (2017) 12(1):11. doi: 10.1186/s13014-016-0752-1

8. Amos EH, Mendenhall WM, McCarty PJ, Gage JO, Emlet JL, Lowrey GC, et al. Postoperative radiotherapy for locally advanced colon cancer. *Ann Surg Oncol* (1996) 3 (5):431–6. doi: 10.1007/BF02305760

9. Willett CG, Goldberg S, Shellito PC, Grossbard M, Clark J, Fung C, et al. Does postoperative irradiation play a role in the adjuvant therapy of stage T4 colon cancer? *Cancer J Sci Am* (1999) 5(4):242–7.

10. Martenson JA Jr., Willett CG, Sargent DJ, Mailliard JA, Donohue JH, Gunderson LL, et al. Phase III study of adjuvant chemotherapy and radiation therapy compared with chemotherapy alone in the surgical adjuvant treatment of colon cancer: results of intergroup protocol 0130. *J Clin Oncol* (2004) 22(16):3277–83. doi: 10.1200/JCO.2004.01.029

11. Hawkins AT, Ford MM, Geiger TM, Hopkins MB, Kachnic LA, Muldoon RL, et al. Neoadjuvant radiation for clinical T4 colon cancer: A potential improvement to overall survival. *Surgery* (2019) 165(2):469–75. doi: 10.1016/j.surg.2018.06.015

12. Margalit O, Mamtani R, Lawrence YR, Yang YX, Reiss KA, Golan T, et al. Postoperative radiation for pathologic stage T4 colon cancers receiving adjuvant chemotherapy. *Clin Colorectal Cancer* (2019) 18(2):226–30.e2. doi: 10.1016/j.clcc.2019.04.004

13. Sebastian NA-O, Tan Y, Miller ED, Williams TM, Diaz DA. Surgery with and without adjuvant radiotherapy is associated with similar survival in T4 colon cancer. *Colorectal Dis* (2020) 22(7):779–89. doi: 10.1111/codi.14953

14. Huang Y, Gu X, Ge K, Fu G, Chu J, Wei W. The survival benefit of adjuvant radiotherapy for pathological T4N2M0 colon cancer in the Modern Chemotherapy Era: evidence from the SEER database 2004-2015. *Artif Cells Nanomed Biotechnol* (2020) 48 (1):834–40. doi: 10.1080/21691401.2020.1770270

15. Siegel RA-O, Miller KA-O, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin* (2020) 70(3):145–64. doi: 10.3322/caac.21601

16. Buunen M, Veldkamp R, Hop WCJ, Kuhry E, Jeekel J, Haglind E, et al. Survival after laparoscopic surgery versus open surgery for colon cancer: long-term outcome of a randomised clinical trial. *Lancet Oncol* (2009) 10(1):44–52. doi: 10.1016/S1470-2045(08)70310-3

17. Wang L, Hirano Y, Ishii T, Kondo H, Hara K, Obara N, et al. Left colon as a novel high-risk factor for postoperative recurrence of stage II colon cancer. *World J Surg Oncol* (2020) 18(1):54. doi: 10.1186/s12957-020-01818-7

18. Vergara-Fernandez O, Navarro-Navarro A, Rangel-Ríos HA, Salgado-Nesme N, Reyes-Monroy JA, Velázquez-Fernández D. Oncological implications of lymph nodes retrieval and perineural invasion in colorectal cancer: outcomes from a referral center. *Rev Invest Clin* (2018) 70:291–300. doi: 10.24875/RIC.18002505

19. McLaughlin C, Kim NK, Bandyopadhyay D, Deng X, Kaplan B, Matin K, et al. Adjuvant radiation therapy for T4 non-rectal colon adenocarcinoma provides a cause-specific survival advantage: A SEER database analysis. *Radiother Oncol* (2019) 133:50–3. doi: 10.1016/j.radonc.2018.11.026

20. Kim CG, Ahn JB, Jung M, Beom SH, Heo SJ, Kim JH, et al. Preoperative serum carcinoembryonic antigen level as a prognostic factor for recurrence and survival after curative resection followed by adjuvant chemotherapy in stage III colon cancer. *Ann Surg Oncol* (2017) 24(1):227–35. doi: 10.1245/s10434-016-5613-5

21. Saito G, Sadahiro S, Kamata H, Miyakita H, Okada K, Tanaka A, et al. Monitoring of Serum Carcinoembryonic Antigen Levels after Curative Resection of Colon Cancer: Cutoff Values Determined according to Preoperative Levels Enhance the Diagnostic Accuracy for Recurrence. *Oncology* (2017) 92(5):276–82. doi: 10.1159/000456075

22. Magaji BA, Moy FA-O, Roslani AC, Law CW. Survival rates and predictors of survival among colorectal cancer patients in a Malaysian tertiary hospital. *BMC Cancer* (2017) 17(1):339. doi: 10.1186/s12885-017-3336-z

23. Ren D, Wang WL, Wang G, Chen WW, Li XK, Li GD, et al. Development and internal validation of a nomogram-based model to predict three-year and five-year overall survival in patients with stage II/III colon cancer. *Cancer Manag Res* (2022) 14:225–36. doi: 10.2147/CMAR.S335665

24. Orsenigo EA-O, Gasparini GA-O, Carlucci M. Clinicopathological factors influencing lymph node yield in colorectal cancer: A retrospective study. *Gastroenterol Res Pract* (2019) 2019:5197914. doi: 10.1155/2019/5197914

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Predicting colorectal cancer risk: a novel approach using anemia and blood test markers

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Background and objectives: Colorectal cancer remains an important public health problem in the context of the COVID-19 (Corona virus disease 2019) pandemic. The decline in detection rates and delayed diagnosis of the disease necessitate the exploration of novel approaches to identify individuals with a heightened risk of developing colorectal cancer. The study aids clinicians in the rational allocation and utilization of healthcare resources, thereby benefiting patients, physicians, and the healthcare system.

Methods: The present study retrospectively analyzed the clinical data of colorectal cancer cases diagnosed at the Affiliated Hospital of Guilin Medical University from September 2022 to September 2023, along with a control group. The study employed univariate and multivariate logistic regression as well as LASSO (Least absolute shrinkage and selection operator) regression to screen for predictors of colorectal cancer risk. The optimal predictors were selected based on the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. These predictors were then utilized in constructing a Nomogram Model for predicting colorectal cancer risk. The accuracy of the risk prediction Nomogram Model was assessed through calibration curves, ROC curves, and decision curve analysis (DCA) curves.

Results: Clinical data of 719 patients (302 in the case group and 417 in the control group) were included in this study. Based on univariate logistic regression analysis, there is a correlation between Body Mass Index (BMI), red blood cell count (RBC), anemia, Mean Corpuscular Volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), Red Cell Distribution Width-Standard Deviation (RDW-SD), and the incidence of colorectal cancer. Based on the findings of multivariate logistic regression analysis, the variables of BMI and RBC exhibit a decrease, while anemia and PLT demonstrate an increase, all of which are identified as risk factors for the occurrence of colorectal cancer. LASSO regression selected BMI, RBC, anemia, and PLT as prediction factors. LASSO regression and multivariate logistic regression analysis yielded the same results. A nomogram was constructed based on the 4 prediction factors identified by LASSO regression analysis to predict the risk of colorectal cancer. The AUC of the nomogram was 0.751 (95% CI, OR: 0.708-0.793). The calibration curves in the validation and training sets showed

good performance, indicating that the constructed nomogram model has good predictive ability. Additionally, the DCA demonstrated that the nomogram model has diagnostic accuracy.

Conclusion: The Nomogram Model offers precise prognostications regarding the likelihood of Colorectal Cancer in patients, thereby helping healthcare professionals in their decision-making processes and promoting the rational categorization of patients as well as the allocation of medical resources.

KEYWORDS

anemia, colorectal cancer, risk prediction, nomogram, machine learning

1 Introduction

The COVID-19 global pandemic has seemingly led to a reduction in the overall prevalence of cancer; however, it is imperative to acknowledge that cancer continues to pose a significant public health concern (1). Colorectal cancer is positioned as the third most prevalent form of cancer worldwide, exhibiting a comparatively elevated fatality rate (2). Moreover, colorectal cancer is a prominent contributor to mortality rates in both developed and developing nations, imposing a substantial societal and economic burden (3–5). The prevalence of colorectal cancer in the Guangxi Zhuang Autonomous Region of China has exhibited a consistent upward trend over the years. The northern region of Guangxi exhibits a high prevalence of colorectal cancer, with a notably elevated disease burden compared to other cancer types, as indicated by a DALYs (Disability adjusted life years) rate of 218.20 per 100,000 person-years (6). Presently, two efficacious screening techniques for colorectal cancer exist, namely the Fecal Occult Blood Test (FOBT) and the Fecal Immunochemical Test (FIT). In comparison to FOBT, FIT exhibits greater specificity as a screening modality, necessitates a reduced number of fecal sample collections, and is more amenable to widespread adoption. Nevertheless, the adoption rates for both screening methods remain suboptimal, and the implementation of colorectal cancer screening encounters certain challenges (7). Moreover, the emergence of the COVID-19 pandemic has precipitated a postponement in the identification of colorectal cancer, consequently yielding a diminished rate of detection and frequently culminating in the identification of advanced stages and severe complications. The challenges encountered in clinical management, coupled with the healthcare system's incomplete recuperation, will exert detrimental consequences on the disease's prognosis. Hence, there is an imperative need for an effective and uncomplicated approach to screen individuals at high risk for colorectal cancer (2, 8).

Machine learning techniques have significantly contributed to the evaluation of metastasis and prognosis in contemporary studies on colorectal cancer, exemplified by the utilization of the nomogram model (9), the 9-gene COX regression model (10), the random forest model (11), and the social ecological model (SEM) (12). These models employ a comprehensive approach to assess the pre-onset or post-onset condition of colorectal cancer in a population by simultaneously considering multiple risk factors. This approach can significantly aid clinical practitioners in promptly identifying patients and devising suitable treatment strategies, consequently enhancing prognosis and survival rates. Nevertheless, existing research falls short in providing a more precise easy to use prediction model of developing colorectal cancer.

In the realm of clinical research, it was observed that individuals afflicted with colorectal cancer experienced a noteworthy reduction in anemia indicators, namely hemoglobin, MCV, and RBC, prior to their diagnosis. Furthermore, these indicators exhibited a discernible correlation with the patients' survival outcomes (13). Apart from that, previous research has demonstrated a correlation between reduced levels of hemoglobin, diminished MCV, and decreased MCH with an escalation in the T stage of colorectal cancer (14). Hence, the utilization of anemia and blood-related indicators as prediction factors for the initiation of colorectal cancer holds promise, and through the utilization of a nomogram that incorporates anemia and blood-related clinical indicators as risk factors, the potential to forecast and quantify the probability of disease development in individual patients is attainable (15).

This study retrospectively gathered anemia and blood-related clinical indicators from patients diagnosed with colorectal cancer and control patients. Subsequently, nomogram Model were constructed to forecast the probability of colorectal cancer development among patients. The primary objective of this analysis was to facilitate clinical practitioners in rational resource allocation and enhance patient survival rates.

2 Materials and methods

The data utilized in this research was acquired via a retrospective survey conducted by an investigator, encompassing

clinical data from newly admitted inpatient cases at the Affiliated Hospital of Guilin Medical University, spanning from September 2022 to September 2023.The inclusion criteria for the cases in this study are as follows: (1) patients diagnosed with colorectal cancer for the first time between September 2022 and September 2023; (2) demographic indicators including age, gender, smoking, drinking, and BMI; blood test indicators including RBC, anemia, MCV, MCH, MCHC, RDW-SD, platelet distribution width (PDW), and platelet-large cell ratio (P-LCR),PLT; (3) newly diagnosed colorectal cancer patients with primary colorectal cancer; (4) newly diagnosed colorectal cancer patients should have been confirmed by at least two imaging examinations or histopathological diagnosis; (5) patients over 18 years old. The exclusion criteria for the cases in this study are as follows: (1) newly diagnosed colorectal cancer patients who are not primary colorectal cancer patients; (2) Incomplete information, including demographic and blood test indicators; (3) Patients who have received radiotherapy or chemotherapy as adjuvant therapy before obtaining blood test indicators.

The inclusion criteria for control in this study are: (1) patients admitted from September 2022 to September 2023; (2) Patients with demographic indicators including age, gender, smoking, drinking, and BMI; blood test indicators including RBC, anemia, MCV, MCH, MCHC, RDW-SD, PDW, P-LCR, PLT; (3) Patients who have not had colorectal cancer or other malignant tumors; (4) Patients over 18 years old. The exclusion criteria for control in this study are: (1) Patients with or who have had malignant tumors;(2) Incomplete information, including demographic and blood test indicators. (3) Patients who have received radiotherapy or chemotherapy as adjuvant therapy before obtaining blood test indicators.

This study included 302 cases and 417 controls. The allocation of training set and validation set followed a complete randomization process, resulting in a 7:3 ratio. Specifically, 70% of the cases and controls were assigned to the training set, while the remaining 30% were assigned to the validation set. The cases in the training set were used to construct nomogram Model, while the cases in the validation set were used to validate the nomogram Model (Supplementary Figure S1). This study was a retrospective study conducted with the approval of the Ethics Committee of Guilin Medical College. The ethics number is (GYLL2022056).

3 Data processing and analysis

This study used Excel 2021 to input data, establish a database. R software was then used for descriptive analysis, conducting differential tests on all factors between the case group and the control group. Differential tests were also performed on the training and validation sets to ensure the reliability of data splitting. For quantitative data, the or Median (interquartile range) were used for description, and differential tests were conducted using t-tests, Wilcoxon rank-sum tests, or Kolmogorov-Smirnov tests. Frequency or percentage was used to represent count or ordinal data, and differential tests were conducted using chi-square tests or Fisher's exact tests. In the differential analysis, $P<0.05$ was considered statistically significant. logistic analysis and LASSO regression analysis were applied using R software to screen for risk factors. Variables with $P<0.1$ in the univariate logistic analysis were included in the multivariate logistic regression analysis to identify independent risk factors for colorectal cancer. LASSO regression was also used to screen for prediction factors. The prediction factors selected by the three methods were evaluated based on ROC curves and AUC to establish the optimal model, and a visual nomogram was created (16, 17).

4 Results

4.1 Clinical characteristics of patients

Based on the predetermined inclusion and exclusion criteria, a comprehensive cohort of 719 patients was selected for participation in this study, comprising 302 individuals in the case group and 417 individuals in the control group (Table 1). The patients in both the case group and the control group were randomly assigned to either the training set or the validation set in a ratio of 7:3. The training set consisted of 504 cases, while the validation set comprised 215 cases (Table 2). Differential analysis showed no significant differences ($P>0.05$) between the training set and the validation set in various indicators.

Statistical analysis of the clinical data of the 719 patients showed that in the case group and the control group, age ($P=0.547$), Sex ($P=0.704$), smoking ($P=0.557$), drinking ($P=0.822$), MCV ($P=0.052$), RDW-SD ($P=0.307$), PDW ($P=0.715$), and P-LCR ($P=0.95$) had no statistical significance. However, BMI ($P<0.001$), RBC ($P<0.001$), HGB ($P<0.001$), MCH ($P=0.005$), MCHC ($P=0.002$), and PLT ($P<0.001$) were statistically significant.

4.2 Logistic regression for screening prediction factors

This study employed Univariate logistic regression analysis to examine 14 risk factors in order to ascertain the factors linked to the occurrence of colorectal cancer (Table 3). The results indicate that there are 8 prediction factors associated with the incidence of colorectal cancer, including BMI ($P<0.001$), RBC ($P<0.001$), Anemia ($P<0.001$), MCV ($P=0.073$), MCH ($P=0.002$), MCHC ($P<0.001$), RDW-SD ($P=0.018$), PLT ($P<0.001$). Furthermore, this study conducted Multivariate logistic regression analysis on the 8 factors, revealing that BMI ($P=0.009$), RBC ($P=0.001$), Anemia ($P<0.001$), and PLT ($P<0.001$) are independent predictive factors for the incidence of colorectal cancer, as shown in Table 3.

4.3 LASSO regression for prediction factors

The 14 prediction factors mentioned above using LASSO regression. The relationship between the binomial deviation curve and $\log(\lambda)$ is shown in Figure 1, where λ is the tuning parameter. In Figure 1, the vertical solid line represents the binomial deviation ±

TABLE 1 Clinical characteristics of cases in the case and control groups.

Characteristics		Case (n=302)	Control (n=417)	P
Age		63.34(56.71,71.20)	63.81(57.01,71.99)	0.547
Sex				0.704
	Male	187(61.92%)	264(63.31%)	
	Female	115(38.08%)	153(36.69%)	
BMI (Weight (kg)/Height (m) ^ 2)		22.68(20.31,24.88)	23.97(21.24,26.35)	<0.001*
Smoking				0.557
	Yes	71(23.51%)	106(25.42%)	
	No	231(76.49%)	311(74.58%)	
Drinking				0.822
	Yes	46(15.23%)	61(14.63%)	
	No	256(84.77%)	356(85.37%)	
RBC (10^12/L)		4.14(3.72,4.54)	4.51(4.13,4.93)	<0.001*
Anemia				<0.001*
	Yes	194(64.2%)	157(37.6%)	
	No	108(35.7%)	260(62.3%)	
MCV (fl)		87.03(82.93,93.08)	88.39(86.10,93.10)	0.052
MCH (pg)		28.22(26.80,30.80)	29.05(28.40,30.90)	0.005*
MCHC (g/L)		323.07(316.00,334.75)	327.62(320.00,337.00)	0.002*
RDW-SD (fl)		44.02(40.03,45.28)	42.90(39.80,45.20)	0.307
PDW (fl)		10.92(9.60,11.90)	10.94(9.40,12.00)	0.715
P-LCR (fl)		0.24(0.19,0.29)	0.24(0.19,0.29)	0.945
PLT (10^9/L)		285.24(220.00,336.25)	235.33(189.00,271.00)	<0.001*

standard error (SE), and the vertical dashed line is drawn through the minimum standard deviation of λ and 1-SE standard. According to the logarithm of λ (Figure 1) and the best simplification of the model, the value of λ selected through the 1-SE standard is 0.04536598. Therefore, this method selects 4 predictive factors from the training set: BMI, RBC, Anemia, and PLT (Supplementary Table S2).

4.4 Established a predictive model

The models were constructed using a combination of eight predictive factors (BMI, RBC, Anemia, MCV, MCH, MCHC, RDE-SD, PLT) identified through Univariate logistic regression analysis, four predictive factors (BMI, RBC, Anemia, PLT) identified through Multivariate logistic regression analysis, and four predictive factors (BMI, RBC, Anemia, PLT) identified through LASSO regression. Since the predictive factors selected by Multivariate logistic regression analysis and LASSO regression are the same, we established two models named Model1 and Model2 based on the 8 factors and 4 factors. We used the AUC and ROC curve (Figure 2) to evaluate whether there were differences between the two models. DeLong's test (Supplementary Table S1) showed that there was no significant difference between Model1 and Model2 in the validation set (P=0.846) and training set (P=0.672). Since the Logistic regression result was an 8-factor model, in order to make the model as simple as possible, a nomogram Model for predicting the incidence of colorectal cancer was constructed and visualized (Figure 3) based on 4 predictors (BMI, RBC, HCT, PLT) through LASSO regression for the prediction of the incidence of colorectal cancer.

4.5 Validation of nomogram in training and validation sets

There are 504 patients in the training set, of which 219 patients have colorectal cancer and 285 patients do not have colorectal cancer. We used the ROC curve and AUC area under the curve to evaluate the discrimination ability of the nomogram. The ROC curve of the training set (Figure 4) shows that the area under the curve of the training set nomogram is 0.751 (95% CI, 0.708-0.793). This study used a calibration curve (Figure 5) to evaluate the calibration of the model and Hosmer-Lemeshow test

TABLE 2 Clinical characterization of training and validation sets.

Characteristics		Training set (n=504)	Validation set (n=215)	P
Colorectal Cancer				0.261
	Yes	219(43.40%)	83(38.6%)	
	No	285(56.50%)	132(61.3)	
Age		63.56(56.94,71.13)	66.22(56.93,74.37)	0.128
Sex				0.783
	Male	314(62.30%)	137(63.70%)	
	Female	190(37.60%)	78(36.20%)	
BMI (Weight (kg)/Height (m) ^ 2)		23.08(20.76,26.04)	22.94(20.91,25.81)	0.822
Smoking				0.499
	Yes	120(23.80%)	57(26.50%)	
	No	384(76.10%)	158(73.40%)	
Drinking				0.423
	Yes	71(14.0%)	36(16.70%)	
	No	433(85.90%)	179(83.20%)	
RBC (10^12/L)		4.36(3.93,4.85)	4.37(3.94,4.73)	0.893
Anemia				0.866
	Yes	241(47.80%)	105(48.8%)	
	No	263(52.10%)	110(51.1%)	
MCV (fl)		89.40(84.80,93.00)	90.30(88.90,93.65)	0.139
MCH (pg)		29.60(27.60,30.80)	29.90(28.05,31.00)	0.203
MCHC (g/L)		327.00(318.00,336.00)	327.00(319.00,336.00)	0.727
RDW-SD (fl)		42.50(39.80,45.10)	42.30(39.80,45.30)	0.990
PDW (fl)		10.50(9.40,12.00)	10.80(9.70,11.80)	0.242
P-LCR (fl)		0.23(0.18,0.29)	0.25(0.20,0.29)	0.052
PLT (10^9/L)		249.00(205.80,299.00)	236.00(185.50,284.00)	0.081

(Supplementary Table S3, P=0.639>0.05) indicates that the model consistency is good. The DCA curve (Figure 6) shows that the nomogram can be used as a prediction tool for the occurrence of colorectal cancer in patients.

There are 215 patients in the validation set, of which 83 patients have colorectal cancer and 132 patients do not have colorectal cancer. Based on the data of the test set, we established a ROC curve. The nomogram of the test set (Figure 4) has an AUC of 0.694 (95% CI, 0.623-0.765). The calibration curve (Figure 5) indicates that the model is stable, and Hosmer-Lemeshow test (Supplementary Table S3, P=0.448>0.05) indicates that the model consistency is good. The DCA curve (Figure 6) indicates that the nomogram can be used as a prediction tool for the occurrence of colorectal cancer in patients.

Additionally, we developed a clinical impact curve (CIC) to plot to evaluate the clinical usefulness and applicability net benefits of the model with the best diagnostic value (Figure 7).

4.6 ROC curves for each risk factor in the training and validation sets

This study compared the area under the ROC curve of each predictor with Nomogram Model on the training and validation sets (Figure 8). The results showed that the AUCs of all predictors were lower than that of the Nomogram Model, both on the training and validation sets. This implies that the Nomogram exhibits a high degree of reliability.

5 Discussion

This study retrospectively analysed the clinical data of 719 patients, comprising 302 cases in the case group and 417 cases in the control group. LASSO regression was employed to screen risk

TABLE 3 Logistic analysis results in the training set.

Characteristics	OR	CI	P	OR	CI	P
	Univariate logistic regression			Multivariate logistic regression		
Age	0.99	0.98-1.01	0.266	–	–	–
Sex (Male)	0.92	0.64-1.32	0.651	–	–	–
BMI	0.91	0.87-0.96	<0.001*	0.93	0.88-0.98	0.009*
Smoking (Yes)	0.91	0.6-1.38	0.651	–	–	–
Drinking (Yes)	0.83	0.49-1.38	0.462	–	–	–
RBC	0.44	0.34-0.58	<0.001*	0.6	0.44-0.81	0.001*
Anemia (Yes)	3.59	2.48-5.2	<0.001*	2.19	1.42-3.39	<0.001*
MCV	0.98	0.96-1	0.073*	–	–	–
MCH	0.93	0.88-0.97	0.002*	–	–	–
MCHC	0.97	0.96-0.99	<0.001*	–	–	–
RDWSD	1.04	1.01-1.07	0.018*	–	–	–
PDW	1	0.92-1.09	0.922	–	–	–
P-LCR	1.13	0.11-11.25	0.916	–	–	–
PLT	1.01	1-1.01	<0.001*	1.01	1-1.01	<0.001*

*Mean the P are significant.

factors and develop a nomogram for predicting the risk of colorectal cancer. The results of the univariate logistic regression analysis indicate that BMI, RBC, Anemia, MCV, MCH, MCHC, RDW-SD, and PLT exhibit significant associations with the development of colorectal cancer. Specifically, a decrease in BMI, RBC, and the presence of anemia, along with an increase in PLT, are identified as independent risk factors for the development of colorectal cancer.

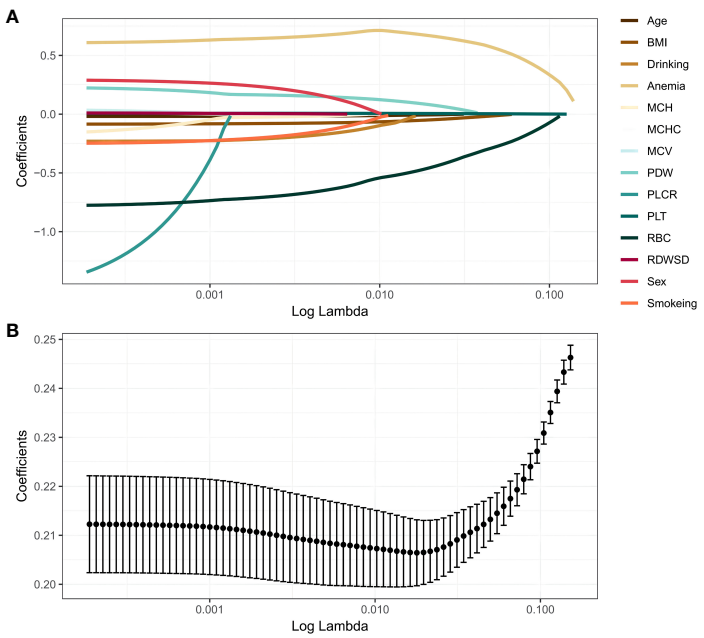

FIGURE 1
LASSO regression analysis. **(A)** LASSO coefficient profiles. The LASSO is commonly employed for regression analysis involving predictors. This method incorporates an L1 penalty to effectively reduce specific regression coefficients to zero. In order to visualize the impact of the tuning parameter (λ), the binomial deviation curve is plotted against the logarithm of λ. **(B)** Ten time cross-validation for tuning parameter selection in the LASSO. The vertical solid line represents the binomial deviation ± standard error (SE), and the vertical dashed line is drawn through the minimum standard deviation of λ and 1-SE standard.

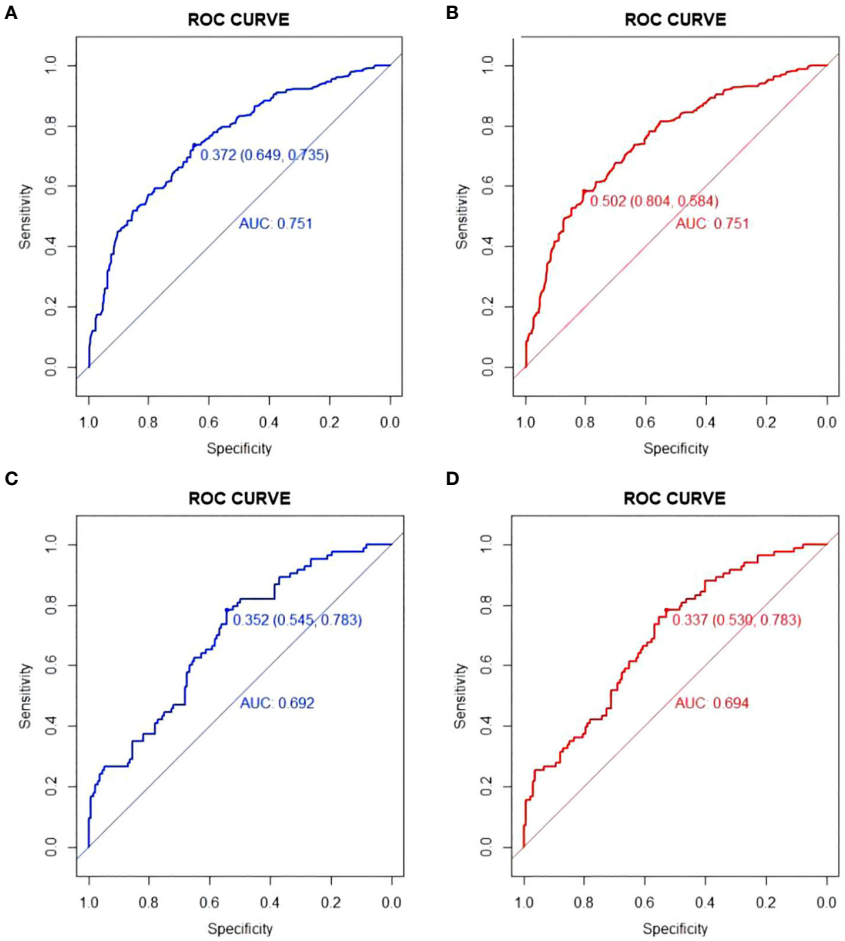

FIGURE 2
ROC curve of Model1 and Model2. **(A)** ROC curve of Model1in training set; **(B)** ROC curve of Model2 in training set; **(C)** ROC curve of Model1in validation set; **(D)** ROC curve of Model2in validation set.

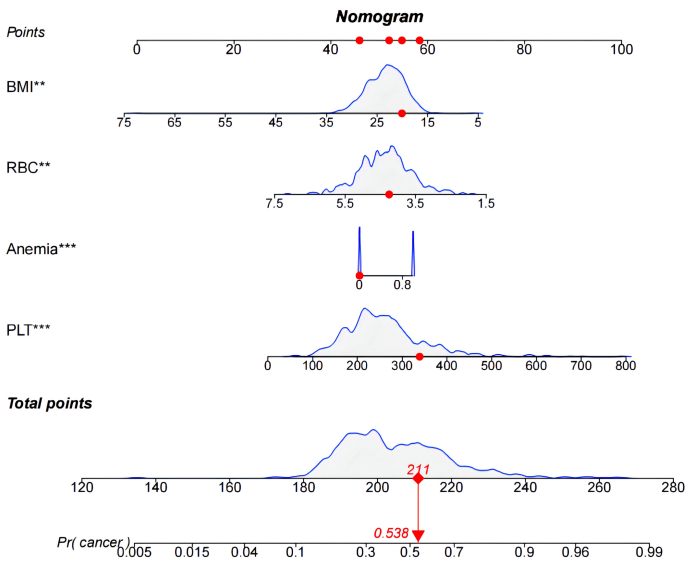

FIGURE 3
Nomogram used to predict colorectal cancer incidence in patients. The predicted colorectal cancer incidence for patient #5.

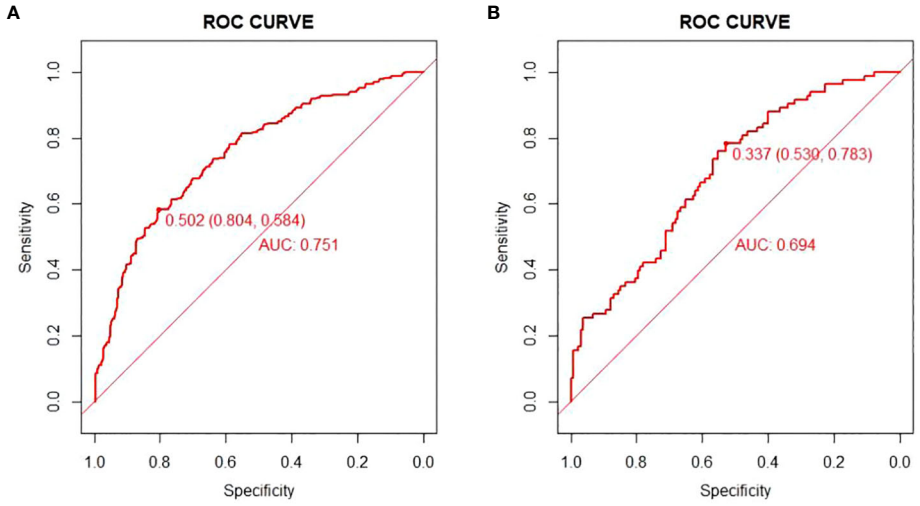

FIGURE 4
Nomogram model roc curve in training and validation sets. **(A)** ROC curve of Nomogram Model in training set; **(B)** ROC curve of Nomogram Model in validation set.

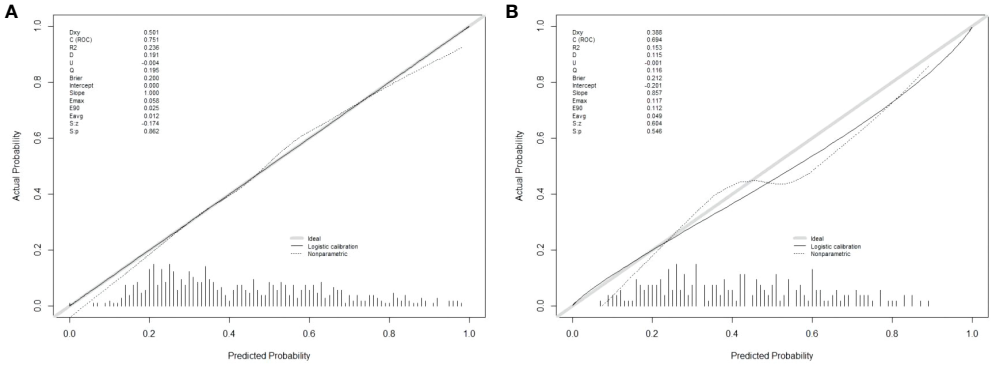

FIGURE 5
Nomogram model calibration curve in training and validation sets. **(A)** Calibration curve of Nomogram Model in training set; **(B)** Calibration curve of Nomogram Model in validation set.

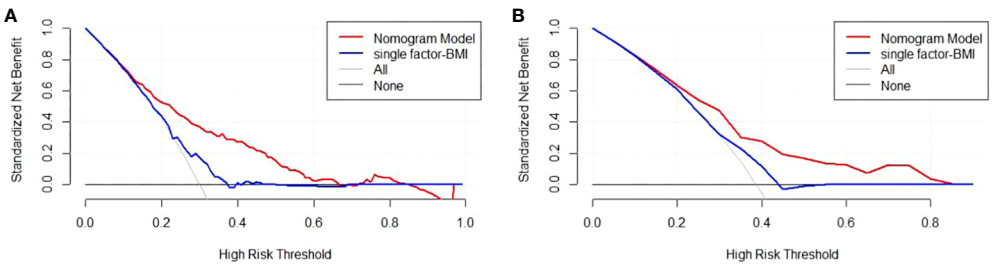

FIGURE 6
Nomogram model DCA curve in training and validation sets. **(A)** DCA curve of Nomogram Model in training set; **(B)** DCA curve of Nomogram Model in validation set.

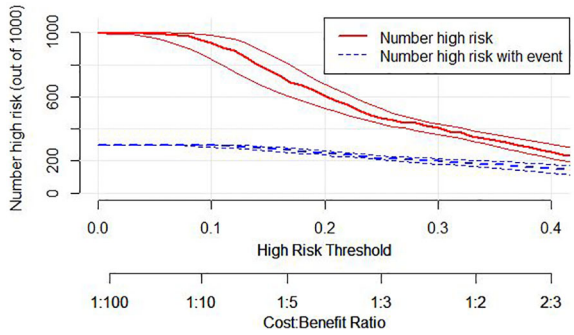

FIGURE 7
Clinical Impact Curve (CIC) of nomogram model. evaluate clinical applicability of risk prediction nomogram. CIC visually showed that the nomogram had a superior overall net benefit within the wide and practical ranges of threshold probabilities and impacted patient outcomes, which indicates that the Nomogram Model possesses significant predictive value.

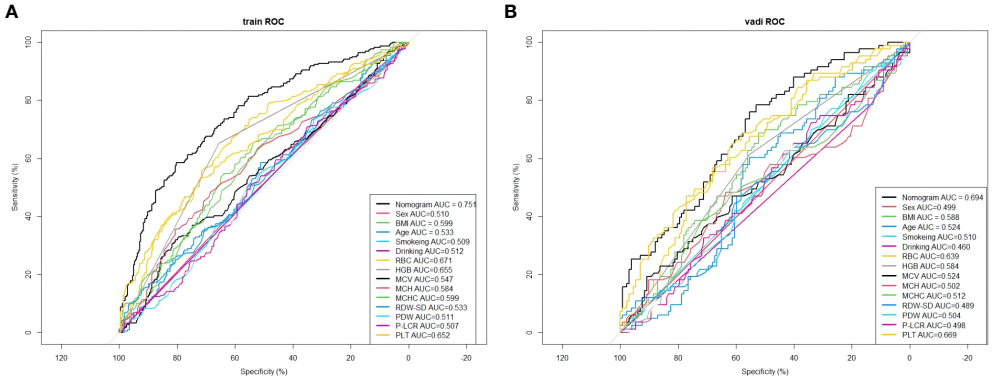

FIGURE 8
Comparison of the area under the ROC curve for each independent factor and the Nomogram Model in the training set. **(A)** In the training set; **(B)** In the validation set.

This study incorporates LASSO regression to identify four predictive factors, namely BMI, RBC, Anemia, and PLT. Unlike conventional logistic regression, LASSO regression effectively mitigates overfitting by reducing the regression coefficients of independent variables to zero, thereby enhancing its variable selection capabilities (18–22). However, the findings of this study demonstrate that both LASSO regression and multivariate logistic regression yielded consistent results, thereby enhancing the robustness of the factor selection outcomes. In this study, a nomogram was constructed utilizing the variables chosen through LASSO regression. The model was then visually represented using a patient No. 5 from the training group. Furthermore, a variety of metrics were utilized to evaluate the discriminatory power, calibration, and clinical usefulness of the nomogram model. The findings demonstrate that the nomogram model demonstrates favorable discrimination (AUC=0.751), effectively forecasting the probability of colorectal cancer occurrence in patients [as indicated by the Hosmer-Lemeshow test (P>0.05)]. Moreover, the DCA and CIC curves suggest that the model holds potential for delivering valuable clinical advantages to patients.

In this study, anemia was defined as hemoglobin levels below 130g/L in males and 120g/L in females (23). There were 74 cases of anemia (64.34%) in the female case group, compared to 52 cases (33.99%) in the female control group. In the male case group, there were 120 cases (64.17%) of anemia, compared to 105 cases (39.77%) in the male control group. Regardless of gender disparities, the prevalence of anemia among individuals diagnosed with colorectal cancer exhibited a notably higher proportion compared to the control group (P<0.001), aligning with the prevailing observations in clinical research (13, 24). The clinical data for this study were gathered prior to patient diagnosis, suggesting that the occurrence of anemia precede the emergence of colorectal cancer. A systematic review study reveals that individuals with colorectal cancer exhibit a decrease in red blood cell count, hemoglobin concentration, and mean corpuscular volume upon assessment of their complete blood count, meanwhile, the red blood cell distribution width, white blood cell count, and platelet levels are higher (25). In line with our investigation, a systematic review revealed that blood measurements were typically conducted within one year following diagnosis in the examined research (26). All reports consistently indicated that

individuals diagnosed with colorectal cancer exhibited lower levels of red blood cells and hemoglobin compared to non-cancer patients within the initial year post-diagnosis. This implies that colorectal cancer exerts an influence on blood constituents, and alterations in one or multiple constituents within the blood may serve as indicators for the initiation of colorectal cancer. Moreover, research has demonstrated that patients exhibiting anemia as a distinctive manifestation of colorectal cancer exhibit a comparatively elevated mortality rate, with anemia being linked to an unfavorable prognosis (27). In the context of colorectal cancer, the majority of full blood count (FBC) parameters exhibit alterations upon the onset of the event (26). It is plausible that prior investigations have overlooked the potential utility of these alterations in the detection of colorectal cancer, as blood levels may persist within the confines of the normal reference range. Through our analysis, we have successfully identified the association between anemia, blood-related indicators, and the risk of colorectal cancer in patients. Furthermore, our prediction model exhibits commendable predictive performance. The existing body of research is insufficient in providing conclusive evidence on the chronological order of anemia and the initiation of colorectal cancer, as well as the potential causative association between the two. Consequently, it is imperative to conduct cohort studies to obtain more robust evidence.

It is worth mentioning that our observations indicate a lower body mass index (BMI) in individuals newly diagnosed with colorectal cancer, as compared to the control group. This finding aligns with a previous investigation on early-onset colorectal cancer, and it is notable that certain colorectal cancer patients experienced weight loss prior to their diagnosis (28). Moreover, some studies suggest that the weight loss within two years prior to diagnosis has the most significant impact on BMI and the risk of colorectal cancer (29). However, past studies have suggested that higher BMI is a risk factor for colorectal cancer (30). It is evident that the aforementioned studies may have underestimated the correlation between BMI and the risk of colorectal cancer (BMI demonstrates distinct attributes at various stages of colorectal cancer). This correlation between BMI and colorectal cancer has the potential to result in an underestimation or even a reversal of the direction of the correlation as presented in existing studies. The influence of being overweight or obese on the risk of colorectal cancer may be more significant than what is currently indicated by epidemiological evidence (31). However, given that the data utilized in this study pertains exclusively to individuals recently diagnosed with colorectal cancer, there exists the possibility of bias stemming from the timing of disease development preceding diagnosis. Consequently, it is imperative for future investigations to acknowledge potential biases in the correlation between BMI and colorectal cancer, as well as the connection between BMI and distinct stages of colorectal cancer advancement. This endeavor holds the potential to unveil the genuine association between BMI and the risk of developing colorectal cancer.

Conventional population-based screening initiatives have historically employed a uniform methodology, primarily relying on age as the key determinant for screening. However, a comprehensive evaluation indicates that incorporating colorectal cancer-associated risk factors can enhance the identification of individuals harboring colorectal cancer tumors (32). According to the risk prediction model for colorectal cancer, patients can be categorized based on their likelihood of developing the disease. Those identified as high-risk can derive greater advantages from colonoscopy examinations, thereby optimizing the efficiency of this diagnostic procedure (33). On the contrary, individuals with a low risk profile have the option to select non-invasive screening tests, such as FIT, for the purpose of detection. These tests are comparatively simpler to administer than colonoscopy and entail reduced risks and medical expenses. It is worth noting that cancer screening tends to yield substantial clinical advantages for a limited subset of individuals, while potentially imposing medical burdens and risks on a larger population. The examination of cost-effectiveness reveals that a screening approach reliant on risk factors must possess an area under the curve (AUC) value of no less than 0.65 to surpass the cost-effectiveness of a conventional screening program (34, 35). Within the context of our research, an AUC value of 0.751 meant a comparatively advantageous outcome.

In a recent study, a limited number of predictive factors (hemoglobin, MCV, platelets) were employed in joint models to forecast the likelihood of colorectal cancer development within a two-year timeframe for patients (36). Despite the utilization of a relatively small set of predictive factors, the model exhibited commendable predictive efficacy (AUC=0.751). Conversely, the ColonFlag model integrated twenty blood-based factors to construct a predictive model, yielding a not obvious enhancement in predictive capability (AUC=0.78) (37). The incorporation of additional predictive factors did not result in a discernible enhancement in the accuracy of the model, despite the heightened intricacy. In contrast to prior studies, our implementation of a machine learning model enables the visualization of an individual patient's susceptibility to developing the disease. Moreover, the indicators we have chosen possess greater acceptance and comprehension within the healthcare domain. Consequently, these indicators facilitate the explication of colorectal cancer risk to patients, thereby furnishing a justifiable foundation for subsequent screening and follow-up procedures.In brief, this study has developed a nomogram Model utilizing clinical data indicators, including the patient's anemia and blood indices, with the objective of forecasting the likelihood of colorectal cancer occurrence in patients. By employing various clinically accessible factors, the nomogram enables the computation of a patient's score, thereby quantifying their individual risk of developing colorectal cancer. Consequently, this tool aids clinicians in making informed clinical decisions and rational resource allocation. Despite the nomogram model's commendable AUC, it lacks the capacity to accurately predict cancer staging in patients. Our present sample exhibits a greater prevalence of stage I and II cancer in comparison to stage III and IV cancer, thus indicating a higher proportion of early-stage patients relative to late-stage patients. However, in order to fulfill the criteria for prediction model construction, a larger cohort of patients at various stages is still necessary to effectively identify early-stage tumors. In subsequent research endeavors, we intend to gather additional clinical data pertaining to colorectal cancer patients and classify them into distinct subgroups according

to tumor characteristics, thereby facilitating the development of a prognostic model for colorectal cancer staging. Furthermore, the integration of the predictive capacity for staging into the existing model presents a promising avenue for future investigation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was a retrospective study conducted with the approval of the Ethics Committee of Guilin Medical College. The ethics number is (GYLL2022056). The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from The data utilized in this research was acquired via a retrospective survey conducted by an investigator, encompassing clinical data from newly admitted inpatient cases at the Affiliated Hospital of Guilin Medical University, spanning from September 2022 to September 2023. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

ZZ: Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. TZ: Formal analysis, Writing – original draft, Writing – review & editing. RZ: Methodology, Writing – original draft, Writing – review & editing. XZ: Formal analysis, Writing – original draft. XW: Formal analysis, Writing – original draft. ST: Conceptualization, Supervision, Writing – original draft. ZJ: Conceptualization, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1347058/full#supplementary-material

References

1. Negoita S, Chen HS, Sanchez PV, Sherman RL, Henley SJ, Siegel RL, et al. Annual report to the nation on the status of cancer, part 2: early assessment of the Covid-19 pandemic's impact on cancer diagnosis. *Cancer* (2023) 130(1):117–27. doi: 10.1002/cncr.35026

2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA: Cancer J Clin* (2023) 73(1):17–48. doi: 10.3322/caac.21763

3. Li YJ, Cao MD, Wang X, Lei L, Peng J, Shi J. [Thirty-year changes in disability adjusted life years for colorectal cancer in China: A screening perspective analysis]. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi* (2022) 43(9):1381–7. doi: 10.3760/cma.j.cn112338-20220504-00377

4. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. *CA: Cancer J Clin* (2020) 70(3):145–64. doi: 10.3322/caac.21601

5. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (Risk factors, screening, and treatment) to reduce future rates. *Cancer* (2010) 116(3):544–73. doi: 10.1002/cncr.24760

6. Zhou Z, Li Q, Yu J, Rong M, Cao J, Ge L, et al. Epidemiological characteristics and disease burden of cancer in Guangxi cancer registration areas, 2017. *Chin J Oncol Prev Treat* (2022) 14(3):264–73. doi: 10.3969/j.issn.1674-5671.2022.03.04

7. Navarro M, Nicolas A, Ferrandez A, Lanas A. Colorectal cancer population screening programs worldwide in 2016: an update. *World J Gastroenterol* (2017) 23 (20):3632–42. doi: 10.3748/wjg.v23.i20.3632

8. Ghoshal S, Rigney G, Cheng D, Brumit R, Gee MS, Hodin RA, et al. Institutional surgical response and associated volume trends throughout the Covid-19 pandemic and postvaccination recovery period. *JAMA network Open* (2022) 5(8):e2227443. doi: 10.1001/jamanetworkopen.2022.27443

9. He JH, Cao C, Ding Y, Yi Y, Lv YQ, Wang C, et al. A nomogram model for predicting distant metastasis of newly diagnosed colorectal cancer based on clinical features. *Front Oncol* (2023) 13:1186298. doi: 10.3389/fonc.2023.1186298

10. Zhao J, Wang Y, Gao J, Wang Y, Zhong X, Wu X, et al. A nine-gene signature to improve prognosis prediction of colon carcinoma. *Cell Cycle (Georgetown Tex)* (2021) 20(10):1021–32. doi: 10.1080/15384101.2021.1919827

11. Zhang B, Xu S, Xu W, Chen Q, Chen Z, Yan C, et al. Leveraging fecal bacterial survey data to predict colorectal tumors. *Front Genet* (2019) 10:447. doi: 10.3389/fgene.2019.00447

12. Petrik AF, Johnson ES, Mummadi R, Slaughter M, Coronado GD, Lin SC, et al. The use of individual and multilevel data in the development of a risk prediction model to predict patients' Likelihood of completing colorectal cancer screening. *Prev Med Rep* (2023) 36:102366. doi: 10.1016/j.pmedr.2023.102366

13. Schneider C, Bodmer M, Jick SS, Meier CR. Colorectal cancer and markers of anemia. *Eur J Cancer Prev* (2018) 27(6):530–8. doi: 10.1097/cej.0000000000000397

14. McSorley ST, Tham A, Steele CW, Dolan RD, Roxburgh CS, Horgan PG, et al. Quantitative data on red cell measures of iron status and their relation to the magnitude of the systemic inflammatory response and survival in patients with colorectal cancer. *Eur J Surg Oncol* (2019) 45(7):1205–11. doi: 10.1016/j.ejso.2019.02.027

15. Luo Y, Lai Q, Huang H, Luo J, Miao J, Liao R, et al. Risk factor analysis and nomogram construction for predicting suicidal ideation in patients with cancer. *BMC Psychiatry* (2022) 22(1):353. doi: 10.1186/s12888-022-03987-z

16. Vickers AJ, Elkin EB. Decision curve analysis: A novel method for evaluating prediction models. *Med decision making* (2006) 26(6):565–74. doi: 10.1177/0272989x06295361

17. Heagerty PJ, Lumley T, Pepe MS. Time-dependent roc curves for censored survival data and a diagnostic marker. *Biometrics* (2000) 56(2):337–44. doi: 10.1111/j.0006-341x.2000.00337.x

18. Xu Y, Han D, Huang T, Zhang X, Lu H, Shen S, et al. Predicting icu mortality in rheumatic heart disease: comparison of xgboost and logistic regression. *Front Cardiovasc Med* (2022) 9:847206. doi: 10.3389/fcvm.2022.847206

19. Varshney K, Glodjo T, Adalbert J. Overcrowded housing increases risk for Covid-19 mortality: an ecological study. *BMC Res Notes* (2022) 15(1):126. doi: 10.1186/s13104-022-06015-1

20. Li Y, Lu F, Yin Y. Applying logistic lasso regression for the diagnosis of atypical Crohn's disease. *Sci Rep* (2022) 12(1):11340. doi: 10.1038/s41598-022-15609-5

21. McNeish DM. Using lasso for predictor selection and to assuage overfitting: A method long overlooked in behavioral sciences. *Multivariate Behav Res* (2015) 50 (5):471–84. doi: 10.1080/00273171.2015.1036965

22. Tibshirani R. Regression shrinkage and selection via the lasso. *J R Stat Soc Ser B: Stat Method* (1996) 58(1):267–88. doi: 10.1111/j.2517-6161.1996.tb02080.x

23. Nutritional anaemias. Report of a Who scientific group. *World Health Organ Tech Rep Ser* (1968) 405:5–37.

24. Devon KM, McLeod RS. Pre and peri-operative erythropoietin for reducing allogeneic blood transfusions in colorectal cancer surgery. *Cochrane Database Sys Rev* (2009) (1):CD007148. doi: 10.1002/14651858.CD007148.pub2

25. Virdee PS, Marian IR, Mansouri A, Elhussein L, Kirtley S, Holt T, et al. The full blood count blood test for colorectal cancer detection: A systematic review, meta-analysis, and critical appraisal. *Cancers* (2020) 12(9):2348.. doi: 10.3390/cancers12092348

26. Virdee PS, Patnick J, Watkinson P, Birks J, Holt TA. Trends in the full blood count blood test and colorectal cancer detection: A longitudinal, case-control study of UK primary care patient data. *NIHR Open Res* (2022) 2:32. doi: 10.3310/nihropenres.13266.2

27. Gonzalez-Hermoso F, Perez-Palma J, Marchena-Gomez J, Lorenzo-Rocha N, Medina-Arana V. Can early diagnosis of symptomatic colorectal cancer improve the prognosis? *World J Surg* (2004) 28(7):716–20. doi: 10.1007/s00268-004-7232-8

28. Low EE, Demb J, Liu L, Earles A, Bustamante R, Williams CD, et al. Risk factors for early-onset colorectal cancer. *Gastroenterology* (2020) 159(2):492–501.e7. doi: 10.1053/j.gastro.2020.01.004

29. Mandic M, Safizadeh F, Niedermaier T, Hoffmeister M, Brenner H. Association of overweight, obesity, and recent weight loss with colorectal cancer risk. *JAMA network Open* (2023) 6(4):e239556. doi: 10.1001/jamanetworkopen.2023.9556

30. Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, et al. Meta-analyses of colorectal cancer risk factors. *Cancer causes control: CCC* (2013) 24 (6):1207–22. doi: 10.1007/s10552-013-0201-5

31. Mandic M, Li H, Safizadeh F, Niedermaier T, Hoffmeister M, Brenner H. Is the association of overweight and obesity with colorectal cancer underestimated? An umbrella review of systematic reviews and meta-analyses. *Eur J Epidemiol* (2023) 38 (2):135–44. doi: 10.1007/s10654-022-00954-6

32. Kastrinos F, Kupfer SS, Gupta S. Colorectal cancer risk assessment and precision approaches to screening: brave new world or worlds apart? *Gastroenterology* (2023) 164 (5):812–27. doi: 10.1053/j.gastro.2023.02.021

33. Imperiale TF, Monahan PO, Stump TE, Ransohoff DF. Derivation and validation of a predictive model for advanced colorectal neoplasia in asymptomatic adults. *Gut* (2021) 70(6):1155–61. doi: 10.1136/gutjnl-2020-321698

34. Naber SK, Kundu S, Kuntz KM, Dotson WD, Williams MS, Zauber AG, et al. Cost-effectiveness of risk-stratified colorectal cancer screening based on polygenic risk: current status and future potential. *JNCI Cancer Spectr* (2020) 4(1):pkz086. doi: 10.1093/jncics/pkz086

35. Cooper JA, Moss SM, Smith S, Seaman HE, Taylor-Phillips S, Parsons N, et al. Fit for the future: A case for risk-based colorectal cancer screening using the faecal immunochemical test. *Colorectal Dis* (2016) 18(7):650–3. doi: 10.1111/codi.13365

36. Virdee PS, Patnick J, Watkinson P, Holt T, Birks J. Full blood count trends for colorectal cancer detection in primary care: development and validation of a dynamic prediction model. *Cancers* (2022) 14(19):4779. doi: 10.3390/cancers14194779

37. Kinar Y, Kalkstein N, Akiva P, Levin B, Half EE, Goldshtein I, et al. Development and validation of a predictive model for detection of colorectal cancer in primary care by analysis of complete blood counts: A binational retrospective study. *J Am Med Inf Association: JAMIA* (2016) 23(5):879–90. doi: 10.1093/jamia/ocv195

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Quantitative analysis of contrast-enhanced ultrasound in neoadjuvant treatment of locally advanced rectal cancer: a retrospective study

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Purpose: To explore the clinical value of contrast-enhanced ultrasound (CEUS) quantitative analysis in the evaluation and prognosis of neoadjuvant chemoradiotherapy for locally advanced rectal cancer (LARC).

Methods: Eighty-three consecutive patients undergoing neoadjuvant chemoradiotherapy and total mesorectal excision for LARC were retrospectively included. According to pathological results, patients were categorized into complete or incomplete response groups. Differences in ultrasonic parameters, pathological results, and clinical data between groups were evaluated. The cutoff point for a complete response as determined by quantitative analysis of CEUS was assessed using a receiver operating characteristic curve; additionally, overall survival (OS) and progression-free survival (PFS) were analyzed.

Results: Of the 83 patients, 12 (14.5%) achieved a complete response and 71 (85.5%) did not. There were significant between-group differences in carcinoembryonic antigen (CEA) levels, differentiation degree, proportion of tumor occupying the lumen, anterior-posterior and superior-inferior diameters of the lesion, and intensity of enhancement (P<0.05). CEUS quantitative analysis showed significant between-group differences in peak intensity (PI) and area under the curve (AUC) values (P<0.05). The OS and PFS of patients with high PI, high AUC value, and poorly differentiated cancer were significantly worse than those with low PI, low AUC values, and moderately to highly differentiated cancer (P<0.05). High CEA levels (hazard ratio: 1.02, 95% confidence interval: 1.01–1.04; P=0.002) and low differentiation (2.72, 1.12–6.62; P=0.028) were independent risk factors for PFS and OS.

Conclusions: CEUS can predict the response to neoadjuvant treatment in patients with LARC. CEUS quantitative analysis is helpful for clinical prognosis.

KEYWORDS

ultrasonography, neoadjuvant therapy, locally advanced rectal cancer, prognosis, colorectal cancer

Introduction

Colorectal cancer ranks high in both morbidity and mortality rates worldwide (1, 2). In recent years, total mesorectal excision (TME) has become the standard treatment for rectal cancer, while neoadjuvant chemoradiotherapy (NCRT) has substantially improved the control of local lesions in patients with locally advanced rectal cancer (LARC). This has led to increased survival rates for patients with rectal cancer (3, 4). Therefore, the efficacy of neoadjuvant treatment and its impact on patient prognosis have garnered much clinical attention. Timely observation of the efficacy of neoadjuvant treatment is of great importance for selecting appropriate clinical treatment measures; some strictly selected patients can even achieve a complete response after NCRT with a "wait and see" policy and avoid surgical treatment (5, 6).

In patients with LARC, the use of magnetic resonance imaging (MRI)-based radiomics has demonstrated a certain effect in predicting a complete response as well as survival outcomes after chemoradiotherapy (7, 8). Transrectal ultrasound has been utilized in staging rectal cancer and in assessing responses to neoadjuvant treatment. Several studies have reported that sequential endorectal ultrasonography examinations can predict the effectiveness of preoperative chemoradiotherapy as a treatment for LARC (9–11). Contrast-enhanced ultrasound (CEUS) is a widely accepted and extensively used imaging modality that can quantitatively evaluate tumor microvascular blood-flow perfusion information (12, 13). However, the role of CEUS-derived blood-flow information in assessing the efficacy of chemoradiotherapy and predicting survival outcomes in patients with LARC has not yet been reported.

Thus, this study aimed to investigate the relationship between post-neoadjuvant treatment CEUS parameters of LARC and the pathological results and clinical data of these patients to evaluate the effect of quantitative parameters on the prognosis of patients after TME surgery.

Materials and methods

Study population

We retrospectively reviewed 83 consecutive patients diagnosed with rectal cancer based on pathology at our hospital between May 2017 and December 2021. We collected the patients' ultrasound parameters, pathological results, and clinical data and conducted follow-ups to ascertain survival outcomes. Moreover, all ultrasound parameter data were collected after neoadjuvant treatment and 1 week prior to surgery. Inclusion criteria were a clinical diagnosis of LARC with neoadjuvant treatment prior to surgery, a distance of 12cm from the lower margin of the tumor to the anal margin, and the availability of complete and analyzable CEUS data of the lesion. Exclusion criteria were failure to complete the planned neoadjuvant treatment or radical surgery, the simultaneous presence of multiple primary malignant tumors, or loss to follow-up.

The protocol of this study was approved by the Institutional Review Board of our hospital Clinical Research Ethics Committee (protocol number: 201901–03; date of approval: January 29, 2019). The trial was registered at Chinese Clinical Trials.gov: No. ChiCTR1900022298.

Treatment methods

All enrolled patients received NCRT, with a total radiation dosage of 50–55 Gy delivered in 1.8–2.0 Gy fractions over 25–28 sessions, and concomitant chemotherapy consisting of 5-fluorouracil or capecitabine. Radical surgery treatment was performed at 6–8 weeks after completion of neoadjuvant treatment.

Follow-up definitions

Progression-free survival (PFS) was defined as the time/duration from the initial diagnosis at our hospital until local recurrence, distant metastasis, or death prior to surgery. Overall survival (OS) was defined as the duration from the initial diagnosis to death or the end of the follow-up period. All patients underwent routine clinical examinations every 3 months during the first-year post-surgery and every 6 months thereafter. Each examination included a review of the clinical data, serum testing, and chest-abdomen-pelvis computed tomography (CT).

Instrument and methods

A LOGIQ E9 ultrasound scanner (GE Healthcare, Chicago, IL), equipped with low mechanical index ultrasound imaging technology, was used with a transrectal endoscopic probe with a frequency of 5–9 MHz and SonoVue contrast agent (Bracco, Milan, Italy). The patient was given an enema 1 h prior to the examination and then placed in a left lateral position with the hip and knee in flexion. The location of the lesion was determined under two-dimensional ultrasound, and the thickness, cumulative length, and percentage of the intestinal lumen occupied by the tumor were recorded by repeated multi-section scanning. Subsequently, when the blood flow was most abundant in the lesion and some normal intestinal wall was displayed simultaneously, the CEUS mode was switched on, and 2.4 mL of ultrasound contrast agent microbubble suspension was injected into the cubital vein cluster, followed by rapid injection of 5 mL of saline to allow CEUS examination of the primary tumor lesion. Using the dual-phase contrast interface, the enhancement of the contrast agent was observed in real-time, and images were continuously stored and recorded for 90 s each. The instrument's integral measurement software was used to obtain values for contrast-related parameters, and the rectal tumor was selected as the region of interest (ROI). The ROI, where the contrast agent was uniformly and steadily distributed, was manually adjusted, and the mucosal layer of the normal intestinal wall at 1 cm away from the tumor was selected as the control area. The software automatically draws the time-intensity curve of the contrast agent perfusion in the ROI, including its rise time (RT), time to peak enhancement (TTP), peak intensity (PI), ascending slope (AS), and area under the curve (AUC). The time-intensity

curve was measured continuously five times, and the average values were obtained. The enhancement mode was divided into high enhancement, iso-enhancement, and low enhancement, based on the contrast between the lesion and the normal mucosal layer of the rectal wall. Image analysis was performed in a blinded fashion by two ultrasound physicians with over 10 years of experience.

Statistical analysis

SPSS version 26.0 (IBM, Armonk, NY) was employed for statistical analyses. According to the pathological results, the patients were divided into complete and incomplete pathological response groups. Categorical variables were compared between groups using the chi-squared test. For differences in measures between groups, we used the *t*-test when the data conformed to a normal distribution. We used the Mann–Whitney U test for non-normally distributed parameters and plotted the receiver operating characteristic (ROC) curve to evaluate the diagnostic efficacy. Kaplan–Meier survival and Cox regression analyses were performed based on pathological results, clinical data, and CEUS parameters. A p-value of less than 0.05 was considered to be statistically significant.

Results

Comparison of baseline characteristics and CEUS methods

The 83 patients with LARC who underwent neoadjuvant treatment had adenocarcinoma confirmed by postoperative pathology and included 32 (37.3%) cases of poorly differentiated carcinoma and 51 (57.8%) cases of moderately to highly differentiated carcinoma. Moreover, 25 (30.1%) cases were lymph node-positive and 58 (69.9%) were lymph node-negative. Twelve (14.5%) patients achieved a complete response, while 71 (85.5%) patients had a partial response. Complete response to neoadjuvant treatment for rectal cancer was related to carcinoembryonic antigen (CEA) levels and tumor differentiation, with significant differences (P=0.041 and 0.045, respectively). However, it was not associated with sex or age. Complete response to neoadjuvant treatment for rectal cancer was significantly associated with the proportion of the bowel lumen occupied by the tumor, the anterior-posterior and superior-inferior diameters of the lesion, and the intensity of enhancement (all p<0.05) (Table 1).

Quantitative analysis of CEUS images in neoadjuvant treatment for rectal cancer

The results of the CEUS quantitative analysis indicated that the AUC and PI values in the group with an incomplete pathological response following neoadjuvant treatment for rectal cancer were significantly higher than those in the group with a complete response (both P<0.05). However, no significant differences were observed in RT, TTP, or AS between the two groups (all P>0.05) (Table 2; Figure 1).

PI and AUC evaluation for complete response after neoadjuvant treatment for rectal cancer

Using ROC analysis, cutoff values of 23.1 dB and 938.56 dB were selected for the PI and AUC, respectively, to evaluate the sensitivity and specificity of a complete response after neoadjuvant treatment for rectal cancer. The sensitivity and specificity were 76.4% and 83.3% for PI, and 64.6% and 83.3% for AUC (Figure 2).

Prognostic analysis

Among the 83 patients with LARC, the median follow-up time was 27 months, with a maximum follow-up time of 63 months. At the last follow-up, 19 patients died, 3 had *in situ* recurrence, and 21 had distant metastasis, mainly to the liver, lungs, and pelvic lymph nodes. Based on the PI grouping by ROC curve, Kaplan–Meier analysis showed that the PFS and OS of the low-PI group were significantly better than those of the high-PI group (P=0.014 and 0.019, respectively). Moreover, significant differences were observed in PFS and OS between the low- and high-AUC groups (P=0.042 and 0.012, respectively). Furthermore, the PFS and OS of the moderately to highly differentiated group were significantly superior to those of the poorly differentiated group (P=0.001 and 0.034, respectively) (Figure 3). In the Cox regression analysis, the univariate analysis results indicated that CEA, differentiation degree, lymph node metastasis, AUC, and PI were key predictors of PFS and OS. The multivariate analysis revealed that high CEA levels (hazard ratio [HR]: 1.02, 95% confidence interval [CI]: 1.01–1.04; P=0.002) and low differentiation (HR: 2.72, 95% CI: 1.12–6.62; P=0.028) were independent risk factors for PFS and OS (Table 3).

Discussion

For the treatment of LARC, current guidelines recommend a comprehensive strategy of preoperative NCRT and radical TME. Previous studies have reported that 15–20% of patients with rectal cancer who undergo neoadjuvant treatment achieve a pathological complete response (pCR) (14). In the present study, the pCR rate reached 14.5%, which was consistent with the values reported in the literature, indicating that NCRT has a significant therapeutic effect on progressive rectal cancer and can help change or determine the subsequent treatment strategy to some extent. The degree of tumor response to NCRT is an indicator of clinical efficacy. Therefore, accurately evaluating the therapeutic effect of neoadjuvant treatment, particularly in patients with pCR, is a challenging topic in clinical research.

Currently, the clinical imaging methods that have been used to evaluate the efficacy of neoadjuvant treatment for rectal cancer primarily include CT, MRI, and positron emission tomography-

TABLE 1 Comparison of baseline characteristics and CEUS methods.

Characteristic		Complete response (n=12)	Incomplete response (n=71)	x^2/Z	P-value
Sex					
	Male	4 (33.3)	45 (63.4)	2.690	0.101
	Female	8 (66.7)	26 (36.6)		
Age, years					
	<60	6 (50.0)	39 (54.9)	0.100	0.751
	≥60	6 (50.0)	32 (45.1)		
CEA (ug/L)					
	<5	12 (100)	47 (66.2)	4.180	0.041
	≥5	0 (0)	24 (33.8)		
Differentiation					
	Poorly differentiated	2 (16.7)	30 (42.3)	4.020	0.045
	Moderately to highly differentiated	10 (83.3)	41 (57.7)		
Extent of tumor infiltration					
	<1/2	12 (100)	47 (66.2)	4.18	0.041
	≥1/2	0 (0)	24 (33.8)		
Intensity of enhancement					
	Low enhancement	8 (66.7)	21 (29.6)	4.687	0.030
	High enhancement	4 (33.3)	50 (70.4)		
Anterior-posterior diameter (cm)*		0.60 ± 0.32	1.11 ± 0.50	-3.685	<0.001
Superior-inferior diameter (cm) *		1.75 ± 0.98	2.60 ± 1.50	-3.980	<0.001

Data are n (%) unless otherwise indicated.
*Data are medians and interquartile range, and Mann–Whitney U test was used.
CEUS, contrast-enhanced ultrasound; CEA, carcinoembryonic antigen.

CT, with MRI with different sequences being the primary choice. Studies have confirmed that both MRI functional parameters, such as apparent diffusion coefficient values and vascular perfusion parameters, are reliable predictors of prognosis in patients with rectal cancer (15–17). However, rectal MRI requires special coil preparation, particular postures, and involves a cumbersome operation, as well as discomfort to the patient due to the confined space, which limits the use of MRI to examine rectal cancer. The literature has revealed that transrectal ultrasound is highly accurate in the preoperative staging of rectal cancer (18). However, due to swelling, inflammation, fibrosis, and necrosis of the rectal tumor and the surrounding structures induced by radiotherapy and

TABLE 2 Quantitative analysis of CEUS in NCRT for rectal cancer.

Parameter	RT*	TTP#	AS*	AUC#	PI*
Complete response (n=12)	9.00 ± 3.50	27.17 ± 8.05	2.39 ± 1.20	829.63 ± 131.91	21.25 ± 4.22
Incomplete response (n=71)	9.00 ± 6.00	27.04 ± 7.19	2.28 ± 1.42	966.00 ± 117.22	24.50 ± 3.40
***t*/Z**	-0.560	0.003	-0.123	13.408	-3.737
P-value	0.576	0.957	0.902	0.001	<0.001

*Data are medians and interquartile range, and Mann–Whitney U test was used.
#Data are mean ± standard deviation, and Student's t-test was used.
CEUS, contrast-enhanced ultrasound; NCRT, neoadjuvant chemoradiotherapy, RT, rise time; TTP, time to peak enhancement; AS, ascending slope; AUC, area under the curve; PI, peak intensity.

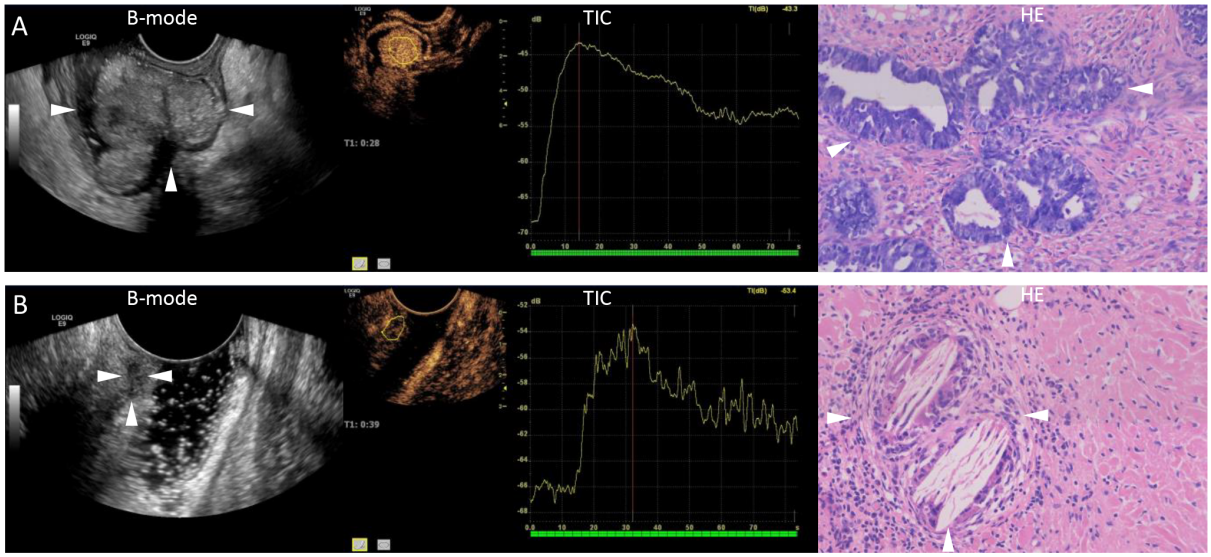

FIGURE 1
Representative images of ultrasound and pathology after neoadjuvant chemoradiotherapy. **(A)** A 76-year-old male patient with a B-mode ultrasound showing the extent of rectal lesions (arrows). Quantitative analysis of contrast-enhanced ultrasound shows high enhancement, with a peak intensity of 24.9 dB and an area under the curve of 1065.70 dB. Pathology showing incomplete remission (arrows, hematoxylin stain, original magnification ×20). **(B)** A 52-year-old female patient with a B-mode ultrasound showing the extent of rectal lesions (arrows). Quantitative analysis of contrast-enhanced ultrasound shows low enhancement, with a peak intensity of 13.6 dB and an area under the curve of 524.58 dB. Pathology showing complete remission (arrows, hematoxylin stain, original magnification ×20). B-mode, brightness-mode; TIC, time-intensity curve; HE, hematoxylin stain.

chemotherapy, transrectal ultrasound cannot accurately identify the tumor margin. Hence, it is difficult to assess the efficacy of neoadjuvant treatment for rectal cancer accurately by ultrasound. Antitumor therapies, such as radiotherapy and chemotherapy, can alter the hemodynamic parameters related to blood-flow perfusion within the tumor (19). Ultrasound contrast agent microbubbles are pure blood-pool contrast agents that always flow in the blood circulation after intravenous injection and do not penetrate outside blood vessels, making them an ideal tracer for studying tissue blood perfusion (20, 21). In this study, ultrasound contrast and quantitative analysis were used to evaluate the efficacy of neoadjuvant treatment for rectal cancer. Our results showed that the proportion of the tumor occupying the intestinal lumen, the anterior-posterior and superior-inferior diameters of the lesion, and the enhancement intensity of the ultrasound contrast after neoadjuvant treatment were smaller in the complete response group than in the incomplete response group, indicating that the treatment effect was better. The tumor shrinkage was more obvious in the complete response group. The quantitative analysis results revealed that the PI and AUC values of rectal cancer lesions after neoadjuvant treatment were significantly lower in the complete response group than in the incomplete response group. This indicated that the pathological microscopic changes after radiotherapy mainly involved neovascularization and necrosis of tumor cells, while CEUS could reflect changes in hemodynamic parameters related to blood-flow perfusion in tumor tissues, regardless of the enhancement mode or quantitative analysis. ROC curve analysis showed that the sensitivity of PI and AUC values in evaluating complete response following neoadjuvant treatment for LARC was 76.4% and 64.6%, respectively, while the specificity was 83.3% for both (Figure 2). Accordingly, the use of CEUS and quantitative measurement of PI and AUC values, in addition to routine ultrasound examination, demonstrated high sensitivity and specificity for distinguishing a complete response after neoadjuvant

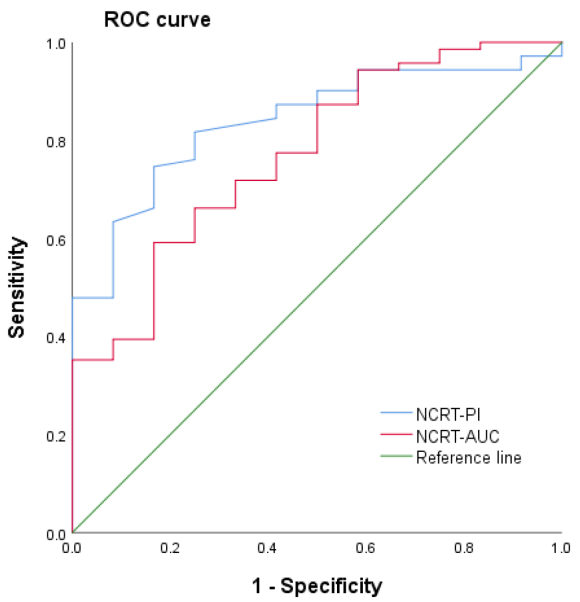

FIGURE 2
Receiver operating characteristic (ROC) curve of contrast-enhanced ultrasound quantitative analysis for evaluating a complete response following neoadjuvant treatment for rectal cancer. NCRT, neoadjuvant chemoradiotherapy; AUC, area under the curve; PI, peak intensity.

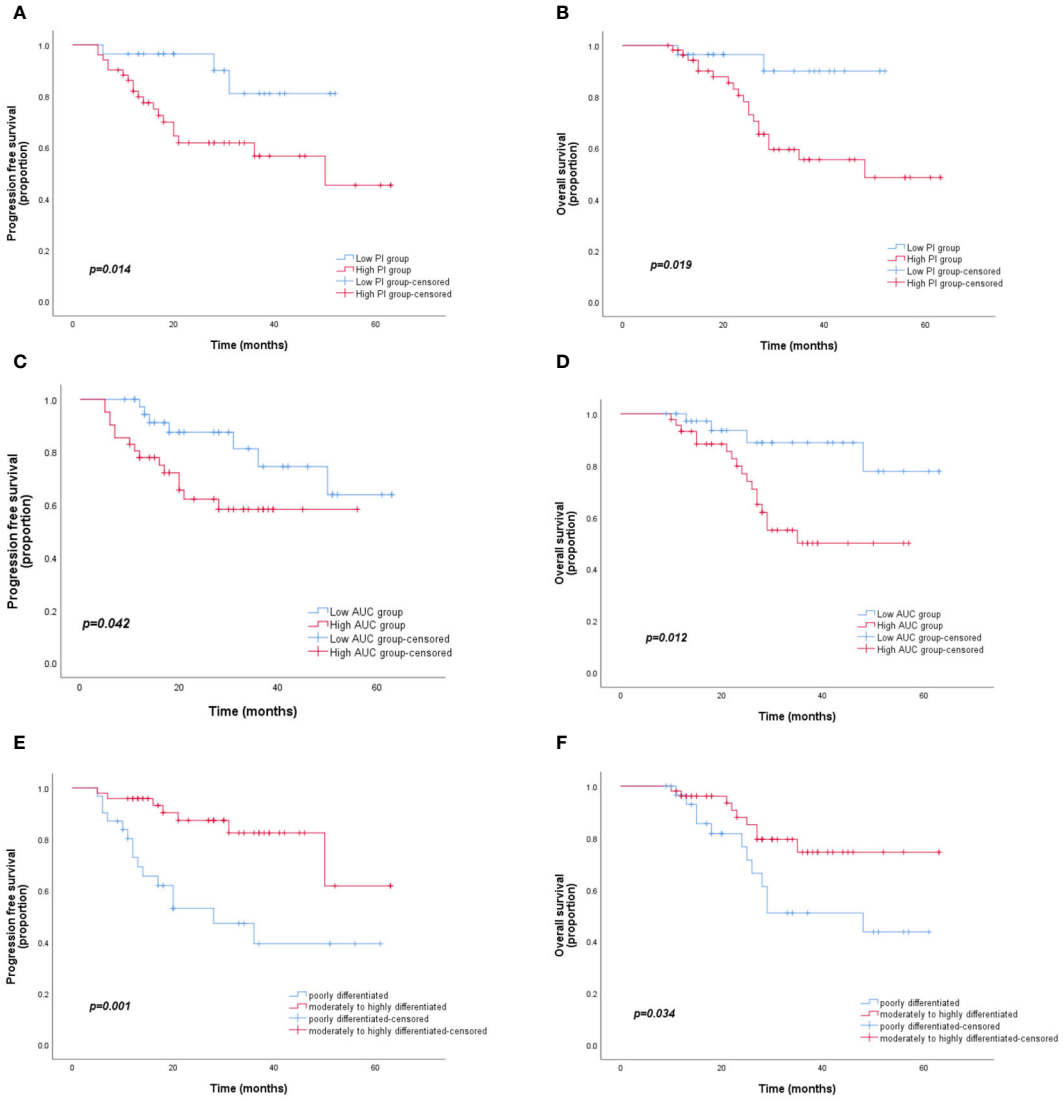

FIGURE 3
Kaplan–Meier curves for PFS and OS according to the AUC, PI, and differentiation degree. **(A, B)** OS and PFS for different PI groups. **(C, D)** OS and PFS for different AUC groups. **(E, F)** OS and PFS for different degrees of differentiation. PFS, progression-free survival; OS, overall survival; AUC, area under the curve; PI, peak intensity.

treatment for LARC, which can serve as a reference for clinical judgment of the efficacy of neoadjuvant treatment.

The prognosis of LARC treated with neoadjuvant treatment and TME is of great concern to clinicians and patients. Early evaluation and analysis of complete response or partial regression of tumors following neoadjuvant treatment play a vital role in improving the long-term prognosis of patients with LARC. The literature shows that CEA, the degree of differentiation, and the extent of tumor infiltration are independent risk factors for rectal cancer (22, 23), and that CEA and the degree of differentiation are also poor prognostic factors for combined neoadjuvant treatment and TME in LARC (24–26). Our results were essentially consistent with these findings (Table 3; Figure 3). The Kaplan–Meier analysis demonstrated that the PFS and OS of patients with rectal cancer with low PI and AUC values who underwent neoadjuvant treatment combined with TME were significantly better than those of rectal cancer patients with high PI and AUC values.

This study has several limitations. First, our study only discussed the difference between patients with complete and incomplete responses. In patients with incomplete response, there are individuals who have a good or poor response to neoadjuvant therapy, but we have not conducted further studies. At the same time, compared with the number of patients with incomplete response, the number of patients with complete response was smaller and there was a quantitative imbalance between the two. In order to reduce allocation bias, we will again subdivide and classify patients with incomplete response in future studies. Secondly, because ultrasound is affected by human factors, we used the same sonographer who has been in the field for more than 10 years to collect images, and asked for measurements at the same level, and took five measurements to calculate the average.

In conclusion, our results indicate that quantitative analysis of CEUS can be used to evaluate the efficacy of neoadjuvant treatment

TABLE 3 Univariate and multivariate analyses of prognostic factors for PFS and OS.

Analysis	PFS		OS	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Univariable				
CEA (ug/L)	1.03 (1.01, 1.05)	<0.001	1.02 (1.01, 1.03)	0.004
Differentiation (poorly vs. moderately to highly)	0.19 (0.07, 0.48)	<0.001	0.34 (0.14, 0.82)	0.017
Lymph node metastasis (no vs. yes)	3.85 (1.64, 9.07)	0.002	3.43 (1.44, 8.16)	0.005
Anterior-posterior diameter (cm)	0.88 (0.51, 1.51)	0.649	0.98 (0.66, 1.48)	0.939
Superior-inferior diameter (cm)	1.01 (0.77, 1.33)	0.944	1.04 (0.79, 1.38)	0.758
AUC	1.01 (1.00, 1.01)	0.017	1.01 (1.00, 1.01)	0.011
PI	1.22 (1.03, 1.43)	0.018	1.21 (1.02, 1.44)	0.031
Multivariable				
CEA (ug/L)	1.03 (1.01, 1.05)	0.001	1.02 (1.01, 1.04)	0.011
Differentiation (moderately to highly vs. poorly)	5.15 (1.98, 13.39)	0.003	2.70 (1.12, 6.62)	0.031
AUC	1.00 (1.00, 1.01)	0.062	1.00 (1.00, 1.01)	0.045

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen; AUC, area under the curve; PI, peak intensity.

in patients with progressive rectal cancer and that this may become a new reference index for assessing the degree of relief and changes in the effectiveness of neoadjuvant treatment in clinical practice. In future studies, more sensitive CEUS parameters should be explored, and the sample size should be increased to verify the utility of CEUS quantitative parameters in the clinical evaluation of the effectiveness of neoadjuvant treatment for rectal cancer.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Institutional Review Board of Tangdu Hospital, Fourth Military Medical University Clinical Research Ethics Committee (protocol number: 201901–03; date of approval: January 29, 2019). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

GB: Conceptualization, Writing – original draft. CW: Data curation, Formal Analysis, Writing – original draft. YS: Data curation, Writing – review & editing, Resources. JL: Data curation, Writing – review & editing, Resources. XS: Data curation, Resources, Writing – review & editing. WZ: Data curation, Resources, Writing – review & editing. RY: Writing – review & editing, Conceptualization, Project administration, Supervision, Writing – original draft. YY: Project administration, Supervision, Writing – original draft, Writing – review & editing, Methodology, Validation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A , et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 713:209–49. doi: 10.3322/caac.21660

2. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J Clin* (2023) 73:233–54. doi: 10.3322/caac.21772

3. Oronsky B, Reid T, Larson C, Knox SJ. Locally advanced rectal cancer: The past, present, and future. *Semin Oncol* (2020) 471:85–92. doi: 10.1053/j.seminoncol.2020.02.001

4. Feeney G, Sehgal R, Sheehan M, Hogan A, Regan M, Joyce M, et al. Neoadjuvant radiotherapy for rectal cancer management. *World J Gastroenterol* (2019) 2533:4850–69. doi: 10.3748/wjg.v25.i33.4850

5. Fernandez LM, São Julião GP, Figueiredo NL, Beets GL, van der Valk MJM, Bahadoer RR, et al. Conditional recurrence-free survival of clinical complete responders managed by watch and wait after neoadjuvant chemoradiotherapy for rectal cancer in the international watch & wait database: A retrospective, international, multicentre registry study. *Lancet Oncol* (2021) 221:43–50. doi: 10.1016/s1470-2045(20)30557-x

6. Dossa F, Chesney TR, Acuna SA, Baxter NN. A watch-and-wait approach for locally advanced rectal cancer after a clinical complete response following neoadjuvant chemoradiation: A systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* (2017) 27:501–13. doi: 10.1016/s2468-1253(17)30074-2

7. Shin J, Seo N, Baek SE, Son NH, Lim JS, Kim N, et al. Mri radiomics model predicts pathologic complete response of rectal cancer following chemoradiotherapy. *Radiology* (2022) 3032:351–8. doi: 10.1148/radiol.211986

8. Wei Q, Chen Z, Tang Y, Chen W, Zhong L, Mao L, et al. External validation and comparison of MR-based radiomics models for predicting pathological complete response in locally advanced rectal cancer: a two-centre, multi-vendor study. *Eur Radiol* (2023) 33:1906–17. doi: 10.1007/s00330-022-09204-5

9. Luglio G, Pagano G, Tropeano FP, Spina E, Maione R, Chini A, et al. Endorectal ultrasonography and pelvic magnetic resonance imaging show similar diagnostic accuracy in local staging of rectal cancer: An update systematic review and meta-analysis. *Diagnostics (Basel)* (2021) 12(1):5. doi: 10.3390/diagnostics12010005

10. Reginelli A, Clemente A, Sangiovanni A, Nardone V, Selvaggi F, Sciaudone G, et al. Endorectal ultrasound and magnetic resonance imaging for rectal cancer staging: A modern multimodality approach. *J Clin Med* (2021) 10(4):641. doi: 10.3390/jcm10040641

11. Li N, Dou L, Zhang Y, Jin J, Wang G, Xiao Q, et al. Use of sequential endorectal us to predict the tumor response of preoperative chemoradiotherapy in rectal cancer. *Gastrointest Endosc* (2017) 853:669–74. doi: 10.1016/j.gie.2016.06.042

12. Sidhu PS, Cantisani V, Dietrich CF, Gilja OH, Saftoiu A, Bartels E, et al. The efsumb guidelines and recommendations for the clinical practice of contrast-enhanced ultrasound (ceus) in non-hepatic applications: Update 2017 (long version). *Ultraschall Med* (2018) 392:e2–e44. doi: 10.1055/a-0586-1107

13. Lassau N, Coiffier B, Faivre L, Benatsou B, Bidault S, Girard E, et al. Study of intrapatient variability and reproducibility of quantitative tumor perfusion parameters evaluated with dynamic contrast-enhanced ultrasonography. *Invest Radiol* (2017) 523:148–54. doi: 10.1097/rli.0000000000000324

14. Maas M, Nelemans PJ, Valentini V, Das P, Rödel C, Kuo LJ, et al. Long-term outcome in patients with a pathological complete response after chemoradiation for rectal cancer: A pooled analysis of individual patient data. *Lancet Oncol* (2010) 119:835–44. doi: 10.1016/s1470-2045(10)70172-8

15. Gollub MJ, Blazic I, Felder S, Knezevic A, Gonen M, Garcia-Aguilar J, et al. Value of adding dynamic contrast-enhanced mri visual assessment to conventional mri and clinical assessment in the diagnosis of complete tumour response to chemoradiotherapy for rectal cancer. *Eur Radiol* (2019) 293:1104–13. doi: 10.1007/s00330-018-5719-1

16. Yang L, Qiu M, Xia C, Li Z, Wang Z, Zhou X, et al. Value of high-resolution dwi in combination with texture analysis for the evaluation of tumor response after preoperative chemoradiotherapy for locally advanced rectal cancer. *AJR Am J Roentgenol* (2019) 2126:1279–86. doi: 10.2214/ajr.18.20689

17. Zhang G, Xu Z, Zheng J, Wang M, Ren J, Wei X, et al. Prognostic value of multi b-value DWI in patients with locally advanced rectal cancer. *Eur Radiol* (2023) 33:1928–37. doi: 10.1007/s00330-022-09159-7

18. Nasseri Y, Langenfeld SJ. Imaging for colorectal cancer. *Surg Clin North Am* (2017) 973:503–13. doi: 10.1016/j.suc.2017.01.002

19. Graham K, Unger E. Overcoming tumor hypoxia as a barrier to radiotherapy, chemotherapy and immunotherapy in cancer treatment. *Int J Nanomedicine* (2018) 13:6049–58. doi: 10.2147/ijn.S140462

20. Averkiou MA, Bruce MF, Powers JE, Sheeran PS, Burns PN. Imaging methods for ultrasound contrast agents. *Ultrasound Med Biol* (2020) 463:498–517. doi: 10.1016/j.ultrasmedbio.2019.11.004

21. Amioka A, Masumoto N, Gouda N, Kajitani K, Shigematsu H, Emi A, et al. Ability of contrast-enhanced ultrasonography to determine clinical responses of breast cancer to neoadjuvant chemotherapy. *Jpn J Clin Oncol* (2016) 464:303–9. doi: 10.1093/jjco/hyv215

22. Vuik FER, Nieuwenburg SAV, Nagtegaal ID, Kuipers EJ, Spaander MCW. Clinicopathological characteristics of early onset colorectal cancer. *Aliment Pharmacol Ther* (2021) 5411-12:1463–71. doi: 10.1111/apt.16638

23. Choi MS, Huh JW, Shin JK, Park YA, Cho YB, Kim HC, et al. Prognostic factors and treatment of recurrence after local excision of rectal cancer. *Yonsei Med J* (2021) 6212:1107–16. doi: 10.3349/ymj.2021.62.12.1107

24. MaChado Carvalho JV, Dutoit V, Corrò C, Koessler T. Promises and challenges of predictive blood biomarkers for locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy. *Cells* (2023) 12:413. doi: 10.3390/cells12030413

25. Cai Z, Huang L, Chen Y, Xie X, Zou Y, Lan P, et al. Cea decline predicts tumor regression and prognosis in locally advanced rectal cancer patients with elevated baseline cea. *J Cancer* (2020) 1122:6565–70. doi: 10.7150/jca.49252

26. Park H. Predictive factors for early distant metastasis after neoadjuvant chemoradiotherapy in locally advanced rectal cancer. *World J Gastrointest Oncol* (2021) 134:252–64. doi: 10.4251/wjgo.v13.i4.252

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Combined effects of exercise and immuno-chemotherapy treatments on tumor growth in MC38 colorectal cancer-bearing mice

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Acute exercise induces transient modifications in the tumor microenvironment and has been linked to reduced tumor growth along with increased infiltration of immune cells within the tumor in mouse models. In this study, we aimed to evaluate the impact of acute exercise before treatment administration on tumor growth in a mice model of MC38 colorectal cancer receiving an immune checkpoint inhibitor (ICI) and chemotherapy. Six-week-old mice injected with colorectal cancer cells (MC38) were randomized in 4 groups: control (CTRL), immuno-chemotherapy (TRT), exercise (EXE) and combined intervention (TRT/EXE). Both TRT and TRT-EXE received ICI: anti-PD1-1 (1 injection/week) and capecitabine + oxaliplatin (5 times a week) for 1 week (experimentation 1), 3 weeks (experimentation 2). TRT-EXE and EXE groups were submitted to 50 minutes of treadmill exercise before each treatment administration. Over the protocol duration, tumor size has been monitored daily. Tumor growth and microenvironment parameters were measured after the intervention on Day 7 (D7) and Day 16 (D16). From day 4 to day 7, tumor volumes decreased in the EXE/TRT group while remaining stable in the TRT group (p=0.0213). From day 7 until day 16 tumor volume decreased with no significant difference between TRT and TRT/EXE. At D7 the TRT/EXE group exhibited a higher total infiltrate T cell (p=0.0118) and CD8+ cytotoxic T cell (p=0.0031). At D16, tumor marker of apoptosis, vascular integrity and inflammation were not significantly different between TRT and TRT/EXE. Our main result was that acute exercise before

immuno-chemotherapy administration significantly decreased early-phase tumor growth (D0 to D4). Additionally, exercise led to immune cell infiltration changes during the first week after exercise, while no significant molecular alterations in the tumor were observed 3 weeks after exercise.

KEYWORDS

colorectal cancer, acute exercise, immune check point inhibitor, immune cell, cancer immunotherapy

1 Introduction

The introduction of new therapies that combine immunotherapy, such as Immune Checkpoint Inhibitors (ICIs) with chemotherapy, has led to a significant improvement in patient survival compared to conventional treatments (1). Despite its acceptable clinical therapeutic efficacy, the primary limitation of immunotherapy lies in innate or acquired resistance to ICIs, which promotes cancer progression and the risk of relapse (2, 3). While chemotherapy acts directly on the tumor, the goal of immunotherapy is to boost the immune system and to restore an effective response against tumor cells. Indeed, promoting a comprehensive physiological environment that enhances immune response could augment the efficacy of immunotherapy.

Physical activity (PA) is now recognized for its benefits on immunity and the reduction of complications related to chemotherapy (4–6). Moreover, post-diagnosis PA is associated with a reduced risk of mortality in cancer patients (7). While the precise mechanisms underlying this beneficial effect are not yet fully understood, several hypotheses have been proposed. It is suggested that PA may enhance tumor vascularization (8–10), and alleviate inhibitory metabolic conditions within the tumor microenvironment, including hypoxia, acidosis, lactate accumulation, and decreased glucose levels (11). Additionally, PA may promote an increased immune infiltration (12–14), contributing to the observed reduction in mortality. Recently, physical exercise has been proposed to enhance the effectiveness of immunotherapy by modulating immune checkpoint inhibitors like PD-1 (Programmed cell death 1) and PD-L1 (Programmed cell death-Ligand 1) (15, 16). Furthermore, studies have observed that exercise conducted immediately before or during chemotherapy infusion in animal cancer models can trigger several advantageous mechanisms, including transient improvements in solid tumor perfusion, reductions in tumor hypoxia, and enhanced drug delivery to tumors (10, 17).

Most of the available evidence on the benefits of physical exercise in cancer patients have been observed in interventions performed either after the treatment or during the interval between the chemotherapy cycles. While published murine model studies have demonstrated a reduction in tumor growth through aerobic exercise training (18, 19), only a limited few have investigated the combination of exercise with immune-chemotherapeutic treatments and no study has evaluated the effect of exercise immediately before treatment administration. Yet, molecular mechanisms underlying the potential direct acute effects of exercise on the immune-chemotherapy effectiveness on tumor remain poorly understood and are still to be studied.

Considering the effects of exercise on both the tumor itself and the systemic immune system, we aimed to evaluate the impact of a pre-treatment administration of acute exercise on tumor development in a mouse model (C57BL/6) of MC38 colorectal cancer treated by ICI and chemotherapy.

2 Materials and methods

2.1 Cell culture

MC38 murine colon cancer cell line was obtained from Kerafast (USA) and was negative for mycoplasma assays. MC38 cells were cultured in DMEM medium complemented with 10% fetal bovine serum, 100 U/mL penicillin and streptomycin. Cells were incubated in a humidified incubator with 5% CO2 at 37°C.

2.2 Animals

All experiments and protocols were compliant with the ARRIVE guidelines and were approved by the Animal Ethics Committee of the University Claude Bernard of Lyon (CEEA: DR2021-44v2) and the Ethics Committee of Centre Léon Bérard Comprehensive Cancer Center (Lyon) (2021-SCAR-107). Six-week-old female C57BL/6 mice (n = 120; Janvier Labs, Le Genest-St-Isle, France) placed in a box equipped with 12:12 inverted light cycle at 22 ± 2°C, were distributed in enriched cages with nest and paper (5-6 mice/cage), with *ad libitum* access to food and water. After reception, mice followed an acclimatization period of one week in the animal house.

2.3 MC38 tumor models and tumor monitoring

We adopted the methodology and treatment described in the study by Grasselly et al. (2018). MC38 cells were injected in 6-week-

old C57BL/6 mice. Suspensions of exponentially growing cancer cells diluted in 0.2 mL of PBS were injected subcutaneously into the right flank of mice (5.10^6 cells for MC38). When tumor volume reached 200 mm^3 (around 5 days), mice were randomized in one of the four groups (1) Control (CRTL), (2) Treatment (TRT), (3) Exercise (EXE) and (4) Treatment plus Exercise (TRT/EXE) and the first treatment was administered (D0). Tumor growths were measured by manual measurement length x width (in mm^3) using a calliper, every 2 days. To account for a possible loss of body mass, the mice were also weighed 3 times a week. The tumor volume was determined using the formula: $V = 4/3 \times \pi \times r^3$. In the case of excessive tumor volume (>1600 mm^3) or if weight loss was too great (15 to 20% in a few days compared with the weight at the start of treatment), the animals were excluded and sacrificed.

2.4 Treatment

The combination regimen used for the MC38 colon adenocarcinoma mouse model was composed of capecitabine (Accord), administered *per os* 5 days/week at a dose of 250mg/kg. Oxaliplatin (Accord 5mg/mL) was injected i.p. once a week at a dose of 5 mg/kg. Anti-PD-1 (RMP1-14, BioXCell) was injected i.p. once a week at a dose of 12.5 mg/kg (20).

2.5 Exercise protocol

2.5.1 Exercise familiarization and maximal speed assessment

After a one-week acclimatation, mice were familiarized with running on a treadmill (Ugo Basile, Gemonio, Italy) using the following protocol: *day 1*: 5 minutes at 8m/min; *day 2 and 3*: 5 minutes at 8m/min and 10 minutes at 10m/min with no slope. Then, mice performed an incremental speed test to determine their maximum aerobic speed (MAS). The test began with a warm-up period at a speed of 5, 9, 12, and 15 m/min with 15° slope for 5 minutes for each speed. After this period, speed was increased by 2 m/min each 2 minutes. MAS was reached when the mouse stopped running and remained immobile for 5 consecutive seconds on an electrical grid.

2.5.2 Exercise protocol

Mice of the EXE and TRT/EXE groups followed a running program on the treadmill five days per week at 60% MAS for 50 min with no treadmill inclination. The submaximal exercise was scheduled to terminate 15-20 min prior to infusion in TRT/EXE group. CTRL and TRT groups were placed in the room where treadmill running sessions took place, to be exposed to the same stress as EXE mice.

2.6 Experiment

In the first set of experiments, mice were trained for 5 days and were sacrificed at 14 days after cancer cell injection. Tumors were dissected and analyzed by flow cytometry (n=20/groups).

In the second set of experimentation, non-treated EXE group (n=8) were trained for 1 week whereas TRT/EXE groups were trained for 16 days. CTRL group (n=8) and EXE group were sacrificed after 12 days of cancer cell injection. TRT (n=12) and TRT/EXE (n=12) groups were sacrificed at 25 days after cancer cell injection. Tumors were dissected and cut in two equal parts at the center; half were frozen in liquid nitrogen and the other half were embedded in OCT prior to being frozen. All samples were stored at −80 °C.

2.7 Immune cell phenotyping analyses by flow cytometry

Flow cytometry was used to analyze the tumor immune microenvironment. All tumor tissue samples per group were collected. Cells from the tumors were counted and cell surface markers were stained with the following fluorescently conjugated antibodies: anti-CD45 (30F11, BD Biosciences), anti-CD4 (RM4-5, BD Biosciences), anti-CD8 (53-6.7, Miltenyi Biotec), anti-CD3 (17A2, BD Biosciences), antiCD11b (M1/70, Invitrogen), anti-PD-1 (10F.9G2, BioLegend), anti-CD25 (PC61, Biolegend), anti-CD49b (DX5, BD Biosciences), anti-Granzyme b/Cryofix (GB11, Invitrogen), Viability UV Zombie. Flow cytometry data were acquired on the BD LSRFortessa X20 cytometer and FlowJo software (Ashland, OR, USA) was used for analyses.

2.8 Western blot analyses

Tumor samples were lysed in buffer pH 7.4 (10 mM Tris Base, 0.5M sucrose, 50 mM NaCl, 5 mM EDTA, 30 mM Sodium pyrophosphate, 1% NP40, 0.25% sodium deoxycholate, 5µl/ml of inhibitors of proteases cocktail, 50mM NaF and 100µM sodium orthovanadate) and centrifuged at 12 000 g for 12 min (4°C). Protein concentration was determined using Lowry protein assay (Lowry et al., 1951). Proteins (50 µg) were separated by SDS-PolyAcrylamide Gel Electrophoresis (PAGE) and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% BSA or non-fat dried milk in TBS-Tween (0.05%) and were incubated overnight at 4°C with the appropriate primary antibodies (Table 1). Then, the membranes

TABLE 1 List of western blot antibodies.

Protein	Molecular weight	Reference
p-ERK1/2	42, 44 kDa	Cell signaling 4376
ERK1/2	42, 44 kDa	Santa Cruz sc-514302
p-AKT	60 kDa	Cell signaling 9271
AKT	60 kDa	Cell signaling 9272
Cleaved caspase 3	17 kDa	Cell signaling 9661
BAX	20 kDa	Cell signaling 2772
BCL-2	28 kDa	Abcam ab7973
HSC70	70 kDa	Santa Cruz sc-7298

were washed three times with TBS-Tween (0.05%) and were incubated with secondary antibodies for 1 hour at room temperature. Immunoreactive bands were visualized with Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA) and protein loading was normalized to HSC70 expression.

2.9 RNA extraction and RT-qPCR

Total RNA was extracted from frozen tumor tissue samples using TRIzol® reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). RNA amounts and purity were determined on a microplate reader (Varioskan Lux, ThermoScientific, Waltham, MA, USA) with a µDrop® plate (ThermoScientific, Waltham, MA, USA) and RNA integrity was controlled on 1.2% agarose gel using the FlashGel electrophoresis system (Lonza, Rockland, ME, USA). Reverse transcription reaction was carried out on 1 µg of total RNA using the iScript Supermix (Bio-Rad, Hercules, CA, USA). QPCR was performed on a CFX-96 Real Time System (Bio-Rad, Hercules, CA, USA) using Sybrgreen method (SsoAdvanced Universal Sybr® Green Supermix, Bio-Rad, Hercules, CA, USA). The expression of target genes was normalized to reference genes (HPRT1, RPL4 and RPL19) and the relative expression was calculated using the ΔΔCt method (Table 2).

2.10 Statistical analysis

All statistical analyses were performed in Prism9 (GraphPad software, San Diego, CA, USA). All variables were tested for normality using the Shapiro-Wilk test, which tests for normal distribution of the data. The tumor volume data were analyzed using a two-way (Exercise and Treatment) repeated measures ANOVA for experiment 1 and using repeated measures ANOVA (group effect: TRT vs. TRT/EXE) for experiment 2, followed by a Fisher's Least Significant Difference (LSD) test. Flow Cytometry data (experiment 1) were analyzed using a two-way (Exercise and Treatment) ANOVA, followed by a Fisher's LSD test. Western Blot and RT-qPCR (experiment 2), independent t-test or Mann-Whitney test was utilized to compare TRT vs. TRT/EXE because distribution was not normal. The comparisons were considered statistically significant for $p < 0.05$. Results were expressed as mean ± SEM (Standard Error of the Mean).

3 Results

3.1 Exercise prior to administration of immuno-chemotherapy shows an early decrease in tumor growth in the MC38 model of colorectal cancer

To investigate the impact of acute exercise prior to the administration of a combination of immune checkpoint inhibitors (anti-PD1) and platinum-based chemotherapy, we initially monitored tumor growth over a 7-day period (Figure 1A). At Day 0 (D0), the measured tumor volumes were similar among all groups (Figure 1B). By Day 7 (D7), exercise alone did not exhibit any effect on tumor growth when compared to the control group (EXE vs. CTRL). In contrast, both the control and exercise groups (CTRL and EXE) showed significantly larger tumors at D7 (Figure 1B). However, it is worth noting that a trend toward a more substantial reduction in tumor volume was observed in the EXE/TRT group compared to the TRT group. Specifically, on the final day of Experiment 1 (D7), the mean tumor volume in the TRT group measured 463.5 ± 134.5 mm3, while the EXE/TRT group had tumors measuring 185.5 ± 62.2 mm3 (p=0.0747, Figure 1B). Intriguingly, between D4 and D7, tumor volumes remained stable in the TRT group but decreased in the EXE/TRT group, with a significant reduction in tumor volume observed between these two time points (p=0.0213, Figure 1B).

3.2 Exercise prior to administration of immuno-chemotherapy modulates the infiltration of immune cells in the MC38 colorectal cancer model

We characterized the tumor immune infiltrate profiles by flow cytometry for each group at D7. This characterization encompassed total immune infiltrate CD45+ cells and the total count of CD3+ T

TABLE 2 List of primers used for RT-qPCR analysis.

Gene	Forward (5′ → 3′)	Reverse (5′ → 3′)
HPRT1	AGGCCAGACTTTGTTGGATTT	CAGGACTCCTCGTATTTGCAG
RPL4	CGCAACATCCCTGGTATTACT	TGTGCATGGGCAGGTTATAGT
RPL19	GAAGGTCAAAGGGAATGTGTTCA	CCTTGTCTGCCTTCAGCTTGT
Angpt1	CGTGGAGCCGGATTTCTCTT	TTAGTACCTGGGTCTCAACATCTG
Angpt2	TCATCACCCAACTCCAAGAGC	CGGTGTTGGATGACTGTCCA
IL2rb	GTCCATGCCAAGTCGAACCT	AGGCGAAGGTTGTCAAAGGG
IL-6	ACTTCCATCCAGTTGCCTTCT	GAATTGCCATTGCACAACTCT
TNFa	GCCTCTTCTCATTCCTGCTTG	CTGATGAGAGGGAGGCCATT
CHOP	CCTGAGGAGAGAGTGTTCCAG	CTCCTGCAGATCCTCATACCA

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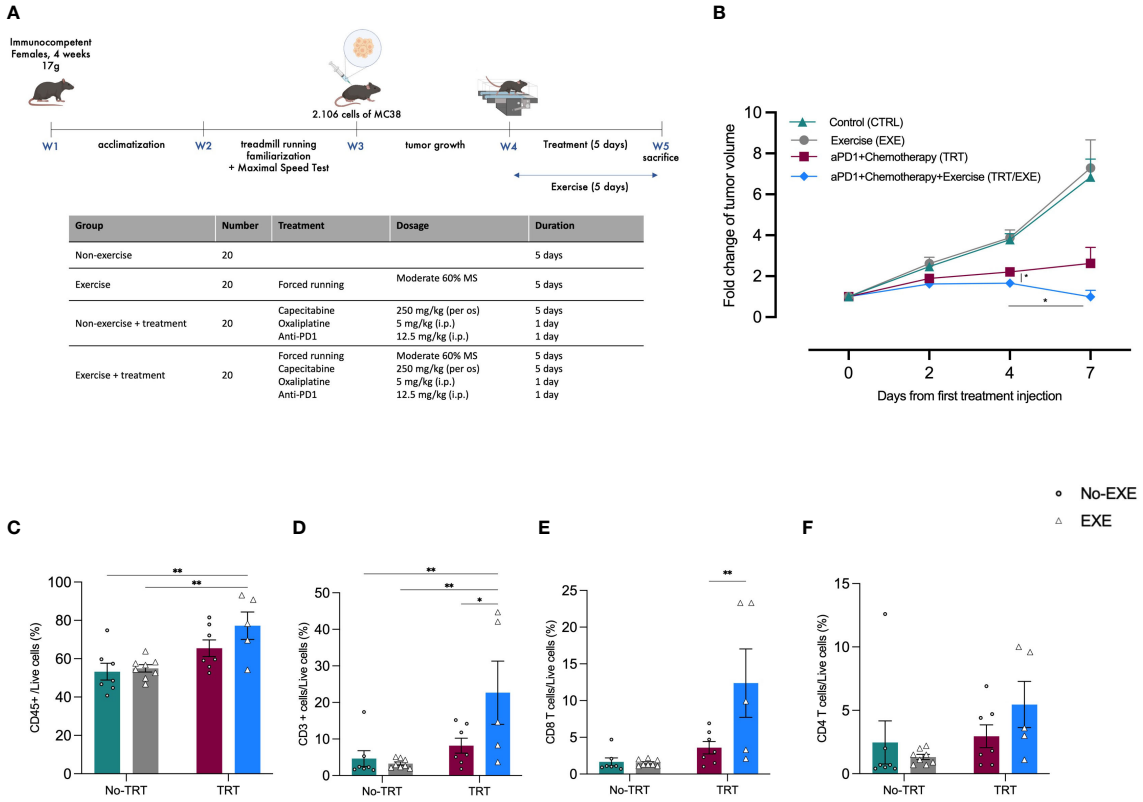

Group	Number	Treatment	Dosage	Duration
Non-exercise	20			5 days
Exercise	20	Forced running	Moderate 60% MS	5 days
Non-exercise + treatment	20	Capecitabine Oxaliplatine Anti-PD1	250 mg/kg (per os) 5 mg/kg (i.p.) 12.5 mg/kg (i.p.)	5 days 1 day 1 day
Exercise + treatment	20	Forced running Capecitabine Oxaliplatine Anti-PD1	Moderate 60% MS 250 mg/kg (per os) 5 mg/kg (i.p.) 12.5 mg/kg (i.p.)	5 days 5 days 1 day 1 day

FIGURE 1
Experimental design and tumor volume change and post intervention intratumoral immune cell infiltration in Experiment 1 **(A)** Experimental study design. N=20 mice/group **(B)** The average fold changes in tumor volume at each measurement were calculated as follows: the ratio of the tumor volume on the corresponding day to the tumor volume on day 0 (Mean ± SEM) **(C-F)** Flow cytometric characterization of MC38 tumor infiltrating immune cell populations: proportion of total in immune infiltration CD45+ cells **(C)**, proportion of total lymphocyte infiltration CD3+ cells on total CD45+ cells (CD45+CD3$^+$) **(D)**, proportion of CD4+ T cells on CD3+ (CD45+CD3+CD4$^+$) **(E)**, proportion of CD8+ T cells on CD3+ (CD45+CD3+CD8$^+$) **(F)** **p<0.01, *p<0.05, TRT: Treatment; EXE: Exercise.

cells, including effector cells like CD4+ T cells and CD8+ cytotoxic T cells. The following results are presented as the mean percentages of cells within each treatment group, normalized to the total live cells count.

3.2.1 Total leukocyte infiltrate

As shown in Figure 1C, the total leucocyte infiltrate, evaluated by CD45 labeling, was significantly higher in tumors in the TRT/EXE group compared to the CTRL group (72.2 vs 53.2%; *p=0.0063)* and the EXE group (54.9%; *p=0.0094)*. There was no difference between the TRT group (65.9%), EXE group and TRT group.

3.2.2 Total lymphocyte T cells

The total T cells infiltrate, evaluated by CD3 labeling in the TRT/EXE group was significantly higher to the two non-treated groups (p<0.01) and the TRT group (p=0.0118) whereas TRT group was not significantly different than the two non-treated groups (Figure 1D). Similarly, CD8+ T cell subpopulations in the TRT/EXE group was significantly higher to the three other groups (p<0.01) whereas TRT group was not significantly different than the two non-treated groups (Figure 1E). We found no effect of TRT/EXE on CD4+ subpopulations (Figure 1F). The TRT group had significantly less CD8+PD-1+ T cells compared to the CTRL and EXE groups (Supplementary Data). EXE also tended to have less CD8+PD-1+ T cells compared to the CTRL group (p=0.06). However, exercise did not have an additive effect on the reduction of CD8+PD-1+ T cells in the TRT/EXE group.

3.3 A longer period of repeated bouts of acute exercise prior to administration of immuno-chemotherapy has no additional benefit compared with treatment alone

Considering the trends observed in the reduction of tumor growth during Experiment 1, Experiment 2 extended the exercise period in the treated groups to evaluate the kinetics of tumor growth with and without pre-treatment exercise (Figure 2A). Similar to Experiment 1, tumor volume was regularly assessed throughout Experiment 2 to evaluate the tumor growth. Data from the CTRL and EXE groups compared to TRT and TRT/EXE confirm Experiment 1, with similar tumor growth between D0 and D7 and the same treatment efficacy (Supplementary Data). In the TRT/EXE group, tumor volume tended to increase less compared to the TRT group, with a significant difference observed on D2 (p=0.0245, Figure 2B). Additionally, both groups showed a return

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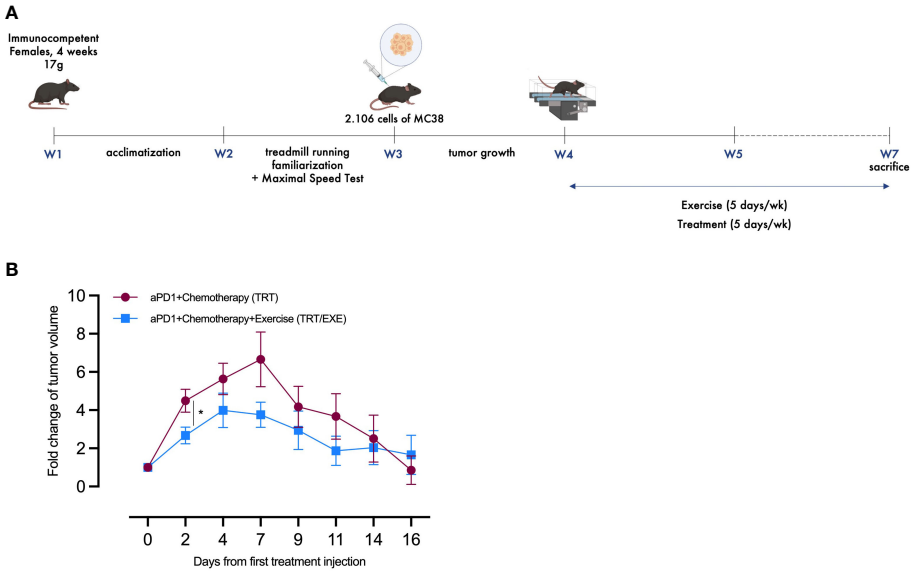

FIGURE 2
Design and tumor volume change of Experiment 2 **(A)** Experimental study design. N=20 mice/group **(B)** The average fold changes in tumor volume at each measurement were calculated as follows: the ratio of the tumor volume on the corresponding day to the tumor volume on day 0 (Mean ± SEM). All data are presented as mean ± SEM. *p<0.05. TRT: Treatment; EXE: Exercise.

to volumes close to those at day 0 by day 16, with this effect occurring by day 7 for the TRT group and by day 4 for the TRT/EXE group.

3.4 Physical exercise prior to administration of immuno-chemotherapy does not significantly induce changes in the molecular tumor markers

To evaluate the impact of the exercise pre-treatment administration on cell death, we measured apoptosis by immunoblotting of the cleaved caspase-3 (cCASP3). Apoptotic effector cCASP3 protein amount remained unchanged with TRT/EXE compared to TRT (Figure 3A). The activation of AKT through its phosphorylation on MC38 tumor cells was evaluated, to assess its promotion of proliferative and survival pathways. The pAKT/AKT ratio showed no change in the group of animals running prior to immune-chemotherapy administration (Figure 3B).

Angiopoietin-1 (ANGPT1) and Angiopoietin 2 (ANGPT2) involved in promoting vascular hyperpermeability and weakening of the vascular barrier were not different between TRT and TRT/EXE (Figure 3C). We observed that the ANGPT2:1 ratio tended to be lower in the TRT/EXE group than in the TRT group (Figure 3D). Il2rβ, a subunit of IL-2 receptor essential for regulation of immune response and expressed by NK cells, was not affected by the combination of exercise-treatment (vs treatment alone). Similar results were observed for Tumor Necrosis Factor alpha (TNF-α) and Interleukine-6 (IL-6) within tumor, 2 cytokine genes coding for pro-inflammatory. Genic expression of CHOP (C/EBP homologous protein-10), a key transcription factor for initiation of apoptotic program under extreme Endoplasmic Reticulum (ER) stress conditions was not different between TRT and TRT/EXE (Figure 3C).

4 Discussion

Our study is the first to investigate the impact of physical exercise performed just before the administration of immunotherapy with immune checkpoint inhibitor anti PD-1, combined with a platinum-based chemotherapy (oxaliplatin+capecitabine) on the tumor growth in a MC38 colorectal murine model. The primary finding was that, during the early phase of treatment, exercise prior to the administration of immunochemotherapy significantly reduced tumor growth, and significantly increased the total intra-tumoral T-cell infiltrate and CD8+ T cell subpopulations compared to immune-chemotherapy alone. Yet, there was no difference in the final tumor volume (D16) and tumor markers of apoptosis, vascular integrity and inflammation did not significantly differ between these two treatment groups.

4.1 A promising antitumor effect with exercise prior to administration of capecitabine-oxaliplatin plus anti-PD1 combination in a MC38 murine model

The significantly slower kinetics of tumor growth in the early stage of MC38 cell development displayed in both experiments by the group receiving exercise in addition to immuno-chemotherapy compared to the group receiving immuno-chemotherapy only, suggests that exercise may enhance the effects of immuno-chemotherapy. However, our second experiment did not support the hypothesis of sustained exercise-induced effects on tumor

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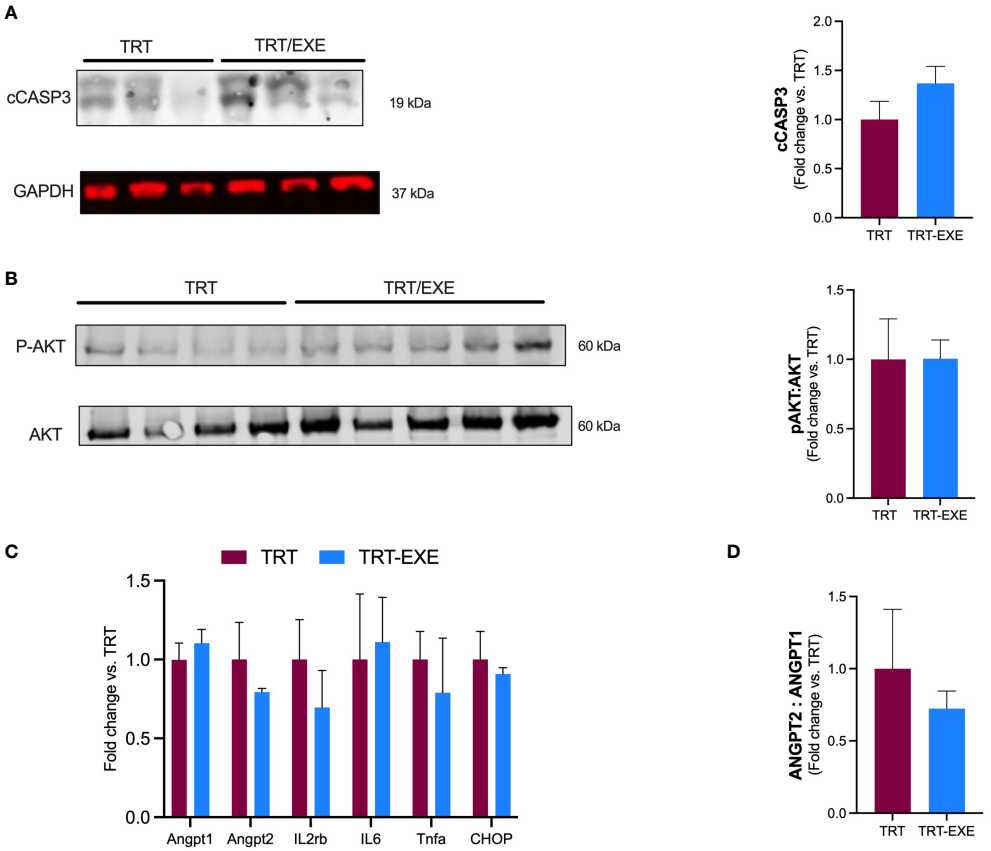

FIGURE 3
Tumor apoptosis, inflammation, vascular permeability, and growth after 16 days of treatment and exercise **(A, B)** Western blot analysis. Representative and quantification of western blots for cCASP3, GAPDH, pAKT and Akt. **(C)** mRNA expression of angiopoietin-1 (ANGPT1), angiopoietin-2 (ANGPT2), Tumor Necrose Factor alpha (Tnfa), Interleukin 6 (IL6), (CHOP) and Interleukin 2 receptor beta chain (IL2rb) in the tumor. **(D)** ANGPT2: ANGPT 1 ratio. Data are represented as means ± SEM. *p<0.05. TRT: Treatment; EXE: Exercise.

growth in addition to immunotherapy beyond D7, as the tumor volumes in both groups returned to their initial sizes (D0) on D16. This observation could be the result of the particularly strong effect of this specific immune-chemotherapy combination on MC38 tumor growth, as previously demonstrated, that may overshadow the exercise effect (20). MC38 is a syngeneic colorectal cancer model which has been extensively used in the context of immune checkpoint inhibitor therapy as it has shown robust responses to anti PD1 and anti PDL1 antibodies. Although the subcutaneous (SC) and orthotopic localizations have been shown to differ in terms of tumor immune microenvironment, the SC tumors remain largely used, in particular for the description of resistance mechanisms to immune checkpoint inhibitors (21, 22).

Furthermore, only a few rodent studies combined exercise with immuno-chemotherapy in various clinical cancer settings. Several murine studies have investigated the combination of immunotherapy with exercise in different types of cancer including breast cancer, melanoma liver and lung carcinoma with divergent results (23, 24). Overall, the tumor models (i.e. syngeneic transplanted mouse models, drug-induced or transgenic models) and exercise modalities (i.e. pre-injection *vs* post-injection exercise, programmed *vs* voluntary exercise, intensity) were heterogeneous. In their Patient-Derived Xenograft (PDX) model of non-small cell lung cancer, Martin Ruiz et al. found that combining immunotherapy (specifically nivolumab, a PD-1 inhibitor) with programmed exercise post-injection (aerobic and resistance training, 8 weeks) did not exhibit any additional effect on the inhibition of tumor growth compared to immunotherapy alone (25). In addition, the final tumor volume resulting from the nivolumab + exercise intervention tended to be larger than that observed in the other study groups. The authors attribute this outcome to a 'paradoxical' or 'unconventional' response associated with the immune cell recruitment and the intratumoral inflammatory environment triggered by these cells. Bay et al. showed an additive effect of a combination of 2 weeks of voluntary wheel running with anti-PD-1 treatment on tumor growth in a B16 melanoma model (26). Gomes-Santos et al. investigated how physical activity, either in conjunction with anti-PD-1 treatment alone or when combined with anti-CTLA-4 treatment, influenced tumor growth in three murine breast cancer models (14). The authors observed that ICI treatment alone had minimal impact on orthotopic breast cancers, whereas combining ICI with programmed treadmill running led to a delay in tumor growth and a reduction in the final tumor volume. Wennerberg et al., found in mice with established 4T1 triple negative mammary carcinoma that combining anti-PD1 and radiotherapy with

programmed treadmill exercise significantly slowed tumor growth compared to anti-PD1 and radiotherapy alone (27).

In the present study, the groups without immuno-chemotherapy treatment (CTRL and EXE) exhibited larger tumor size than the treated groups from D4 up to D16, without difference between the two groups. These results confirmed the successful tumor cell implementation and the efficiency of the immuno-chemotherapy treatment previously reported (20). Moreover, the lack of a difference in tumor volume and intratumoral T-cell infiltrate between the control and the exercise only groups suggests that the observed beneficial effect of combined exercise-immuno-chemotherapy might be explained by a potentiation effect of exercise rather than a proper effect of exercise itself.

4.2 Exercise before each immuno-chemotherapy administration increases intratumoral T-cell infiltrates

Similar to Grasselly et al. (2020), we reported here that tumor volume reached its peak volume at D7 after treatment initiation and decreased afterwards, in both the TRT and TRT/EXE groups. At D7, we observed a significant increase in total CD45+ leukocyte, CD3+ T lymphocyte and CD8+ T cytotoxic cells infiltration in the combined exercise-immuno-chemotherapy group tumors compared to immune-chemotherapy alone, suggesting that that one week of exercise before immuno-chemotherapy administration is sufficient to increase total leukocyte and T lymphocyte tumor infiltration included the CD8+ cytotoxic T cells. As previously mentioned, exercise is known to have a profound influence on the human immune system (28). Currently, it remains unclear whether the anticancer effects of physical activity are mediated by the acute mobilization and redistribution of cytotoxic effector cells in response to acute exercise sessions or through an adaptive training effect that enhances tissue-associated T cells. In our study, we demonstrated that acute exercise leads to an increase in intratumoral immune cell infiltration, which may explain our observations of reduced tumor growth after 7 days of acute exercise prior to immuno-chemotherapy administration.

To date, several studies in different murine models have observed that exercise without anti-tumor treatment increases intratumoral immune infiltrates after several weeks of treadmill or voluntary wheel running training (12, 27, 29, 30). The results by Wang et al. suggested that a minimum of exercise intensity, comparable to the present protocol, is needed to observe beneficial effects on intratumoral immune cell infiltration (31). Of note, we did not observe an effect of pre-treatment exercise on the infiltration of CD4+ and CD8+ lymphocyte subpopulations. A study showed that training an ApcMin/+ murine model of intestinal cancer on a treadmill for 12 weeks reduced tumor burden and increased CD8+ expression in the tumor (29). Martín-Ruiz et al. (2020) observed that neutrophil tumor infiltration trended to be higher in the exercise group in combination with nivolumab compared to the nivolumab alone group (25).

We hypothesized that exercise combined with anti-PD1 could decrease checkpoint molecule PD-1 expression by CD8+ cells. Our results showed that exercise alone decreases CD8+ cells expressing PD-1+ compared to the control group. However, we found no additive effect on the decrease in CD8+ PD-1+ cell expression when exercise was combined with anti-PD1. Some of the previously mentioned preclinical studies have explored the effects of exercise training on levels of the checkpoint molecule PD-1 expression linked to T cell exhaustion, however results are heterogenous. Wennerberg et al., showed that exercise training, when combined with radiotherapy and anti-PD1 treatment following the injection of 4T1 triple negative breast tumors, reduced the proportion of splenic PD1+CD8+ T cells, which was not achieved by anti-cancer therapy alone, and resulted in reduced tumor growth (27). In their study, Bay et al. showed that voluntary wheel running led to enhanced expression levels of the checkpoint molecule PD-1 and its two ligands PD-L1 and PD-L2 in their B16 melanoma mouse model, without effect on tumor growth with the combination of exercise and anti-PD-1 therapy (26).

Overall, these results suggest that the effects of physical exercise on PD1+ T cells and the efficacy of anti-PD1 immunotherapy are inconclusive. Further research is needed to determine whether the anticancer effects of physical activity are mediated by PD1-dependent or independent pathways.

4.3 No molecular changes in MC38 cells with combined exercise and immuno-chemotherapy after 3 weeks of training

Other mechanisms might explain the observed effects of exercise on reducing tumor growth, such as the improvement of tumor vascularization and perfusion, which can lead to a more favorable metabolic profile within the tumor, including the mitigation of hypoxia (8–10, 32–34). Angiopoietin-1 (Angpt1) is an activator of tyrosine kinase receptor TEK expressed mainly on endothelial cells. TEK activation and phosphorylation promote vascular maturity and endothelial cell survival. While Angpt1 counteracts hyperpermeability, Angiopoietin 2 (Angpt2) is upregulated in human cancer and its activation weakens the vascular barrier. Increased ANGPT2/ANGPT1 ratio at the mRNA level has been reported to correlate with neo-angiogenesis and poor prognosis in many cancer types (35). Unfortunately, we did not find any effect of exercise on the expression of these genes alone, but we have observed a trend towards an increase in the ANGPT2/ANGPT1 ratio. A recent review attributed the difficulty to come to a conclusion on the effect of exercise on vascular remodeling is because of the methodological heterogeneity among preclinical studies (36).

Moreover, McCullough et al. suggested that exercise induces a transient increase in tumor perfusion, potentially leading to enhanced immune infiltration and chemotherapy infiltration within the tumor (10). In the present study, we were not able to explore this hypothesis. Future studies exploring the effect of acute exercise just before or during anti-cancer treatment should plan to measure transient tumor perfusion during exercise.

Furthermore, in the present study, the combination of exercise with immuno-chemotherapy had no effect on apoptosis at D16, whereas we expected an increase in cell death markers such as

Caspase 3. One study found a potentiation of apoptosis when exercise was combined with radiotherapy compared with radiotherapy alone in a prostate cancer model (34, 37). In a patient-derived xenograft (PDX) non-small-cell lung cancer model, Martín-Ruiz et al. also found that exercise in combination with PD-1 blockade reduced tumor growth, exhibiting diminished tumor cell proliferation and increase of tumor necrosis. The lack of exercise effect (TRT vs TRT/EXE) on tumor markers of apoptosis, vascular integrity and inflammation is consistent with the absence of a difference in tumor volume at D16.

We did not find any difference in the pAKT/AKT ratio in the tumor, a tumor survival pathway, when exercise was combined with immuno-chemotherapy compared to treatment alone at D16. These results of intratumoral molecular analysis at D16 are consistent with the lack of difference in tumoral volumes observed at that time between the TRT and TRT/EXE groups.

The present study has several limitations. Firstly, the MC38 subcutaneous murine model of colorectal cancer that was used in this study exhibited aggressive tumor growth (22). Most untreated animals reached ethical endpoint criteria, namely a tumor size > 1600 mm3 and tumor necrosis, within 14 days of subcutaneous tumor implantation. Additionally, tumor volumes in the treated groups reached their maximum after 7 days of treatment and exercise. Moreover, it is possible that the impact of the capecitabine +oxaliplatin+anti-PD1 combination was already substantial after a few days, making it difficult to observe additional benefits of exercise when administered alone. For future studies, it would be relevant to investigate the combination of ICI with exercise in less aggressive models of colorectal cancer characterized by slower tumor growth and lower treatment doses. Such models would better mimic the clinical conditions observed in humans.

5 Conclusion

We hypothesized that combining PD-1 inhibitors with programmed treadmill running could enhance tumor growth control beyond the effects of immune-chemotherapy alone. Our findings demonstrate that exercise in combination with immune-chemotherapy effectively slows tumor growth in early-stage of MC38 development for up to 7 days after treatment initiation, accompanied by increased tumor immune cell infiltration and particularly CD8+ cytotoxic T cells. However, it is important to note that this study did not provide sufficient evidence to support an additional benefit of exercise when combined with immunotherapy-chemotherapy after a 3-week pre-treatment administration exercise intervention. This may be attributed to the significant anti-tumor impact of the combined therapy used in this study. Consequently, further pre-clinical investigations are warranted to explore the effects of acute physical exercise combined with anti-tumor treatments on tumor growth.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Animal Ethics Committee of the University Claude Bernard of Lyon (CEEA: DR2021-44v2) and the Ethics Committee of Centre Léon Bérard Comprehensive Cancer Center (Lyon) (2021-SCAR-107). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Writing – original draft, Writing – review & editing. AR: Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. AT: Conceptualization, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. SB: Data curation, Investigation, Resources, Software, Writing – review & editing. E-LM: Methodology, Writing – review & editing. EG: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. LO: Investigation, Methodology, Resources, Writing – review & editing. BM: Data curation, Investigation, Resources, Writing – review & editing. OP: Funding acquisition, Supervision, Writing – review & editing. CC: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. EC: Resources, Validation, Writing – review & editing. CD: Conceptualization, Funding acquisition, Methodology, Validation, Writing – original draft, Writing – review & editing. BF: Conceptualization, Funding acquisition, Supervision, Validation, Writing – original draft, Writing – review & editing. VP: Methodology, Writing – review & editing, Conceptualization, Funding acquisition, Supervision, Validation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. Yan Y, Kumar AB, Finnes H, Markovic SN, Park S, Dronca RS, et al. Combining immune checkpoint inhibitors with conventional cancer therapy. *Front Immunol* (2018) 9:1739. doi: 10.3389/fimmu.2018.01739

2. Shin D, Garcia-Diaz A, Zaretsky J, Escuin-Ordinas H, Hu-Lieskovan S, Palaskas NJ, et al. Innate resistance of PD-1 blockade through loss of function mutations in JAK resulting in inability to express PD-L1 upon interferon exposure. *J Immunother Cancer* (2015) 3:P311. doi: 10.1186/2051-1426-3-S2-P311

3. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive and acquired resistance to cancer immunotherapy. *Cell* (2017) 168(4):707–23. doi: 10.1016/j.cell.2017.01.017

4. Wang J, Liu S, Li G, Xiao J. Exercise regulates the immune system. *Adv Exp Med Biol* (2020) 1228:395–408. doi: 10.1007/978-981-15-1792-1_27

5. Koelwyn GJ, Wennerberg E, Demaria S, Jones LW. Exercise in regulation of inflammation-immune axis function in cancer initiation and progression. *Oncology (Williston Park)* (2016) 29(12):214800.

6. Wang Q, Zhou W. Roles and molecular mechanisms of physical exercise in cancer prevention and treatment. *J Sport Health Sci* (2021) 10(2):201–10. doi: 10.1016/j.jshs.2020.07.008

7. Friedenreich CM, Stone CR, Cheung WY, Hayes SC. Physical activity and mortality in cancer survivors: A systematic review and meta-analysis. *JNCI Cancer Spectr* (2019) 4(1). doi: 10.1093/jncics/pkz080

8. SChadler KL, Thomas NJ, Galie PA, Bhang DH, Roby KC, Addai P, et al. Tumor vessel normalization after aerobic exercise enhances chemotherapeutic efficacy. *Oncotarget* (2016) 7(40):65429–40. doi: 10.18632/oncotarget.11748

9. Betof AS, Lascola CD, Weitzel D, Landon C, Scarbrough PM, Devi GR, et al. Modulation of murine breast tumor vascularity, hypoxia, and chemotherapeutic response by exercise. *J Natl Cancer Inst* (2015) 107(5). doi: 10.1093/jnci/djv040

10. McCullough DJ, Stabley JN, Siemann DW, Behnke BJ. Modulation of blood flow, hypoxia, and vascular function in orthotopic prostate tumors during exercise. *J Natl Cancer Inst* (2014) 106(4). doi: 10.1093/jnci/dju036

11. Zhang X, Ashcraft KA, Betof Warner A, Nair SK, Dewhirst MW. Can exercise-induced modulation of the tumor physiologic microenvironment improve antitumor immunity? *Cancer Res* (2019) 79(10):2447–56. doi: 10.1158/0008-5472.CAN-18-2468

12. Pedersen L, Idorn M, Olofsson GH, Lauenborg B, Nookaew I, Hansen RH, et al. Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. *Cell Metab* (2016) 23(3):554–62. doi: 10.1016/j.cmet.2016.01.011

13. Bigley AB, Rezvani K, Chew C, Sekine T, Pistillo M, Crucian B, et al. Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells. *Brain Behavior Immunity* (2014) 39:160–71. doi: 10.1016/j.bbi.2013.10.030

14. Gomes-Santos IL, Amoozgar Z, Kumar AS, Ho WW, Roh K, Talele NP, et al. Exercise training improves tumor control by increasing CD8+ T-cell infiltration *via* CXCR3 signaling and sensitizes breast cancer to immune checkpoint blockade. *Cancer Immunol Res* (2021) 9(7):765–78. doi: 10.1158/2326-6066.CIR-20-0499

15. Wadley AJ, Cullen T, Vautrinot J, Keane G, Bishop NC, Coles SJ. High intensity interval exercise increases the frequency of peripheral PD-1+ CD8+ central memory T-cells and soluble PD-L1 in humans. *Brain Behavior Immun - Health* (2020) 3:100049. doi: 10.1016/j.bbih.2020.100049

16. Gustafson MP, DiCostanzo AC, Wheatley CM, Kim CH, Bornschlegl S, Gastineau DA, et al. A systems biology approach to investigating the influence of exercise and fitness on the composition of leukocytes in peripheral blood. *J Immunother Cancer* (2017) 5:30. doi: 10.1186/s40425-017-0231-8

17. Wiggins JM, Opoku-Acheampong AB, Baumfalk DR, Siemann DW, Behnke BJ. Exercise and the tumor microenvironment: potential therapeutic implications. *Exercise Sport Sci Rev* (2018) 46(1):56–64. doi: 10.1249/JES.0000000000000137

18. Hojman P, Gehl J, Christensen JF, Pedersen BK. Molecular mechanisms linking exercise to cancer prevention and treatment. *Cell Metab* (2018) 27(1):10–21. doi: 10.1016/j.cmet.2017.09.015

19. Ashcraft KA, Warner AB, Jones LW, Dewhirst MW. Exercise as adjunct therapy in cancer. *Semin Radiat Oncol* (2018) 29(1):16–24. doi: 10.1016/j.semradonc.2018.10.001

20. Grasselly C, Denis M, Bourguignon A, Talhi N, Mathe D, Tourette A, et al. The antitumor activity of combinations of cytotoxic chemotherapy and immune checkpoint inhibitors is model-dependent. *Front Immunol* (2018) 9:2100. doi: 10.3389/fimmu.2018.02100

21. Denis M, Grasselly C, Choffour PA, Wierinckx A, Mathé D, Chettab K, et al. *In vivo* syngeneic tumor models with acquired resistance to anti-PD-1/PD-L1 therapies. *Cancer Immunol Res* (2022) 10(8):1013–27. doi: 10.1158/2326-6066.CIR-21-0802

22. Denis M, Mathé D, Micoud M, Choffour PA, Grasselly C, Matera EL, et al. Impact of mouse model tumor implantation site on acquired resistance to anti-PD-1 immune checkpoint therapy. *Front Immunol* (2022) 13:1011943. doi: 10.3389/fimmu.2022.1011943

23. Handford J, Chen M, Rai R, Moss CL, Enting D, Peat N, et al. Is there a role for exercise when treating patients with cancer with immune checkpoint inhibitors? A scoping review. *Cancers (Basel)* (2022) 14(20):5039. doi: 10.3390/cancers14205039

24. Brummer C, Pukrop T, Wiskemann J, Bruss C, Ugele I, Renner K. Can exercise enhance the efficacy of checkpoint inhibition by modulating anti-tumor immunity? *Cancers* (2023) 15(18):4668. doi: 10.3390/cancers15184668

25. Martín-Ruiz A, Fiuza-Luces C, Rincón-Castanedo C, Fernández-Moreno D, Martínez-Martínez E, Martín-Acosta P, et al. Benefits of exercise and immunotherapy in a murine model of human non–small-cell lung carcinoma. *Exerc Immunol Rev* (2020) 26:100–15.

26. Bay ML, Unterrainer N, Stagaard R, Pedersen KS, Schauer T, Staffeldt MM, et al. Voluntary wheel running can lead to modulation of immune checkpoint molecule expression. *Acta Oncologica* (2020) 59(12):1447–54. doi: 10.1080/0284186X.2020.1817550

27. Wennerberg E, Lhuillier C, Rybstein MD, Dannenberg K, Rudqvist NP, Koelwyn GJ, et al. Exercise reduces immune suppression and breast cancer progression in a preclinical model. *Oncotarget* (2020) 11(4):452–61. doi: 10.18632/oncotarget.27464

28. Walsh N. Immune function and exercise. *Eur J Sport Sci* (2011) 4(3):52–66.

29. McClellan JL, Steiner JL, Day SD, Enos RT, Davis MJ, Singh UP, et al. Exercise effects on polyp burden and immune markers in the ApcMin/+ mouse model of intestinal tumorigenesis. *Int J Oncol* (2014) 45(2):861–8. doi: 10.3892/ijo.2014.2457

30. Rundqvist H, Veliça P, Barbieri L, Gameiro PA, Bargiela D, Gojkovic M, et al. Cytotoxic T-cells mediate exercise-induced reductions in tumor growth. *Elife* (2020) 9. doi: 10.7554/eLife.59996

31. Wang B, Xu H, Hu X, Ma W, Zhang J, Li Y, et al. Synergetic inhibition of daidzein and regular exercise on breast cancer in bearing-4T1 mice by regulating NK cells and apoptosis pathway. *Life Sci* (2020) 245:117387. doi: 10.1016/j.lfs.2020.117387

32. Jones LW, Antonelli J, Masko EM, Broadwater G, Lascola CD, Fels D, et al. Exercise modulation of the host-tumor interaction in an orthotopic model of murine prostate cancer. *J Appl Physiol (1985)* (2012) 113(2):263–72. doi: 10.1152/japplphysiol.01575.2011

33. Jones LW, Viglianti BL, Tashjian JA, Kothadia SM, Keir ST, Freedland SJ, et al. Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer. *J Appl Physiol* (2010) 108(2):343–8. doi: 10.1152/japplphysiol.00424.2009

34. Dufresne S, Guéritat J, Chiavassa S, Noblet C, Assi M, Rioux-Leclercq N, et al. Exercise training improves radiotherapy efficiency in a murine model of prostate cancer. *FASEB J* (2020) 34(4):4984–96. doi: 10.1096/fj.201901728R

35. Tait CR, Jones PF. Angiopoietins in tumours: the angiogenic switch. *J Pathol* (2004) 204(1):1–10. doi: 10.1002/path.1618

36. Emery A, Moore S, Turner JE, Campbell JP. Reframing how physical activity reduces the incidence of clinically-diagnosed cancers: appraising exercise-induced immuno-modulation as an integral mechanism. *Front Oncol* (2022) 12:788113. doi: 10.3389/fonc.2022.788113

37. Dufresne S, Richard C, Dieumegard A, Orfila L, Delpon G, Chiavassa S, et al. Voluntary wheel running does not enhance radiotherapy efficiency in a preclinical model of prostate cancer: the importance of physical activity modalities? *Cancers (Basel)* (2021) 13(21):5402. doi: 10.3390/cancers13215402

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Case report: Successful treatment of advanced colon cancer in an eighty-year-old man with long-term and multi-stage endoscopic minimally invasive therapy

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Background: No previous studies have reported on the use of minimally invasive endoscopic therapy for colon cancer in older patients.

Case presentation: An 80-year-old man was admitted to our hospital with haematochezia and diagnosed with advanced colon cancer in 2018. Traditional surgical care was rejected by his family. We successfully treated the patient with multiple minimally invasive endoscopic therapies, such as argon plasma coagulation, from 2018 to 2021.

Conclusion: Invasive endoscopic therapy is a feasible way to treat colon cancer in older patients.

KEYWORDS

colonic neoplasms, aged, endoscopy, argon plasma coagulation, surgery

Introduction

Colorectal cancer (CRC) is the third most common cause of cancer mortality worldwide and the third leading cause of death in men aged over 80 years (1, 2). Older patients with CRC are often in an advanced stage at the time of diagnosis, losing the opportunity to undergo surgical treatment and substantially limiting their life expectancy. Moreover, many older patients have major comorbidities that may minimize or even negate the benefits of adjuvant chemotherapy or radiotherapy (3, 4). Choosing an appropriate method for treating CRC in older patients is a current challenge in clinical practice. This

case report describes the successful treatment of an 80-year-old patient with advanced colon cancer using multi-stage endoscopic minimally invasive therapy with the intention of providing new ideas for the treatment of colon cancer in older patients.

Case presentation

An 80-year-old Asian man with haematochezia for 10 days was admitted to our hospital in Nov. 2018 and diagnosed with advanced colon cancer, protruded type, clinical stage T2N0M0 (Figure 1). Colonoscopy revealed a mass in the descending colon that blocked the lumen, and blocked passage of the endoscope (Figure 1A). The biopsy specimen was friable and bled easily. Histopathological examination (H&E staining) showed deeply stained irregular nuclei with necrotizing tumour cells and revealed adenocarcinoma (Figure 1B). Contrast-enhanced abdominal computed tomography showed a thickening confined to the descending colon wall and no metastasis to the lymph nodes or abdominal organs (Figures 1C, D). The man had hypertension and coronary artery disease for more than ten years, type 2 diabetes mellitus for more than six years, and had been on long-term medication (unspecified) for years. He did not have a history of surgery or radiation exposure, and his family history was unremarkable. Considering the age and health status of the patient, his family rejected traditional surgery and neoadjuvant therapy because of potential complications like wound infection and kidney injuries, leading to the shortage of lifespan. Therefore, we aimed to prevent luminal obstruction and maintain the patient's quality of life by using a disposable polyp snare, endoscopic resection, and argon plasma coagulation (APC) to remove part of the tumour (Figures 2A, B) and then placing a stent using the colonoscope (Figure 2C). From April 2019 to February 2021, the patient underwent a colonoscopy six times for the tumour in our hospital, including polyp resection, electro-coagulation and electro-section, and APC (Supplementary Figure 1). The tumour size in the colon gradually decreased and no other complications developed (Figure 3). During the entire process, the patient did not receive any other therapy, including chemotherapy, radiotherapy, or traditional Chinese medicine. Surprisingly, a regular examination with colonoscopy in July 2021 showed that only postoperative scars were seen at the original tumour site, and no tumour proliferation was found (Figure 4A). One year later, in May 2022, the patient underwent colonoscopy and still showed no colon cancer recurrence (Figure 4B). And the follow-up examination by abdominal ultrasounds and chest X-ray also showed no metastases or other diseases from 2019 to 2022. However, we did not conduct the follow-up examination in 2023 because the patient could not tolerate the long car rides. Therefore, we phoned his family and were told the activity of daily living (ADL) in this patient was still well, and no warning symptoms of colon cancer had recurred until now.

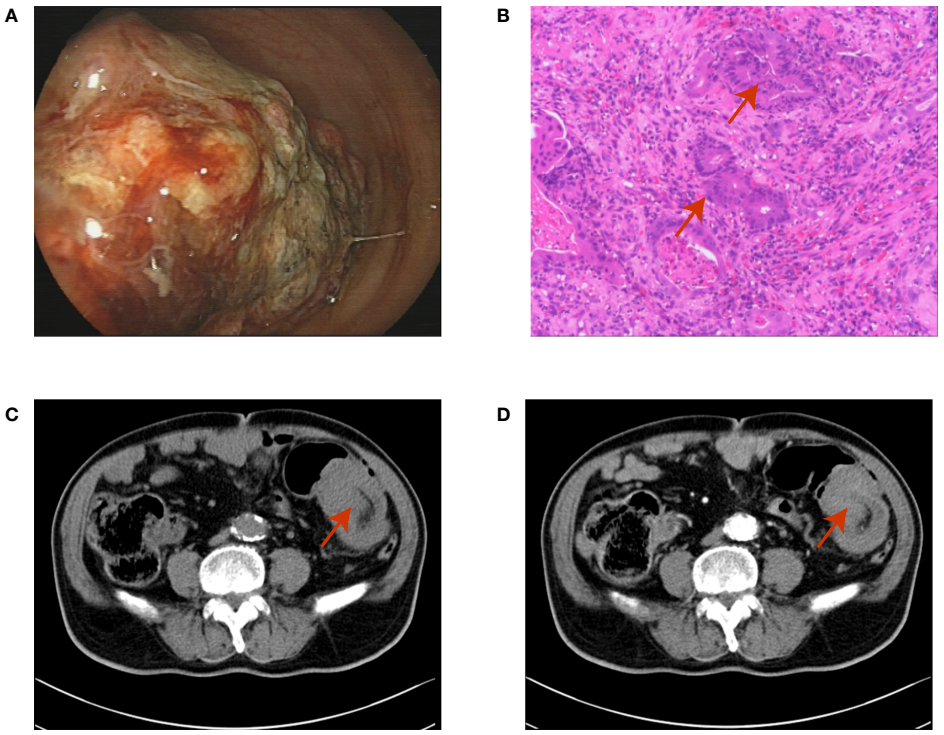

FIGURE 1
(A) The colonoscopy shows a mass blocked the lumen of the descending colon and the endoscope could not pass; **(B)** The haematoxylin-eosin staining of the biopsy shows adenocarcinoma (red arrow); **(C, D)** The contrast-enhanced abdominal computed tomography shows the colon wall thickening and the maximum cross-section of the mass is 4.4×7.0 mm (red arrow).

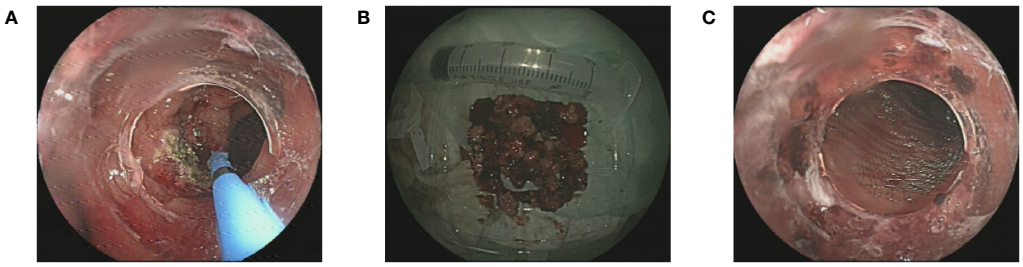

FIGURE 2
(A) The endoscopic resection and argon plasma coagulation of the tumour; **(B)** The excised tumour tissue; **(C)** The stent placed in the lumen.

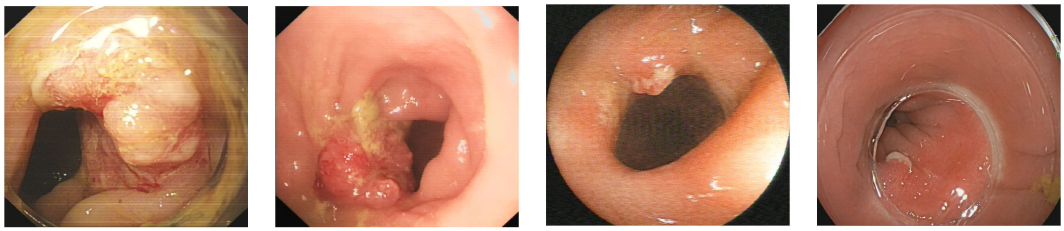

FIGURE 3
The size of the tumour in the colon has gradually shrunk over three years from 2019 to 2021 after endoscopic therapy.

Discussion

To our knowledge, this is the first report of the successful treatment of colon cancer in an older man using multifrequency endoscopic minimally invasive therapy. Older patients diagnosed with advanced CRC are less likely to receive standard antitumour therapies, such as cytotoxic chemotherapy and biological therapy, and the proportion of patients receiving treatment declines with advanced age (5, 6). In this case, we originally aimed to prevent colonic obstruction by the tumour and to maintain the basic quality of life of the patient through endoscopic palliative care. However, the final successful treatment of cancer by multifrequency endoscopic resection provided an easily acceptable and low-risk protocol for the treatment of CRC in older patients.

Relevant literature was reviewed to explore potential reasons. Biller et al. reported that the 5-year survival rate of patients with metastatic CRC is less than 20% (7). The colon cancer in this patient was localised in the lumen when diagnosed and did not metastasise to the lymph nodes or distant sites. The recognition of warning symptoms and accurate diagnosis of colorectal cancer are important, and there were no signs of metastasis during the 4-year follow-up treatment and re-examination, providing an opportunity for long-term endoscopic invasive therapy. Endoscopic invasive therapy is less harmful and safer for older patients than traditional surgical care because of postoperative pain

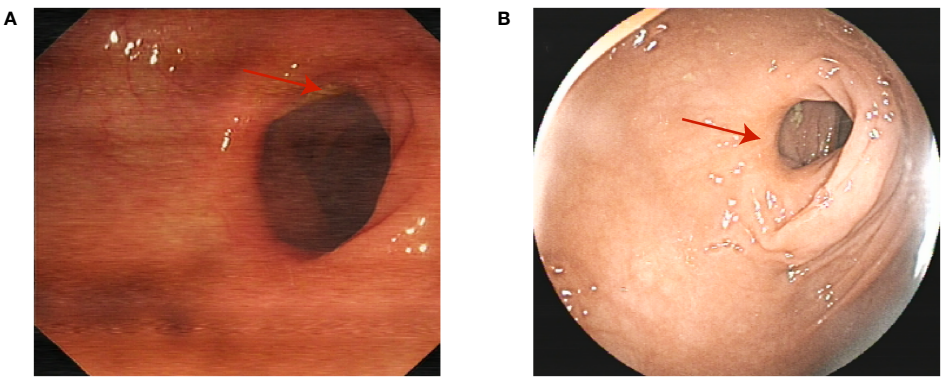

FIGURE 4
Scars are seen at the original tumour site in three **(A)** and four **(B)** years after the original diagnosis in 2018. (red arrow).

and the risk of complications such as wound infection and poor healing caused by the surgery (8). Older patients also are more liable to postoperative surgical site infections and poor recovery after colorectal surgery than young patients because of their poor nutritional absorption and weak resistance to pathogens (9–11). And the complications are reported to be associated with many adverse outcomes like increasing patient costs and length of hospital stay, promoting the incidence of sepsis, or even causing death (12, 13). Recently, minimally invasive endoscopic therapy has been considered for gastrointestinal tumours treatment (14–18). APC has been reported to be effective in treating superficial oesophageal squamous cell carcinoma in patients with severe concomitant disease (15). Endoscopic submucosal dissection (ESD) and endoscopic full-thickness resection (EFTR) have been found as available ways for early colorectal cancer confined to the mucosa or submucosa (16–18). The appropriate frequency of endoscopic therapy for this patient may also be one of the reasons for successful treatment. Moreover, the tumour location of this patient was in the descending colon, and Zhang et al. reported that the prognosis of patients with left-sided colon cancers was better than that of patients with right-sided colon cancers, regardless of stage (19), which may also have contributed to the successful treatment of the patient. Therefore, the primary location of the colon cancer, its staging, the accurate diagnosis and the appropriate choice of therapy were combined to account for the favourable outcome in this patient.

In conclusion, this case implies that invasive endoscopic therapy may be feasible to treat colon cancer in older populations. However, there are also limitations, such as how frequently and to what extent endoscopic resection should be performed and which older patients would benefit. Moreover, the early and accurate diagnosis of colon cancer is also vital to provide the chance for minimally invasive therapy, and some studies have reported deep learning algorithms have the potential to improve the accuracy and efficacy of CRC detection (20, 21). Therefore, further clinical practice and investigations are needed to apply deep learning algorithms to the classification and diagnosis of CRC and invasive endoscopic therapy for the therapy of CRC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by The Ethical Committee of the 909th hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

NZ: Conceptualization, Methodology, Software, Writing – original draft. LZ: Conceptualization, Methodology, Writing – original draft. YL: Conceptualization, Methodology, Writing – review & editing. XC: Writing – review & editing. BZ: Writing – review & editing. CW: Supervision, Writing – review & editing. HZ: Writing – review & editing. QT: Writing – review & editing. MZ: Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1367173/full#supplementary-material

References

1. Wang Z, Dan W, Zhang N, Fang J, Yang Y. Colorectal cancer and gut microbiota studies in China. *Gut Microbes* (2023) 15(1):2236364. doi: 10.1080/19490976.2023.2236364

2. Hathout L, Maloney-Patel N, Malhotra U, Wang SJ, Chokhavatia S, Dalal I, et al. Management of locally advanced rectal cancer in the elderly: a critical review and algorithm. *J Gastrointest Oncol* (2018) 9(2):363–76. doi: 10.21037/jgo.2017.10.10

3. Muss HB, Biganzoli L, Sargent DJ, Aapro M. Adjuvant therapy in the elderly: making the right decision. *J Clin Oncol* (2007) 25(14):1870–5. doi: 10.1200/JCO.2006.10.3457

4. De Marco MF, Janssen-Heijnen ML, van der Heijden LH, Coebergh JW. Comorbidity and colorectal cancer according to subsite and stage: a population-based study. *Eur J Cancer* (2000) 36(1):95–9. doi: 10.1016/s0959-8049(99)00221-x

5. Luo R, Giordano SH, Freeman JL, Zhang D, Goodwin JS. Referral to medical oncology: a crucial step in the treatment of older patients with stage III colon cancer. *Oncologist* (2006) 11(9):1025–33. doi: 10.1634/theoncologist.11-9-1025

6. Schrag D, Gelfand SE, Bach PB, Guillem J, Minsky BD, Begg CB. Who gets adjuvant treatment for stage II and III rectal cancer? Insight from surveillance, epidemiology, and end results–Medicare. *J Clin Oncol* (2001) 19(17):3712–8. doi: 10.1200/JCO.2001.19.17.3712

7. Biller LH, Schrag D. Diagnosis and treatment of metastatic colorectal cancer: A review. *JAMA* (2021) 325(7):669–85. doi: 10.1001/jama.2021.0106

8. Teitelbaum EN, Swanstrom LL. Submucosal surgery: novel interventions in the third space. *Lancet Gastroenterol Hepatol* (2018) 3(2):134–40. doi: 10.1016/S2468-1253(17)30286-8

9. Panos G, Mulita F, Akinosoglou K, Liolis E, Kaplanis C, Tchabashvili L, et al. Risk of surgical site infections after colorectal surgery and the most frequent pathogens isolated: a prospective single-centre observational study. *Med Glas (Zenica)* (2021) 18(2):438–43. doi: 10.17392/1348-21

10. Tian Y, Li R, Wang G, Xu K, Li H, He L. Prediction of postoperative infectious complications in elderly patients with colorectal cancer: a study based on improved machine learning. *BMC Med Inform Decis Mak* (2024) 24(1):11. doi: 10.1186/s12911-023-02411-0

11. Martínez-Escribano C, Arteaga Moreno F, Pérez-López M, Cunha-Pérez C, Belenguer-Varea Á, Cuesta Peredo D, et al. Malnutrition and increased risk of adverse outcomes in elderly patients undergoing elective colorectal cancer surgery: A case-control study nested in a cohort. *Nutrients* (2022) 14(1):207. doi: 10.3390/nu14010207

12. Mulita F, Liolis E, Akinosoglou K, Tchabashvili L, Maroulis I, Kaplanis C, et al. Postoperative sepsis after colorectal surgery: a prospective single-center observational study and review of the literature. *Prz Gastroenterol* (2022) 17(1):47–51. doi: 10.5114/pg.2021.106083

13. Ramanathan ML, MacKay G, Platt J, Horgan PG, McMillan DC. The impact of open versus laparoscopic resection for colon cancer on C-reactive protein concentrations as a predictor of postoperative infective complications. *Ann Surg Oncol* (2015) 22(3):938–43. doi: 10.1245/s10434-014-4065-z

14. Park SU, Min YW, Shin JU, Choi JH, Kim YH, Kim JJ, et al. Endoscopic submucosal dissection or transanal endoscopic microsurgery for nonpolypoid rectal high grade dysplasia and submucosa-invading rectal cancer. *Endoscopy* (2012) 44(11):1031–6. doi: 10.1055/s-0032-1310015

15. Tahara K, Tanabe S, Ishido K, Higuchi K, Sasaki T, Katada C, et al. Argon plasma coagulation for superficial esophageal squamous-cell carcinoma in high-risk patients. *World J Gastroenterol* (2012) 18(38):5412–7. doi: 10.3748/wjg.v18.i38.5412

16. Ebigbo A, Probst A, Messmann H. Endoscopic treatment of early colorectal cancer - just a competition with surgery? *Innov Surg Sci* (2017) 3(1):39–46. doi: 10.1515/iss-2017-0037

17. Hong SW, Byeon JS. Endoscopic diagnosis and treatment of early colorectal cancer. *Intest Res* (2022) 20(3):281–90. doi: 10.5217/ir.2021.00169

18. Silva GL, de Moura EG, Bernardo WM, Leite de Castro V, Morais C, Baba ER, et al. Endoscopic versus surgical resection for early colorectal cancer-a systematic review and meta-analysis. *J Gastrointest Oncol* (2016) 7(3):326–35. doi: 10.21037/jgo.2015.10.02

19. Zheng C, Jiang F, Lin H, Li S. Clinical characteristics and prognosis of different primary tumor location in colorectal cancer: a population-based cohort study. *Clin Transl Oncol* (2019) 21(11):1524–31. doi: 10.1007/s12094-019-02083-1

20. Bousis D, Verras GI, Bouchagier K, Antzoulas A, Panagiotopoulos I, Katinioti A, et al. The role of deep learning in diagnosing colorectal cancer. *Prz Gastroenterol* (2023) 18(3):266–73. doi: 10.5114/pg.2023.129494

21. Chlorogiannis DD, Verras G, Tzelepi V, Chlorogiannis A, Apostolos A, Kotis A, et al. Tissue classification and diagnosis of colorectal cancer histopathology images using deep learning algorithms. Is the time ripe for clinical practice implementation. *Prz Gastroenterol* (2023) 18(4):353–67. doi: 10.5114/pg.2023.130337

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Prediction of pathologic complete response to neoadjuvant chemoradiation in locally advanced rectal cancer

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Purpose: To investigate the predictive factors of pathologic complete response (pCR) in locally advanced rectal cancer (LARC) patients who had been treated with neoadjuvant chemoradiation (nCRT).

Methods and materials: For this retrospective study, 53 LARC patients (37 males and 16 females; age range 25 to 79 years) were selected. Clinical characteristics, baseline mrTNM staging, MR gross tumor volumes (GTV), and pCR were evaluated. The diagnostic accuracy of GTV for predicting pCR was calculated.

Results: Among 53 LARC patients, 15 patients achieved pCR (28.3%), while 38 patients achieved non-pCR. Only three (5.7%) out of 53 patients did not downstage after nCRT. GTV and tumor differentiation were the significant prognostic parameters for predicting pCR. A tumor volume threshold of 21.1 cm^3 was determined as a predictor for pCR, with a sensitivity of 84% and specificity of 47%. In addition, GTV was associated with mrN stage, circumferential resection margin (CRM) status, extramural vascular invasion (EMVI) status, and pretreatment serum CEA level.

Conclusion: Tumor volume and tumor differentiation have significant predictive values in preoperative assessment of pCR among LARC patients. These findings aid clinicians to discriminate those patients who may likely benefit from preoperative regimens and to make optimal treatment plans.

KEYWORDS

LARC, NCRT, PCR, MRI, tumor volume, tumor differentiation

Introduction

Rectal cancer is a common cancer worldwide (1). Due to the widespread use of rectal magnetic resonance imaging (MRI), radiologists' understanding of the main MRI features of rectal cancer, early detection, and improved treatment of rectal cancer, the prognosis of rectal cancer has improved in recent decades (2). However, about half of patients are diagnosed with locally advanced cancer (LARC), which has a higher rate of recurrence and mortality (3). The application of neoadjuvant radiochemotherapy (nCRT) and total rectal mesorectal excision (TME) as a standard treatment has improved the local control for rectal cancer (4). Almost half of patients with LARC After nCRT neoplasms may decrease in stage and one-third of tumors showing pathological complete response (pCR) while TMD TME surgery performed (5, 6). Compared to patients without PCR, those with pCR are related with a better prognosis in local control, distant recurrence, disease-free survival (DFS), and overall survival (OS) (6, 7). A study indicated that an observation approach for LARC after a clinical complete response (cCR) showed no significant differences in non-regrowth cancer recurrence or OS rate between observational and surgical patients (8), which means most cCR patients can avoid the morbidity of radical surgery. Lord et al. evaluated NICE criteria for preoperative radiotherapy in patients with rectal cancer treated only surgically in 2020 and compared them with confirmed MRI prognostic factors. They found that confirmed MRI prognostic factors (extramural venous invasion, tumor deposition, and peripheral margin) were better able to identify high-risk groups (9).

Up to now, accurately predicting pCR or non-pCR to nCRT still remains a challenge, even though it is a crucial prerequisite for making appropriate treatment decisions about whether to make a watch-and-wait strategy for cCR patients, or to intensify treatment for those non-cCR. Therefore, this study attempted to investigate potential preoperative clinical and MRI markers to identify tumor response to nCRT and non-response among LARC patients, thus assisting in determining the optimal treatment planning.

Materials and methods

Patients

We retrospectively analyzed the data of consecutive rectal cancer patients between November 2017 and December 2022. Subsequently, 53 rectal cancer patients who were confirmed by surgical pathology and met the following criteria were enrolled: (a) histopathologically confirmed as rectal adenocarcinoma; (b) diagnosed as LARC, which was defined as clinical stage II (T3/4, node negative) or stage III (node positive) before treatment; (c) had evaluable MR imaging before nCRT; (d) had complete nCRT that was followed by surgical treatment after 5-12 weeks; (e) had complete clinical history; and (f) was free of induction or consolidation chemotherapy before or after the chemoradiation course. The exclusion criteria were patients with stage IV disease, mucinous rectal cancer, previous treatment, recurrent cancer, unavailable clinical or MRI data, or without surgery after nCRT. The process of patients' selection is listed in Figure 1.

MR examination and image analysis

1.5T or 3.0T MR were performed for each patient. Scanners used a pelvic phased-array coil. Patients were not asked to undergo bowel preparation and did not receive anti-peristaltic medication before MRI. Standard T2-weighted image (T2WI) fast spin-echo sequences, including sagittal and axial (perpendicular to the long axis of the intestinal lesion), were performed. Diffusion-weighted imaging (DWI) and contrast enhanced T1-weighted image (CE-T1WI) axial scans were also carried out. The acquisition parameters of MRI scans derived from different devices are summarized in the data supplement. After the collection of MR image data, they were sent to the PACS system.

Rectal cancer MRI staging was based on the American Joint Committee on Cancer (AJCC) TNM staging system for colorectal cancer (8th edition in 2016) (10) and Horvat N et al. (11). The tumor's pretreatment baseline mrTNM staging was evaluated by two board-certified radiologists independently, including tumor location (distance from the anal verge), T category, N category, circumferential resection margin (CRM) status, extramural vascular invasion (EMVI) status, and tumor deposit (TD) status. If there was any disagreement, consensus was reached after the discussion.

The pretreatment gross tumor volume (GTV) was carried out using the ITK-SNAP tool (Version 3.8.0. The tumor on the MR-T2WI axial oblique images was contoured manually slice by slice; non-involved soft tissues, feces, and central lumen were avoided. The GTV measurements were conducted by a single board-certified radiologist and finally revised by another experienced radiologist. The unit of GTV is cm^3. Figure 2 shows representative MRIs.

Neoadjuvant chemoradiotherapy

All patients received concurrent chemoradiotherapy based on oral capecitabine, starting on the first day of radiotherapy, with a dose of 1650 $mg/m^2/d$, divided into morning and evening doses,

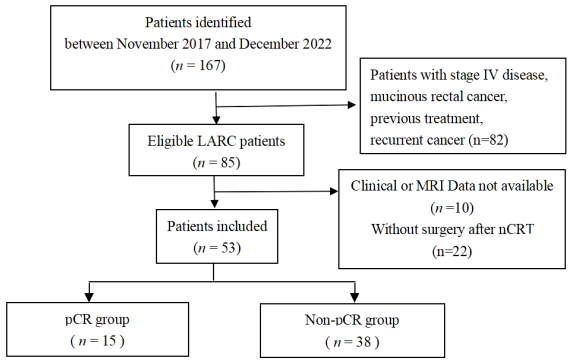

FIGURE 1
Study flow diagram.

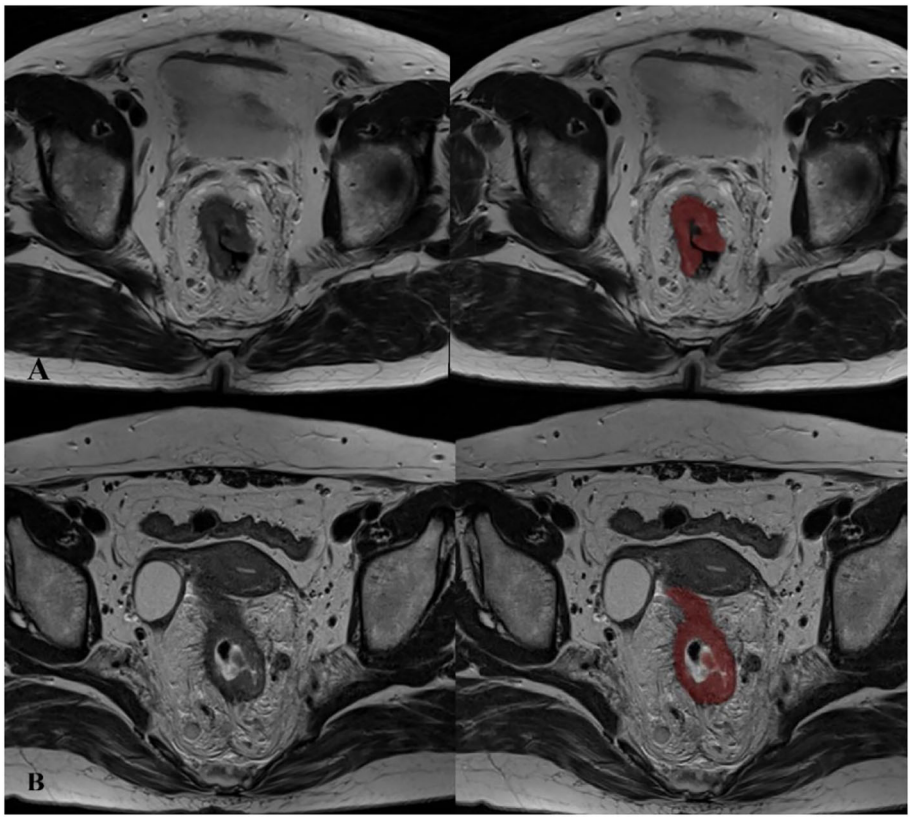

FIGURE 2
Examples for gross tumor volumetry in rectal cancer on axial contiguous MR images. **(A)** A 69-year-old man with a tumor in the mid-rectum, before treatment staged as T3N2, EMVI (+), CRM (+), TD (-). **(B)** A 57-year-old woman with a tumor in the low-rectum, before treatment staged as T4N2, EMVI (+), CRM (+), TD (+).

continuing until the end of radiotherapy. And mFOLFOX6 (oxaliplatin, 5-fluorouracil and calcium folinate) was used as adjuvant chemotherapy. Rectal irradiation was given by lateral opposed fields to the whole pelvis delivered by Varian (6MV). The radiation dose was 50.4Gy in 27 fractions, with 5 days' treatment per week.

Surgery and pathological TRG category

All patients underwent TME surgery 5-12 weeks after nCRT, based on further examinations confirming no surgical contraindications. All postoperative pathology specimens were determined by our hospital's pathology department.

The pathological tumor regression grade (TRG) was based on the classification standard of the American Joint Committee on Cancer/College of American Pathologists (AJCC/CAP) system (12). It is divided into four categories: TRG 0, no tumor cells visible under the microscope; TRG 1, only a single or small cluster of tumor cells remaining; TRG 2, tumor residual with predominant fibrosis; and TRG 3, none or small amount of tumor cell necrosis, extensive tumor residue. Define TRG 0 as the pCR group and TRG 1-3 as the non-pCR group. The TRG category data were independently defined by two experienced pathologists.

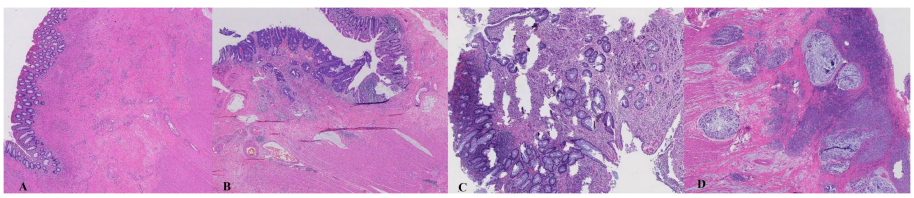

FIGURE 3
Tumor regression in rectal surgical specimens after neoadjuvant chemoradiation. **(A)**: TRG 0, pathological complete response; **(B)**: TRG 1; **(C)**: TRG 2; **(D)** TRG 3.

Figure 3 demonstrates tumor regression in rectal surgical specimens after nCRT.

Statistical analysis

SPSS software (Version 25.0 IBM Corp, Armonk, NY, USA) was adopted. Descriptive statistics such as mean with standard deviation were used for continuous data. Independent *t* tests, Wilcoxon rank-sum tests, or Mann-Whitney tests as appropriate were used to compare continuous variables, while the χ^2 tests were used to compare categorical data. Receiver operating characteristic (ROC) analysis was performed to calculate diagnostic sensitivity and specificity of GTV that predicts for pCR, and a cutoff value was established according to Youden's *J* test. *P* values of.05 were considered statistically significant.

Results

Characteristics of patents

Among fifty-three LARC patients who met the inclusion criteria in this study, there were 37 men (69.8%) and 16 women (30.2%). The mean age was 56.9 ± 12.2 years (range, 25-79). The pretreatment median tumor volume was 33.7cm^3 (range, 5-233cm^3), and the median distance from the anal verge evaluated by MRI was 4.5cm (range, 1.3-9cm). Pretreatment clinical assessment demonstrated only one stage II (1.9%) and 52 stage III (98.1%). Confirmed by surgical pathology after treatment, 15 (28.3%) patients had TRG 0, while 14 (26.4%), 20 (37.8%), and four (7.5%) patients had TRG 1, 2, and 3 respectively. That is, 15 (28.3%) out of 53 patients included in this study achieved pCR, while 38 (71.7%) patients achieved non-pCR. The clinical and pretreatment characteristics of LARC patients in this study are shown in Table 1.

Pretreatment clinical factors between the pCR and non-pCR patients

The mean age was 52.7 ± 13.6 years for pCR group and 58.6 ± 11.4 years for non-pCR group. No significant differences were found between these two groups in terms of age, gender, pretreatment clinical TNM staging, and CEA level, while tumor differentiation was the significant difference between these two groups (P = 0.000) (Table 1), namely that well differentiated rectal carcinomas seemed to attain a better tumor response and higher pCR rate.

Pretreatment MRI status between the pCR and non-pCR patients

Pretreatment mrT stage, mrN stage, tumor location, GTV, CRM, EMVI, and TD status were analyzed for prediction of pCR; GTV was the only statistically significant factor (P = 0.04) (Table 1). Subsequently, ROC analysis of GTV showed that the area under the curve value was 0.68 with asymptotic significance level (P = 0.04) and the tumor volume threshold was 21.1 cm^3 (Figure 4), which showed a sensitivity of 84% and specificity of 47% for predicting pCR (95% CI: 0.525, 0.840).

In addition, GTV was associated with mrN stage, CRM, EMVI, and pretreatment serum CEA level among all 53 LARC patients (Table 2). However, in the non-pCR group, GTV was just associated with CRM and EMVI; no statistically significant association between GTV and other parameters except tumor location was observed in the pCR group.

Posttreatment ypTN status between the pCR and non-pCR patients

After treatment, all 15 pCR patients reached ypT0N0. In the non-pCR group, there were 5/38 (13.1%) patients with ypT1 stage, 13/38 (34.2%) patients with ypT2 stage, 18/38 (47.4%) patients with ypT3 stage, and 2/38 (5.3%) patients with ypT4 stage; there were 26/38 (68.4%) patients with ypN0 stage, 10/38 (26.3%) patients with ypN1 stage, and 2/38 (5.3%) patients with ypN2 stage. In the non-pCR group, there were 12/38 (31.6%) stage I patients, 14/38 (36.8%) II patients, and 12/38 (31.6%) III patients. Only 3/53 (5.7%) patients did not downstage. The posttreatment characteristics of LARC patients in this study are shown in Table 3.

Discussion

In the current study, we found some evidence that gross tumor volume (GTV) and tumor differentiation were the significant prognostic parameters for predicting pCR. And we have found that pCR was in a rate of 28.3% among LARC patients treated with nCRT, which was similar to previous research. Factors such as age, gender, tumor location from anal verge, pretreatment CEA level, clinical TNM stage, and MRI parameters including mrTD, mrEMVI, and mrCRM failed to predict pCR. Additionally, GTV was associated with mrN stage, mrCRM, mrEMVI, and pretreatment serum CEA level among all the patients. These results help select individuals who may likely benefit from preoperative therapy.

The prediction of pCR in LARC patients has always been challenging. In earlier research, De Felice et al. found that pretherapeutic tumor size less than 5 cm could be considered as a significant predictor for pCR (13). And Reggiani et al. revealed tumor length larger than 3cm would be an independent prognostic factor, which tended to have worse DFS and cancer-specific survival (CSS) (14). Jankowski et al. argued that watch-and-wait strategy in patients with tumor length more than 7 cm was undetermined (15). However, it is easy to measure tumor length while not comprehensively reflecting the characteristics of tumor itself.

Recently, several studies have elucidated the value of tumor volume in predicting prognosis. Martens et al. reviewed literature

TABLE 1 Characteristics of 53 patients with locally advanced rectal cancer.

	pCR (n=15), n (%)	Non-pCR (n=38), n (%)	*P* value
Sex			0.333
Female	6 (40)	10 (26.3)	
Male	9 (60)	28 (73.7)	
Age (y)			0.114
Mean ± SD	52.7 ± 13.6	58.6 ± 11.4	
CEA level			0.230
Normal	11 (73.3)	21 (55.3)	
Abnormal	4 (26.7)	17 (44.7)	
Gross tumor volume (cm^3)			0.040*
Mean ± SD	27.0 ± 14.0	46.8 ± 40.7	
Tumor location (from anal verge)			0.765
Low	8 (53.3)	22 (57.9)	
Middle	7 (46.7)	16 (42.1)	
High	0	0	
Tumor differentiation			0.000*
Poor	1 (6.7)	11 (28.9)	
Moderate	5 (33.3)	24 (63.2)	
Well	9 (60)	3 (7.9)	
cTNM stage at baseline			0.111
I-II	1 (6.7)	0	
III	14 (93.3)	38 (100)	
mrT stage at baseline			0.072
T1-2	2 (13.3)	2 (5.3)	
T3	11 (73.4)	22 (57.9)	
T4	2 (13.3)	14 (36.8)	
mrN stage at baseline			0.483
N0-1	2 (13.3)	9 (23.7)	
N2	13 (86.7)	29 (76.3)	
mrTD status at baseline			0.243
Negative	11 (73.4)	33 (86.8)	
Positive	4 (26.7)	5 (13.2)	
mrEMVI status at baseline			0.314
Negative	2 (13.3)	10 (26.3)	
Positive	13 (86.7)	28 (73.7)	
mrCRM status at baseline			0.509
Negative	4 (26.7)	7 (18.4)	
Positive	11 (73.3)	31 (81.6)	

pCR, pathologic complete response; SD, standard deviation; TD, tumor deposit; EVMI, extramural vascular invasion; CRM, circumferential resection margin; CEA, carcinoembryonic antigen.
*Signifies a significant difference between pCR and non-pCR groups (P < 0.05).

on tumor measurements on MRI and validated tumor length or 3-dimensional tumor size were not accurate enough to assess the tumor response after chemoradiotherapy (16). They found tumor volume measured by MR achieved up to 80% accuracy to assess a complete tumor response. Jiang et al. reported a tumor volume less than 9.49 cm^3 was significantly correlated with DFS and local recurrence-free survival (LRFS) in earlier rectal cancer patients who had been operated on with radical surgery (17). And the tumor volume was significantly associated with pretherapeutic CEA level, Hb level, and the number of lymph nodes. Lutsyk et al. found tumor volume less than 39.5 cm^3 was a significant predictor for achieving pCR among 187 LARC patients (18). Similarly, Yang et al. demonstrated tumor volume less than 37.3 cm^3 could be predictive for pCR in 412 LARC patients receiving nCRT (19).

Considering the tumor volumetry might result in bias from different MR devices, we used a new computational algorithm, which is based on MRI spatial voxels, that makes a more precise measurement of GTV. This voxel-based approach for GTV utilizing MR scans is an improvement from conventional rough 1-dimensional and 3-dimensional tumor measurements, regardless of volume data from different modalities. To some extent, this may be the reason why tumor volume in our study was smaller than other studies. Moreover, Maas et al. had compared the accuracy of 3T and 1.5T MR scanners to discriminate between T2 and borderline T3 rectal cancers when performing exams on the same group of patients and found no significant differences between the two MRI scanners (20), which suggested that it could not be a confounder impacting the tumor volume estimation. And all the patients in our study were performed on a standardized imaging protocol, which allows for accurate and reproducible interpretations in the evaluation of rectal cancer.

Tumor differentiations are found more frequently to associate with prognosis. Poor differentiated tumors are more commonly found to be aggressive, by invading blood vessels and nerves and adjacent histological boundaries. Al-Sukhni et al. identified lower tumor grade was correlated with higher odds of pCR among 23,747 patients with rectal cancer who received nCRT (21). A recent retrospective study of 325 patients demonstrated that poor differentiation was recognized as an independent risk factor for tumor local recurrence and 3-year overall survival (22). However, Huang et al. did not discuss the assessments of the tumor itself, neither the length nor volume. Reggiani et al. also suggested that a

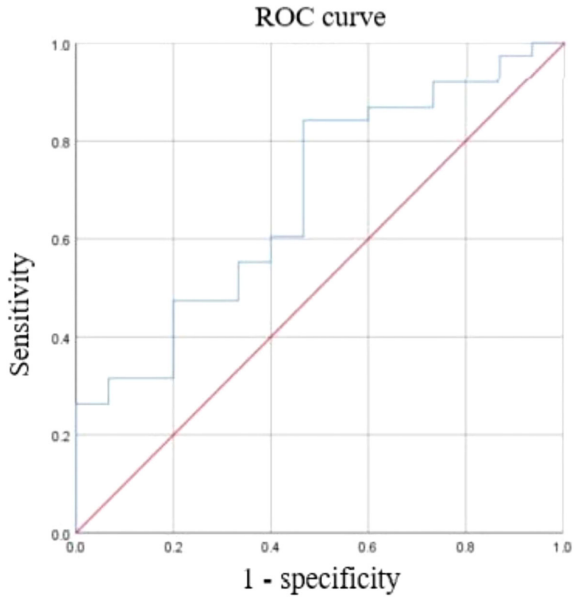

FIGURE 4
Receiver operating characteristic (ROC) curve with tumor volume cutoff threshold for pathological complete response. Area under the curve (AUC) = 0.682, 95% CI: 0.525, 0.84.

comprehensive approach should be applied to rectal cancer patients with poor differentiation (14).

Some studies have identified the significance of several clinical and radiological markers in predicting pCR and non-pCR among LARC patients. Huh JW et al. reported that pretreatment tumor circumference, tumor ulceration, and CEA level should be considered when attaining pCR (23). However, they did not evaluate pretreatment tumor volume. Zhao et al. used mrDEC score to predict tumor response to nCRT and showed that mrTDs and mrEMVI were statistically significant but not mrCRM and mrDEC (24). We also tried to use mrDEC scoring system to detect pCR but found no significant difference in our study. In addition to the commonly used comprehensive assessments, functional imaging is also increasingly being applied to evaluate prediction in LARC patients. Lambregts et al. demonstrated that diffusion-weighted MRI (DWI) helped to identify complete tumor response after CRT by qualitative evaluation (25). Joe et al. systematically reviewed the data on the role of DWI and ^{18}F-FDG PET/CT when attaining pCR, and they revealed that DWI and ^{18}F-FDG PET/CT were not accurate enough to stratify patients for conservative approaches (26). Lian et al. found that the mean T1 and T2 values were significantly lower in pCR patients and those T-downstage patients by pretreatment quantitative synthetic MRI (27). However, proton density (PD) and ADC values failed to identify pCR and T-downstaging. Iafrate et al. showed that pretreatment ADC values were significantly lower in pCR patients when compared with those non-pCR patients, but they failed to identify pretreatment tumor volume associated with pathological response, with the median value of 21.3 cm^3 and 24 cm^3 respectively (28). In the current study, we did not evaluate ADC values as it was generated by variate equipment which might be unreliable.

Research based on radiomics has been emerging recently to evaluate the tumor response to nCRT in LARC patients. Zhou et al. indicated that pretreatment, multiparametric MRI radiomic features played an important role in predicting non-response to nCRT (29). Ren et al. developed nomograms for predicting pCR probability and showed the significance of neoadjuvant therapy options, tumor differentiation, MRF status, and tumor length (30). Chiloiro G et al. and Shin J et al. used radiomics models and showed a good performance for predicting pCR after nCRT (31, 32). Moreover, Chiloiro G et al. found that the best performing two-

TABLE 2 Factors associated with gross tumor volume.

	53 LARC patients and *P* value	pCR group and *P* value	Non-pCR group and *P* value
mrT stage at baseline	0.15	0.47	0.47
mrN stage at baseline	0.03	0.09	0.06
mrTD status at baseline	0.85	0.36	0.65
mrEMVI status at baseline	0.006	0.23	0.005
mrCRM status at baseline	0.002	0.24	0.007
CEA level	0.04	1	0.06
Tumor location (from anal verge)	0.35	0.04	0.84
Tumor differentiation	0.22	0.4	0.74

TABLE 3 Characteristics of patients in the pCR and non-pCR groups after treatment.

	pCR (n=15), n (%)	Non-pCR (n=38), n (%)
ypTNM stage		
I	NA	12 (31.6%)
II	NA	14 (36.8%)
III	NA	12 (31.6%)
ypT status		
T0	NA	0
T1	NA	5 (13.1%)
T2	NA	13 (34.2%)
T3	NA	18 (47.4%)
T4	NA	2 (5.3%)
ypN status		
N0	NA	26 (68.4%)
N1	NA	10 (26.3%)
N2	NA	2 (5.3%)

year DFS prediction model was developed on the basis of tumor volume as well as mesorectal features (33).

This study was limited by its small sample size and retrospective nature. When assessing pCR in LARC patients, lymph node status did not play a significant predictive role in this study, which is due to the fact that those enrolled LARC patients were mostly associated with lymph node positive when they arrived at our hospital, thus resulting in a similar preoperative lymph node status between the two groups. Furthermore, an increase in the number of T4 patients in the non-pCR group may interfere with the significance, which marginally showed no significant difference in T stage between the two groups. And we could not provide enough data to use multivariable logistic regression models to investigate factors that may have an independent influence on tumor response. If the dataset is small, it is not conducive to obtaining a better training mode when splitting the same dataset in training and evaluation subsets. This is why we did not run an external validation study. Regardless, these results in the current study have demonstrated potential predictors based on clinical characteristics and MRI markers. Further large and prospective studies are on the way to validate these findings.

Conclusion

The current study shows that preoperative gross tumor volume and tumor differentiation can be potential predictors for pCR in LARC. These findings help clinicians to stratify those patients who may benefit from a conservative rather than aggressive therapeutic approach after nCRT. When evaluating the clinical response, clinicians can make a more personalized regimen for rectal cancer patients based on personal characteristics and patient's risk factors.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the ethics committee review board of Shenzhen People's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XZ: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. GZ: Data curation, Formal analysis, Writing – original draft. LZ: Data curation, Formal analysis, Writing – original draft. SY: Data curation, Formal analysis, Writing – original draft. YF: Data curation, Formal analysis, Investigation, Writing – review & editing. WH: Conceptualization, Investigation, Methodology, Writing – review & editing. GL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Kamath SD, Torrejon N, Wei W, Tullio K, Nair KG, Liska D, et al. Racial disparities negatively impact outcomes in early-onset colorectal cancer independent of socioeconomic status. *Cancer Med*. (2021) 10:7542–50. doi: 10.1002/cam4.4276

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. (2018) 68:7–30. doi: 10.3322/caac.21442

3. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin*. (2020) 70:145–64. doi: 10.3322/caac.21601

4. You YN, Hardiman KM, Bafford A, Poylin V, Francone TD, Davis K, et al. The american society of colon and rectal surgeons clinical practice guidelines for the management of rectal cancer. *Dis Colon Rectum*. (2020) 63:1191–222. doi: 10.1097/DCR.0000000000001762

5. Schrag D, Shi Q, Weiser MR, Gollub MJ, Saltz LB, Musher BL, et al. Preoperative treatment of locally advanced rectal cancer. *N Engl J Med*. (2023) 389:322–34. doi: 10.1056/NEJMoa2303269

6. Maas M, Nelemans PJ, Valentini V, Das P, Rödel C, Kuo LJ, et al. Long-term outcome in patients with a pathological complete response after chemoradiation for rectal cancer: a pooled analysis of individual patient data. *Lancet Oncol*. (2010) 11:835–44. doi: 10.1016/S1470-2045(10)70172-8

7. Martin ST, Heneghan HM, Winter DC. Systematic review and meta-analysis of outcomes following pathological complete response to neoadjuvant chemoradiotherapy for rectal cancer. *Br J Surg*. (2012) 99:918–28. doi: 10.1002/bjs.8702

8. Dossa F, Chesney TR, Acuna SA, Baxter NN. A watch-and-wait approach for locally advanced rectal cancer after a clinical complete response following neoadjuvant chemoradiation: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. (2017) 2:501–13. doi: 10.1016/S2468-1253(17)30074-2

9. Lord AC, Corr A, Chandramohan A, Hodges N, Pring E, Airo-Farulla C, et al. Assessment of the 2020 NICE criteria for preoperative radiotherapy in patients with rectal cancer treated by surgery alone in comparison with proven MRI prognostic factors: a retrospective cohort study. *Lancet Oncol*. (2022) 23:793–801. doi: 10.1016/S1470-2045(22)00214-5

10. Amin MB, Greene FL, Edge SB, Byrd DR, Brookland RK, Washington MK, et al. *AJCC Cancer Staging Manual. 8th ed*. New York: Springer (2016).

11. Horvat N, Carlos Tavares Rocha C, Clemente Oliveira B, Petkovska I, Gollub MJ. MRI of rectal cancer: tumor staging, imaging techniques, and management. *Radiographics*. (2019) 39:367–87. doi: 10.1148/rg.2019180114

12. Benson AB, Venook AP, Al-Hawary MM, Azad N, Chen YJ, Ciombor KK, et al. Rectal cancer, version 2.2022, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. (2022) 20:1139–67. doi: 10.6004/jnccn.2022.0051

13. De Felice F, Izzo L, Musio D, Magnante AL, Bulzonetti N, Pugliese F, et al. Clinical predictive factors of pathologic complete response in locally advanced rectal cancer. *Oncotarget*. (2016) 7:33374–80. doi: 10.18632/oncotarget.8133

14. Reggiani Bonetti L, Lionti S, Domati F, Barresi V. Do pathological variables have prognostic significance in rectal adenocarcinoma treated with neoadjuvant chemoradiotherapy and surgery? *World J Gastroenterol*. (2017) 23:1412–23. doi: 10.3748/wjg.v23.i8.1412

15. Jankowski M, Pietrzak L, Rupiński M, Michalski W, Hołdakowska A, Paciorek K, et al. Watch-and-wait strategy in rectal cancer: Is there a tumour size limit? Results from two pooled prospective studies. *Radiother Oncol*. (2021) 160:229–35. doi: 10.1016/j.radonc.2021.05.014

16. Martens MH, van Heeswijk MM, van den Broek JJ, Rao SX, Vandecaveye V, Vliegen RA, et al. Prospective, multicenter validation study of magnetic resonance volumetry for response assessment after preoperative chemoradiation in rectal cancer: can the results in the literature be reproduced? *Int J Radiat Oncol Biol Phys*. (2015) 93:1005–14. doi: 10.1016/j.ijrobp.2015.09.008

17. Jiang Y, You K, Qiu X, Bi Z, Mo H, Li L, et al. Tumor volume predicts local recurrence in early rectal cancer treated with radical resection: a retrospective observational study of 270 patients. *Int J Surg*. (2018) 49:68–73. doi: 10.1016/j.ijsu.2017.11.052

18. Lutsyk M, Awawda M, Gourevich K, Yosef RB. Tumor volume as predictor of pathologic complete response following neoadjuvant chemoradiation in locally advanced rectal cancer. *Am J Clin Oncol*. (2021) 44:482–6. doi: 10.1097/COC.0000000000000846

19. Yang F, Hill J, Abraham A, Ghosh S, Steed T, Kurtz C, et al. Tumor volume predicts for pathologic complete response in rectal cancer patients treated with neoadjuvant chemoradiation. *Am J Clin Oncol*. (2022) 45:405–9. doi: 10.1097/COC.0000000000000942

20. Maas M, Lambregts DM, Lahaye MJ, Beets GL, Backes W, Vliegen RF, et al. T-staging of rectal cancer: accuracy of 3.0 Tesla MRI compared with 1.5 Tesla. *Abdom Imaging*. (2012) 37:475–81. doi: 10.1007/s00261-011-9770-5

21. Al-Sukhni E, Attwood K, Mattson DM, Gabriel E, Nurkin SJ. Predictors of pathologic complete response following neoadjuvant chemoradiotherapy for rectal cancer. *Ann Surg Oncol*. (2016) 23:1177–86. doi: 10.1245/s10434-015-5017-y

22. Huang Q, Qin H, Xiao J, He X, Xie M, He X, et al. Association of tumor differentiation and prognosis in patients with rectal cancer undergoing neoadjuvant chemoradiation therapy. *Gastroenterol Rep (Oxf)*. (2019) 7:283–90. doi: 10.1093/gastro/goy045

23. Huh JW, Kim HR, Kim YJ. Clinical prediction of pathological complete response after preoperative chemoradiotherapy for rectal cancer. *Dis Colon Rectum*. (2013) 56:698–703. doi: 10.1097/DCR.0b013e3182837e5b

24. Zhao M, Feng L, Zhao K, Cui Y, Li Z, Ke C, et al. An MRI-based scoring system for pretreatment risk stratification in locally advanced rectal cancer. *Br J Cancer*. (2023) 129:1095–104. doi: 10.1038/s41416-023-02384-x

25. Lambregts DM, Vandecaveye V, Barbaro B, Bakers FC, Lambrecht M, Maas M, et al. Diffusion-weighted MRI for selection of complete responders after chemoradiation for locally advanced rectal cancer: a multicenter study. *Ann Surg Oncol*. (2011) 18:2224–31. doi: 10.1245/s10434-011-1607-5

26. Joe I, Deroose CM, Vandecaveye V, Haustermans K. The role of diffusion-weighted MRI and (18)F-FDG PET/CT in the prediction of pathologic complete response after radiochemotherapy for rectal cancer: a systematic review. *Radiother Oncol*. (2014) 113:158–65. doi: 10.1016/j.radonc.2014.11.026

27. Lian S, Liu H, Meng T, Ma L, Zeng W, Xie C. Quantitative synthetic MRI for predicting locally advanced rectal cancer response to neoadjuvant chemoradiotherapy. *Eur Radiol*. (2023) 33:1737–45. doi: 10.1007/s00330-022-09191-7

28. Iafrate F, Ciccarelli F, Masci GM, Grasso D, Marruzzo F, De Felice F, et al. Predictive role of diffusion-weighted MRI in the assessment of response to total neoadjuvant therapy in locally advanced rectal cancer. *Eur Radiol*. (2023) 33:854–62. doi: 10.1007/s00330-022-09086-7

29. Zhou X, Yi Y, Liu Z, Cao W, Lai B, Sun K, et al. Radiomics-based pretherapeutic prediction of non-response to neoadjuvant therapy in locally advanced rectal cancer. *Ann Surg Oncol*. (2019) 26:1676–84. doi: 10.1245/s10434-019-07300-3

30. Ren DL, Li J, Yu HC, Peng SY, Lin WD, Wang XL, et al. Nomograms for predicting pathological response to neoadjuvant treatments in patients with rectal cancer. *World J Gastroenterol*. (2019) 25:118–37. doi: 10.3748/wjg.v25.i1.118

31. Chiloiro G, Cusumano D, de Franco P, Lenkowicz J, Boldrini L, Carano D, et al. Does restaging MRI radiomics analysis improve pathological complete response prediction in rectal cancer patients? A prognostic model development. *Radiol Med*. (2022) 127:11–20. doi: 10.1007/s11547-021-01421-0

32. Shin J, Seo N, Baek SE, Son NH, Lim JS, Kim NK, et al. MRI radiomics model predicts pathologic complete response of rectal cancer following chemoradiotherapy. *Radiology*. (2022) 303:351–8. doi: 10.1148/radiol.211986

33. Chiloiro G, Cusumano D, Romano A, Boldrini L, Nicolì G, Votta C, et al. Delta radiomic analysis of mesorectum to predict treatment response and prognosis in locally advanced rectal cancer. *Cancers (Basel)*. (2023) 15:3082. doi: 10.3390/cancers15123082

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Development and validation of a nomogram to predict the risk factors of major complications after radical rectal cancer surgery

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Purpose: The aim of this study was to establish a validated nomogram to predict risk factors for major post-operative complications in patients with rectal cancer (RC) by analyzing the factors contributing to major post-operative complications in RC patients.

Methods: We retrospectively collected baseline and surgical information on patients who underwent RC surgery between December 2012 and December 2022 at a single-center teaching hospital. The entire cohort was randomly divided into two subsets (60% of the data for development, 40% for validation). Independent risk factors for major post-operative complications were identified using multivariate logistic regression analyses, and predictive models were developed. Area under the curve (AUC) was calculated using receiver operating characteristic curve (ROC) to assess predictive probability, calibration curves were plotted to compare the predicted probability of the nomogram with the actual probability, and the clinical efficacy of the nomogram was assessed using decision curve analysis (DCA).

Results: Our study included 3151 patients who underwent radical surgery for RC, including 1892 in the development set and 1259 in the validation set. Forty (2.1%) patients in the development set and 26 (2.1%) patients in the validation set experienced major post-operative complications. Through multivariate logistic regression analysis, age (p<0.01, OR=1.044, 95% CI=1.016-1.074), pre-operative albumin (p<0.01, OR=0.913, 95% CI=0.866-0.964), and open surgery (p<0.01, OR=2.461, 95% CI=1.284-4.761) were identified as independent risk factors for major post-operative complications in RC, and a nomogram prediction model was established. The AUC of the ROC plot for the development set was 0.7161 (95% CI=0.6397-0.7924), and the AUC of the ROC plot for the validation set was 0.7191 (95% CI=0.6182-0.8199). The predicted probabilities in the calibration curves were highly consistent with the actual probabilities, which indicated that the prediction model had good predictive ability. The DCA also confirmed the good clinical performance of the nomogram.

Conclusion: In this study, a validated nomogram containing three predictors was created to identify risk factors for major complications after radical RC surgery. Due to its accuracy and convenience, it could contribute to personalized management of patients in the perioperative period.

KEYWORDS

rectal cancer, surgery, complications, nomogram, risk factors

Introduction

Colorectal cancer is one of the most prevalent cancers in the world and is a serious threat to human health, with an estimated 1.9 million new cases and 935,000 deaths in 2020 (1). In recent years, with the rapid development of laparoscopic instruments and techniques, transabdominal low anterior resection (LAR) combined with total mesorectal excision (TME) has become the standard approach for the treatment of low and intermediate rectal cancer (RC). Laparoscopic rectal surgery (LRS) has been widely used for the treatment of RC because of its low trauma rate and fast recovery (2). A clear surgical field and full exposure of anatomical structures enabled LRS to achieve radical resection of RC, reduce surgical trauma, and improve the post-operative quality of life (3). However, post-operative complications remained a major concern. Previous studies have reported that the incidence of post-operative complications in RC was 20%-30%, the incidence of serious complications was 5%-12%, and the mortality rate was approximately 2% (4, 5). Anastomotic leakage (AL), a common serious complication after radical resection for RC, had an incidence of 2.4% to 27.0% and a mortality rate of 18% (6–8). These complications and bowel dysfunction might affect the patients' quality of life and long-term prognosis.

In recent years, anastomotic devices and surgical techniques have improved considerably, however, the incidence of complications has not decreased significantly (9–12). Many previous randomized controlled studies have explored the risk factors for post-operative complications in RC, including age (13), pre-operative albumin (14), pre-operative neoadjuvant therapy (15) and body mass index (BMI) (16). Tumor-related factors included tumor size and the distance of the tumor from the anal verge (17, 18). Surgery-related factors included the duration of surgery and intraoperative blood loss (19). There were conflicting reports on the risk factors for complications after radical RC.

The Clavien-Dindo system has been widely used to classify post-operative complications. Clavien-Dindo III-IV complications requiring re-operation and endoscopic or radiological intervention were defined as serious complications (20), which always led to catastrophic consequences such as organ failure or even death, as well as high medical costs.

Therefore, the aim of this study was to establish a validated nomogram to predict risk factors for major post-operative complications in patients with RC by analyzing the factors contributing to post-operative complications in RC patients, and to provide a reference point for the prevention and treatment of post-operative complications for RC and provides timely and effective interventions in the peri-operative period.

Materials and methods

Patient selection

We retrospectively collected baseline and surgical information on patients who underwent radical RC surgery between December 2012 and December 2022 at a single-center teaching hospital. The inclusion criteria were that patients with a pathologically confirmed preoperative diagnosis of rectal malignancy who underwent radical surgery for RC. The exclusion criteria were as follows: 1. patients who underwent RC after recurrence; 2. patients with metastatic RC; 3. patients who underwent emergency surgery, including bowel obstruction and bleeding; and 4. patients with incomplete baseline or surgical information. Ultimately, 3151 patients with complete information were finally enrolled in the study, who were randomly assigned in a 6:4 ratio to the development set (n=1892) and validation set (n=1259) based on computer-generated random numbers.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (K2024-002-01). It complied with the principles of medical ethics and the Declaration of Helsinki, and all patients participating in the study signed an informed consent form.

Data elements

We retrospectively collected baseline and surgical information of the patients. The baseline information included age, sex, BMI, smoking and drinking history, previous abdominal surgery (PAS), and preoperative comorbidities. Clinical information included preoperative albumin and hemoglobin, tumor stage, and tumor size. Surgical information included surgical methods, surgical time, blood loss, and major post-operative complications. The pre-

operative comorbidities included hypertension, type 2 diabetes mellitus (T2DM), and chronic heart disease (CHD).

Surgery management

All patients who underwent radical resection according to the guidelines of the Chinese Society of Clinical Oncology (CSCO) for colorectal cancer, that's total mesorectal excision or complete mesocolic excision, and the post-operative pathology was confirmed R0 resection.

Definition

Tumors were staged according to the 8th edition of the American Joint Committee on Cancer (AJCC) guidelines and were classified as stages I-IV (21).

Major post-operative complications within 30 days of surgery were assessed using the Clavien-Dindo scale (22). Clavien-Dindo III/IV complications requiring surgical, endoscopic, or radiological intervention were defined as major complications.

Statistical analysis

All data in this study were processed using SPSS (version 22.0) and R (version 4.1.2). Continuous variables that followed a normal distribution were expressed as mean ± standard deviation (SD) and comparisons were made using the t-test; categorical variables were expressed as numbers and percentages, and chi-squared or Fisher's exact test was used. Univariate logistic regression analysis was performed on all variables, and variables with $P<0.05$ were considered potential risk factors for the occurrence of major post-operative complications in RC patients. The screened potential risk factors were subjected to multivariate logistic regression analysis to identify independent predictors of complications after RC surgery. Finally, multivariate logistic regression analysis included variables with $P<0.05$, and a nomogram was created to predict the risk of major post-operative complications in RC.

The predictive models were evaluated in three ways. First, the predictive value of the risk factors was verified using receiver operating characteristic curve (ROC), and the performance of the nomogram was assessed by calculating the area under the curve (AUC) for the development and validation sets. The AUCs ranged from 0 and 1, with 1 indicating perfect agreement, 0.5 indicating no better than chance, and greater than 0.7 indicating that the model had relatively good predictive power (23, 24). Second, prediction curves were plotted to test the calibration of major post-operative complication risk map, and the predicted and actual probabilities of the nomogram for the development and validation sets were analyzed and compared, using the 45-degree line as the perfect model with 100% accuracy (25). Finally, decision curve analysis (DCA) was used to analyze the net benefits of the development and validation sets based on different threshold probabilities to determine the clinical applicability of the nomogram (26, 27).

Results

Baseline information

Based on the above inclusion and exclusion criteria, 3151 patients who underwent radical RC surgery were included in this study. This included 1892 patients in the development set and 1259 patients in the validation set. Forty (2.1%) patients in the development set and 26 (2.1%) patients in the validation set experienced major post-operative complications. Baseline information was comparable between the two groups ($P>0.05$) (Table 1).

Nomogram variable screening

Univariate and multivariate logistic regression analyses, including baseline, and surgical information, were performed to identify the risk factors influencing the occurrence of major post-operative complications in RC. The results of univariate logistic regression analysis showed that age ($p<0.01$, OR=1.044, 95% CI=1.016-1.074), preoperative albumin ($p<0.01$, OR=0.913, 95% CI=0.866-0.964), and open surgery ($p<0.01$, OR=2.461, 95% CI=1.284-4.761) were potential risk factors for major post-operative complications of RC. Further multivariate logistic regression analysis of the three potential risk factors showed that age ($p=0.023$, OR=1.033, 95% CI=1.005-1.062), pre-operative albumin ($p=0.032$, OR=0.940, 95% CI=0.888-0.995), and open surgery ($p=0.049$, OR=1.992, 95% CI=1.003-3.956) were independent risk factors for the occurrence of major post-operative complications in RC (Table 2).

Development of a nomogram to predict the occurrence of major complications after RC surgery

Using the three independent risk factors identified by the multivariate logistic regression analysis, a nomogram model was constructed to predict the risk of major post-operative complications in RC patients. As shown in Figure 1, the corresponding scores for each factor were derived from the patients' own actual situation, and the three scores were added to derive the total score. The final predicted risk of major post-operative complications was the probability corresponding to the patient's individual total score.

Validation of a nomogram for predicting major complications after RC surgery

The ROC curve was used to assess the predictive accuracy of the nomogram. The results showed that the area under the ROC curve for the development set was 0.7161 (95% Cl=0.6397-0.7924), and that of the validation set was 0.7191 (95% CI=0.6182-0.8199). (Figure 2) The calibration curve showed a high degree of agreement between the predicted and observed results of the nomogram model constructed in this study. (Figure 3) Finally,

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TABLE 1 Baseline information between the development and validation cohorts.

Characteristics	Development (1892)	Validation (1259)	P value
Age, year	63.5 ± 12.8	63.5 ± 12.8	0.881
Sex			0.438
Male	1054 (55.7%)	719 (57.1%)	
Female	838 (44.3%)	540 (42.9%)	
BMI, kg/m^2	22.5 ± 3.1	22.5 ± 3.4	0.912
Smoking	685 (36.2%)	470 (37.3%)	0.521
Drinking	570 (30.1%)	376 (29.9%)	0.875
Hypertension	474 (25.1%)	341 (27.1%)	0.202
T2DM	231 (12.2%)	146 (11.6%)	0.604
CHD	94 (5.0%)	65 (5.2%)	0.807
PAS	531 (28.1%)	347 (27.6%)	0.757
Albumin, g/L	38.8 ± 5.7	38.7 ± 5.9	0.714
Hemoglobin, g/L	113.7 ± 26.4	113.9 ± 25.6	0.825
TNM stage			0.769
I	257 (13.6%)	160 (12.7%)	
II	939 (49.6%)	628 (49.9%)	
III	696 (36.8%)	471 (37.4%)	
Tumor size			0.077
< 5cm	943 (49.8%)	587 (46.6%)	
≥ 5cm	949 (50.2%)	672 (53.4%)	
Surgical methods			0.850
Open	378 (20.0%)	255 (20.3%)	
Laparoscopic	1514 (80.0%)	1004 (79.7%)	
Surgical time, min	214.9 ± 79.1	218.5 ± 79.2	0.217
Blood loss, mL	98.7 ± 137.3	107.9 ± 190.7	0.114
Major complications	40 (2.1%)	26 (2.1%)	0.925

Variables are expressed as the mean ± SD, n (%), *P-value <0.05.
T2DM, type 2 diabetes mellitus; BMI, body mass index; CHD, chronic heart disease; PAS, previous abdominal surgery.

DCA was used to evaluate the clinical application value of the prediction model, as shown in Figure 4.

TABLE 2 Univariate and multivariate logistic regression analysis of the major complications.

Risk factors	Univariate logistic regression analysis		Multivariate logistic regression analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Age, year	1.044 (1.016-1.074)	<0.01*	1.033 (1.005-1.062)	0.023*
Sex (female/male)	0.672 (0.349-1.295)	0.235		
BMI, Kg/m^2	0.983 (0.889-1.087)	0.742		
Smoking (yes/no)	1.059 (0.554-2.022)	0.863		
Drinking (yes/no)	1.119 (0.573-2.186)	0.741		
Hypertension (yes/no)	1.821 (0.952-3.484)	0.070		
T2DM (yes/no)	1.028 (0.399-2.651)	0.955		
CHD (yes/no)	1.569 (0.475-5.185)	0.460		
PAS (yes/no)	0.972 (0.482-1.959)	0.936		
Albumin, g/L	0.913 (0.866-0.964)	<0.01*	0.940 (0.888-0.995)	0.032*
Hemoglobin, g/L	0.997 (0.986-1.009)	0.673		
Tumor stage (III/II/I)	1.242 (0.769-2.005)	0.376		
Tumor size (≥ 5/ <5), cm	0.994 (0.531-1.859)	0.984		
Surgical methods (open/ laparoscopic)	2.461 (1.284-4.761)	<0.01*	1.992 (1.003-3.956)	0.049*
Surgical time, min	0.997 (0.993-1.002)	0.268		
Blood loss, mL	1.000 (0.998-1.002)	0.777		

*P-value <0.05.
OR, Odds ratio; CI, confidence interval; BMI, body mass index; T2DM, type 2 diabetes mellitus; BMI, body mass index; CHD, chronic heart disease; PAS, previous abdominal surgery.

Discussion

Our study included 3151 patients who underwent radical surgery for RC, including 1892 in the development set and 1259 in the validation set. Forty (2.1%) patients in the development set and 26 (2.1%) patients in the validation set experienced major post-operative complications. Multivariate logistic regression analysis showed that age, pre-operative albumin, and open surgery were independent risk factors for the major post-operative complications of RC. Based on the three independent risk factors, we constructed a nomogram model to predict the risk factors of major post-operative complications in patients for RC.

In recent years, anastomosis and surgical techniques have developed considerably, but the incidence of post-operative complications in RC has not been significantly reduced (9–12). Several previous studies have shown that post-operative complications affected the prognosis of RC (28–30). Therefore, it was necessary to develop a predictive nomogram for major post-operative complications in RC. In our study, 66 (2.1%) patients had

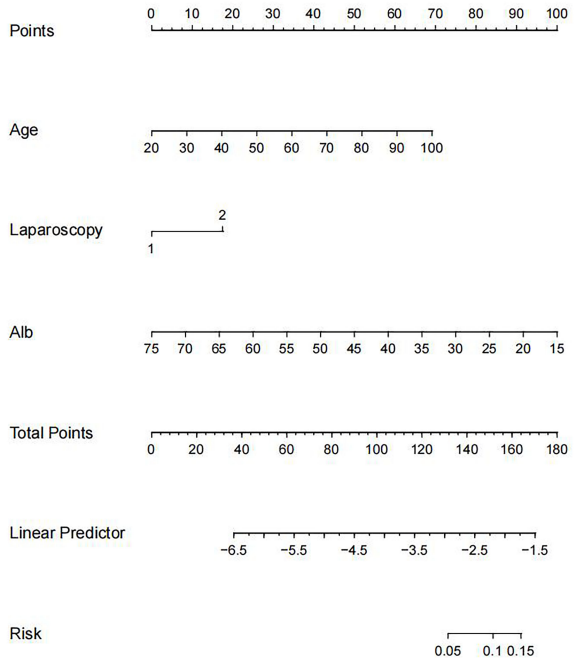

FIGURE 1
Nomogram for predicting the risk of major postoperative complications after RC surgery. RC, rectal cancer.

major post-operative complications, which was significantly lower than those reported in previous studies (31). This might be related to the definition of major post-operative complications.

The results of this study suggested that age was an independent risk factor for major post-operative complications for RC. This was like previous studies (32). Surgery-related comorbidities, including cardiovascular and pulmonary diseases, oncological anemia, and liver or kidney disease were more common in elderly patients (33, 34). In addition, neurological or psychological disorders was often prevalent in elderly patients (35). Previous studies found that elderly patients with limited baseline performance status (defined by Eastern Cooperative Group performance statuses 2-4, abnormalities in activities of daily living and instrumental activities of daily living) were less likely to tolerate the procedure and had worse outcomes than younger patients (36).

Studies have shown that low pre-operative albumin negatively affected wound healing and disease severity (37). In RC surgery, low pre-operative albumin levels significantly increased the incidence of post-operative complications (38, 39). Albumin has been reported to play a variety of roles, including stabilization of cell growth, DNA replication, maintenance of sex hormone balance and modulation of systemic inflammation (40). In addition, albumin levels were

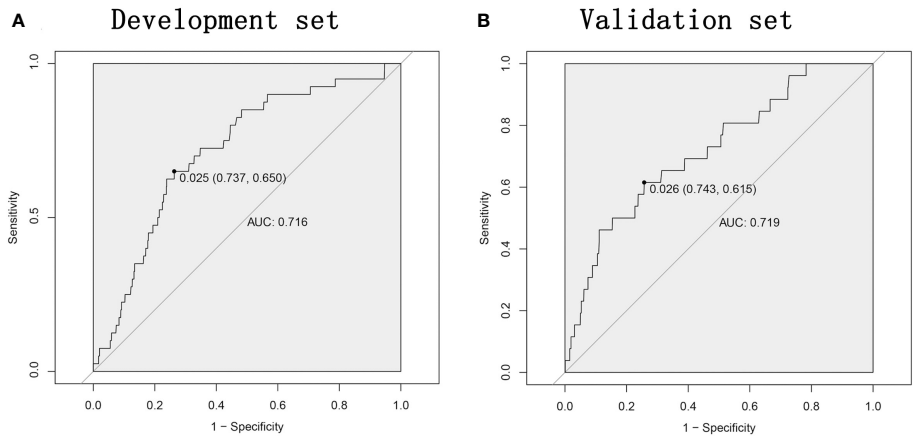

FIGURE 2
The nomogram model predicts the receiver operating characteristic ROC curve for major complications after rectal cancer surgery. **(A)** The area under the curve of the development set is 0.7161. **(B)** The area under the curve of the validation set is 0.7191. ROC, receiver operating characteristic; AUC, area under the curve.

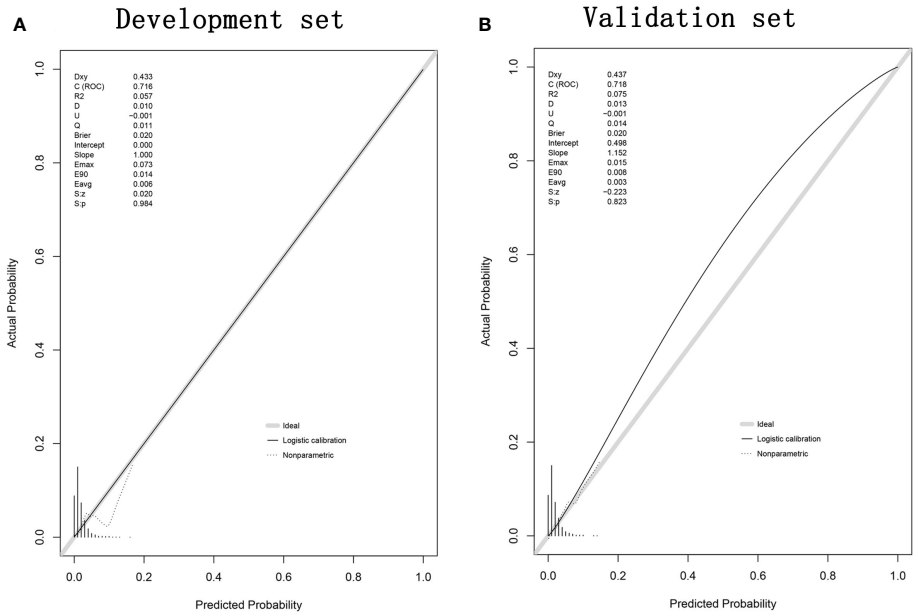

FIGURE 3
Calibration curves for development set **(A)** and validation set **(B)** nomograms.

widely used as a variety of prognostic indicators, including the prognostic nutritional index (41), the systemic inflammation index (42) and played an important role in maintaining colloid osmolality, scavenging free radicals, and altering capillary membrane permeability (43). The important physiological function of serum albumin might be an important reason why albumin was prediction of serious post-operative complications.

The current study suggested that open surgery was an independent risk factor for major post-operative complications for RC. Compared with open surgery, laparoscopic rectal surgery was widely used in the treatment of RC because of its less trauma and faster recovery (2). Clear surgical vision and full exposure of anatomical structures enable laparoscopic RC surgery to achieve radical resection, reduce surgical trauma, and improve post-operative quality of life (3). Which had similar results with previous studies (44, 45).

The application of these three risk predictors to our model was crucial. Despite the good performance of our nomogram, this study had some limitations. First, this was a single-center retrospective study. Second, the pre-operative baseline and clinical information included were imperfect, including pre-operative neoadjuvant

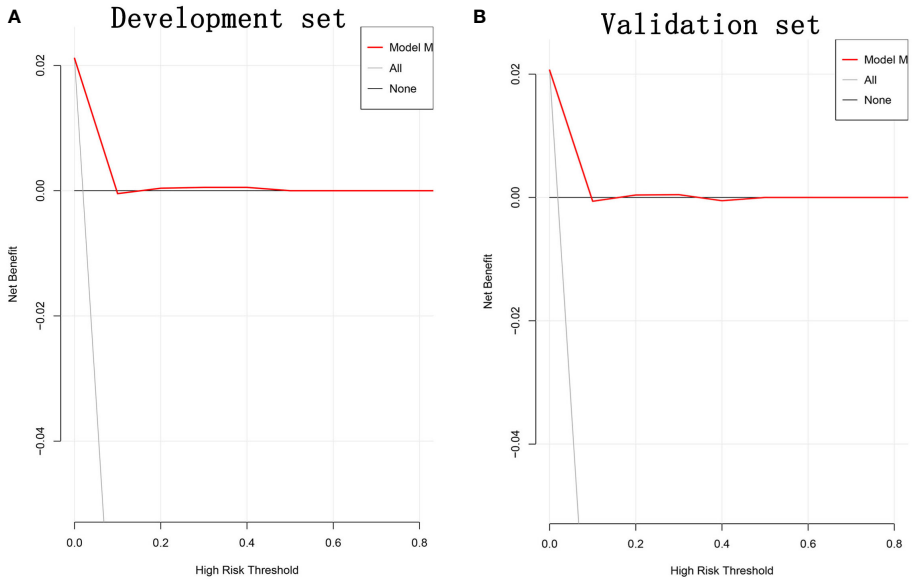

FIGURE 4
DCA for development set **(A)** and validation set **(B)**. DCA, decision curve analysis.

chemo-radiotherapy, the relationship to the rectal mesenteric fascia, the final distance from the anal verge, and the long-term efficacy of RC surgery. Third, in this study, a subset of patients underwent protective ileostomies. The results might be affected by the protective ileostomy. Fourth, because this study focused on post-operative major complications in RC, it was lacking types of surgery. Finally, the nomogram prediction model established in this study has not been internally validated, and we will continue to collect clinical data from relevant patients to further improve the internal validation. In the future, we hope that our study will be a joint effort of multiple centers to collect as many variables as possible and to continuously test and revise the prediction model in clinical practice.

In this study, a validated nomogram containing three predictors was created to identify risk factors for major complications after radical RC surgery. Due to its accuracy and convenience, it could contribute to personalized management of patients in the perioperative period.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (K2024-002-01). It complied with the principles of medical ethics and the Declaration of Helsinki, and all patients participating in the study signed an informed consent form. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

QL: Conceptualization, Data curation, Resources, Validation, Writing – original draft. YY: Resources, Writing – original draft. S-PQ: Data curation, Formal analysis, Investigation, Validation, Writing – original draft. Y-HD: Conceptualization, Investigation, Methodology, Resources, Writing – original draft. Z-XH: Data curation, Investigation, Project administration, Writing – original draft. ZX: Data curation, Methodology, Validation, Writing – review & editing. DP: Data curation, Funding acquisition, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. (2021) 71:209–49. doi: 10.3322/caac.21660

2. van der Pas MH, Haglind E, Cuesta MA, Fürst A, Lacy AM, Hop WC, et al. COlorectal cancer Laparoscopic or Open Resection II (COLOR II) Study Group. Laparoscopic versus open surgery for rectal cancer (COLOR II): short-term outcomes of a randomised, phase 3 trial. *Lancet Oncol*. (2013) 14:210–8. doi: 10.1016/S1470-2045(13)70016-0

3. Akiyoshi T. Technical feasibility of laparoscopic extended surgery beyond total mesorectal excision for primary or recurrent rectal cancer. *World J Gastroenterol*. (2016) 22:718–26. doi: 10.3748/wjg.v22.i2.718

4. Paun BC, Cassie S, MacLean AR, Dixon E, Buie WD. Postoperative complications following surgery for rectal cancer. *Ann Surg*. (2010) 251:807–18. doi: 10.1097/SLA.0b013e3181dae4ed

5. McSorley ST, Horgan PG, McMillan DC. The impact of the type and severity of postoperative complications on long-term outcomes following surgery for colorectal cancer: A systematic review and meta-analysis. *Crit Rev Oncol Hematol*. (2016) 97:168–77. doi: 10.1016/j.critrevonc.2015.08.013

6. Matsubara N, Miyata H, Gotoh M, Tomita N, Baba H, Kimura W, et al. Mortality after common rectal surgery in Japan: a study on low anterior resection from a newly established nationwide large-scale clinical database. *Dis Colon Rectum*. (2014) 57:1075–81. doi: 10.1097/DCR.0000000000000176

7. Matthiessen P, Hallböök O, Rutegård J, Simert G, Sjödahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. *Ann Surg*. (2007) 246:207–14. doi: 10.1097/SLA.0b013e3180603024

8. Liu XR, Liu F, Zhang W, Peng D. The aortic calcification is a risk factor for colorectal anastomotic leakage. *Updates Surg*. (2023) 75:1857–65. doi: 10.1007/s13304-023-01630-4

9. Arnarson Ö, Butt-Tuna S, Syk I. Postoperative complications following colonic resection for cancer are associated with impaired long-term survival. *Color Dis*. (2019) 21:805–15. doi: 10.1111/codi.14613

10. Planellas P, Farrés R, Cornejo L, Rodríguez-Hermosa JI, Pigem A, Timoteo A, et al. Randomized clinical trial comparing side to end vs end to end techniques for colorectal anastomosis. *Int J Surg*. (2020) 83:220–9. doi: 10.1016/j.ijsu.2020.09.039

11. Zeman M, Czarnecki M, Chmielik E, Idasiak A, Skałba W, Strączyński M, et al. The assessment of risk factors for long-term survival outcome in ypN0 patients with rectal cancer after neoadjuvant therapy and radical anterior resection. *World J Surg Oncol*. (2021) 19:154. doi: 10.1186/s12957-021-02262-x

12. Chen TY, Wiltink LM, Nout RA, Meershoek-Klein Kranenbarg E, Laurberg S, Marijnen CA, et al. Bowel function 14 years after preoperative short-course radiotherapy and total mesorectal excision for rectal cancer: report of a multicenter randomized trial. *Clin Colorectal Cancer*. (2015) 14:106–14. doi: 10.1016/j.clcc.2014.12.007

13. Guo F, Sun Z, Wang Z, Gao J, Pan J, Zhang Q, et al. Nomogram for predicting prolonged postoperative ileus after laparoscopic low anterior resection for rectal cancer. *World J Surg Oncol*. (2023) 21:380. doi: 10.1186/s12957-023-03265-6

14. Lohsiriwat V, Lohsiriwat D, Boonnuch W, Chinswangwatanakul V, Akaraviputh T, Lert-Akayamanee N. Pre-operative hypoalbuminemia is a major risk factor for postoperative complications following rectal cancer surgery. *World J Gastroenterol*. (2008) 14:1248–51. doi: 10.3748/wjg.14.1248

15. Yang J, Luo Y, Tian T, Dong P, Fu Z. Effects of neoadjuvant radiotherapy on postoperative complications in rectal cancer: A meta-analysis. *J Oncol*. (2022) 2022:8197701. doi: 10.1155/2022/8197701

16. Alqarni A, Aljehaiman F, Almousa SA, Almarshad SA, Alrzouq FK. The relationship between BMI and postoperative complications among colorectal cancer patients undergoing surgery. *Cureus*. (2023) 15:e48715. doi: 10.7759/cureus.48715

17. Wang K, Li M, Liu R, Ji Y, Yan J. Analysis of Risk Factors for Anastomotic Leakage After Laparoscopic Anterior Resection of Rectal Cancer and Construction of a Nomogram Prediction Model. *Cancer Manag Res*. (2022) 14:2243–52. doi: 10.2147/CMAR.S364875

18. Tao W, Liu F, Cheng YX, Zhang B, Liu XY, Zhang W, et al. Comparison of postoperative outcome and prognosis among laparoscopic left colectomy and laparoscopic sigmoidectomy in sigmoid colon cancer patients: A propensity score matching study. *Cancer Control*. (2023) 30:10732748231210676. doi: 10.1177/10732748231210676

19. Lu SQ, Chang XF, Yang XD, Yu DC, Huang QG, Wang F. [Establishment of a nomogram predicting risk factors of postoperative perineal wound complications after abdominoperineal resection for rectal cancer]. *Zhonghua Wei Chang Wai Ke Za Zhi*. (2019) 22:357–63. doi: 10.3760/cma.j.issn

20. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg*. (2004) 240:205–13. doi: 10.1097/01.sla.0000133083.54934.ae

21. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin*. (2017) 67:93–9. doi: 10.3322/caac.21388

22. Bolliger M, Kroehnert JA, Molineus F, Kandioler D, Schindl M, Riss P. Experiences with the standardized classification of surgical complications (Clavien-Dindo) in general surgery patients. *Eur Surg*. (2018) 50:256–61. doi: 10.1007/s10353-018-0551-z

23. Liu Q, Zhou Q, Song M, Zhao F, Yang J, Feng X, et al. A nomogram for predicting the risk of sepsis in patients with acute cholangitis. *J Int Med Res*. (2020) 48:300060519866100. doi: 10.1177/0300060519866100

24. Yu P, Kan R, Meng X, Wang Z, Xiang Y, Mao B, et al. A nomogram for predicting the risk of CKD based on cardiometabolic risk factors. *Int J Gen Med*. (2023) 16:4143–54. doi: 10.2147/IJGM.S425122

25. Lin Z, Li Y, Wu J, Zheng H, Yang C. Nomogram for prediction of prolonged postoperative ileus after colorectal resection. *BMC Cancer*. (2022) 22:1273. doi: 10.1186/s12885-022-10377-x

26. Shi B, Shen L, Huang W, Cai L, Yang S, Zhang Y, et al. A nomogram for predicting surgical timing in neonates with necrotizing enterocolitis. *J Clin Med*. (2023) 12:3062. doi: 10.3390/jcm12093062

27. Huai J, Ye X, Ding J. Nomogram for the prediction of delayed colorectal post-polypectomy bleeding. *Turk J Gastroenterol*. (2021) 32:727–34. doi: 10.5152/tjg.2021.20842

28. Liu XY, Li ZW, Zhang B, Liu F, Zhang W, Peng D. Effects of preoperative bicarbonate and lactate levels on short-term outcomes and prognosis in elderly patients with colorectal cancer. *BMC Surg*. (2023) 23:127. doi: 10.1186/s12893-023-02039-x

29. Nowakowski M, Pisarska M, Rubinkiewicz M, Torbicz G, Gajewska N, Mizera M, et al. Postoperative complications are associated with worse survival after laparoscopic surgery for non-metastatic colorectal cancer - interim analysis of 3-year overall survival. *Wideochir Inne Tech Maloinwazyjne*. (2018) 13:326–32. doi: 10.5114/wiitm.2018.76179

30. Liu XY, Zhang B, Kang B, Cheng YX, Yuan C, Tao W, et al. The effect of complications on oncological outcomes of colorectal cancer patients after primary surgery: A propensity score matching analysis. *Front Oncol*. (2022) 12:857062. doi: 10.3389/fonc.2022.857062

31. Liang LC, Liu DL, Liu SJ, Hu L, He YR, Wan X, et al. Risk factors for severe complications after laparoscopic surgery for T3 or T4 rectal cancer for Chinese patients: experience from a single center. *Med Sci Monit*. (2020) 26:e920604. doi: 10.12659/MSM.920604

32. Turri G, Caliskan G, Conti C, Martinelli L, De Giulio E, Ruzzenente A, et al. Impact of age and comorbidities on short- and long-term outcomes of patients undergoing surgery for colorectal cancer. *Front Oncol*. (2022) 12:959650. doi: 10.3389/fonc.2022.959650

33. Gröne J, Kreis ME. Individualisierte Therapie des kolorektalen Karzinoms im hohen Alter [Personalized treatment of colorectal cancer in old age]. *Chirurg*. (2013) 84:305–9. doi: 10.1007/s00104-012-2452-1

34. Forman DE, Maurer MS, Boyd C, Brindis R, Salive ME, Horne FM, et al. Multimorbidity in older adults with cardiovascular disease. *J Am Coll Cardiol*. (2018) 71:2149–61. doi: 10.1016/j.jacc.2018.03.022

35. Person H, Keefer L. Psychological comorbidity in gastrointestinal diseases: Update on the brain-gut-microbiome axis. *Prog Neuropsychopharmacol Biol Psychiatry*. (2021) 107:110209. doi: 10.1016/j.pnpbp.2020.110209

36. Pope D, Ramesh H, Gennari R, Corsini G, Maffezzini M, Hoekstra HJ, et al. Pre-operative assessment of cancer in the elderly (PACE): a comprehensive assessment of underlying characteristics of elderly cancer patients prior to elective surgery. *Surg Oncol*. (2006) 15:189–97. doi: 10.1016/j.suronc.2007.04.009

37. Sofić A, Rašić I, Halilović E, Mujić A, Muslić D. Is preoperative hypoproteinemia associated with colorectal cancer stage and postoperative complications? *Med Glas (Zenica)*. (2021) 18:450–5. doi: 10.17392/1353-21

38. Kang B, Zhao ZQ, Liu XY, Cheng YX, Tao W, Wei ZQ, et al. Effect of hypoalbuminemia on short-term outcomes after colorectal cancer surgery: A propensity score matching analysis. *Front Nutr*. (2022) 9:925086. doi: 10.3389/fnut.2022.925086

39. Liu ZJ, Ge XL, Ai SC, Wang HK, Sun F, Chen L, et al. Postoperative decrease of serum albumin predicts short-term complications in patients undergoing gastric cancer resection. *World J Gastroenterol*. (2017) 23:4978–85. doi: 10.3748/wjg.v23.i27.4978

40. Gupta D, Lis CG. Pretreatment serum albumin as a predictor of cancer survival: a systematic review of the epidemiological literature. *Nutr J*. (2010) 9:69. doi: 10.1186/1475-2891-9-69

41. Tokunaga R, Sakamoto Y, Nakagawa S, Miyamoto Y, Yoshida N, Oki E, et al. Prognostic nutritional index predicts severe complications, recurrence, and poor prognosis in patients with colorectal cancer undergoing primary tumor resection. *Dis Colon Rectum*. (2015) 58:1048–57. doi: 10.1097/DCR.0000000000000458

42. Hong T, Shen D, Chen X, Cai D, Wu X, Hua D. A novel systematic inflammation related index is prognostic in curatively resected non-metastatic colorectal cancer. *Am J Surg*. (2018) 216:450–7. doi: 10.1016/j.amjsurg.2017.07.021

43. Caraceni P, O'Brien A, Gines P. Long-term albumin treatment in patients with cirrhosis and ascites. *J Hepatol*. (2022) 76:1306–17. doi: 10.1016/j.jhep.2022.03.005

44. Miyakawa T, Michihata N, Kumazawa R, Matsui H, Honda M, Yasunaga H. Short-term surgical outcomes of laparoscopic and open surgery for rectal cancer: A nationwide retrospective analysis. *Asian J Endosc Surg*. (2023) 16:376–85. doi: 10.1111/ases.13166

45. Jiang JB, Jiang K, Dai Y, Wang RX, Wu WZ, Wang JJ, et al. Laparoscopic versus open surgery for mid-low rectal cancer: a systematic review and meta-analysis on short- and long-term outcomes. *J Gastrointest Surg*. (2015) 19:1497–512. doi: 10.1007/s11605-015-2857-5

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Effect of different anaesthetic techniques on the prognosis of patients with colorectal cancer after resection: a systematic review and meta-analysis

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Background: The effect of total intravenous anaesthesia (TIVA) and inhalation anaesthesia (IA) on the prognosis of patients with colorectal cancer after resection is controversial. This study aimed to explore the effects of different anaesthesia methods on the postoperative prognosis of colorectal cancer.

Methods: PubMed, Embase and Cochrane Library databases were searched for relevant literature from each database's inception until 18 November 2023. The literature topic was to compare the effects of TIVA and IA on the prognosis of patients undergoing colorectal cancer resection.

Results: Six studies were selected for meta-analysis. The studies involved 111043 patients, with a trial size of 1001–88184 people. A statistically significant difference was observed in the overall survival (OS) between colorectal cancer patients administered TIVA and IA (hazard ratio [HR], 0.83; 95% confidence interval [CI], 0.70–0.99), but none in recurrence-free survival (RFS) (HR, 0.99; 95% CI, 0.90–1.08). In the subgroup analysis of OS, no statistically significant difference was observed between colorectal cancer patients administered TIVA and IA in Asia (HR, 0.77; 95% CI, 0.57–1.05), and not in Europe (HR, 0.99; 95% CI, 0.93–1.06). Regarding tumour location, no significant association was found between TIVA and IA in the colon, rectum and colorectum ((HR, 0.70; 95% CI, 0.38–1.28), (HR, 0.95; 95% CI, 0.83–1.08) and (HR, 0.99; 95% CI, 0.93–1.06), respectively).

Conclusion: OS differed significantly between patients administered TIVA and IA when undergoing colorectal cancer resection, but no difference was observed in RFS. The prognostic effects of TIVA and IA differed.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42023453185, identifier CRD42023453185.

KEYWORDS

colorectal cancer, inhalation anaesthesia, recurrence, survival, total intravenous anaesthesia

Introduction

Between 2008 and 2018, the number of patients with cancer increased by >25% globally (1, 2), and excisional surgery remains one of the main treatments for solid organ tumours in cancer patients (3). Particularly, colorectal cancer is the fourth deadliest cancer worldwide, with approximately 900000 cases of mortality yearly (4). Surgery is the cornerstone of many treatment options for colorectal cancer. Although it is usually aimed at healing, the removal of tumours is also a risk factor for metastasis. Tumour cells can enter the bloodstream before, during or after surgery, leading to distant organ metastasis (5). The mechanism of metastasis includes carcinomas escaping the immune system, proliferating and invading tissues. Surgery creates a tumorigenic physiological environment that may directly or indirectly affect tumour cell survival.

Multiple perioperative factors collectively contribute to a relatively immunosuppressive state, including surgical stress response and surgical inflammatory response, as well as the direct effects of aneasthetics, opioids and other perioperative drugs. Research has shown that volatile anaesthetics used in inhalation anaesthesia (IA) promote tumour metastasis, which may include direct promotion of carcinoma survival, inhibition of immune cell function and tumour cell-killing function (6–9). Propofol used in total intravenous anaesthesia (TIVA) is the most commonly used intravenous inducer, and some preclinical evidence suggests that it may have anti-tumour effects. Propofol exerts anti-tumour effects by directly regulating key ribonucleic acid pathways and signal transduction in carcinomas (10). It also has anti-inflammatory and anti-oxidant effects (11–16), preventing immune suppression during the perioperative period.

The impact of TIVA and IA on the prognosis of patients with colorectal cancer has always been controversial. Previous research results showed inconsistent trends. A retrospective analysis showed that volatile anaesthetics slightly increased the cancer recurrence rate in patients undergoing colorectal cancer surgery compared with TIVA using propofol (17). Another study (18) showed that there was no difference in overall survival (OS) or recurrence-free survival (RFS) between the two anaesthesia methods for colorectal cancer.

Based on the above controversy, this study aimed to explore the impact of TIVA and IA on the prognosis of patients with colorectal cancer after resection through meta-analysis.

Methods

Protocol and guidance

This study was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses reporting guidelines (19). This study did not require ethical approval or informed consent. The protocol for this review has been registered with PROSPERO (CRD42023453185).

Search strategy

We searched PubMed, EMBASE and the Cochrane Library electronic databases for research written in English from its establishment until 18 November 2023, with keywords including ('Colorectal Cancer', 'colon' or 'rectal'), ('analgesia', 'Anesthesia', 'Inhalation' or 'intravenous') and ('Desflurane', 'Propofol', 'dexmedetomidine' or 'Sevoflurane'). Other studies were searched for by reviewing reference lists and qualified publications of potential qualified studies. All searches were conducted independently by two authors, and differences were discussed after the search process.

Inclusion and exclusion criteria

If the retrieved studies (1) were cohort studies, (2) investigated patients with colorectal cancer, (3) compared clinical studies on long-term all-cause mortality and recurrence after TIVA or IA and (4) provided hazard ratios (HRs) or risk ratios and their 95% confidence intervals (CIs), they were eligible for qualitative and quantitative analyses.

If study participants (1) had malignant tumours other than colorectal cancer and (2) lacked measurements of cancer recurrence or mortality, the study was excluded.

Data extraction and quality assessment

Two reviewers independently extracted data from the included studies. This review introduced the following details: the name of the first author, year of publication, country, number of participants, tumour location, research type, intervention measures and main research indicators.

The quality of all selected studies was checked according to the Newcastle–Ottawa cohort study quality assessment scale (20). This semi-quantitative scale uses a star rating system to evaluate the quality of eight items in three fields: selection (four items, one star each), comparability (one item, up to two stars) and exposure (three items, one star each). In this meta-analysis, we classified quality as good (≥7 stars), average (4–6 stars) or poor (<4 stars). Differences between the two reviewers were resolved through discussion with the third reviewer.

Data analysis

Based on the effects of TIVA and IA, the results show RFS and OS in patients with cancer. This method is based on the HR obtained from each study with a 95% CI. If HR was unreported, the odds ratio was considered equal to HR. The study selected TIVA rather than IA. If the data included in the study comprised IA rather than TIVA, then it was adjusted (calculate derivative). When there are multiple sets of useful data in the same study, only data from

propensity score matching is selected for analysis. Subgroup analysis was performed based on region (Asia and Europe) and tumour location (colon, rectum and colorectum).

Statistical analysis

Review manager, version 5.4 (Nordic Cochrane Center, Cochrane Collaboration, London, UK) was used for data analysis. The HR was used to measure effectiveness at a 95% CIs. I^2 values were used to describe heterogeneity and were categorised into four levels: no heterogeneity (I^2 < 25%), low heterogeneity (25% ≤ I^2 < 50%), moderate heterogeneity (50% ≤ I^2 < 75%) and high heterogeneity (I^2 ≥ 75%). When the I^2 value was <50%, a fixed model effect was used, whereas when it was >50%, a random model effect was used.

Results

Eligible studies and study characteristics

After identifying 4315 references, 1035 duplicate publications and 3205 irrelevant studies were excluded, leaving 75 potentially eligible studies (Figure 1). Finally, six cohort (17, 18, 21–24) studies conducted between 2014 and 2022 were included in the meta-analysis.

Table 1 lists the general characteristics of the included studies. A total of 111043 patients with cancer participated in the study, with trial sizes of 1001–88184 people. The six studies were retrospective studies using propensity matching scores. The main outcome measures are OS and RFS. Among these studies, two were from Europe and four were from Asia. According to the quality evaluation criteria, all six studies were rated as good quality.

Recurrence-free survival

Three studies investigated the effects of TIVA and IA on the RFS rate of colorectal cancer (Figure 2). The total sample size was 99005 patients. Compared with IA, the use of TIVA was not associated with an improved RFS rate in colorectal cancer (HR, 0.99; 95% CI, 0.90–1.08; p = 0.75).

Overall survival

six studies investigated the effects of TIVA and IA on OS in colorectal cancer patients (Figure 3), involving 111043 patients. Compared with IA, TIVA improved OS (HR, 0.83; 95% CI, 0.70–0.99; p = 0.04).

In these analyses, two studies analysed the colon and rectum, one analysed the colon and the remaining three analysed the colorectum. Subgroup analysis was conducted based on country and cancer location. The results showed no significant correlation between patients from Asia (HR, 0.77; 95% CI, 0.57–1.05; p = 0.09), and not between patients from Europe (HR, 0.99; 95% CI, 0.93–

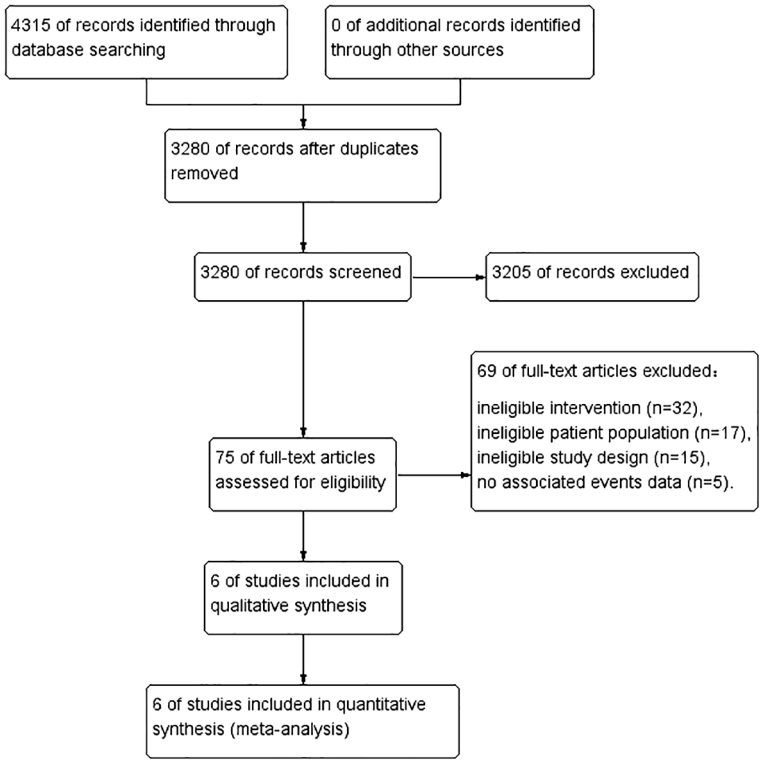

FIGURE 1
PRISMA flow diagram of study selection.

TABLE 1 Characteristics of the included trials.

First Author (Publication year)	Country	Number of participants	Cancer type	Study design	Interventions	Outcomes	Quality assessment
Enlund (2014) (21)	Sweden	1001	Colon, rectal	Retrospective; propensity score matching	Sevoflurane VS Propofol	OS	8
Wu (2018) (22)	China	1158	colon	Retrospective; propensity score matching	Propofol VS Desflurane	OS	7
Makito (2020) (23)	Japan	88184	Colon, rectal	Retrospective; propensity score matching	Desflurane, sevoflurane, or isoflurane with/without nitrous oxide VS Propofol	OS, RFS	9
Hasselager (2021) (17)	Denmark	8694	colorectal	Retrospective; propensity score matching	Sevoflurane VS Propofol	OS, RFS	8
Lee (2022) (18)	Korea	2127	colorectal	Retrospective; propensity score matching	Propofol VS Sevoflurane	OS, RFS	8
Yoon (2022) (24)	Korea	9879	colorectal	Retrospective; propensity score matching	Propofol VS Sevoflurane, desflurane, isoflurane, or enflurane	OS	9

1.06; p = 0.83) (Figure 4). However, in the subgroup analysis of tumour location, no significant associations were found in either the colon, rectum or colorectal tumours (HR, 0.70; 95% CI, 0.38–1.28), (HR, 0.95; 95% CI, 0.83–1.08) and (HR, 0.99; 95% CI, 0.93–1.06) (Figure 5).

Publication bias

In accordance with the criteria in the Cochrane Handbook for systematic reviews of interventions, publication bias was not analysed because none of the groups comprised >10 studies.

Discussion

Our meta-analysis included six retrospective studies for comparing the effects of TIVA and IA on postoperative prognosis after colorectal cancer resection. The data results processed using propensity score matching reduced the impact of selection bias; therefore, conducting a meta-analysis on these data yielded more consistent and less heterogeneous results. We found a statistically significant difference in OS between TIVA and IA for patients with colorectal cancer, but none in RFS. We conducted a subgroup analysis on OS and found no statistically significant difference between TIVA and IA in patients with colorectal cancer in Asia, and not in Europe. Regarding tumour location, no significant association was found between TIVA and IA in colon, rectum or colorectal cancer.

Propofol is the most commonly used intravenous inducer for anaesthesia maintenance. Some preclinical evidence suggests that it may have anti-tumour effects. Laboratory research has shown that propofol exerts anti-tumour effects by directly regulating key ribonucleic acid pathways and signal transduction in carcinomas (10). It also has anti-inflammatory and anti-oxidant effects, preventing immune suppression during the perioperative period. *In vitro* studies have confirmed that propofol has multiple anti-tumour effects in different cancer cell lines. In gastric cancer cell lines, it inhibits cell proliferation, invasion and migration (25). In non-small cell lung cancer (NSCLC), propofol interferes with HIF1A upregulation, thereby reducing carcinoma migration and invasion (26). In a study of breast cancer cell lines, propofol reduced the expression of neuroepithelial transformation gene 1, which promotes adenocarcinoma migration *in vitro* (27).

Laboratory studies have shown that the mechanisms by which volatile anaesthetics promote tumour metastasis may include the

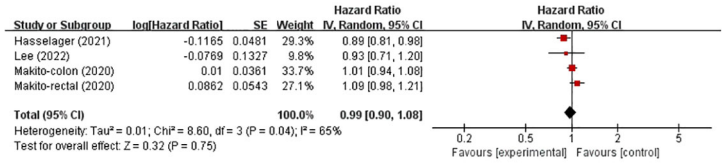

FIGURE 2
TIVA and IA on RFS of colorectal cancer.

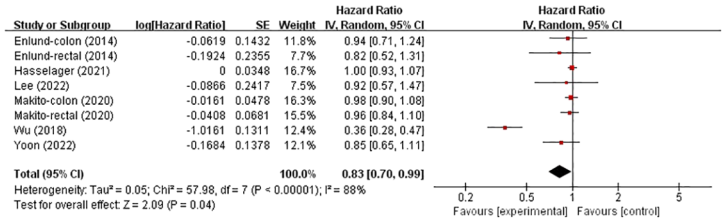

FIGURE 3
TIVA and IA on OS of colorectal cancer.

direct promotion of carcinoma survival and inhibition of immune cell and tumour cell-killing functions. However, the molecular mechanism remains unclear, and the evidence for different inhaled drugs and different cancer cell lines is contradictory. Volatile anaesthetics also have pro-inflammatory effects (28). They may upregulate hypoxia-inducible factor (HIF) and protect carcinomas during the perioperative period (29).

The results of clinical research comparing intravenous and inhaled drugs are inconsistent. Regarding the survival rate, a meta-analysis in 2019 included 6 studies, with >7800 patients with breast cancer, oesophageal cancer or NSCLC undergoing surgery. The results revealed that the RFS of TIVA users was higher than that of IA users (summary HR 0.78, 95% CI 0.65–0.94) (30). Regarding circulating tumour cells, a randomised trial included 210 patients undergoing breast cancer surgery. The results showed that the number of circulating tumour cells after surgery was similar among patients treated with sevoflurane and propofol (31). Regarding immune cells, a randomised trial found a similar proportion of NK cells, helper T cells and cytotoxic T cells in postoperative circulation among 153 patients who underwent

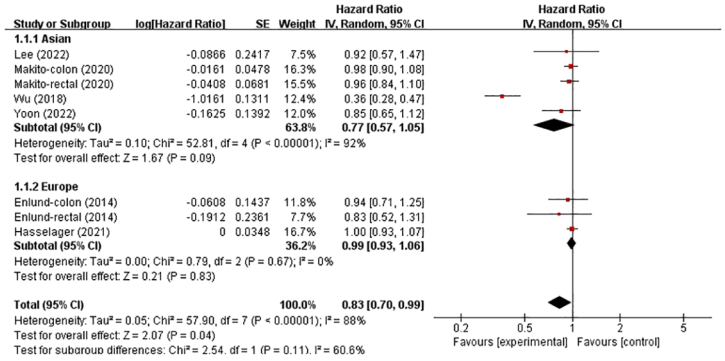

FIGURE 4
Subgroup analysis based on country with OS.

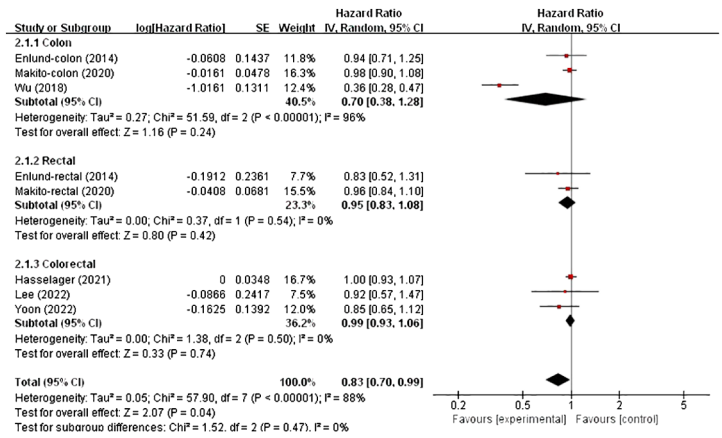

FIGURE 5
Subgroup analysis based on cancer location with OS.

colorectal cancer resection under sevoflurane- and propofol-induced anaesthesia (32). Regarding tumour regulatory factors, *in vivo* studies have not clarified the effects of intravenous and inhaled drugs on these factors. A small study evaluated the expression of oncogenes in patients undergoing head and neck cancer resection and found a significant increase in HIF1A expression among users of volatile anaesthetics (33).

Regardless of the exact mechanism, the choice of TIVA or VA is a potential modifiable factor in the management of colorectal cancer, and our meta-analysis results indicate that TIVA is associated with lower postoperative mortality. Further prospective clinical trials are required to elucidate the role of anaesthetics in cancer prognosis.

In the assessment of bias risk, we noticed the control of confounding factors with the most prominent bias risk. Many studies have not fully considered confounding factors such as patient comorbidities or tumour grading. For any group wishing to conduct further research on this topic, these issues need to be considered. Furthermore, most studies are retrospective and lack prospective randomised controlled trials.

Finally, although our meta-analysis established a possible association, it inferred no causal relationship nor explained potential mechanisms. We believe that further prospective clinical trials are required to elucidate the molecular mechanisms underlying the role of anaesthetics in cancer prognosis.

In conclusion, we conducted a meta-analysis using six studies, which included 111043 patients, and the results showed an association between TIVA and postoperative mortality in cancer surgery, but its impact on RFS remains inconclusive.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

SX: Conceptualization, Data curation, Formal analysis, Software, Writing – original draft, Writing – review & editing. YZ: Data curation, Formal analysis, Software, Writing – original draft. WW: Conceptualization, Funding acquisition, Resources, Writing – original draft, Writing – review & editing. YL: Data curation, Formal analysis, Writing – original draft. LY: Data curation, Software, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Akinyemiju TF, Al Lami FH, Alam T, Alizadeh-Navaei R, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: a systematic analysis for the global burden of disease study. *JAMA Oncol.* (2018) 4(11):1553–68. doi: 10.1001/jamaoncol.2018.2706

2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer.* (2015) 136(5):E359–86. doi: 10.1002/ijc.29210

3. Murphy O, Forget P, Ma D, Buggy DJ. Tumour excisional surgery, anaesthetic-analgesic techniques, and oncologic outcomes: a narrative review. *Br J Anaesth.* (2023) 131:989–1001. doi: 10.1016/j.bja.2023.07.027

4. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet (London England).* (2019) 394(10207):1467–80. doi: 10.1016/S0140-6736(19)32319-0

5. Engilbertsson H, Aaltonen KE, Björnsson S, Kristmundsson T, Patschan O, Rydén L, et al. Transurethral bladder tumor resection can cause seeding of cancer cells into the bloodstream. *J Urol.* (2015) 193(1):53–7. doi: 10.1016/j.juro.2014.06.083

6. Benzonana LL, Perry NJ, Watts HR, Yang B, Perry IA, Coombes C, et al. Isoflurane, a commonly used volatile anesthetic, enhances renal cancer growth and Malignant potential via the hypoxia-inducible factor cellular signaling pathway in vitro. *Anesthesiology.* (2013) 119(3):593–605. doi: 10.1097/ALN.0b013e31829e47fd

7. Buckley A, McQuaid S, Johnson P, Buggy DJ. Effect of anaesthetic technique on the natural killer cell anti-tumour activity of serum from women undergoing breast cancer surgery: a pilot study. *Br J Anaesth.* (2014) 113 Suppl 1:i56–62. doi: 10.1093/bja/aeu200

8. Desmond F, McCormack J, Mulligan N, Stokes M, Buggy DJ. Effect of anaesthetic technique on immune cell infiltration in breast cancer: a follow-up pilot analysis of a prospective, randomised, investigator-masked study. *Anticancer Res.* (2015) 35:1311–9.

9. Ecimovic P, McHugh B, Murray D, Doran P, Buggy DJ. Effects of sevoflurane on breast cancer cell function in vitro. *Anticancer Res.* (2013) 33:4255–60.

10. Jiang S, Liu Y, Huang L, Zhang F, Kang R. Effects of propofol on cancer development and chemotherapy: Potential mechanisms. *Eur J Pharmacol.* (2018) 831:46–51. doi: 10.1016/j.ejphar.2018.04.009

11. Kim R. Anesthetic technique and cancer recurrence in oncologic surgery: unraveling the puzzle. *Cancer Metastasis Rev.* (2017) 36:159–77. doi: 10.1007/s10555-016-9647-8

12. Jaura AI, Flood G, Gallagher HC, Buggy DJ. Differential effects of serum from patients administered distinct anaesthetic techniques on apoptosis in breast cancer cells in *vitro*: a pilot study. *Br J Anaesth.* (2014) 113 Suppl 1:i63–7. doi: 10.1093/bja/aet581

13. Chen Y, Liang M, Zhu Y, Zhou D. [The effect of propofol and sevoflurane on the perioperative immunity in patients under laparoscopic radical resection of colorectal cancer]. *Zhonghua Yi Xue Za Zhi*. (2015) 95:3440.

14. Baki ED, Aldemir M, Kokulu S, Koca HB, Ela Y, Sıvacı RG, et al. Comparison of the effects of desflurane and propofol anesthesia on the inflammatory response and s100β protein during coronary artery bypass grafting. *Inflammation*. (2013) 36 (6):1327–33. doi: 10.1007/s10753-013-9671-6

15. Liu S, Gu X, Zhu L, Wu G, Zhou H, Song Y, et al. Effects of propofol and sevoflurane on perioperative immune response in patients undergoing laparoscopic radical hysterectomy for cervical cancer. *Med (Baltimore)*. (2016) 95(49):e5479. doi: 10.1097/MD.0000000000005479

16. Liu TC. Influence of propofol, isoflurane and enflurance on levels of serum interleukin-8 and interleukin-10 in cancer patients. *Asian Pac J Cancer Prev*. (2014) 15:6703–7. doi: 10.7314/apjcp.2014.15.16.6703

17. Hasselager RP, Hallas J, Gögenur I. Inhalation or total intravenous anaesthesia and recurrence after colorectal cancer surgery: a propensity score matched Danish registry-based study. *Br J Anaesth*. (2021) 126:921–30. doi: 10.1016/j.bja.2020.11.019

18. Lee S, Pyo DH, Sim WS, Lee WY, Park M. Early and long-term outcomes after propofol-and sevoflurane-based anesthesia in colorectal cancer surgery: A retrospective study. *J Clin Med*. (2022) 11:2648. doi: 10.3390/jcm11092648

19. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PloS Med*. (2009) 6 (7):e1000100. doi: 10.1371/journal.pmed.1000100

20. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol*. (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z

21. Enlund M, Berglund A, Andreasson K, Cicek C, Enlund A, Bergkvist L. The choice of anaesthetic–sevoflurane or propofol–and outcome from cancer surgery: a retrospective analysis. *Ups J Med Sci*. (2014) 119:251–61. doi: 10.3109/03009734.2014.922649

22. Wu ZF, Lee MS, Wong CS, Lu CH, Huang YS, Lin KT, et al. Propofol-based total intravenous anesthesia is associated with better survival than desflurane anesthesia in colon cancer surgery. *Anesthesiology*. (2018) 129(5):932–41. doi: 10.1097/ALN.0000000000002357

23. Makito K, Matsui H, Fushimi K, Yasunaga H. Volatile versus total intravenous anesthesia for cancer prognosis in patients having digestive cancer surgery. *Anesthesiology*. (2020) 133:764–73. doi: 10.1097/ALN.0000000000003440

24. Yoon S, Jung SY, Kim MS, Yoon D, Cho Y, Jeon Y. Impact of Propofol-based Total Intravenous Anesthesia Versus Inhalation Anesthesia on Long-term Survival after Cancer Surgery in a Nationwide Cohort. *Ann Surg*. (2023) 278(6):1024–31. doi: 10.1097/SLA.0000000000005568

25. Yang C, Gao J, Yan N, Wu B, Ren Y, Li H, et al. Propofol inhibits the growth and survival of gastric cancer cells in *vitro* through the upregulation of ING3. *Oncol Rep*. (2017) 37(1):587–93. doi: 10.3892/or.2016.5218

26. Yang N, Liang Y, Yang P, Ji F. Propofol suppresses LPS-induced nuclear accumulation of HIF-1α and tumor aggressiveness in non-small cell lung cancer. *Oncol Rep*. (2017) 37:2611–9. doi: 10.3892/or.2017.5514

27. Ecimovic P, Murray D, Doran P, Buggy DJ. Propofol and bupivacaine in breast cancer cell function in *vitro* - role of the NET1 gene. *Anticancer Res*. (2014) 34:1321–31.

28. Kurosawa S, Kato M. Anesthetics, immune cells, and immune responses. *J Anesth*. (2008) 22:263–77. doi: 10.1007/s00540-008-0626-2

29. Tavare AN, Perry NJ, Benzonana LL, Takata M, Ma D. Cancer recurrence after surgery: direct and indirect effects of anesthetic agents. *Int J Cancer*. (2012) 130:1237–50. doi: 10.1002/ijc.26448

30. Yap A, Lopez-Olivo MA, Dubowitz J, Hiller J, Riedel BGlobal Onco-Anesthesia Research Collaboration Group. Anesthetic technique and cancer outcomes: a meta-analysis of total intravenous versus volatile anesthesia [published correction appears in Can J Anaesth. 2019 Aug;66(8):1007-1008]. *Can J Anaesth*. (2019) 66:546–61. doi: 10.1007/s12630-019-01330-x

31. Hovaguimian F, Braun J, Z'graggen BR, Schläpfer M, Dumrese C, Ewald C, et al. Anesthesia and circulating tumor cells in primary breast cancer patients: A randomized controlled trial. *Anesthesiology*. (2020) 133(3):548–58. doi: 10.1097/ALN.0000000000003409

32. Oh CS, Park HJ, Piao L, Sohn KM, Koh SE, Hwang DY, et al. Expression profiles of immune cells after propofol or sevoflurane anesthesia for colorectal cancer surgery: a prospective double-blind randomized trial. *Anesthesiology*. (2022) 136(3):448–58. doi: 10.1097/ALN.0000000000004119

33. Ferrell JK, Cattano D, Brown RE, Patel CB, Karni RJ. The effects of anesthesia on the morphoproteomic expression of head and neck squamous cell carcinoma: a pilot study. *Transl Res*. (2015) 166:674–82. doi: 10.1016/j.trsl.2015.09.001

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LGR6 is a prognostic biomarker for less differentiated tumors in lymph nodes of colon cancer patients

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Introduction: The aim was to investigate whether the stem cell marker LGR6 has prognostic value in colon cancer, alone or in combination with the prognostic biomarkers CEA and CXCL16.

Methods: LGR6 mRNA levels were determined in 370 half lymph nodes of 121 colon cancer patients. Ability to predict relapse after curative surgery was estimated by Kaplan-Meier survival model and Cox regression analyses.

Results: Patients with high LGR6 levels [LGR6(+)] had a decreased mean survival time of 11 months at 5-year follow-up and 47 months at 12-year follow-up, respectively, with hazard ratios of 3.2 and 2.8. LGR6 mRNA analysis added prognostic value to CEA and CXCL16 mRNA analysis. In the poor prognosis groups CEA(+) and CXCL16(+), further division was achieved by LGR6 analysis. LGR6(+) patients had a very poor prognosis. LGR6 also identified a small number of CEA(-), TNM stage I patients who relapsed suggesting stem cell origin of these tumors. LGR6 and LGR5 levels correlated strongly in lymph nodes of stage I and IV patients but not in stage II patients, suggesting that these stem cell markers are differentially regulated.

Conclusion: This study highlights LGR6 as a useful prognostic biomarker independently and in combination with CEA, CXCL16 or LGR5 identifying different risk groups.

KEYWORDS

colon cancer, regional lymph nodes, cancer stem cells, LGR6, LGR5, CEA, CXCL16, qRT-PCR

1 Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide and a form of cancer that is increasing in frequency (1). The main treatment modality for CRC is surgery with its risk of postoperative complications of which surgical site infection (SSI) is the most common (2, 3). Unfortunately, approximately 25% of patients having curative surgery will relapse and most of them will die from cancer (4, 5). Since the standard methods are not able to identify this group of patients there is an urgent need to develop methods that can accomplish this aim. The standard method to determine if the tumor has spread to the regional lymphatic field is histopathology. Although still considered the most important method to identify patients with tumors that will relapse, the method is far from perfect. Thus, a significant fraction of patients judged to be free of tumors in their lymph nodes (TNM stage I and II patients) actually contain tumor cells that are missed by histopathology. The main reasons for this are that only a small fraction of the lymph node (LN) volume is analyzed, and that histopathology is a subjective method requiring a trained pathologist. Biomarker mRNA analysis is a very promising alternative allowing analysis of the entire LN volume and analysis of a combination of biomarkers that characterize different properties of the tumors that can be combined in a kit. ColoNode, which combines analysis of mRNAs of CEA (CEACAM5), Kallikrein Related Peptidase 6 (KLK6), Solute Carrier Family 35 Member D3 (SLC35D3), Mucin 2 (MUC2) and Periostin (POSTN) of half the LN volume is a successful colon cancer (CC) prognostic test that surpasses histopathology in identifying patients that will relapse and in addition grades patients with different degrees of risk (6). The study identified two distinct group of patients, one which should be recommended postoperative adjuvant treatment and another which should be left untreated. There is, however, a small group who are tumor cell positive in the LNs but the tumor cells do not demonstrate all aggressiveness factors. For this group no clear treatment recommendation can be given. Analysis of markers for cancer stem cells (CSC) may help in dividing this group further.

CRC may originate from epithelial stem cells or from more mature epithelial cells, and tumors in a patient may be a mixture of tumor cells of both origins. Moreover, different patients are likely to differ in the proportion of stem cell derived tumors. CSC are considered to be more aggressive than other cancer cells and have self-renewal and multi-lineage differentiation capacities and play important roles in tumor initiation, progression, metastasis, drug and radiation resistance (7–9). CSC can be identified by biomarkers. We have recently studied the CSC biomarkers leucine-rich repeat-containing G protein-coupled receptor 4 and 5 (LGR4 and LGR5) in CC and found both markers to be associated with poor prognosis after curative surgery when applied to LN mRNA analysis (10). Additionally, we found that the chemokine CXCL17 and the G protein-coupled receptor 35 (GPR35) were associated with stem cell-like features, detecting undifferentiated CC tumor cells (11–13).

The LGR subfamily contains three members, LGR4, LGR5 and LGR6. They are members of the glycoprotein hormone receptor subfamily of rhodopsin-like, seven transmembrane domain receptors (14). All three LGRs function as receptors for the R-spondin family of stem cell factors to potentiate Wnt/β-catenin signaling (15–18). The R-spondins (RSPO1-4) are secreted proteins. For example, LGR6 is a high affinity receptor for RSPO1-3 and binding has a positive effect on Wnt/β-catenin signaling (18). Not only do the LGRs interact with RSPOs but also with each other - interaction score between LGR6 and LGR5 or LGR4 respectively >0.905 (= very high confidence) (19). A recent study showed that LGR6 also activates the PI3K/AKT pathway in CRC (20). Several groups have studied the prognostic value of LGR6 in cancer. In esophageal squamous cell carcinoma patients high levels of LGR6 in the primary tumor indicated significantly worse prognosis than patients with low levels (19). In CRC one study gave the same result, that is, that patients with high levels of LGR6 have significantly shorter overall survival rates than patients with low levels (20), while another study showed the opposite result (21). Targeting CSC in CRC may constitute a new and effective treatment strategy (22).

Here, we have studied the prognostic value of the CSC marker LGR6 for analysis of regional LNs of CC patients. Analyses have been performed at the mRNA level using a novel highly specific quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay for LGR6, that detects all 3 splice forms of LGR6 mRNA. The same clinical material of LNs from CC patients as has been investigated earlier for expression of CEA-, LGR4-, LGR5- and the chemokine CXCL16- mRNAs has been used (10, 23). The utility of combining expression levels of the different biomarkers was also investigated. We found that high mRNA levels in lymph nodes of LGR6 predict shortened disease-free survival and that determinations of LGR6 together with the CC prognostic markers CEA and CXCL16 significantly enhances prognostic effectiveness.

2 Materials and methods

2.1 Patients and tissue specimens for mRNA analysis

Primary tumor specimens were gathered from 66 CC patients (30 men and 36 women; median age 74 years, range 42–88 years). Patients belonged TNM stages as follows: 14 patients in stage I, 30 patients in stage II, 17 patients in stage III, and 5 patients in stage IV. None of the patients received preoperative therapy. The specimens were collected immediately after resection, frozen and preserved at -70°C until RNA extraction. Normal colon specimens were taken from the resection margins of tumors of 30 CC patients (17 men and 13 women; median age 72 years, range 57–85 years).

Half LNs were gathered from 121 CC patients (55 men and 66 women; median age 74 years, range 42–89 years). Of these, 69 LNs came from 23 patients in stage I, 186 LNs from 52 patients in stage II, 85 LNs from 37 patients in stage III, and 30 LNs from 9 patients in stage IV. According to routine histopathology, disseminated tumor cells were detected in 20 LNs [H&E(+)] and 350 LNs were H&E(-). Thirteen non-cancer patients (10 males and 3 women; median age 23 years, range 9–32 years) provided 77 control LNs. One control patient had lipoma, 1 had Crohn's disease, and 11 had ulcerative colitis. The half LNs were collected immediately after resection, frozen and preserved at -70°C until RNA extraction.

2.2 Cell lines

RNA from 5 human CC cell lines (HT29, LS174T, Caco2, T84, HCT8), 1 T cell line (Jurkat), 2 B cell lines (CNB6, KR4), 1 monocyte cell line (U937), 1 endothelial cell line (HUVEC) and primary foreskin fibroblasts (FSU) were from previous studies (24–29).

2.3 Real-time qRT-PCR

For absolute quantification of LGR6 mRNA, we constructed a real time qRT-PCR assay using specific primers placed in different exons and a reporter dye-labeled probe hybridizing over the exon boundary in the amplicon and specific RNA copy standard. The LGR6 mRNA assay detects all three known transcript variants (NM_001017403.2, NM_021636.3, NM_001017404.2). The primer and probe sequences were: forward primer 5′-AGCTGGAGATGGAGGACTCAAA-3′, reverse primer 5′-CCAG CTTTCAAAGAGGTACTCACA-3′, and probe 5′-TACTCCAGG CCCCTTC-3′. MGB served as the quencher dye and FAM as the reporter dye. The amplicon measured 95 bases. The qRT-PCR profile was 60°C for 5 min and 95°C for 1 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. The RNA copy standard was a custom synthesized RNA oligonucleotide (Dharmacon, Lafayette, CO, USA) with identical sequence to the area amplified in the qRT-PCR assay. Real-time qRT-PCR assays for CEA, CXCL16, LGR4 and LGR5 mRNAs were described previously (10, 23, 24). Each qRT-PCR run included serial dilutions of the respective RNA copy standard at concentrations ranging from 10^3 to 10^8 copies/µL. Concentrations in unknown samples were determined from the standard curve and expressed as copies of mRNA/µL. The concentration of 18S rRNA was expressed as arbitrary units from a standard curve of serial dilutions of a preparation of total RNA from human peripheral blood mononuclear cells. One unit was defined as the amount of 18S rRNA in 10 pg RNA (30). Expression levels were expressed as mRNA copies/18S rRNA unit.

2.4 Statistical analysis

The statistical significance of differences between LGR6 mRNA levels in primary CC tumors compared to normal colon tissues, H&E(+) LNs compared to H&E (-) LNs, LNs of patients in different TNM stage groups, and LNs in the CEA(+), CEA(int) and CEA(-) groups were analyzed using two-tailed Mann-Whitney rank sum test. Correlations between LGR6 mRNA levels and CEA, CXCL16, LGR4 and LGR5 mRNA levels were analyzed using the nonparametric Spearman correlation coefficient. The software utilized for statistical calculations was GraphPad Prism 9 (Graph pad Software, San Diego, CA, USA). The SPSS software (IBM Corporation, Armonk, NY, USA) was used for statistical analyses of differences between patient groups in disease-free survival time and analyses of risk for recurrent disease after surgery, according to the Kaplan-Meier survival model in combination with the log-rank test and univariate Cox regression analysis. A p-value of ≤ 0.05 was considered statistically significant.

2.5 Ethical considerations

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments and comparable ethical standards. Tumor samples and LNs were collected after patients' written, informed consent. The study was approved by the Local Ethics Research Committee of the Medical Faculty, Umeå University, Umeå, Sweden (registration number: 03-503; date of approval: 3 December 2003 and registration number: 2023-01396-01; date of approval; 3rd of May 2023).

3 Results

3.1 Expression levels of LGR6 mRNA in primary colon cancer tumor, normal colon tissue, colon cancer cell lines and immune cell lines

The LGR6 mRNA median expression level was ten times higher in primary tumor (CC) than in normal colon tissues (NC) (0.7 and 0.07 mRNA copies/18S rRNA unit, respectively; $p < 0.0001$. Figure 1). The expression levels in four of five CC cell lines (T84, LS174T, HT29, CaCo2) were similar to those of primary CC tumors. In the fifth CC cell line (HCT8) the level was almost 100 times higher. Immune cell lines expressed clearly lower levels of LGR6 mRNA than CC cell lines of which the T cell line, Jurkat, expressed the highest level (about 0.03 mRNA copies/18S rRNA unit). Only very low levels of LGR6 mRNA were expressed in an endothelial cell line (HUVEC) and no LGR6 mRNA was detected in foreskin fibroblasts (FSU) (Figure 1). In a LN context, LGR6 mRNA

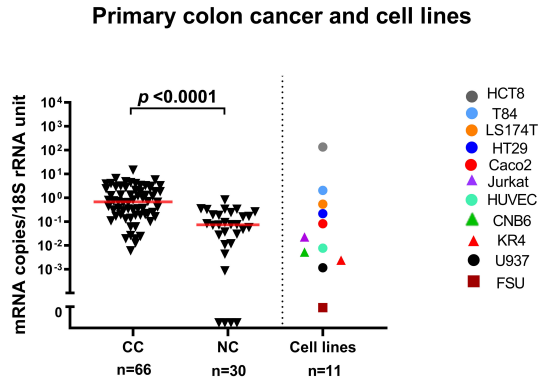

FIGURE 1
LGR6 mRNA expression levels in primary colon cancer tissues (CC), resected normal colon tissues (NC), and in a panel of colon cancer cell lines (HCT8, T84, LS174T, HT29, Caco2), a T-cell line (Jurkat), an endothelial cell line (HUVEC), two B-cell lines (CNB6 and KR4), a monocyte cell line (U937), and primary foreskin fibroblast cells (FSU). The median values are indicated by the red horizontal lines. The n-values show the number of analyzed primary CC tumors, normal colon samples and cell lines. Two-tailed Mann-Whitney rank sum test was used to determine the p-value.

can therefore be classified as epithelial cell specific with minimal influence of other cells that occurs in this organ.

3.2 Expression levels of LGR6 mRNA in regional lymph nodes of colon cancer patients

The mRNA expression levels of LGR6 were evaluated in a panel of 370 regional LNs from 121 CC patients representing all four TNM-stages and 77 LNs from 13 patients with non-cancerous disease. LGR6 mRNA median expression levels were 0.006, 0.007, 0.011 and 0.011 mRNA copies/18S rRNA unit in TNM stages I, II, III and IV, respectively. Notably, the LGR6 mRNA median expression level was 0.011 mRNA copies/18S rRNA unit in LNs of control patients. There was a significant difference of LGR6 mRNA expression level between LNs of stage I and stage III (p=0.01), between stage I and stage IV (p=0.02), between stage II and stage III (p=0.02), and a significant difference between LNs of stage II and stage IV (p=0.02) (Figure 2A).

LGR6 mRNA expression levels were then compared with CEA mRNA levels previously determined for the same 370 LNs (24). Figure 2B shows the result. LNs were divided into 3 groups depending on the CEA mRNA levels. CEA(+) >3.67 copies/18S rRNA unit, CEA(int) <3.67 and >0.013 copies/18S rRNA unit, and CEA(-) <0.013 copies/18S rRNA unit. The LGR6 mRNA median expression levels were 0.09, 0.007 and 0.007 mRNA copies/18S rRNA unit in the CEA(+), CEA(int) and CEA(-) LN groups, respectively. A highly significant difference between the expression levels of the CEA(+) and both the CEA(int) and CEA(-) groups was seen (p<0.0001).

In Figure 2C the LGR6 mRNA expression levels were compared with the results of examination for presence of tumor cells of H&E stained LN tissue sections of the same 370 LNs of CC patients and in 77 LNs of controls. Twenty LNs had metastases [H&E(+)] and 350 LNs were H&E(-). The LGR6 mRNA median expression level was 9 times higher in H&E(+) than H&E(-) LNs (0.06 and 0.007 mRNA copies/18S rRNA unit, respectively). There was a highly significant difference between H&E(+) LNs and both H&E(-) LNs and LNs of control patients (p<0.0001 and p<0.0001, respectively).

In order to make the LGR6 mRNA expression data directly comparable with the survival data as determined by Cox regression and Kaplan-Meier analysis (see below) we used the LGR6 mRNA level of the LN expressing the highest level to represent each patient. Figures 2D-F show the results for the 121 CC patients and the 13 control patients. As can be seen the expression pattern was closely similar to that found when all LNs were analyzed. One difference was, however, that control LNs did not differ significantly from H&E(+) or H&E(-) LNs, although the trend was the same, that is, to express levels in-between the other two groups (Figure 2F).

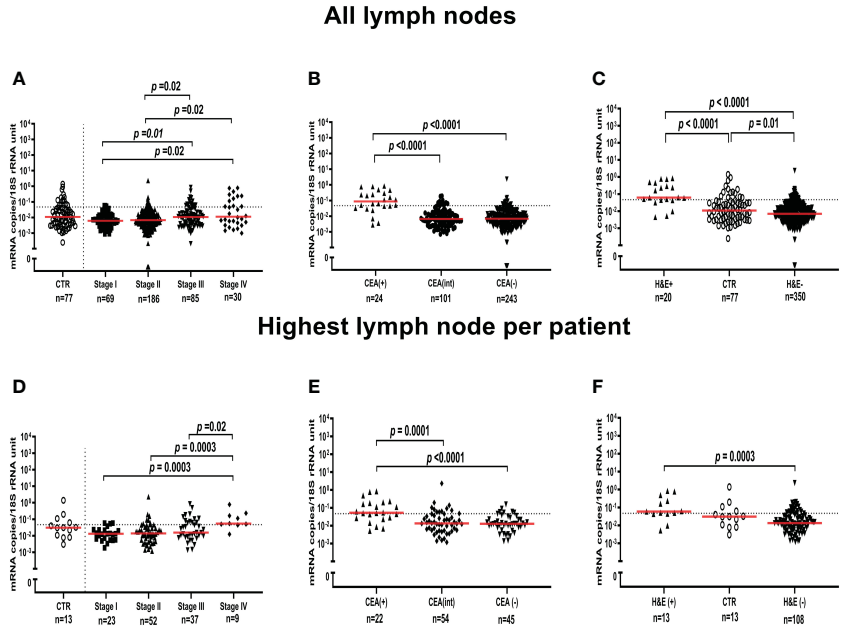

FIGURE 2
LGR6 mRNA expression levels in all lymph nodes **(A-C)** and the lymph node with the highest level for each patient **(D-F)**. **(A, D)** show LGR6 levels in lymph nodes from non-cancerous disease patients (CTR) and from colon cancer patients of different TNM stages (Stage I-IV). **(B, E)** show LGR6 levels in lymph nodes grouped according to their level of CEA mRNA, CEA(+) (> 3.67 copies/18S rRNA unit), CEA(int) (between 0.013 and 3.67 copies/18S rRNA unit) and CEA(-) (<0.013 copies/18S rRNA unit). **(C, F)** shows LGR6 levels in metastatic lymph nodes [H&E(+)], non-metastatic lymph nodes [H&E(-)] and non-cancerous disease patients (CTR). Dashed horizontal line indicates the 75th percentile (0.0471 LGR6 mRNA copies/18S rRNA unit) which was used as cut-off for LGR6(+) and LGR6(-) categories in analyses of prognostic value. The n-values shown the number of lymph node samples analyzed. Two-tailed Mann-Whitney rank sum test was used to determine the p-values.

3.3 Correlation between mRNA expression levels of LGR6 and of LGR4, LGR5, CEA and CXCL16 in regional lymph nodes of colon cancer patients

The mRNA expression levels of LGR4, LGR5, CEA and CXCL16 have previously been determined in the same 370 LNs studied in this work (10, 23, 24). Table 1 shows the correlation coefficients (r) and the degree of significance of the correlation between the biomarker mRNAs for all 121 CC patients both for the patients as one group and for the different TNM stage groups. The highest correlation coefficient was seen between LGR6 and CEA (r=0.73), followed by LGR6 and CXCL16 (r=0.66), and thereafter LGR6 and LGR5 (r=0.53) in LNs of stage IV patients. All three correlations were highly significant. Significant correlation between LGR6 and CEA and between LGR6 and CXCL16 was also seen in LNs of stage III patients. These data indicate that LGR6, CEA, CXCL16 and LGR5 to a large extent identifies the same tumor cell population which is enriched in LNs of stage III and IV patients.

3.4 Clinical utility of expression level analysis of LGR6 mRNA alone or in combination with CEA or CXCL16 mRNA in lymph nodes to predict colon cancer recurrence after surgery

To evaluate the significance of using the expression levels of LGR6 mRNA in regional LNs of CC patients for prediction of disease recurrence after surgery, we used Cox regression analysis to calculate the hazard risk ratio for recurrence and Kaplan-Meier survival model combined with the log-rank test to evaluate differences in disease-free survival time after surgery. Each patient was represented by the LN with the highest expression level of LGR6 mRNA. A cut-off level discriminating between patients with high and low risk for recurrence was analytically determined, dividing the patients into two categories, LGR6(+) and LGR6(−). The cutoff used to divide the patients into a LGR6(+) category and a LGR6(-) category was the 75th percentile (0.0471 LGR6 mRNA copies/18S rRNA unit). The prognostic value of the LGR6 level in the CEA(+)-, CEA(int)-, CEA(-)-, and CXCL16 (+) groups, as well as in a of group of patients that were CEA(+)-, CEA(int)- and CXCL16(+) was also investigated. These survival analyses are shown in Table 2 and Kaplan–Meier cumulative survival curves in Figure 3.

Patients in the LGR6(+) category (n = 30) showed a 3.2-fold higher risk of recurrence compared to the LGR6(-) category (n = 91) when followed for five years and a 2.8-fold higher risk at a follow-up time of 12 years (p=0.001 and p=0.003, respectively). According to Kaplan-Meier survival analysis the associated decrease in mean disease-free survival time was 11 months at 5 years and 47 months at 12 years after surgery (p<0.001 and p=0.002, respectively, Figure 3A).

A clear-cut division of the patients in terms of survival was seen if LGR6 mRNA analysis was combined with CEA mRNA analysis. Thus, when patients in the CEA(+) group were divided into a LGR6(+) category (n = 13) and a LGR6(-) category (n = 9) a markedly increased risk for recurrence with a hazard ratio (HR) of 3.7 was seen for the positive category when followed for five years (p=0.05). Note, that no patients were alive in the LGR6(+) group 90 months after surgery. Note also that patient survival in the LGR6(-) category was poor although not as poor as in the LGR6(+) category of CEA(+) patients. The associated decrease in mean survival time was 17 months in 5 years (p=0.03; Figure 3C). In contrast, no significant difference in recurrence risk or mean survival time between the LGR6(+) and LGR6(-) categories was observed when analysis was confined to LNs of the CEA(int) group (Figure 3E). For the CEA(-) group there was a small but significant difference with the LGR6(+) category having a worse outcome than the LGR6(-) category at 5-years follow-up. (Figure 3B).

Subdivision of CC patients belonging to the CXCL16(+) category could also be achieved by LGR6 mRNA analysis (Figure 3D). The LGR6(+) category (n = 17) showed a 3.9-fold higher recurrence risk compared to the LGR6(-) category (n = 31) when followed for 5 years and 3.7 when followed for 12 years

TABLE 1 Correlations between LGR6 mRNA expression levels and expression levels of LGR4, LGR5, CEA and CXCL16 mRNAs in lymph nodes of colon cancer patients.

			LGR4	LGR5	CEA	CXCL16
LGR6	All CC LNs	r	0.28	0.22	0.12	0.33
		p-value	<0.0001	<0.0001	0.02	<0.0001
	TNM Stage I LNs	r	0.37	0.37	-0.09	0.34
		p-value	0.002	0.002	0.49	0.004
	TNM Stage II LNs	r	0.23	0.04	-0.11	0.26
		p-value	0.002	0.55	0.15	0.0003
	TNM Stage III LNs	r	0.19	0.26	0.35	0.34
		p-value	0.08	0.02	0.001	0.002
	TNM Stage IV LNs	r	0.40	0.53	0.73	0.66
		p-value	0.03	0.003	<0.0001	<0.0001

The correlation coefficients (r) and the p-values were calculated by two-tailed Spearman's rank order correlation test.

TABLE 2 Comparative analysis of average survival time after surgery and risk of recurrence of disease in colon cancer patients with LGR6(+) or LGR6(-) lymph nodes.

Patient Group	Category[a]	Number of patients	5-Year Follow Up after Surgery					12-Year Follow Up after Surgery				
			Disease-free survival time[b]			Risk of recurrence of CC[c]		Disease-free survival time[b]			Risk of recurrence of CC[c]	
			Average (months)	Difference (months)	*p*-value	Hazard Ratio (95% CI)	*p*- value	Average (months)	Difference (months)	*p*-value	Hazard Ratio (95% CI)	*p*- value
All CC Patients	LGR6(-)	91	54	11	<0.001	3.2 (1.6-6.4)	0.001	119	47	0.002	2.8 (1.1-5.4)	0.003
	LGR6(+)	30	43					72				
CEA(+) patients[d]	LGR6(-)	9	49	17	0.03	3.7 (1.0-13.6)	0.05					
	LGR6(+)	13	32									
CEA (int) patients[e]	LGR6(-)	43	54	1	0.9	0.9 (0.2-4.1)	0.9	122	23	1.0	1.0 (0.2-4.7)	1.0
	LGR6(+)	11	53					99				
CEA (-) patients[f]	LGR6(-)	39	55	8	0.04	3.8 (1.0-15.4)	0.05	112	21	0.4	1.7 (0.5-6.3)	0.4
	LGR6(+)	6	47					91				
CXCL16 (+) patients[g]	LGR6(-)	31	53	18	0.002	3.9 (1.5-9.7)	0.004	104	48	0.002	3.7 (1.5-9.2)	0.004
	LGR6(+)	17	35					56				
CEA(int)/ CEA(+)/ CXCL16 (+) patients[h]	LGR6(-)	22	52	17	0.01	3.5 (1.2-9.8)	0.02	103	57	0.008	3.8 (1.3-10.7)	0.01
	LGR6(+)	14	35					46				

[a]CC patients were divided into categories according to LGR6 mRNA level. LGR6(-): the highest lymph node had <0.0471 mRNA copies/18S rRNA unit; LGR6(+): the highest lymph node had ≥0.0471 mRNA copies/18S rRNA unit.
[b]Mean survival time after surgery calculated by cumulative survival analysis according to the Kaplan-Meier model.
[c]Hazard ratio with 95% confidence interval (CI) calculated according to univariate Cox regression analysis.
[d]CC patients with CEA mRNA levels above 3.67 mRNA copies /18S rRNA unit.
[e]CC patients with CEA mRNA levels between 0.013 and 3.67 mRNA copies /18S rRNA unit.
[f]CC patients with CEA mRNA levels below 0.013 mRNA copies /18S rRNA unit.
[g]CC patients with CXCL16 mRNA levels above 7.2 mRNA copies /18S rRNA unit.
[h]CC patients with CEA mRNA levels above 0.013 mRNA copies /18S rRNA unit and CXCL16 mRNA levels above 7.2 mRNA copies /18S rRNA unit.

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(p=0.004 at both timepoints; Table 2). Corresponding figures for decrease in mean survival time was 18 months at 5 years and 48 months at 12 years after surgery (p=0.002 at both timepoints).

Finally, we used LGR6 mRNA analysis to further divide a patient group expressing high or intermediate levels of CEA mRNA in their LNs as well as high levels of CXCL16 (CEA (+)/CEA(int)/CXCL16(+) group). Patients in the LGR6(+) category (n = 14) showed a 3.5-fold higher recurrence risk compared to the LGR6(-) category (n = 22) when followed for 5 years and 3.8-fold at a follow-up time of 12 years (p=0.02 and p=0.01, respectively). The Kaplan-Meier survival analysis was associated with a decrease in mean survival time of 17 months in 5 years and 57 months in 12 years after surgery (p=0.01 and p=0.008, respectively; Figure 3F).

3.5 LGR6 mRNA expression levels of lymph nodes of colon cancer patients in relation to TNM stage

Table 3 shows how LGR6(+) and LGR6(-) patients are distributed in relation to different TNM stages and Figures 4A, B show Kaplan Meier analysis of TNM stage I and II patients, respectively. As can be seen, there are 3 stage I patients which are LGR6(+) and 20 which are LGR6(-). Two of the LGR6(+) patients have died from their cancer and the third patient from other causes. After 110 months no LGR6 (-) patient had died from cancer (Figure 4A; Δ = 23 months at 5 years, p<0.001). It can safely be concluded that two of these patients are missed by histopathology and also missed by CEA mRNA analysis since they were found to belong to the CEA(-) group (Table 3). Thus,

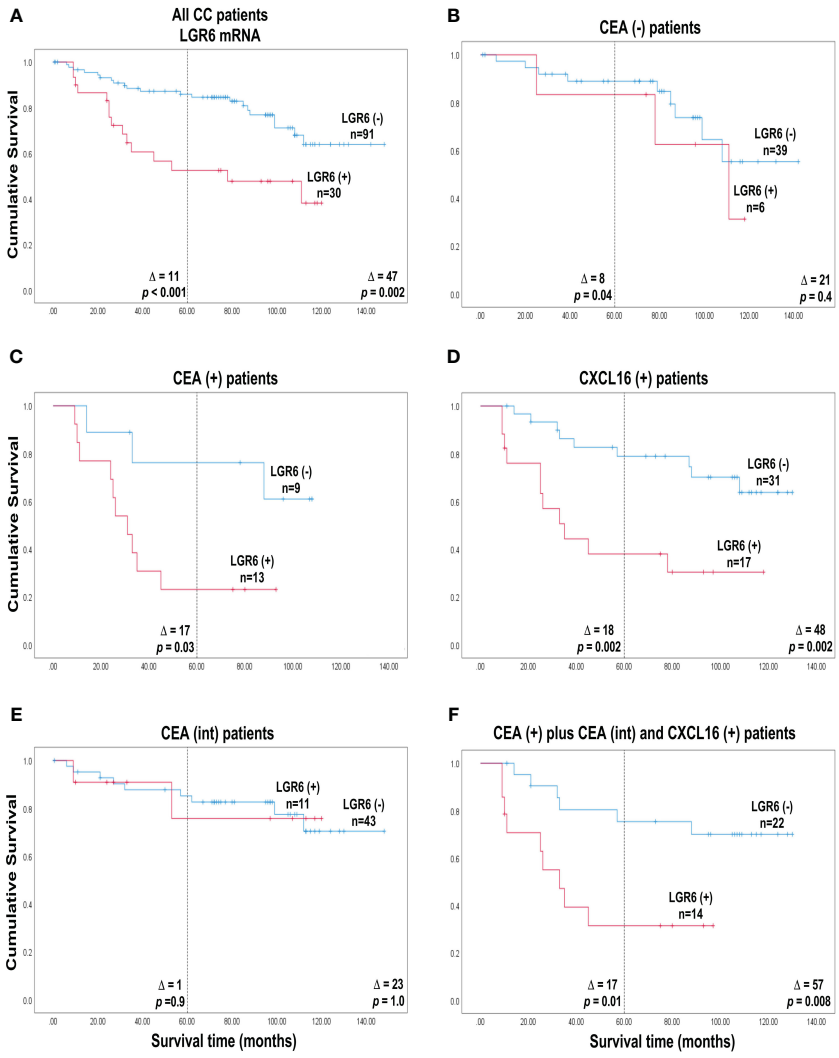

FIGURE 3
Cumulative survival curves according to Kaplan-Meier of colon cancer patients belonging to either of the two categories LGR6(+) and LGR6(-) defined as patients with a LGR6 mRNA level in the highest lymph node above respectively below the cut-off 0.0471 LGR6 mRNA copies/18S rRNA unit. **(A)** all 121 colon cancer patients. **(B)** the 45 CEA(-) patients who had CEA mRNA levels <0.013 copies/18S rRNA unit. **(C)** the 22 CEA(+) patients who had CEA mRNA levels >3.67 copies/18S rRNA unit. **(D)** the 48 CXCL16(+) patients who had CXCL16 mRNA levels >7.2 copies/18S rRNA unit. **(E)** the 54 CEA(int) patients who had CEA mRNA levels between 0.013 and 3.67 copies/18S rRNA unit. **(F)** the 36 patients who had CEA mRNA levels above 0.013 copies/ 18S rRNA unit and CXCL16 levels >7.2 copies/18S rRNA unit. The numbers next to the curves indicate the number of patients in the category. The difference between mean survival time without recurrence between the categories are given as Δ-values. The p-values are from log rank test analysis of survival data. The dashed line indicates 5 years of observation after surgery.

LGR6 analysis adds to histopathology and CEA mRNA analysis. There are 8 stage II patients that fall into the LGR6(+) category and 44 patients in the LGR6(-) category of which 4 were found to belong to the CEA(int) and 4 to the CEA(-) groups (Table 3). However, no significant difference in recurrence risk or mean survival time was observed between the LGR6(+) and LGR6(-) categories when analysis was confined to LNs of TNM stage II (Figure 4B).

3.6 No correlation between recurrence risk or survival after surgery and levels of LGR6 mRNA in primary tumors of colon cancer

When CC patients were divided to LGR6(+) and LGR6(−) categories based on the median mRNA level of the primary CC tumors (0.7 mRNA copies/18S rRNA unit) or the 75th percentile as cutoffs, no differences were found between the groups in either the survival time or recurrence risk.

4 Discussion

The most important finding of this study is that LGR6 can be used as a complementary biomarker to CEA and CXCL16 to detect CC patients that relapse after surgery who are missed by these markers and by histopathology. LGR6 is useful as a complementary biomarker in mRNA analysis of LNs of patients with CC but not for analysis of the primary tumor. LGR6 mRNA analysis has prognostic value in two different situations 1) if the CC patient has LNs expressing high levels of CEA mRNA and 2) if the CC patient has LNs that do not express CEA mRNA (that is CEA mRNA levels below the cut of level for LNs of control patients). In the former situation LGR6 mRNA levels discriminate between patients with very bad prognosis and those with less bad prognosis. In the latter situation high levels of LGR6 mRNA reveal those relatively few patients that relapse but only express minimal levels or no CEA at all, the CEA(-) group. Of particular interest is that these patients also are missed by histopathology i.e. they belong to TNM stage I and II. In this study, LGR6(+) stage I and II patients constituted 11 patients which is equal to 9% of all patients. LGR6 could detect CC patients at risk in stage I but not in stage II, indicating that the size of the primary tumor does not necessarily reflect the aggressivity of the cancer. Probably genetic features of the tumor have a greater impact. Why does LGR6 detect patients with bad prognosis who are not detected by CEA or CXCL16? We hypothesize that this is due to that LGR6 detect colonic epithelial stem cells which are poorly detected by CEA and CXCL16. Such stem cells also occur to a variable degree in LNs of CC patients. However, LGR6 is not a stem cell specific marker in humans as revealed by studies with monoclonal antibodies (31). LGR6 is also expressed in tumor cells that are more mature, the difference being that CSC express higher levels than more mature cancer cells. Moreover, our cell line studies indicate that LGR6 mRNA is highly expressed in colonic CSC. The CC cell line HCT8 expressed very high levels of LGR6 mRNA, which is in line with the findings by Yan et al., 2016 who found that CSC could easily be isolated from this cell line (32). Another important observation is that LGR6 detects a subpopulation of tumor cells that is not detected by LGR5 since LGR6 identified CC patients at high risk with low CEA levels that were not identified by LGR5 (10). Despite the

TABLE 3 Number of LGR6(+) and LGR6(-) colon cancer patients in different TNM stages divided into different patients groups.

Patient Group	Category[a]	Number of LGR6(+) patients			
		Stage I	Stage II	Stage III	Stage IV
All CC Patients n=121	LGR6(-)	20	44	25	2
	LGR6(+)	3	8	12	7
CEA(+) patients[b] n=22	LGR6(-)	2	1	5	1
	LGR6(+)	0	0	6	7
CEA(int) patients[c] n=54	LGR6(-)	10	25	7	1
	LGR6(+)	1	4	6	0
CEA(-) patients[d] n=45	LGR6(-)	8	18	13	0
	LGR6(+)	2	4	0	0
CXCL16(+) patients[e] n=48	LGR6(-)	8	13	9	1
	LGR6(+)	2	2	7	6
CEA(int)/CEA(+)/ CXCL16(+) patients[f] n=36	LGR6(-)	5	9	7	1
	LGR6(+)	0	1	7	6

[a]CC patients were divided into categories according to LGR6 mRNA level. LGR6(-): the highest lymph node had <0.0471 mRNA copies/18S rRNA unit; LGR6(+): the highest lymph node had ≥0.0471 mRNA copies/18S rRNA unit.
[b]CC patients with CEA mRNA levels above 3.67 mRNA copies /18S rRNA unit.
[c]CC patients with CEA mRNA levels between 0.013 and 3.67 mRNA copies /18S rRNA unit.
[d]CC patients with CEA mRNA levels below 0.013 mRNA copies /18S rRNA unit.
[e]CC patients with CXCL16 mRNA levels above 7.2 mRNA copies /18S rRNA unit.
[f]CC patients with CEA mRNA levels above 0.013 mRNA copies /18S rRNA unit and CXCL16 mRNA levels above 7.2 mRNA copies /18S rRNA unit.

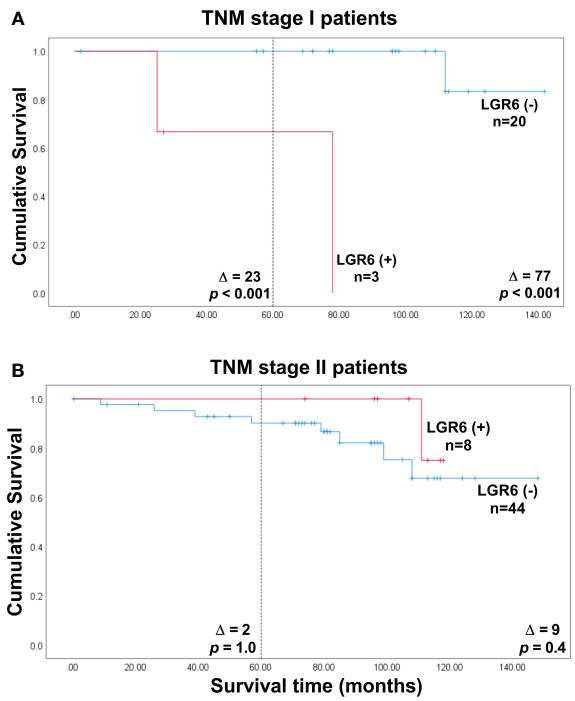

FIGURE 4
Cumulative survival curves according to Kaplan-Meier of colon cancer patients belonging to either of the two categories LGR6(+) and LGR6(-) defined as patients with a LGR6 mRNA level in the highest lymph node above respectively below the cut-off 0.0471 LGR6 mRNA copies/18S rRNA unit. **(A)** the 23 TNM stage I colon cancer patients. **(B)** the 52 TNM stage II patients. The numbers next to the curves indicate the number of patients in the category. The difference between mean survival time without recurrence between the categories are given as Δ-values. The *p*-values are from log rank test analysis of survival data. The dashed line indicates 5 years of observation after surgery.

similarity between the structure of LGR6 and LGR5 (33, 34), LGR6 was barely detected in fibroblasts, in contrast to LGR5, which was expressed at high levels suggesting differences in function between the two CSC markers. In this study we have used PCR primers and a probe that detect all three splice forms of LGR6 but do not cross-react with mRNA for LGR5 or LGR4. An interesting possibility, that has not been explored, is that any of the three splice forms could have a different specificity pattern.

LGR6 mRNA levels correlate with CEA and CXCL16 mRNA levels in stage III and IV patients and LGR6 correlates significantly, but less strongly, with LGR5 and LGR4 and in nearly all TNM stages. The relationship between the three LGRs is complex and is not fully understood. LGR4 and LGR6 show a closer expression pattern than LGR5 and LGR6 as shown in this study and our previous study (10). It was noted that LGR6 protein can bind to LGR4 protein and LGR5 protein possibly indicating that LGRs form complexes with each other (19) that can positively or negatively regulate the Wnt/β-catenin pathway. Complex formation between LGRs may be responsible for the contradictory results seen by different groups using LGRs as prognostic marker in cancer including CC.

LGR6 promotes CRC cell proliferation and migration *in vitro* by activating the PI3K/AKT signaling pathway and was suggested to serve as a predictive biomarker of CRC primary tumors for bad prognosis and a therapeutic target for patients with advanced stages of CRC (20). Moreover, LGR6 is implicated in the growth and proliferation of several cancer types, including gastric and colon cancer and is also attributed with cancer therapy resistance (20, 35–38).

It is unlikely that the results presented here would have been possible to obtain by histopathology or even immunohistochemistry with specific antibodies supported by artificial intelligence (AI) deep learning algorithms, because only a small portion of the LN volume is analyzed by these methods (39, 40). We showed in a previous study that disseminated tumor cells are heterogeneously distributed in the LN and metastases can be missed if only a small volume is analyzed (41). In the present study the molecular technique qRT-PCR was used to analyze extracts from as much as half the LN thereby strongly increasing the probability of detecting LGR6 mRNA from cancer stem cells. Another complicating factor for the microscopic methods is selection of LNs for examination of presence of stem cells and assessment of stem cell numbers. The LNs of a single patient can differ considerably regarding the number of tumor cells and the risk factors these cells express (6). The fact that current guidelines for determination of metastasis status (pN-stage) requires examination of a minimum of 12 LNs points out the fact that LNs of one patient vary considerably in tumor burden (42, 43). Determination of LGR6 mRNA levels is readily done in several LNs in a fast and objective manner.

The novel results of this study, obtained with our well-studied clinical material of CC patients, need to be validated in a larger clinical material, which should also include patients with rectal cancer. Moreover, preferably all LNs collected from a patient should be included in the study.

5 Conclusion

We conclude that LGR6 mRNA analysis of LNs from CC patients can serve as an important complement to CEA- or CXCL16 mRNA analysis detecting cancer stem cells which express very low levels or no mRNA for these two markers. Moreover, it appears to be difficult to identify cancer cells in these LNs by histopathology either because the number of cancer cell is very low or that the CSCs are very unevenly distributed in the LN tissue. LGR6 has a different expression pattern than the CSC marker LRG5 and could detect other patients at risk. Using LGR6 mRNA analysis will help to identify additional patients which would benefit from adjunct therapy.

Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Ethics statement

The study was approved by the Local Ethics Research Committee of the Medical Faculty, Umeå University, Umeå,

Sweden (registration number: 03-503; date of approval: 3 December 2003 and registration number: 2023-01396-01; date of approval; 3rd of Mai 2023). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HE: Writing – original draft, Data curation. MA: Writing – review & editing, Data curation. HI: Writing – review & editing, Data curation. FZ: Writing – review & editing, Supervision. AG: Writing – review & editing, Supervision. LO: Writing – review & editing, Data curation. GL: Writing – review & editing, Data curation. MH: Writing – review & editing, Writing – original draft, Resources, Data curation, Conceptualization. SH: Writing – review & editing, Writing – original draft, Visualization, Data curation, Conceptualization. BS: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Data curation, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Global Cancer Observatory Cancer Today. Available online at: https://Gco.Iarc.Fr/ (Accessed Feburary 8, 2024).

2. Panos G, Mulita F, Akinosoglou K, Liolis E, Kaplanis C, Tchabashvili L, et al. Risk of surgical site infections after colorectal surgery and the most frequent pathogens isolated: a prospective single-centre observational study. *Med Glas (Zenica)*. (2021) 18:438–43. doi: 10.17392/1348-21

3. Mulita F, Liolis E, Akinosoglou K, Tchabashvili L, Maroulis I, Kaplanis C, et al. Postoperative sepsis after colorectal surgery: a prospective single-center observational study and review of the literature. *Gastroenterol Rev*. (2022) 17:47–51. doi: 10.5114/pg.2021.106083

4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. (2018) 68:7–30. doi: 10.3322/caac.21442

5. Böckelman C, Engelmann BE, Kaprio T, Hansen TF, Glimelius B. Risk of recurrence in patients with colon cancer stage II and III: a systematic review and meta-analysis of recent literature. *Acta Oncol*. (2015) 54:5–16. doi: 10.3109/0284186X.2014.975839

6. Lindmark G, Olsson L, Sitohy B, Israelsson A, Blomqvist J, Kero S, et al. qRT-PCR analysis of CEACAM5, KLK6, SLC35D3, MUC2 and POSTN in colon cancer lymph nodes-an improved method for assessment of tumor stage and prognosis. *Int J Cancer*. (2023) 154:573–84. doi: 10.1002/ijc.34718

7. Yadav AK, Desai NS. Cancer stem cells: acquisition, characteristics, therapeutic implications, targeting strategies and future prospects. *Stem Cell Rev Rep*. (2019) 15:331–55. doi: 10.1007/s12015-019-09887-2

8. Chaudhary A, Raza SS, Haque R. Transcriptional factors targeting in cancer stem cells for tumor modulation. *Semin Cancer Biol*. (2023) 88:123–37. doi: 10.1016/j.semcancer.2022.12.010

9. Huang T, Song X, Xu D, Tiek D, Goenka A, Wu B, et al. Stem cell programs in cancer initiation, progression, and therapy resistance. *Theranostics*. (2020) 10:8721–43. doi: 10.7150/thno.41648

10. AbdelMageed M, Ismail HTH, Olsson L, Lindmark G, Hammarström ML, Hammarström S, et al. Clinical Significance of stem cell biomarkers EPCAM, LGR5 and LGR4 mRNA levels in lymph nodes of colon cancer patients. *Int J Mol Sci*. (2021) 23:403. doi: 10.3390/ijms23010403

11. Ohlsson L, Hammarström ML, Lindmark G, Hammarström S, Sitohy B. Ectopic expression of the chemokine CXCL17 in colon cancer cells. *Br J Cancer*. (2016) 114:697–703. doi: 10.1038/bjc.2016.4

12. Rashad Y, Olsson L, Israelsson A, Öberg Å, Lindmark G, Hammarström ML, et al. Lymph node CXCL17 messenger RNA: A new prognostic biomarker for colon cancer. *Tumour Biol*. (2018) 40:1010428318799251. doi: 10.1177/1010428318799251

13. Ali H, AbdelMageed M, Olsson L, Israelsson A, Lindmark G, Hammarström ML, et al. Utility of G protein-coupled receptor 35 expression for predicting outcome in colon cancer. *Tumour Biol*. (2019) 41:1010428319858885. doi: 10.1177/1010428319858885

14. Hsu SY, Kudo M, Chen T, Nakabayashi K, Bhalla A, van der Spek PJ, et al. The three subfamilies of leucine-rich repeat-containing G protein-coupled receptors (LGR): identification of LGR6 and LGR7 and the signaling mechanism for LGR7. *Mol Endocrinol*. (2000) 14:1257–71. doi: 10.1210/mend.14.8.0510

15. de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, et al. LGR5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature*. (2011) 476:293–7. doi: 10.1038/nature10337

16. Carmon KS, Gong X, Lin Q, Thomas A, Liu Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc Natl Acad Sci U.S.A*. (2011) 108:11452–7. doi: 10.1073/pnas.1106083108

17. Glinka A, Dolde C, Kirsch N, Huang YL, Kazanskaya O, Ingelfinger D, et al. LGR4 and LGR5 are R-spondin receptors mediating Wnt/β-catenin and Wnt/PCP signalling. *EMBO Rep*. (2011) 12:1055–61. doi: 10.1038/embor.2011.175

18. Gong X, Carmon KS, Lin Q, Thomas A, Yi J, Liu Q. LGR6 is a high affinity receptor of R-spondins and potentially functions as a tumor suppressor. *PloS One*. (2012) 7:e37137. doi: 10.1371/journal.pone.0037137

19. Chai T, Shen Z, Zhang Z, Chen S, Gao L, Zhang P, et al. LGR6 is a potential diagnostic and prognostic marker for esophageal squamous cell carcinoma. *J Clin Lab Anal*. (2020) 34:e23121. doi: 10.1002/jcla.23121

20. Wang F, Dai CQ, Zhang LR, Bing C, Qin J, Liu YF. Downregulation of LGR6 inhibits proliferation and invasion and increases apoptosis in human colorectal cancer. *Int J Mol Med*. (2018) 42:625–32. doi: 10.3892/ijmm.2018.3633

21. Wang W, Ding S, Zhang H, Li J, Zhan J, Zhang H. G protein-coupled receptor LGR6 is an independent risk factor for colon adenocarcinoma. *Front Med*. (2019) 13:482–91. doi: 10.1007/s11684-018-0633-0

22. Frank MH, Wilson BJ, Gold JS, Frank NY. Clinical implications of colorectal cancer stem cells in the age of single-cell omics and targeted therapies. *Gastroenterology*. (2021) 160:1947–60. doi: 10.1053/j.gastro.2020.12.080

23. AbdelMageed M, Ali H, Olsson L, Lindmark G, Hammarström ML, Hammarström S, et al. The chemokine CXCL16 is a new biomarker for lymph node analysis of colon cancer outcome. *Int J Mol Sci*. (2019) 20:5793. doi: 10.3390/ijms20225793

24. Ohlsson L, Israelsson A, Öberg Å, Palmqvist R, Stenlund H, Hammarström ML, et al. Lymph node CEA and MUC2 mRNA as useful predictors of outcome in colorectal cancer. *Int J Cancer*. (2012) 130:1833–43. doi: 10.1002/ijc.26182

25. Ali H, Olsson L, Lindmark G, Hammarström ML, Hammarström S, Sitohy B. The myeloid cell biomarker EMR1 is ectopically expressed in colon cancer. *Tumour Biol*. (2021) 43:209–23. doi: 10.3233/TUB-200082

26. Olsson L, Lindmark G, Hammarström ML, Hammarström S, Sitohy B. Evaluating macrophage migration inhibitory factor 1 expression as a prognostic biomarker in colon cancer. *Tumour Biol*. (2020) 42:1010428320924524. doi: 10.1177/1010428320924524

27. Ali H, AbdelMageed M, Olsson L, Lindmark G, Hammarström ML, Hammarström S, et al. Detection of lymph node metastasis in colon cancer by ectopically expressed fibroblast markers FOXQ1 and THBS2. *Front Oncol*. (2023) 13:1297324. doi: 10.3389/fonc.2023.1297324

28. Ohlsson L, Hammarström ML, Israelsson A, Näslund L, Öberg A, Lindmark G, et al. Biomarker selection for detection of occult tumour cells in lymph nodes of colorectal cancer patients using real-time quantitative RT-PCR. *Br J Cancer*. (2006) 95:218–25. doi: 10.1038/sj.bjc.6603206

29. Ismail HTH, AbdelMageed M, Lindmark G, Hammarström ML, Hammarström S, Sitohy B. Prognostic significance of GPR55 mRNA Expression in colon cancer. *Int J Mol Sci*. (2022) 23:4556. doi: 10.3390/ijms23094556

30. Bas A, Forsberg G, Hammarström S, Hammarström ML. Utility of the housekeeping genes 18S rRNA, beta-actin and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) for normalization in real-time quantitative RT-PCR analysis of gene expression in human T lymphocytes. *Scand J Immunol*. (2004) 59:566–73. doi: 10.1111/j.0300-9475.2004.01440.x

31. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Tissue-based map of the human proteome. *Science*. (2015) 347:1260419. doi: 10.1126/science.1260419

32. Yan C, Luo L, Guo CY, Goto S, Urata Y, Shao JH, et al. Doxorubicin-induced mitophagy contributes to drug resistance in cancer stem cells from HCT8 human colorectal cancer cells. *Cancer Lett*. (2017) 388:34–42. doi: 10.1016/j.canlet.2016.11.018

33. Raslan AA, Yoon JK. R-spondins: Multi-mode WNT signaling regulators in adult stem cells. *Int J Biochem Cell Biol*. (2019) 106:26–34. doi: 10.1016/j.biocel.2018.11.005

34. Schindler AJ, Watanabe A, Howell SB. LGR5 and LGR6 in stem cell biology and ovarian cancer. *Oncotarget*. (2018) 9:1346–55. doi: 10.18632/oncotarget.20178

35. Ogunwobi OO, Mahmood F, Akingboye A. Biomarkers in colorectal cancer: current research and future prospects. *Int J Mol Sci*. (2020) 21:5311. doi: 10.3390/ijms21155311

36. van de Glind GC, Rebel HG, Out-Luiting JJ, Zoutman W, Tensen CP, de Gruijl FR. Lgr6+ stem cells and their progeny in mouse epidermis under regimens of exogenous skin carcinogenesis, and their absence in ensuing skin tumors. *Oncotarget*. (2016) 7:86740–54. doi: 10.18632/oncotarget.13436

37. Garcia-Closas M, Couch FJ, Lindstrom S, Michailidou K, Schmidt MK, Brook MN, et al. Genome-wide association studies identify four ER negative-specific breast cancer risk loci. *Nat Genet*. (2013) 45:392–8. doi: 10.1038/ng.2561

38. Colak S, Medema JP. Cancer stem cells–important players in tumor therapy resistance. *FEBS J*. (2014) 281:4779–91. doi: 10.1111/febs.13023

39. Bousis D, Verras GI, Bouchagier K, Antzoulas A, Panagiotopoulos I, Katinioti A, et al. The role of deep learning in diagnostic colorectal cancer. *Gastroenterol Rev*. (2023) 18:266–73. doi: 10.5114/pg.2023.129494

40. Chlorogiannis DD, Verras GI, Tzelepi V, Chlorogiannis A, apostolos A, Kotis K, et al. Tissue classification and diagnosis of colorectal cancer histopathology images using deep learning algorithms. Is the time ripe for clinical practice implementation? *Gastroenterol Rev*. (2023) 18:353–67. doi: 10.5114/pg.2023.130337

41. Olsson LM, Lindmark GE, Israelsson ACE, Korkocic D, Hammarström SG, Hammarström MLKC. CEACAM5, KLK6, SLC35D3, POSTN and MUC2 mRNA analysis improves detection and allows characterization of tumor cells in lymph nodes of colon cancer patients. *Dis Colon Rectum*. (2021) 64:1354–63. doi: 10.1097/DCR.0000000000002151

42. Chang GJ, Rodriguez-Bigas MA, Skibber JM, Moyer VA. Lymph node evaluation and survival after curative resection of colon cancer: systematic review. *J Natl Cancer Inst*. (2007) 99:433–41. doi: 10.1093/jnci/djk092

43. Tsai HL, Huang CW, Yeh YS, Ma CJ, Chen CW, Lu CY, et al. Factors affecting number of lymph nodes harvested and the impact of examining a minimum of 12 lymph nodes in stage I-III colorectal cancer patients: a retrospective single institution cohort study of 1167 consecutive patients. *BMC Surg*. (2016) 16:17. doi: 10.1186/s12893-016-0132-7

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Diagnostic performance of volatile organic compounds analysis and electronic noses for detecting colorectal cancer: a systematic review and meta-analysis

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Introduction: The detection of Volatile Organic Compounds (VOCs) could provide a potential diagnostic modality for the early detection and surveillance of colorectal cancers. However, the overall diagnostic accuracy of the proposed tests remains uncertain.

Objective: This systematic review is to ascertain the diagnostic accuracy of using VOC analysis techniques and electronic noses (e-noses) as noninvasive diagnostic methods for colorectal cancer within the realm of clinical practice.

Methods: A systematic search was undertaken on PubMed, EMBASE, Web of Science, and the Cochrane Library to scrutinize pertinent studies published from their inception to September 1, 2023. Only studies conducted on human subjects were included. Meta-analysis was performed using a bivariate model to obtain summary estimates of sensitivity, specificity, and positive and negative likelihood ratios. The Quality Assessment of Diagnostic Accuracy Studies 2 tool was deployed for quality assessment. The protocol for this systematic review was registered in PROSPERO, and PRISMA guidelines were used for the identification, screening, eligibility, and selection process.

Results: This review encompassed 32 studies, 22 studies for VOC analysis and 9 studies for e-nose, one for both, with a total of 4688 subjects in the analysis. The pooled sensitivity and specificity of VOC analysis for CRC detection were 0.88 (95% CI, 0.83-0.92) and 0.85 (95% CI, 0.78-0.90), respectively. In the case of e-nose, the pooled sensitivity was 0.87 (95% CI, 0.83-0.90), and the pooled specificity was 0.78 (95% CI, 0.62-0.88). The area under the receiver operating characteristic analysis (ROC) curve for VOC analysis and e-noses were 0.93 (95% CI, 0.90-0.95) and 0.90 (95% CI, 0.87-0.92), respectively.

Conclusion: The outcomes of this review substantiate the commendable accuracy of VOC analysis and e-nose technology in detecting CRC. VOC analysis has a higher specificity than e-nose for the diagnosis of CRC and a sensitivity comparable to that of e-nose. However, numerous limitations, including a modest sample size, absence of standardized collection methods,

lack of external validation, and a notable risk of bias, were identified. Consequently, there exists an imperative need for expansive, multi-center clinical studies to elucidate the applicability and reproducibility of VOC analysis or e-nose in the noninvasive diagnosis of colorectal cancer.

Systematic review registration: https://www.crd.york.ac.uk/prospero/#recordDetails, identifier CRD42023398465.

KEYWORDS

volatile organic compounds, VOCs, electronic nose, E-nose, colorectal cancer, diagnosis

1 Introduction

Colorectal carcinoma (CRC) stands as a substantial global public health concern, with an estimated 1.93 million new cases and 0.93 million deaths in 2020 (1). CRC is known to develop from precursor lesions, in most cases adenomas, through the adenoma-carcinoma sequence (2) which can be diagnosed earlier through screening even in its early stages. Through standardized early diagnosis and treatment, the 5-year survival rate for early-stage CRC could exceed 90% (1). Fecal immunochemical test (FIT) and colonoscopy screening for colorectal cancer are pivotal tools for early diagnosis of colorectal cancer (3). However, the detection performance of FIT falls short, with a miss detection rate of 9-29% for CRC and 60-75% for advanced CRC (4). FIT-positive patients are recommended to undergo colonoscopy, but colonoscopy is painful, expensive, and invasive, with the risk of complications such as perforation and bleeding. So not all FIT-positive individuals undergo regular colonoscopy follow-up (5, 6). Therefore, there is an urgent need for convenient, non-invasive, reliable, simple, and cost-effective diagnostic methods to enhance early diagnosis and screening of colorectal cancer.

The analysis of Volatile organic compounds (VOCs) has been applied as a novel and promising diagnostic technique for exploration of non-invasive colorectal neoplasia biomarker. VOCs constitute the by-products of biochemical processes within the human body and typically mirror metabolic states (7, 8). Pathological conditions precipitate aberrant metabolic processes, resulting in a marked increase in VOC production (9). Investigations into cancer-related VOCs have explored various matrices, including breath, blood, urine, saliva, and feces (10–13). Many studies have demonstrated that the applicability of VOC analysis could be used in cancer diagnosis (14–20).

The electronic nose (e-nose) emerges as an instrument equipped with a suite of sensors endowed with specificity and an adept pattern recognition system capable of discerning both simple and complex odors (21). As a relatively recent development, the e-nose has become widely accepted for detecting diseases, owing to its portability, expeditious, cost-effective, and user-friendly diagnostic capabilities, rendering it particularly suited for routine clinical applications. Multiple researchers (22–24) have substantiated the commendable diagnostic accuracy of available e-nose technologies across diverse indications. Notably, van Keulen et al. (25) analyzed exhaled breath from patients with CRC and advanced adenomas (AAs), proving that the Aeonose electronic nose can distinguish CRC and AAs from controls. Additionally, de Meij et al. (26) reported an e-nose sensitivity of 0.85 and a specificity of 0.87 in CRC detection.

Our article aims to systematically review published studies on VOC analysis and e-nose technology concerning colorectal cancer (CRC) detection. Furthermore, we aim to compare their diagnostic performance, with the aspiration of offering a valuable reference for the application of diagnostic techniques in CRC diagnosis.

2 Methods

2.1 Registration

This systematic review has been registered with PROSPERO, under registration number CRD42023398465. The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines were adhered to in both the identification and reporting phases of this review (27).

2.2 Search strategy

A comprehensive literature search encompassing PubMed, Embase, Cochrane Library, and Web of Science was conducted from inception up to September 1, 2023. This search, void of language or data publication restrictions, utilized keywords such as “Volatile Organic Compounds,” “VOCs,” “electronic nose,” “e-nose,” “Colorectal neoplasms,” and “diagnosis” or “diagnostic” as search strategy terms. A detailed search strategy is provided in the Supplement.

2.3 Study selection

A total of 192 articles were retrieved. The eligibility of each article was assessed through a meticulous examination of titles and abstracts by two independent reviewers (Y.F. and S.Y.T.). Inclusion criteria were as follows: (1) studies conducted on adult subjects; (2) studies involving colorectal patients; and (3) studies that identified evaluating the diagnostic accuracy of using VOC analysis or e-nose technology. Exclusion criteria encompassed: (1) studies lacking information on the number of cases, controls, sensitivity, and specificity; and (2) studies published as review articles or case reports. Discrepancies between reviewers were resolved through consensus or, if necessary, with the involvement of a third investigator (Q.L.W.). A total of 32 articles met the inclusion criteria and were subsequently included in this systematic review.

2.4 Data collection process

The data extraction and tabulation process from the selected studies was undertaken by two reviewers (S.Y.T. and R.Y.Z.). Tables 1, 2 summarized basic study characteristics, including authorship, country and year of publication, study type, detection medium, analysis method, sample size, CRC stage, statistical analysis methodology, sampler, sensitivity, specificity, and the area under the curve (AUC), as well as accuracy.

2.5 Quality assessment

The Quality Assessment of Diagnostic Studies 2 tool (QUADAS-2) (56) was conducted to assess the quality of the included studies. This evaluation encompassed four domains: patient selection, index test, reference standard, and patient flow and timing. Ratings were assigned as "low risk," "unclear," or "high risk". The assessment was conducted independently by two investigators (Y.F.J. and Z.H.L.), and any disparities were resolved through the involvement of a third investigator (X.P.H). The complete QUADAS-2 version can be found in Supplement.

2.6 Statistical analysis

This meta-analysis was performed by a bivariate model to obtain summary estimates of sensitivity, specificity, and positive and negative likelihood ratios. The Deeks funnel plot asymmetry test was employed to discern publication bias (57). A two-sided $P<0.10$ was deemed statistically significant. Statistical heterogeneity was evaluated among pooled studies using I^2 index. STATA software (version 16 SE; Stata Corporation, College Station, TX, USA) was used to aggregate analysis and the statistical package MIDAS was used for bivariate meta-analysis and summary receiving operate characteristic (SROC) curve calculation with 95% confidence region. Subgroup analyses were performed by Open Meta-Analyst software to explore sources of heterogeneity based on the characteristics of the included articles.

3 Results

3.1 Study selection

The literature search strategy yielded an initial pool of 192 articles. Following review, 110 articles were excluded based on title and abstract screening. Subsequently, 59 full-text articles, with a total of 4688 subjects underwent scrutiny against the inclusion criteria. Ultimately, 32 studies fulfilled the inclusion criteria for this review. The selection process of the studies is shown in the PRISMA diagram-Figure 1.

3.2 Study characteristics

All thirty-two studies included in this review were published in English (7, 25, 26, 28–55, 58). Among them, 22 studies employed VOC analysis for the diagnosis of colorectal cancer (7, 28–46, 48, 49), 9 studies utilized e-nose technology (25, 26, 50–55, 58), and one study used both VOC analysis and e-nose (47). In the VOC studies, 10 studies used breath samples (7, 28–30, 38, 39, 41–43, 49), 6 studies used urine samples (32, 37, 44–46, 48), 5 studies used fecal samples (31, 33, 35, 36, 40), and one study used salivary sample (34). Most studies used MS-based techniques, principally GC-MS (n=7), TD-GC-MS (n=4), FAIM (n=4), and SIFT-MS (n=2). In E-nose studies, 5 studies used breath samples (25, 50–52, 58), two studies used urine samples (53, 54), and two studies used fecal samples (26, 55). One study used both VOC analysis and e-nose technology in testing urine samples (47). The most commonly used e-noses were Aeonose (n=3), PEN3 (n=2), and WOLF (n=2). All studies were prospective, 25 were case-control studies, and 7 employed cross-sectional studies. Logistic regression analysis (LRA) and partial least squares discriminant analysis (PLS-DA) emerged as the most frequently reported analytical methods. Other reported analytical methods encompassed artificial neural network (ANN), support vector machine (SVM), linear discriminant analysis (LDA), random forest (RF), probabilistic neural network (PNN), discriminant function analysis (DFA), and neural network (NN). The majority of studies were conducted in hospital settings, with 29 studies in Europe, two in Asia, and one with an undisclosed location. Tables 1, 2 provides an overview of the fundamental characteristics of the studies.

3.3 Risk of bias

The quality appraisal of all incorporated literature was conducted according to the QUADAS-2 scale through Review Manager 5.4 software. The results of the risk of bias assessment are visually presented in Figures 2A, B.

In the aggregate, a few studies exhibited a high risk of bias. Concerning 'patient selection' seven studies (32, 34, 39, 42, 46, 53, 54) (21.9%) incurred a high risk of bias. The primary contributor to this high risk pertained to the absence of a detailed description of the sampling process and the implementation of a case-control study design. Regarding the 'index test' while most studies employed reference diagnostic tests to delineate the definition of a positive

TABLE 1 Basic characteristics and outcomes of VOC studies in the analysis.

Study	year	country	Type of Study	Detection medium	Analysis method	No of CRC patients	No of controls	Stage of CRC	Statistical method	Sampler	Sensitivity, %	Specificity, %	AUC, %	Accuracy, %
Altomare et al. (28)	2013	Italy	case-control	breath	GC-MS	37	41 (healthy)	I/II:19 III/IV:18	PNN	Tedlar bag	86	83	85.2	85
Altomare et al. (29)	2015	Italy	case-control	breath	TD-GC-MS	48	55 (healthy)	I/II:28 III/IV:20	PNN	Tedlar bag	100	97.72	100	98.75
Altomare et al. (30)	2020	Italy	case-control	breath	GC-MS	83	90 (non-cancer)	I/II:38 III/IV:42	LRA	ReCIVA	90	93	97.9	NR
Alustiza et al. (31)	2023	Spain	case-control	feces	TD-GC-MS	24	32(healthy) 24 (Adenomas)	I/II:7 III/IV:17	ANOVA	plastic container	83	82	85	NR
Arasaradnam et al. (32)	2014	UK	case-control	urine	FAIMS	83	50 (healthy)	NR	FDA	ATLAS sampler	88	60	NR	NR
Batty et al. (33)	2015	UK	case-control	feces	SIFT-MS	31	31 (healthy)	NR	PLS-DA	Nalophan sampler	72	78	NR	75
Bel'skaya et al. (34)	2020	Russia	case-control	salivary	capillary GS	18	16 (noncancer)	I/II:25 III/IV:38	CRT	NR	92.3	100	NR	NR
Bond et al. (35)	2019	UK	case-control	feces	GC-MS	21	60 (non-cancer)	NR	PLS-DA, LRA	OdoReader box	87.9	84.6	82	NR
Bosch et al. (36)	2020	Netherlands	case-control	feces	GC-IMS	14	227 (healthy)	AA:24	LRA, RF, SVM,NN	NR	100	100	96.1	NR
Boulind et al. (37)	2022	UK	cross-sectional	urine	GC-MS	558 (suspected)	NR	NR	ANN	NR	87.8	88.2	89.6	NR
Cheng et al. (38)	2022	Netherlands	cross-sectional	breath	TD-GC-TOF-MS	30	84 (negative colonoscopy)	AA:138	RF	Tedlar bag	80	70	NR	NR
Depalma et al. (39)	2014	Italy	case-control	breath	TD-GC-MS	15	15 (healthy)	NR	LDA	Tedlar bag	96.5	100	NR	NR
Ishibe et al. (40)	2018	Japan	case-control	feces	GC-MS	30	26 (healthy)	NR	PLS-DA	Tedlar bag	90	57.7	NR	75
Leja et al. (41)	2015	NR	case-control	breath	GC-MS	71	131 (healthy)	NR	NR	NR	85	90	NR	88
Lena et al. (42)	2012	Italy	case-control	breath	TD-GC-MS	34	36 (healthy)	NR	SVM	Tedlar bag	83	88	94.4	80

(Continued)

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TABLE 1 Continued

Study	year	country	Type of Study	Detection medium	Analysis method	No of CRC patients	No of controls	Stage of CRC	Statistical method	Sampler	Sensitivity, %	Specificity, %	AUC, %	Accuracy, %
Markar et al. (43)	2019	UK	case-control	breath	SIFT-MS	50	100 (healthy)	NR	LRA	NR	96	76	NR	NR
McFarlane et al. (44)	2019	UK	case-control	urine	FAIMS	56	82 (non-cancer)	NR	RF	NR	69	69	72	NR
Mozdiak et al. (45)	2019	UK	cross-sectional	urine	FAIMS and GC-IMS	163 (positive FOBT)	NR	NR	SLR, GPC	NR	100	100	98	NR
Politi et al. (7)	2021	Italy	case-control	breath	IMR-MS	52	45 (healthy)	NR	LRA	NR	96	93	NR	NR
Psutka et al. (46)	2017	JAPAN	case-control	urine	FAIMS	139	78 (healthy)	NR	PCA	NR	67.4	82.1	NR	NR
Tyagi et al. (47)	2021	UK	case-control	urine	GC-TOF-MS	58	38 (healthy)	I/II:24 III/IV:34	RF, NN	NR	86	81	93	NR
Widlak et al. (48)	2018	UK	cross-sectional	urine	FAIMS	562 (Completed colonoscopy)	NR	NR	PCA	NR	63	63	NR	NR
Zambrana et al. (49)	2012	Spain	case-control	breath	GC-MS	38	43 (healthy)	NR	NR	NR	87.06	76.85	NR	NR

AA, advanced adenomas; ANN, artificial neural network; CRC, colorectal cancer; CRT, Classification and Regression Tree; FDA, Fisher Discriminant Analysis; FOBT, fecal occult blood testing; GPC, Gaussian process classifier; LDA, linear discriminant analysis; LRA, logistic regression analysis; NR, Not reported; NN, Neural Network; PCA, Principal Component Analysis, PLS-DA, partial least squares discriminant analysis; PNN, Probabilistic Neural Network; RF, Random Forest; SLR, Sparse Logistic Regression, SVM, support vector machine.

10.3389/fonc.2024.1397259

TABLE 2 Basic characteristics and outcomes of e-nose studies in the analysis.

source	year	country	Type of Study	Detection medium	E-Nose type	NO of CRC patients	No of controls	Stage of CRC	Statistical method	Sampler	Sensitivity, %	Specificity, %	AUC, %	Accuracy, %
Amal et al. (14)	2015	Latvia	case-control	breath	Prototype: 6 nanomaterial sensors (GNP and SWCNTs)	65	122 (healthy)	AA:22	DFA	NR	85	94	NR	91
Altomare et al. (50)	2016	Italy	case-control	breath	PEN3:10 MOS	15	15 (healthy)	I/II:1 III/IV:14	PNN	Tedlar bag	93.3	10	NR	37.78
de Meij et al. (26)	2014	Netherlands	case-control	feces	Cyranose 320:32 conducting polymer sensors	40	57 (healthy)	AA:60	CDA	BD box	85	87	NR	92
Steenhuis et al. (51)	2020	Netherlands	cross-sectional	breath	Aeonose:3 MOS	62	NR	I/II:25 III/IV:37	ANN	NR	88	75	NR	NR
Tyagi et al. (47)	2021	UK	case-control	urine	PEN3:10 MOS	58	38 (healthy)	I/II:24 III/IV:34	RF, NN	NR	91	55	81	NR
van de Goor et al. (52)	2017	Netherlands	case-control	breath	Aeonose:3 MOS	28	100 (HNSCC)	NR	ANN	NR	79	81	NR	81
van Keulen et al. (25)	2019	Netherlands	cross-sectional	breath	Aeonose:3 MOS	447t (colonoscopy patients)	NR	NR	ANN	NR	95	64	74	84
Westenbrink et al. (53)	2015	UK	case-control	urine	WOLF:13 electro-chemical sensors	39	18 (healthy)	NR	LDA	NR	92	77	NR	NR
Westenbrink et al. (54)	2016	UK	case-control	urine	WOLF:13 electro-chemical sensors	26	23(IBS)	NR	LDA, KNN	sample box	84.1	82.4	NR	NR
Zonta et al. (55)	2020	Italy	cross-sectional	faeces	SCENT A1: 5 semiconductor gas sensors	398 (colonoscopy patients)	NR	NR	SVM	sample box	116	46	22	214

AA, advanced adenomas; ANN, artificial neural network; CDA, canonical discriminant analysis; CRC, colorectal cancer; DFA, discriminant function analysis; HNSCC, head and neck squamous cell carcinoma; IBS, Irritable bowel syndrome patients; KNN, K Nearest Neighbors; LDA, linear discriminant analysis; NR, Not reported; NN, Neural Network; PNN, Probabilistic Neural Network; RF, Random Forest; SVM, support vector machine.

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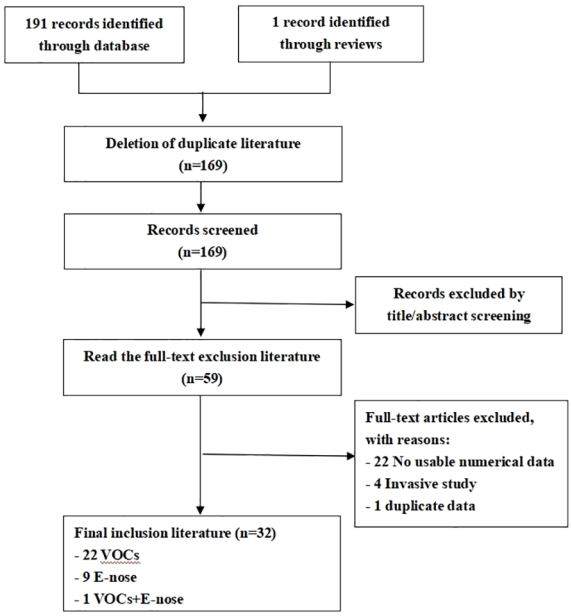

FIGURE 1
Flow chart of the study selection process.

test, only nine studies ensured adequate blinding (26, 29, 30, 35, 43–45, 48, 55), leaving 23 studies with an unspecified risk of bias concerning the 'index test'. Concerning 'reference standard', none of the 13 studies (28, 32, 34, 39, 42, 46–50, 52–54) reported the reference standard test. Concerning 'flow and timing', five studies (39, 40, 42, 46, 52) faced a high risk of bias. The primary reason for this was that these studies do not account for the time interval between the index test and the reference test.

In evaluating clinical applicability, significant concerns in patient selection arose from the absence of matched patient groups, inadequate patient selection criteria, and applicability of the study design to the research question. Six studies exhibited a high applicability concern for patient selection criteria (26, 32, 42, 46, 49, 53). No high-risk concerns were identified regarding the applicability of the index and reference tests to the research questions.

3.4 Diagnostic accuracy

The pooled sensitivity and specificity of VOC analysis for detecting CRC were 0.88 (95% CI, 0.83-0.92) and 0.85 (95% CI, 0.78-0.90), respectively (Figure 3). Similarly, the pooled sensitivity of the e-nose was 0.87 (95% CI, 0.83-0.90), with a specificity of 0.78 (95% CI, 0.62-0.88) (Figure 4). Notably, in VOC studies, the I^2 index was 82.86% for sensitivity and 90.36% for specificity, while for e-nose studies, it was 23.31% for sensitivity and 89.46% for specificity. Pooled receiver operating characteristic analysis of VOC studies resulted in an area under the curve (AUC) of 0.93 (95% CI, 0.90-0.95) (Figure 5). For e-nose studies, the AUC was 0.90 (95% CI, 0.87-0.92) (Figure 6). The Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), and Diagnostic Odds Ratio (DOR) of VOC studies were 5.8 (95% CI, 3.9-8.7), 0.14 (95% CI, 0.09-0.21), and 41 (95% CI, 19-87), respectively. For e-nose studies, the PLR, NLR, and DOR were 3.9 (95% CI, 2.2-6.7), 0.17 (95% CI, 0.13-0.21), and 23 (95% CI, 13-44), respectively.

The funnel plots for publication bias are displayed in Figures 7, 8. The Deeks' regression test for funnel plot asymmetry demonstrated an absence of publication bias among the studies included, with slope coefficients P values of 0.28 and 0.62 for using VOC analysis and e-nose.

3.5 Subgroup analysis

We compared the accuracy of different samples of included studies. A separate pooled analysis of breath VOCs studies exhibited good efficacy, with a sensitivity of 0.819 (95% CI, 0.720-0.888) and a specificity of 0.907 (95% CI, 0.876-0.932) (Table 3). A Separate pooled analysis of GC-MS, TD-GC-MS, and FAIMS methods, showed a sensitivity of 0.732 (95%CI, 0.519-0.874) and a specificity of 0.919 (95%CI, 0.867-0.952) for GC-MS, and a sensitivity of 0.898 (95% CI, 0.756-0.962) and a specificity of 0.889 (95% CI, 0.783-0.947) for TD-GC-MS, and a sensitivity of 0.635 (95% CI, 0.299-0.877) and a specificity of 0.775 (95% CI, 0.568-0.901) for FAIMS (Table 3).

For e-nose studies, exhaled breath samples demonstrated a better specificity of 0.911 (95% CI, 0.859-0.945) but a lower sensitivity of 0.708 (95% CI, 0.543-0.833) (Table 4). A separate pooled analysis for different types of e-Nose demonstrated that Aeonose could detect colorectal with a sensitivity of 0.682 (95% CI, 0.506-0.817) and a specificity of 0.916 (95% CI, 0.832-0.960). Separate pooled analysis for PEN3 showed a sensitivity of 0.654 (95% CI, 0.401-0.843) and a specificity of 0.791 (95% CI, 0.605-

FIGURE 2
(A) Summary and separate outcome of risk of bias and concerns. **(B)** Summary and separate outcome of risk of bias and concerns regarding applicability for included studies using QUADAS-2 tool.

0.903). For WOLF the sensitivity was 0.906 (95%CI, 0.790-0.961) and the specificity was 0.790 (95%CI, 0.359-0.962) (Table 4).

Additional sensitivity analysis for advanced adenomas demonstrated good accuracy in VOC analysis, with a sensitivity of 0.824 (95% CI, 0.770-0.867) and specificity of 0.908 (95% CI, 0.658-0.981) (Table 3). For e-nose studies, the sensitivity and specificity for the detection of advanced adenomas were 0.755 (95% CI, 0.609-0.859) and 0.704 (95% CI, 0.628-0.770), respectively (Table 4).

4 Discussion

We conducted a systematic review and meta-analysis to evaluate VOC analysis and electronic nose in detecting colorectal

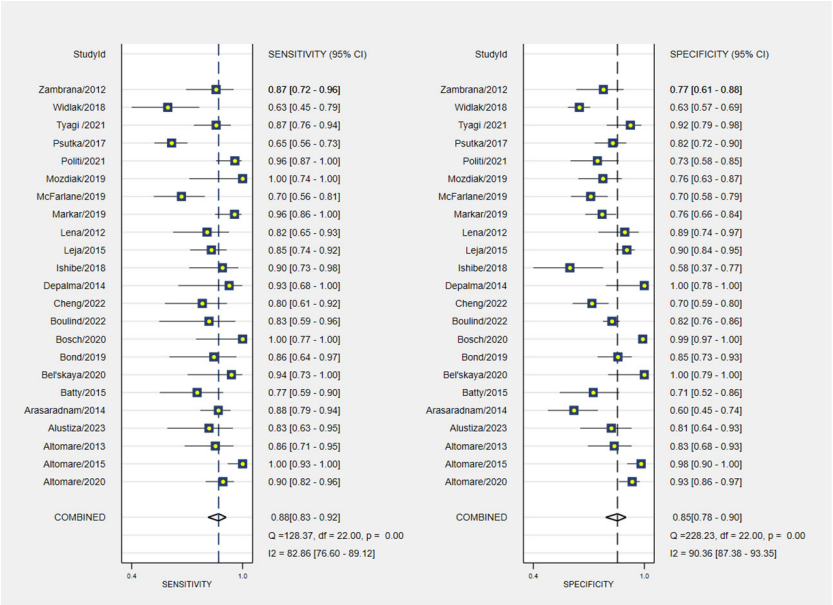

FIGURE 3
Pooled sensitivity and specificity analyses of VOC studies.

cancer, aiming to compare the diagnostic accuracy and clinical application value of these two methods. Pooled analysis of VOC and electronic-nose studies demonstrated high diagnostic accuracy for CRC detection, with a pooled sensitivity of 0.88 and specificity of 0.85 for VOC analysis and a sensitivity of 0.87 and specificity of 0.78 for e-nose studies. The visually assessed SROC curves indicated clinical accuracy, with VOC analysis and e-nose having SROC curves of approximately 0.93 and 0.90, respectively, both close to 1, signifying superior accuracy and diagnostic efficacy in CRC diagnosis. These findings align with prior reviews (22, 59, 60), but the notable heterogeneity between studies and the identified high risk of bias warrant cautious interpretation. The heterogeneity was largely due to the sample media and the analytical methods used.

Subgroup analyses revealed that breath samples in VOC analysis and urine and breath samples in e-nose studies exhibited higher sensitivity or specificity. Breath sampling is easily performed and well-received by patients, and urine samples, boasting high sensitivity and specificity, emerge as valuable alternatives. Recent

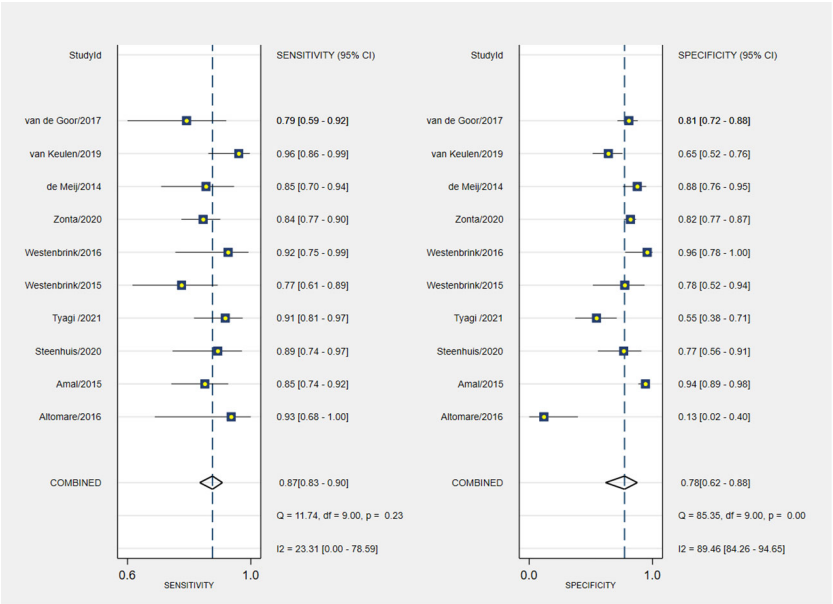

FIGURE 4
Pooled sensitivity and specificity analyses of e-noses studies.

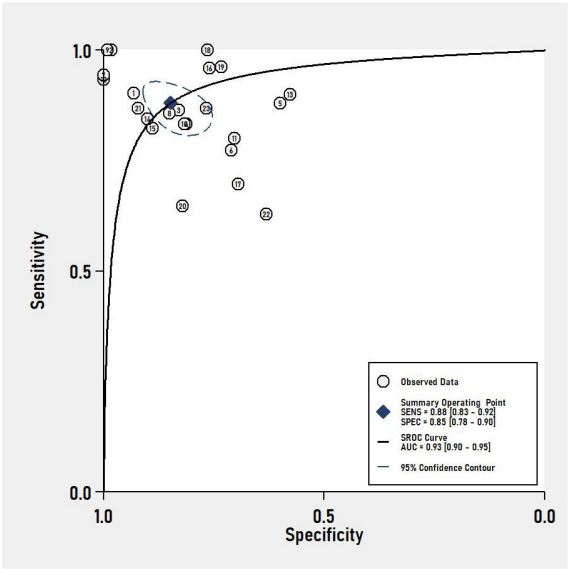

FIGURE 5
Summary receiver operating characteristic (SROC) curve Analysis of VOC studies.

meta-analysis evaluated the performance of the combined FIT and urinary. The findings revealed that the combined FIT-VOC approach could detect 33% more cases of colorectal cancers (60). Chandrapalan S et al. (61) showed that the combination of FIT and VOC can be a better triage tool, for CRC in patients with lower gastrointestinal symptoms than FIT alone.

Due to the lack of standardization in sample collection, handling, and storage, technical barriers exist in measuring and analyzing various VOC characteristics during sampling, whether it involves alveolar air, urine, or feces. In several studies, exhaled

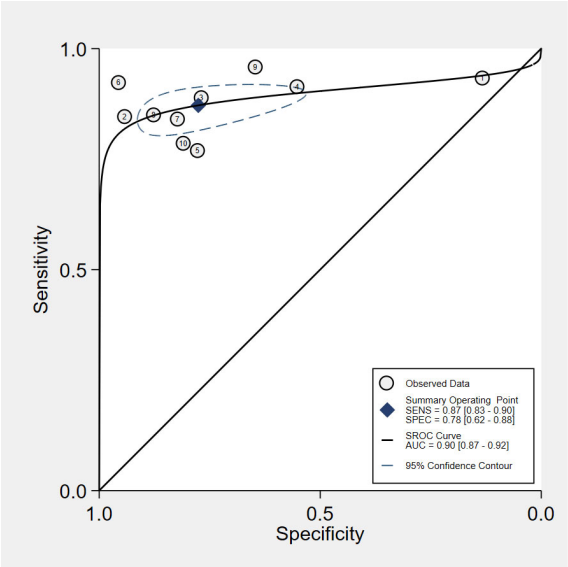

FIGURE 6
Summary receiver operating characteristic (SROC) curve Analysis of e-noses studies.

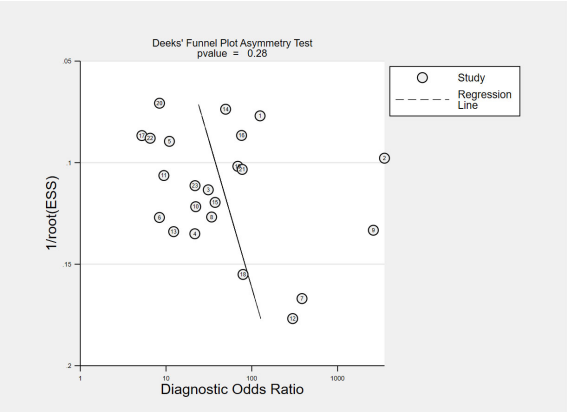

FIGURE 7
Public bias analysis of all the VOC studies.

breath was collected into a bag and subsequently analyzed (28, 29, 33, 38–40, 42, 50). The use of bag collection aligns more closely with real-world medical applications. However, this approach may be influenced by several factors, including interference from ambient VOCs, the material used for collection, and the impact of temperature, humidity, and storage time on specimens (62). For breath samples, it is essential to examine them within 6 hours of the collection's conclusion to ensure test accuracy (63). Therefore, developing methods for the collection, transmission, and handling of breath samples is crucial for the success of this approach. Some studies have indicated that the diagnostic accuracy of fecal and urine VOCs is not significantly affected by storage time (20 months for fecal and 12 months for urine VOCs) (64, 65).

Urine samples are ideal detection medium because they have limited confounding factors compared to breath samples which is influenced by smoking or fecal samples influenced by diet. Further research should standardize the method of collection of such samples and investigate the effects of potential confounding factors.

Among all studies, only six reported on CRC stages, indicating limited generalizability and clinical applicability. Multi-center

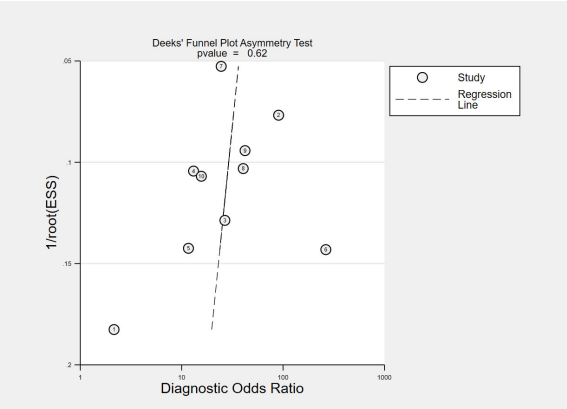

FIGURE 8
Public bias analysis of all the e-nose studies.

TABLE 3 Subgroup analysis in VOC studies.

Subgroup	Sensitivity (95% CI)	I^2	Specificity (95% CI)	I^2
Detects medium				
Breath Samples (n=10)	0.819 (0.720, 0.888)	80.64%	0.907 (0.876, 0.932)	15.58%
Urine Samples (n=7)	0.627(0.365, 0.831)	95.68%	0.862 (0.710, 0.941)	92.09%
Fecal Samples (n=5)	0.730 (0.649, 0.797)	0%	0.905 (0.769, 0.965)	72.52%
The sample analysis method used				
GC-MS (n=7)	0.732 (0.519, 0.874)	0%	0.919 (0.867, 0.952)	60.11%
TD-GC-MS (n=4)	0.898 (0.756, 0.962)	34.83%	0.889 (0.783, 0.947)	30.68%
FAIM (n=4)	0.635 (0.299, 0.877)	3.8%	0.775 (0.568, 0.901)	92.47%
CRC stage				
Advanced adenomas VS. non-cancer control (n=3)	0.824 (0.770, 0.867)	0%	0.908 (0.658, 0.981)	94.03%

validation studies of the diagnostic performance of VOCs on early stages of CRC and its precursor lesions (adenomas or not) is warranted, which could reduce the incidence of CRC.

It has been demonstrated that various factors, such as age, gender, smoking, alcohol consumption, coffee intake, and the consumption of stimulating foods like leeks and garlic, as well as comorbidities and medication, may influence the composition of VOCs in exhaled breath (66). However, only a few studies considered confounding or modifying effects, limiting the validity and reliability of the results. Therefore, future studies should account for the impact of such factors on breath prints during the design phase.

Gas chromatography-mass spectrometry (GC-MS), a traditional method for VOC analysis, is a highly standardized technique providing qualitative and quantitative information on exhaled VOCs (67, 68). In this study, TD-GC-MS demonstrated high sensitivity and specificity in detecting colorectal cancer, while GC-MS exhibited improved specificity but suboptimal sensitivity. The use of GC-MS and newer mass spectrometry technology devices remains the gold standard for identifying specific VOCs for analysis. However, GC-MS technology is costly and complex, with long analysis times, and it demands a high level of expertise from operators.

Based on sensors, electronic nose technology serves as a novel analytical method for disease diagnosis, offering the advantages of being cost-effective, user-friendly, portable, sensitive, and responsive. Nevertheless, there are existing shortcomings that require refinement in the application of e-nose in clinical practice. Unlike GC-MS and other techniques, e-nose lacks the precision to measure specific types and composition ratios of components in VOCs (24). It also cannot identify specific pathophysiological pathways or therapeutic targets. Furthermore, as the e-nose relies on arrays of gas sensors to distinguish and identify response spectra of mixtures composed of multiple VOCs, the diverse sensor types with distinct signal responses prevent the integration of results from one e-nose with different devices or sensor types (69). Van der Sar IG (70) recommends the establishment of a comprehensive worldwide shared database encompassing patient characteristics and other pretest probabilities.

TABLE 4 Subgroup analysis in e-nose studies.

Subgroup	Sensitivity (95% CI)	I^2	Specificity (95% CI)	I^2
Detects medium				
Breath Samples (n=5)	0.708 (0.543, 0.833)	80.64%	0.911 (0.859, 0.945)	15.58%
Urine Samples (n=3)	0.857 (0.689, 0.942)	95.68%	0.786 (0.563, 0.913)	92.09%
Fecal Samples (n=2)	0.758 (0.631, 0.852)	0%	0.904 (0.864, 0.933)	72.52%
E-Nose type				
Aeonose (n=3)	0.682 (0.506, 0.817)	74.62%	0.916 (0.832, 0.960)	37.95%
PEN3 (n=2)	0.654 (0.401, 0.843)	79.99%	0.791 (0.605, 0.903)	0%
WOLF (n=2)	0.906 (0.790, 0.961)	2.06%	0.790 (0.359, 0.962)	80.97%
CRC stage				
Advanced adenomas VS. non-cancer control (n=3)	0.755 (0.609, 0.859)	55.43%	0.704 (0.628, 0.770)	0%

Various algorithms and methods were employed to analyze VOCs in this study, with PLA-DA and logistic regression analysis emerging as the most commonly used approaches. However, the majority of studies fail to elucidate the rationale behind selecting a specific machine learning model for analysis, only reporting the highest accuracy value, thereby impacting the reliability of the results. Additionally, studies with small sample sizes may compromise the reported accuracy. Few studies have conducted external validation to affirm the validity and reliability of these findings. Consequently, large, multi-center external validation studies should be conducted in the future to explore the applicability and reproducibility of the results in different study settings and among diverse target populations.

4.1 Limitation

This study has certain limitations. Heterogeneity was observed among studies, potentially attributed to variations in sample media and analytical methods. Some studies exhibited a high risk of bias, with seven showing concern regarding patient selection and ten having applicability concerns in one or two domains. Furthermore, the study included fewer investigations employing both VOC analysis and e-nose technology, thus impeding an accurate evaluation of the complementary effects of the two methods. In addition, VOC combined with FIT approach could increase the detection of colorectal cancer. However, there are no prospective studies evaluating the positive effect on VOC-FIT for screening prior to the onset of CRC.

5 Conclusion

Based on our meta-analysis, VOC analysis and e-nose technology show promise in the detection of CRC. However, several milestones must be achieved in colorectal cancer detection with these two non-invasive methods before clinical implementation. Firstly, for patients presenting with common non-specific symptoms, which may be an early indication of CRC, an exhaled breath test or a urine test or FIT +VOC could serve as screening tool. Secondly, electronic nose could be utilized in primary care units and community healthcare centers for mass screening of various intestinal diseases due to their portability, ease of use, cost-effectiveness, speed, and independence from specialized technicians. Thirdly, the identification of colorectal cancer-specific VOC biomarkers and combinations of biomarkers for colorectal cancer diagnosis is still necessary. This requires comprehensive metabolomics studies to elucidate the production of endogenous VOCs and the metabolic transformation of exogenous VOCs in colorectal cancer, aiding in the identification of VOC markers for cancer. Finally, large, multi-center external validation trials should be conducted to verify the generalizability and reproducibility of the results in different research settings and at different stages of CRC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

QW: Formal analysis, Funding acquisition, Supervision, Writing – original draft. YF: Investigation, Project administration, Writing – original draft. ST: Methodology, Writing – review & editing. ZL: Data curation, Project administration, Writing – review & editing. RZ: Data curation, Investigation, Writing – review & editing. YR: Methodology, Project administration, Writing – original draft. YJ: Project administration, Software, Writing – original draft. XH: Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1397259/full#supplementary-material

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin*. (2021) 71:209–49. doi: 10.3322/caac.21660

2. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet*. (2019) 394:1467–80. doi: 10.1016/S0140-6736(19)32319-0

3. Shaukat A, Levin TR. Current and future colorectal cancer screening strategies. *Nat Rev Gastroenterol Hepatol*. (2022) 19:521–31. doi: 10.1038/s41575-022-00612-y

4. Imperiale TF, Gruber RN, Stump TE, Emmett TW, Monahan PO. Performance characteristics of fecal immunochemical tests for colorectal cancer and advanced adenomatous polyps: A systematic review and meta-analysis. *Ann Internal Med*. (2019) 170:319–29. doi: 10.7326/M18-2390

5. Bertels L, Lucassen P, van Asselt K, Dekker E, van Weert H, Knottnerus B. Motives for non-adherence to colonoscopy advice after a positive colorectal cancer screening test result: a qualitative study. *Scandinavian J primary Health Care*. (2020) 38:487–98. doi: 10.1080/02813432.2020.1844391

6. Chen H, Li N, Ren J, Feng X, Lyu Z, Wei L, et al. Participation and yield of a population-based colorectal cancer screening programme in China. *Gut*. (2019) 68:1450–7. doi: 10.1136/gutjnl-2018-317124

7. Politi L, Monasta L, Rigressi MN, Princivalle A, Gonfiotti A, Camiciottoli G, et al. Discriminant profiles of volatile compounds in the alveolar air of patients with squamous cell lung cancer, lung adenocarcinoma or colon cancer. *Molecules*. (2021) 26:550. doi: 10.3390/molecules26030550

8. Shirasu M, Touhara K. The scent of disease: volatile organic compounds of the human body related to disease and disorder. *J Biochem*. (2011) 150:257–66. doi: 10.1093/jb/mvr090

9. Hakim M, Broza YY, Barash O, Peled N, Phillips M, Amann A, et al. Volatile organic compounds of lung cancer and possible biochemical pathways. *Chem Rev*. (2012) 112:5949–66. doi: 10.1021/cr300174a

10. Wang C, Li P, Lian A, Sun B, Wang X, Guo L, et al. Blood volatile compounds as biomarkers for colorectal cancer. *Cancer Biol Ther*. (2014) 15:200–6. doi: 10.4161/cbt.26723

11. Sethi S, Nanda R, Chakraborty T. Clinical application of volatile organic compound analysis for detecting infectious diseases. *Clin Microbiol Rev*. (2013) 26:462–75. doi: 10.1128/CMR.00020-13

12. Monedeiro F, Dos Reis RB, Peria FM, Sares CTG, De Martinis BS. Investigation of sweat VOC profiles in assessment of cancer biomarkers using HS-GC-MS. *J Breath Res*. (2020) 14:026009. doi: 10.1088/1752-7163/ab5b3c

13. de Boer NK, de Meij TG, Oort FA, Ben Larbi I, Mulder CJ, van Bodegraven AA, et al. The scent of colorectal cancer: detection by volatile organic compound analysis. *Clin Gastroenterol Hepatol*. (2014) 12:1085–9. doi: 10.1016/j.cgh.2014.05.005

14. Amal H, Shi DY, Ionescu R, Zhang W, Hua QL, Pan YY, et al. Assessment of ovarian cancer conditions from exhaled breath. *Int J Cancer*. (2015) 136:E614–22. doi: 10.1002/ijc.29166

15. Peng G, Hakim M, Broza YY, Billan S, Abdah-Bortnyak R, Kuten A, et al. Detection of lung, breast, colorectal, and prostate cancers from exhaled breath using a single array of nanosensors. *Br J Cancer*. (2010) 103:542–51. doi: 10.1038/sj.bjc.6605810

16. Barash O, Zhang W, Halpern JM, Hua QL, Pan YY, Kayal H, et al. Differentiation between genetic mutations of breast cancer by breath volatolomics. *Oncotarget*. (2015) 6:44864–76. doi: 10.18632/oncotarget.v6i42

17. Corradi M, Poli D, Banda I, Bonini S, Mozzoni P, Pinelli S, et al. Exhaled breath analysis in suspected cases of non-small-cell lung cancer: a cross-sectional study. *J Breath Res*. (2015) 9:027101. doi: 10.1088/1752-7155/9/2/027101

18. Bouza M, Gonzalez-Soto J, Pereiro R, de Vicente JC, Sanz-Medel A. Exhaled breath and oral cavity VOCs as potential biomarkers in oral cancer patients. *J Breath Res*. (2017) 11:016015. doi: 10.1088/1752-7163/aa5e76

19. Guo L, Wang C, Chi C, Wang X, Liu S, Zhao W, et al. Exhaled breath volatile biomarker analysis for thyroid cancer. *Trans Res*. (2015) 166:188–95. doi: 10.1016/j.trsl.2015.01.005

20. Qin T, Liu H, Song Q, Song G, Wang HZ, Pan YY, et al. The screening of volatile markers for hepatocellular carcinoma. *Cancer epidemiology Biomarkers Prev*. (2010) 19:2247–53. doi: 10.1158/1055-9965.EPI-10-0302

21. Gardner JW, Bartlett PN. A brief history of electronic noses. *Sensors Actuators B: Chem*. (1994) 18:210–1. doi: 10.1016/0925-4005(94)87085-3

22. Scheepers M, Al-Difaie Z, Brandts L, Peeters A, van Grinsven B, Bouvy ND. Diagnostic performance of electronic noses in cancer diagnoses using exhaled breath: A systematic review and meta-analysis. *JAMA network Open*. (2022) 5:e2219372. doi: 10.1001/jamanetworkopen.2022.19372

23. Licht JC, Grasemann H. Potential of the electronic nose for the detection of respiratory diseases with and without infection. *Int J Mol Sci*. (2020) 21:9416. doi: 10.3390/ijms21249416

24. van Keulen KE, Jansen ME, Schrauwen RWM, Kolkman JJ, Siersema PD. The smell of lung disease: a review of the current status of electronic nose technology. *Respir Res*. (2021) 22:246. doi: 10.1186/s12931-021-01835-4

25. van Keulen KE, Jansen ME, Schrauwen RWM, Kolkman JJ, Siersema PD. Volatile organic compounds in breath can serve as a non-invasive diagnostic biomarker for the detection of advanced adenomas and colorectal cancer. *Alimentary Pharmacol Ther*. (2019) 51:334–46. doi: 10.1111/apt.15622

26. de Meij TG, Larbi IB, van der Schee MP, Lentferink YE, Paff T, Terhaar Sive Droste JS, et al. Electronic nose can discriminate colorectal carcinoma and advanced adenomas by fecal volatile biomarker analysis: proof of principle study. *Int J Cancer*. (2014) 134:1132–8. doi: 10.1002/ijc.28446

27. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ (Clinical Res ed)*. (2009) 339:b2700. doi: 10.1136/bmj.b2700

28. Altomare DF, Di Lena M, Porcelli F, Travaglio E, Longobardi F, Tutino M, et al. Exhaled volatile organic compounds identify patients with colorectal cancer. *Br J Surg*. (2013) 100:144–50. doi: 10.1002/bjs.8942

29. Altomare DF, Picciariello A, Rotelli MT, De Fazio M, Aresta A, Zambonin CG , et al. Effects of curative colorectal cancer surgery on exhaled volatile organic compounds and potential implications in clinical follow-up. *Ann Surg*. (2015) 262:862–7. doi: 10.1097/SLA.0000000000001471

30. Altomare DF, Porcelli F, Picciariello A, Pinto M, Di Lena M, Caputi Iambrenghi O, et al. Chemical signature of colorectal cancer: case–control study for profiling the breath print. *BJS Open*. (2020) 4:1189–99. doi: 10.1002/bjs5.50354

31. Alustiza M, Ripoll L, Canals A, Murcia O, Martínez-Roca A, García-Heredia A, et al. A novel non-invasive colorectal cancer diagnostic method: Volatile organic compounds as biomarkers. *Clinica Chimica Acta*. (2023) 542:1189–99. doi: 10.1016/j.cca.2023.117273

32. Arasaradnam RP, McFarlane MJ, Ryan-Fisher C, Westenbrink E, Hodges P, Thomas MG, et al. Detection of colorectal cancer (CRC) by urinary volatile organic compound analysis. *PloS One*. (2014) 9. doi: 10.1371/journal.pone.0108750

33. Batty CA, Cauchi M, Lourenço C, Hunter JO, Turner C. Use of the analysis of the volatile faecal metabolome in screening for colorectal cancer. *PLoS One*. (2015) 10. doi: 10.1371/journal.pone.0130301

34. Bel'skaya LV, Sarf EA, Shalygin SP, Postnova TV, Kosenok VK. Identification of salivary volatile organic compounds as potential markers of stomach and colorectal cancer: A pilot study. *J Oral Biosci*. (2020) 62:212–21. doi: 10.1016/j.job.2020.05.002

35. Bond A, Greenwood R, Lewis S, Corfe B, Sarkar S, O'Toole P, et al. Volatile organic compounds emitted from faeces as a biomarker for colorectal cancer. *Alimentary Pharmacol Ther*. (2019) 49:1005–12. doi: 10.1111/apt.15140

36. Bosch S, Bot R, Wicaksono A, Savelkoul E, van der Hulst R, Kuijvenhoven J, et al. Early detection and follow-up of colorectal neoplasia based on faecal volatile organic compounds. *Colorectal Dis*. (2020) 22:1119–29. doi: 10.1111/codi.15009

37. Boulind CE, Gould O, de Lacy Costello B, Allison J, White P, Ewings P, et al. Urinary volatile organic compound testing in fast-track patients with suspected colorectal cancer. *Cancers*. (2022) 14:2127. doi: 10.3390/cancers14092127

38. Cheng HR, van Vorstenbosch RWR, Pachen DM, Meulen LWT, Straathof JWA, Dallinga JW, et al. Detecting colorectal adenomas and cancer using volatile organic compounds in exhaled breath: A proof-of-principle study to improve screening. *Clin Trans Gastroenterol*. (2022) 13. doi: 10.14309/ctg.0000000000000518

39. Depalma N, Di Lena M, Porcelli F, Travaglio E, Longobardi F, Demarinis Loiotile A, et al. Detection of colorectal polyps by exhaled VOCs. Preliminary data. *Techniques Coloproctology*. (2014) 18:92–3. doi: 10.1007/s10151-013-1096-6

40. Ishibe A, Ota M, Takeshita A, Tsuboi H, Kizuka S, Oka H, et al. Detection of gas components as a novel diagnostic method for colorectal cancer. *Ann Gastroenterological Surg*. (2018) 2:147–53. doi: 10.1002/ags3.12056

41. Leja M, Amal H, Funka K, Vanags A, Kikuste I, Sivins A, et al. Nanoarray sensor technology-based volatile marker tests to detect colorectal cancer and colonic adenomas. *Gastroenterology*. (2015) 148. doi: 10.1016/S0016-5085(15)30059-7

42. Lena MD, Porcelli F, Trizio L, Giuratrabocchetta S, Travaglio E, Tutino M, et al. Colorectal cancer screening by breath analysis: A specific pattern of volatile organic compounts (VOCs) can discriminate between patients and healthy controls. *Gastroenterology*. (2012) 142:S528. doi: 10.1016/S0016-5085(12)62029-0

43. Markar SR, Chin S-T, Romano A, Wiggins T, Antonowicz S, Paraskeva P, et al. Breath volatile organic compound profiling of colorectal cancer using selected ion flow-tube mass spectrometry. *Ann Surg*. (2019) 269:903–10. doi: 10.1097/SLA.0000000000002539

44. McFarlane M, Millard A, Hall H, Savage R, Constantinidou C, Arasaradnam R, et al. Urinary volatile organic compounds and faecal microbiome profiles in colorectal cancer. *Colorectal Dis*. (2019) 21:1259–69. doi: 10.1111/codi.14739

45. Mozdiak E, Wicaksono AN, Covington JA, Arasaradnam RP. Colorectal cancer and adenoma screening using urinary volatile organic compound (VOC) detection: early results from a single-centre bowel screening population (UK BCSP). *Techniques Coloproctology*. (2019) 23:343–51. doi: 10.1007/s10151-019-01963-6

46. Psutka C, Yamada M, Matsuda A, Yamahatsu K, Matsumoto S, Kitayama T, et al. Abstract 5303: FAIMS technology in urinary volatile organic compound analysis

to detect colorectal cancer. *Cancer Res.* (2017) 77:5303–. doi: 10.1158/1538-7445.AM2017-5303

47. Tyagi H, Daulton E, Bannaga AS, Arasaradnam RP, Covington JA. Non-invasive detection and staging of colorectal cancer using a portable electronic nose. *Sensors*. (2021) 21:5440. doi: 10.3390/s21165440

48. Widlak MM, Neal M, Daulton E, Thomas CL, Tomkins C, Singh B, et al. Risk stratification of symptomatic patients suspected of colorectal cancer using faecal and urinary markers. *Colorectal Dis*. (2018) 20:O335–O42. doi: 10.1111/codi.14431

49. Zambrana Tevar F, Herrero A, Vidal-de-Miguel G, Bailador G, Criado E, Marquina I, et al. On-line breath analysis of volatile organic compounds as a method for colorectal cancer detection. *J Clin Oncol*. (2012) 30:1570–. doi: 10.1200/jco.2012.30.15_suppl.1570

50. Altomare DF, Porcelli F, Picciariello A, Pinto M, Di Lena M, Caputi Iambrenghi O, et al. The use of the PEN3 e-nose in the screening of colorectal cancer and polyps. *Techniques Coloproctology*. (2016) 20:405–9yang. doi: 10.1007/s10151-016-1457-z

51. Steenhuis EGM, Schoenaker IJH, de Groot JWB, Fiebrich HB, de Graaf JC, Brohet RM, et al. Feasibility of volatile organic compound in breath analysis in the follow-up of colorectal cancer: A pilot study. *Eur J Surg Oncol*. (2020) 46:2068–73. doi: 10.1016/j.ejso.2020.07.028

52. van de Goor RMGE, Leunis N, van Hooren MRA, Francisca E, Masclee A, Kremer B, et al. Feasibility of electronic nose technology for discriminating between head and neck, bladder, and colon carcinomas. *Eur Arch Oto-Rhino-Laryngology*. (2017) 274:1053–60. doi: 10.1007/s00405-016-4320-y

53. Westenbrink E, Arasaradnam RP, O'Connell N, Bailey C, Nwokolo C, Bardhan KD, et al. Development and application of a new electronic nose instrument for the detection of colorectal cancer. *Biosensors Bioelectronics*. (2015) 67:733–8. doi: 10.1016/j.bios.2014.10.044

54. Westenbrink E, O'Connell N, Bailey C, Nwokolo C, Bardhan K, Arasaradnam R, et al. Detection of colorectal cancer from urinary volatile organic compounds using a new chromatograph/electronic-nose instrument – wolf system. *Gut*. (2016) 65:A195.2–A6. doi: 10.1136/gutjnl-2016-312388.361

55. Zonta G, Malagù C, Gherardi S, Giberti A, Pezzoli A, De Togni A, et al. Clinical validation results of an innovative non-invasive device for colorectal cancer preventive screening through fecal exhalation analysis. *Cancers*. (2020) 12:1471. doi: 10.3390/cancers12061471

56. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Internal Med*. (2011) 155:529–36. doi: 10.7326/0003-4819-155-8-201110180-00009

57. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. *J Clin Epidemiol*. (2005) 58:882–93. doi: 10.1016/j.jclinepi.2005.01.016

58. Amal H, Leja M, Funka K, Lasina I, Skapars R, Sivins A, et al. Breath testing as potential colorectal cancer screening tool. *Int J Cancer*. (2016) 138:229–36. doi: 10.1002/ijc.29701

59. Hanna GB, Boshier PR, Markar SR, Romano A. Accuracy and methodologic challenges of volatile organic compound-based exhaled breath tests for cancer diagnosis: A systematic review and meta-analysis. *JAMA Oncol*. (2019) 5:e182815. doi: 10.1001/jamaoncol.2018.2815

60. van Liere E, van Dijk LJ, Bosch S, Vermeulen L, Heymans MW, Burchell GL, et al. Urinary volatile organic compounds for colorectal cancer screening: A systematic review and meta-analysis. *Eur J Cancer (Oxford England: 1990)*. (2023) 186:69–82. doi: 10.1016/j.ejca.2023.03.002

61. Chandrapalan S, Bosch S, Cubiella J, Guardiola J, Kimani P, Mulder C, et al. Systematic review with meta-analysis: volatile organic compound analysis to improve faecal immunochemical testing in the detection of colorectal cancer. *Alimentary Pharmacol Ther*. (2021) 54:14–23. doi: 10.1111/apt.16405

62. Ghimenti S, LoMonaco T, Bellagambi FG, Tabucchi S, Onor M, Trivella MG, et al. Comparison of sampling bags for the analysis of volatile organic compounds in breath. *J Breath Res*. (2015) 9:047110. doi: 10.1088/1752-7155/9/4/047110

63. Mochalski P, King J, Unterkofler K, Amann A. Stability of selected volatile breath constituents in Tedlar, Kynar and Flexfilm sampling bags. *Analyst*. (2013) 138:1405–18. doi: 10.1039/c2an36193k

64. Esfahani S, Sagar NM, Kyrou I, Mozdiak E, O'Connell N, Nwokolo C, et al. Variation in gas and volatile compound emissions from human urine as it ages, measured by an electronic nose. *Biosensors*. (2016) 6:4. doi: 10.3390/bios6010004

65. Chan DK, Leggett CL, Wang KK. Diagnosing gastrointestinal illnesses using fecal headspace volatile organic compounds. *World J Gastroenterol*. (2016) 22:1639–49. doi: 10.3748/wjg.v22.i4.1639

66. Krilaviciute A, Leja M, Kopp-Schneider A, Barash O, Khatib S, Amal H, et al. Associations of diet and lifestyle factors with common volatile organic compounds in exhaled breath of average-risk individuals. *J Breath Res*. (2019) 13:026006. doi: 10.1088/1752-7163/aaf3dc

67. Boots AW, Bos LD, van der Schee MP, van Schooten FJ, Sterk PJ. Exhaled molecular fingerprinting in diagnosis and monitoring: validating volatile promises. *Trends Mol Med*. (2015) 21:633–44. doi: 10.1016/j.molmed.2015.08.001

68. Fiehn O. Metabolomics by gas chromatography–mass spectrometry: combined targeted and untargeted profiling. *Curr Protoc Mol Biol*. (2016) 114:30.4.1–30.4.32. doi: 10.1002/0471142727.mb3004s114

69. Baldini C, Billeci L, Sansone F, Conte R, Domenici C, Tonacci A. Electronic nose as a novel method for diagnosing cancer: A systematic review. *Biosensors*. (2020) 10:84. doi: 10.3390/bios10080084

70. van der Sar IG, Wijsenbeek MS, Moor CC. Exhaled breath analysis in interstitial lung disease. *Curr Opin pulmonary Med*. (2023) 29:443–50. doi: 10.1097/MCP.0000000000000978

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Endoscopic obstruction predominantly occurs in right-side colon cancer and endoscopic obstruction with tumor size ≤ 5 cm seems poor prognosis in colorectal cancer

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Background: Endoscopic obstruction (eOB) is associated with a poor prognosis in colorectal cancer (CRC). Our study aimed to investigate the association between tumor location and eOB, as well as the prognostic differences among non-endoscopic obstruction (N-eOB), eOB with tumor size ≤ 5 cm, and eOB with tumor size > 5 cm in non-elderly patients.

Methods: We retrospectively reviewed the clinicopathological variables of 230 patients with CRC who underwent curative surgery. The multivariable logistic regression model was used to identify risk factors for eOB. The association between eOB with tumor size ≤ 5 cm and disease-free survival (DFS) was evaluated using multivariate cox regression analysis.

Results: A total of 87 patients had eOB while 143 had N-eOB. In multivariate analysis, preoperative carcinoembryonic antigen (p = 0.014), tumor size (p = 0.010), tumor location (left-side colon; p = 0.033; rectum; p < 0.001), and pT stage (T3, p = 0.009; T4, p < 0.001) were significant factors of eOB. The DFS rate for eOB with tumor size ≤ 5 cm was significantly lower (p < 0.001) in survival analysis. The eOB with tumor size ≤ 5 cm (p = 0.012) was an unfavorable independent factor for DFS.

Conclusions: The patients with eOB were significantly associated with right-side colon cancer as opposed to left-side colon cancer and rectal cancer. The eOB with tumor size ≤ 5 cm was an independent poor prognostic factor. Further studies are needed to target these high-risk groups.

KEYWORDS

colorectal cancer, endoscopic obstruction, tumor location, tumor size, disease-free survival

Introduction

Colorectal cancer (CRC), with an estimated 1.9 million new cases and 935,000 deaths globally in 2020, stands as the third most prevalent cancer and the second leading cause of cancer-related mortality, contributing to approximately one in 10 cancer cases and deaths (1). Since circa 2010, the incidence of regional-stage and distant-stage disease has increased by about 3% per year in people younger than 50 years and by 2% and 0.5% per year, respectively, in people aged 50–64 years, while rates in people aged 65 years and older have stabilized since about 2015 after a decade of steep decline (2). This trend may necessitate a revision of current screening programs (3).

Endoscopy, particularly colonoscopy, has evolved to be an integral component of the preoperative assessment in patients with colorectal cancer, not just for its diagnostic capabilities but also for its prognostic implications. The occurrence of endoscopic obstruction (eOB) in individuals with colorectal cancer is not uncommon (4), and eOB is defined as the inability of standard colonoscopy to penetrate the tumor, regardless of clinical signs of intestinal obstruction (abdominal distention, peristalsis abdominal pain, nausea, or vomiting) or imaging findings of intestinal obstruction (dilated intestinal loops). Meanwhile, recent studies indicate that eOB is a marker of poor prognosis in patients with stage II colon cancer and stage III rectal cancer following curative surgery (5, 6). Therefore, early identification of eOB is critical for the patient's treatment. Colorectal obstruction caused by colorectal cancer occurs in 7%–29% of all patients with colorectal cancers (7, 8). Yang et al. indicated that left-side colon cancer was more common than right-side colon cancer in the complete obstructive colorectal cancer compared to the non-obstructive colorectal cancer (9). However, the association between eOB and tumor location has not been investigated. Beyond the influence of tumor location, a distinct variation in tumor size distribution has been noted between eOB and N-eOB. Chalieopanyarwong et al. observed that CRC with N-eOB had a significantly smaller size (4). Smaller tumor size has been reported to be associated with good survival and oncological prognosis in CRC (10). However, several recent studies have reported that tumor size is not associated with survival (11, 12), while others have shown that a smaller size is associated with poorer survival (13, 14). Based on these recent findings, the association between tumor size and oncological prognosis is controversial.

The objectives of this study were to conduct a population-based analysis evaluating the association between primary tumor location and eOB. Additionally, the study aimed to determine the impact of eOB with tumor size ≤ 5 cm on oncological prognosis.

Materials and methods

Patient selection

In this single-center retrospective study, patients with histologically confirmed stage I-III colorectal cancer were included. The patient records were maintained in the colorectal tumor database of Longyan First Hospital Affiliated to Fujian Medical University (Fujian, China) between January 2015 and December 2018. The access and use of clinical data were approved by the Institutional Review Board of Longyan First Hospital Affiliated to Fujian Medical University.

All patients who underwent consecutive curative treatments for colorectal cancer were included in this study, with surgical procedures being carried out by a specialized team. Our treatment policy was curative resection of the primary lesion with sufficient margin and appropriate lymph node dissection, followed by observation or adjuvant chemotherapy based on individual risk features. Exclusion criteria were age of 65 years or older, history of neoadjuvant therapy, diagnosis of multiple primary colorectal cancers, other history of malignant tumor, familial adenomatous polyposis, undergoing emergent surgery, preoperative stent insertion, receiving colonoscopy from another medical institution, missing data, and being lost to follow-up post-operation (Figure 1).

Tumor stages were coded as described by the 8th edition of the AJCC tumor-node-metastasis grading system (15). The tumor location was classified as the right-side colon (including the cecum, the ascending colon, the hepatic flexure and the transverse colon), the left-side colon (including the splenic flexure and the descending, sigmoid colons and rectosigmoid junction) and rectum (16). The surgical techniques include laparoscopic surgery and open surgery, with the latter encompassing conversion to open surgery. Tumor size was categorized into two groups: ≤ 5 cm and > 5 cm (9). Based on this, the eOB-size group was categorized as N-eOB, eOB with tumor size ≤ 5 cm and eOB with tumor size > 5 cm.

Statistical analysis

The chi-square test and Fisher's exact probability test were used to compare categorical data. In order to screen the final predictors of endoscopic obstruction, all candidate predictors with $p < 0.05$ in the univariate analysis were included in the multivariate logistic regression model. In the multivariate analysis, variables with $p < 0.05$ were considered as independent predictors. The Kaplan–Meier method was used to calculate overall survival (OS) and disease-free survival (DFS). And the log-rank test was used to evaluate the difference in survival rate. Univariate and multivariate Cox regression analyses were performed to determine variables related to survival. OS was defined as the time between the date of surgery and the date of death. DFS was defined as the time between the date of surgery and the date of first recurrence of the disease or death. All statistical analyses were carried out using R software (version 4.2.2), and a two-sided p-value below 0.05 was considered statistically significant.

Results

Clinicopathological characteristics of patients

A total of 87 (37.8%) patients were eOB and 143 (62.2%) patients were N-eOB in the study cohort. 56 (24.3%) patients were below 50 years of age. The collected clinical and pathological characteristics of the patients were subjected to

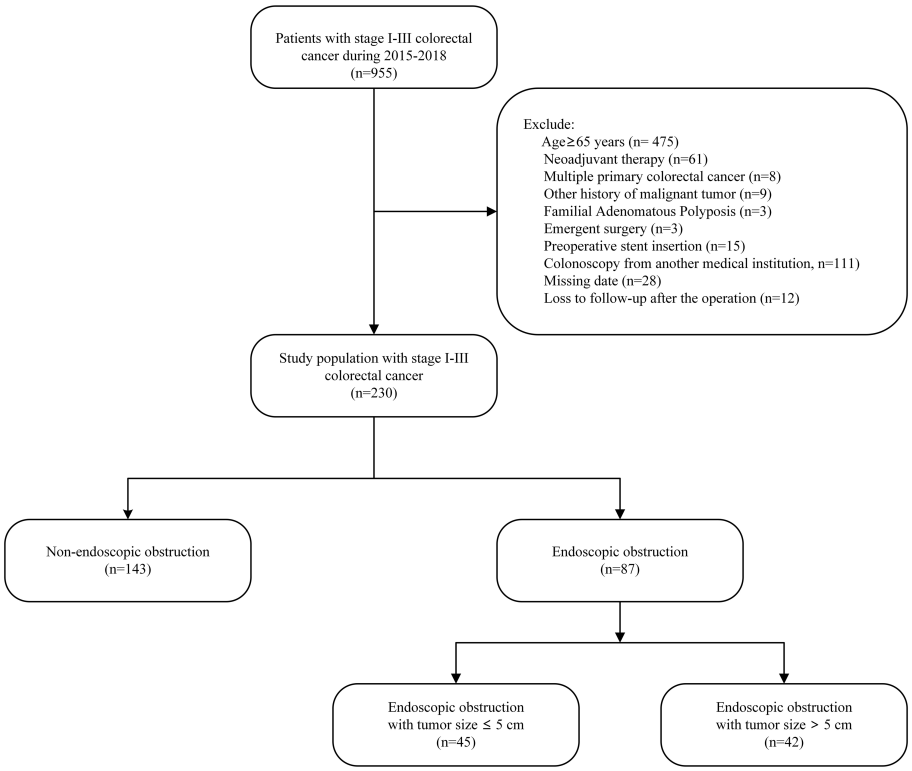

FIGURE 1
Study flow chart.

univariate analysis (Table 1). Sex, BMI, history of smoking, history of drinking, diabetes mellitus, hypertension, history of abdominal surgery, preoperative carbohydrate antigen 19–9 (CA19–9), differentiation, vascular invasion, perineural invasion and pN stage of eOB patients were similar to those of N-eOB patients. Regarding the patient characteristics, patients with younger age or higher preoperative carcinoembryonic antigen (CEA) had a higher risk for eOB (32.2 vs. 19.6%; $p = 0.045$ and 54.0 vs. 25.9%; $p < 0.001$, respectively). Additionally, the patients who experienced eOB had significantly larger number of harvested lymph nodes (98.9 vs. 88.8%; $p = 0.010$) compared with the patients who experienced N-eOB. Regarding tumor characteristics, the highest rate of right-side colon (51.7%) was observed for patients with eOB. Patients with larger tumor size, higher pT stage and pTNM stage had a higher associated risk of eOB in our study ($p < 0.001$, respectively). For treatment factors, including surgical technique, and adjuvant chemotherapy, there were significant differences between eOB patients and N-eOB patients.

In the categorization of patients with eOB, we divided them into two groups based on tumor size: eOB with tumor size ≤ 5 cm and eOB with tumor size > 5 cm. Among the 230 patients reviewed, as illustrated in Table 2, 143 (62.2%) were classified as N-eOB, 45 (19.6%) had eOB with a tumor size of ≤ 5 cm, and 42 (18.3%) had eOB with a tumor size of > 5 cm. Follow-up periods were between 10 and 88 months (median, 58 months). A total number of 45 (19.6%) patients had nodal involvement of eOB with tumor size ≤ 5 cm. Age, sex, history of smoking, history of drinking, hypertension, diabetes mellitus, history of abdominal surgery, preoperative CA19–9, differentiation, vascular invasion, perineural invasion, and pN stage were comparable among patients with N-eOB, eOB with tumor size ≤ 5cm, and eOB with tumor size > 5cm. Statistical differences were observed in the population concerning BMI, preoperative CEA, harvested lymph nodes, tumor location, pT stage, pTNM stage, surgical technique, and adjuvant chemotherapy.

Logistic regression analysis for endoscopic obstruction

To identify predictors of eOB, multivariate analysis was carried out for sample using variables that were available for clinical and pathologic characteristics, including age, preoperative CEA, tumor size, tumor location, and pT stage. Among these factors, preoperative CEA (OR = 2.37; 95% CI: 1.19-4.71, $p = 0.014$), tumor size (OR = 2.56; 95% CI: 1.25-5.24, p = 0.010), tumor location (left-side colon; OR = 0.40; 95% CI: 0.17-0.93, $p = 0.033$; rectum; OR = 0.11; 95% CI: 0.05-0.26, $p < 0.001$), and pT stage (T3; OR = 4.07; 95% CI: 1.43-11.61, $p = 0.009$; T4, OR = 7.45; 95% CI: 2.58-21.55, $p < 0.001$) were found to be independently and significantly correlated with the development of eOB (Table 3).

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TABLE 1 Clinicopathological characteristics of patients with endoscopic obstruction and non-endoscopic obstruction.

Variable	N-eOB (n=143)	eOB (n=87)	*P* value
Age (years, %)			0.045
<50	28 (19.6)	28 (32.2)	
≥50	115 (80.4)	59 (67.8)	
Sex (%)			0.934
Female	58 (40.6)	34 (39.1)	
Male	85 (59.4)	53 (60.9)	
BMI (kg/m², %)			0.132
<18.5	8 (5.6)	8 (9.2)	
18.5–24.9	91 (63.6)	62 (71.3)	
>24.9	44 (30.8)	17 (19.5)	
History of smoking (%)			0.135
No	99 (69.2)	51 (58.6)	
Yes	44 (30.8)	36 (41.4)	
History of drinking (%)			0.749
No	118 (82.5)	74 (85.1)	
Yes	25 (17.5)	13 (14.9)	
Diabetes mellitus (%)			0.077
No	125 (87.4)	83 (95.4)	
Yes	18 (12.6)	4 (4.6)	
Hypertension (%)			0.324
No	111 (77.6)	73 (83.9)	
Yes	32 (22.4)	14 (16.1)	
History of abdominal surgery (%)			0.625
No	122 (85.3)	77 (88.5)	
Yes	21 (14.7)	10 (11.5)	
Preoperative CEA (ng/ml, %)			<0.001
≤5	106 (74.1)	40 (46.0)	
>5	37 (25.9)	47 (54.0)	
Preoperative CA19–9 (U/ml, %)			1.000
≤37	131 (91.6)	79 (90.8)	
>37	12 (8.4)	8 (9.2)	
Differentiation (%)			0.726
Well/Moderate	133 (93.0)	79 (90.8)	
Poor	10 (7.0)	8 (9.2)	
Harvested lymph nodes (%)			0.010
≥12	127 (88.8)	86 (98.9)	
<12	16 (11.2)	1 (1.1)	
Vascular invasion (%)			0.284
No	103 (72.0)	56 (64.4)	
Yes	40 (28.0)	31 (35.6)	
Perineural invasion (%)			0.319
No	122 (85.3)	69 (79.3)	
Yes	21 (14.7)	18 (20.7)	
Tumor location (%)			<0.001
Right-side colon	19 (13.3)	45 (51.7)	
Left-side colon	39 (27.3)	26 (29.9)	
Rectum	85 (59.4)	16 (18.4)	
Tumor size (cm, %)			<0.001
≤5	117 (81.8)	45 (51.7)	
>5	26 (18.2)	42 (48.3)	
pT stage (%)			<0.001
T1–2	56 (39.2)	6 (6.9)	
T3	54 (37.8)	36 (41.4)	
T4	33 (23.1)	45 (51.7)	
pN stage (%)			0.587
N0	84 (58.7)	47 (54.0)	
N1	34 (23.8)	20 (23.0)	
N2	25 (17.5)	20 (23.0)	
pTNM stage (%)			<0.001
stage I	45 (31.5)	5 (5.7)	
stage II	39 (27.3)	42 (48.3)	
stage III	59 (41.3)	40 (46.0)	
Surgical technique (%)			0.002
Open	10 (7.0)	19 (21.8)	
Laparoscopy	133 (93.0)	68 (78.2)	
Adjuvant chemotherapy (%)			0.005
No	64 (44.8)	22 (25.3)	
Yes	79 (55.2)	65 (74.7)	

Survival analysis of overall survival and disease-free survival

There were no significant differences in OS among the three groups: eOB with tumor size ≤ 5 cm, eOB with tumor size > 5 cm and N-eOB (78.8% vs. 88.1% vs. 89.2%, $p = 0.055$) (Figure 2A). The DFS rate for eOB with tumor size ≤ 5 cm was significantly lower compared to that of eOB with tumor size > 5 cm and N-eOB (57.8% vs. 75.5% vs. 84.9%, $p < 0.001$) (Figure 2B).

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TABLE 2 Clinicopathological characteristics of patients with non-endoscopic obstruction, endoscopic obstruction with tumor size ≤ 5 cm and endoscopic obstruction with tumor size > 5 cm.

Variable	N-eOB (n=143)	Endoscopic obstruction with tumor size ≤ 5 cm (n=45)	Endoscopic obstruction with tumor size > 5 cm (n=42)	*P* value
Age (years, %)				0.094
<50	28 (19.6)	14 (31.1)	14 (33.3)	
≥50	115 (80.4)	31 (68.9)	28 (66.7)	
Sex (%)				0.766
Female	58 (40.6)	16 (35.6)	18 (42.9)	
Male	85 (59.4)	29 (64.4)	24 (57.1)	
BMI (kg/m², %)				0.015
<18.5	8 (5.6)	1 (2.2)	7 (16.7)	
18.5–24.9	91 (63.6)	32 (71.1)	30 (71.4)	
>24.9	44 (30.8)	12 (26.7)	5 (11.9)	
History of smoking (%)				0.251
No	99 (69.2)	27 (60.0)	24 (57.1)	
Yes	44 (30.8)	18 (40.0)	18 (42.9)	
History of drinking (%)				0.537
No	118 (82.5)	40 (88.9)	34 (81.0)	
Yes	25 (17.5)	5 (11.1)	8 (19.0)	
Hypertension (%)				0.472
No	111 (77.6)	37 (82.2)	36 (85.7)	
Yes	32 (22.4)	8 (17.8)	6 (14.3)	
Diabetes mellitus (%)				0.108
No	125 (87.4)	42 (93.3)	41 (97.6)	
Yes	18 (12.6)	3 (6.7)	1 (2.4)	
History of abdominal surgery (%)				0.785
No	122 (85.3)	40 (88.9)	37 (88.1)	
Yes	21 (14.7)	5 (11.1)	5 (11.9)	
Preoperative CEA (ng/ml, %)				<0.001
≤5	106 (74.1)	21 (46.7)	19 (45.2)	
>5	37 (25.9)	24 (53.3)	23 (54.8)	
Preoperative CA19–9 (U/ml, %)				0.358
≤37	131 (91.6)	39 (86.7)	40 (95.2)	
>37	12 (8.4)	6 (13.3)	2 (4.8)	
Differentiation (%)				0.194
Well/Moderate	133 (93.0)	43 (95.6)	36 (85.7)	
Poor	10 (7.0)	2 (4.4)	6 (14.3)	
Harvested lymph nodes (%)				0.017
≥12	127 (88.8)	44 (97.8)	42 (100.0)	

(Continued)

TABLE 2 Continued

Variable	N-eOB (n=143)	Endoscopic obstruction with tumor size ≤ 5 cm (n=45)	Endoscopic obstruction with tumor size > 5 cm (n=42)	*P* value
<12	16 (11.2)	1 (2.2)	0 (0.0)	
Vascular invasion (%)				0.475
No	103 (72.0)	29 (64.4)	27 (64.3)	
Yes	40 (28.0)	16 (35.6)	15 (35.7)	
Perineural invasion (%)				0.153
No	122 (85.3)	33 (73.3)	36 (85.7)	
Yes	21 (14.7)	12 (26.7)	6 (14.3)	
Tumor location (%)				<0.001
Right-side colon	19 (13.3)	21 (46.7)	24 (57.1)	
Left-side colon	39 (27.3)	16 (35.6)	10 (23.8)	
Rectum	85 (59.4)	8 (17.8)	8 (19.0)	
pT stage (%)				<0.001
T1–2	56 (39.2)	2 (4.4)	4 (9.5)	
T3	54 (37.8)	19 (42.2)	17 (40.5)	
T4	33 (23.1)	24 (53.3)	21 (50.0)	
pN stage (%)				0.172
N0	84 (58.7)	19 (42.2)	28 (66.7)	
N1	34 (23.8)	13 (28.9)	7 (16.7)	
N2	25 (17.5)	13 (28.9)	7 (16.7)	
pTNM stage (%)				<0.001
stage I	45 (31.5)	2 (4.4)	3 (7.1)	
stage II	39 (27.3)	17 (37.8)	25 (59.5)	
stage III	59 (41.3)	26 (57.8)	14 (33.3)	
Surgical technique (%)				0.004
Open	10 (7.0)	9 (20.0)	10 (23.8)	
Laparoscopy	133 (93.0)	36 (80.0)	32 (76.2)	
Adjuvant chemotherapy (%)				0.012
No	64 (44.8)	11 (24.4)	11 (26.2)	
Yes	79 (55.2)	34 (75.6)	31 (73.8)	

Cox regression analysis for disease-free survival

In the univariate analysis, preoperative CEA, preoperative CA19–9, eOB-size group, differentiation, vascular invasion, perineural invasion, pTNM stage, and adjuvant chemotherapy were all associated with predicting development of DFS (Table 4). These variables were included in a multivariable Cox regression analysis. Preoperative CA19–9 (HR = 2.38, p = 0.026), eOB-size group (eOB with tumor size ≤ 5 cm vs. N-eOB, HR = 2.32, p = 0.012), perineural invasion (HR = 1.97, p = 0.040), and pTNM stage (stage III vs. stage I, HR = 6.06, p = 0.032) remained significantly associated with DFS. While we did not find that preoperative CEA was statistically significant in the multivariate analysis. Differentiation, vascular invasion and adjuvant chemotherapy were also not found to be significantly associated with worse DFS.

Discussion

Few studies have focused on identifying the factors associated with the emergence of eOB, as well as conducting analyses to

TABLE 3 Logistic regression analysis of clinical and pathological predictors for endoscopic obstruction.

Variable	Reference		Univariable analysis			Multivariable analysis		
			OR	95%CI	*P* value	OR	95%CI	*P* value
Age, years	<50	≥50	0.51	0.28–0.94	0.032	0.88	0.40–1.91	0.742
Sex	Female	Male	1.06	0.62–1.83	0.824	–	–	–
BMI, kg/m²	<18.5	18.5–24.9	0.68	0.24–1.91	0.466	–	–	–
		>24.9	0.39	0.12–1.19	0.099	–	–	–
History of smoking	No	Yes	1.59	0.91–2.77	0.102	–	–	–
History of drinking	No	Yes	0.83	0.40–1.72	0.615	–	–	–
Diabetes mellitus	No	Yes	0.33	0.11–1.02	0.055	–	–	–
Hypertension	No	Yes	0.67	0.33–1.33	0.250	–	–	–
History of abdominal surgery	No	Yes	0.75	0.34–1.69	0.493	–	–	–
Preoperative CEA, ng/ml	≤5	>5	3.37	1.92–5.92	<0.001	2.37	1.19–4.71	0.014
Preoperative CA19–9, U/ml	≤37	>37	1.11	0.43–2.82	0.834	–	–	–
Tumor size, cm	≤5	>5	4.20	2.31–7.64	<0.001	2.56	1.25–5.24	0.010
Tumor location	Right-side colon	Left-side colon	0.28	0.14–0.58	0.001	0.40	0.17–0.93	0.033
		Rectum	0.08	0.04–0.17	<0.001	0.11	0.05–0.26	<0.001
Differentiation	Well/Moderate	Poor	1.35	0.51–3.55	0.548	–	–	–
Vascular invasion	No	Yes	1.43	0.81–2.52	0.224	–	–	–
Perineural invasion	No	Yes	1.52	0.76–3.04	0.241	–	–	–
pT stage	T1–2	T3	6.22	2.43–15.95	<0.001	4.07	1.43–11.61	0.009
		T4	12.73	4.90–33.05	<0.001	7.45	2.58–21.55	<0.001
pN stage	N0	N1	1.05	0.54–2.03	0.881	–	–	–
		N2	1.43	0.72–2.84	0.308	–	–	–

evaluate the prognostic implications of eOB with different tumor sizes. This study demonstrated that the incidence of eOB is predominantly higher in the right-side colon compared to the left-side colon in patients diagnosed with non-elderly colorectal cancer. Furthermore, it was noted that eOB with tumor size ≤ 5 cm was associated with a poor survival compared to N-eOB. Conversely, this correlation was not observed for eOB with tumor size > 5 cm.

Unplanned emergency surgeries for colorectal cancer are typically linked with a heightened risk of surgical complications, as well as increased mortality and morbidity rates in emergency scenarios (17–19). A recent study suggested that in instances of luminal obstruction, which is significant enough to impede the further advancement of a colonoscope, physicians should be prompted to contemplate the necessity of urgent surgical intervention, irrespective of the initial symptoms presented (4). Given the importance of preventive interventions, it is crucial to assess the risk factors associated with the emergence of eOB in patients with colorectal cancer. Previous studies presented inconsistent results regarding the relationship between bowel obstruction development and different tumor location. Theoretically, right-side colon cancers are usually ulcerative, and the stool in these regions tends to have a more liquid consistency. Conversely, left-side colon cancers are more likely to present with bowel obstruction, as proliferative lesions are common in this location and the stool is typically of a semisolid consistency. Xinger Lv et al. (20) and Phillips et al. (21) indicated that the left-side colon was more susceptible to bowel obstruction compared with the right-side colon. However, our results demonstrated an increased susceptibility of the right-side colon to eOB, in comparison with the left-side colon and the rectum. Similarly, Kumar et al. observed analogous finding that relatively younger patients present to health center with obstructive colorectal cancer with anatomical shift to the right-side colon (22). In their study, in 54% cases the lesion was in the proximal colon. It is highly plausible that this outcome is attributable to the screening policies. A study indicated that screening for colorectal cancer is associated with lower disease stage (23). Nevertheless, nearly 90% of colorectal cancer patients are diagnosed following the onset of symptoms, exhibiting more advanced disease stages compared to patients identified through asymptomatic screening (24). Furthermore, when presenting with obstruction symptoms, right-side colon

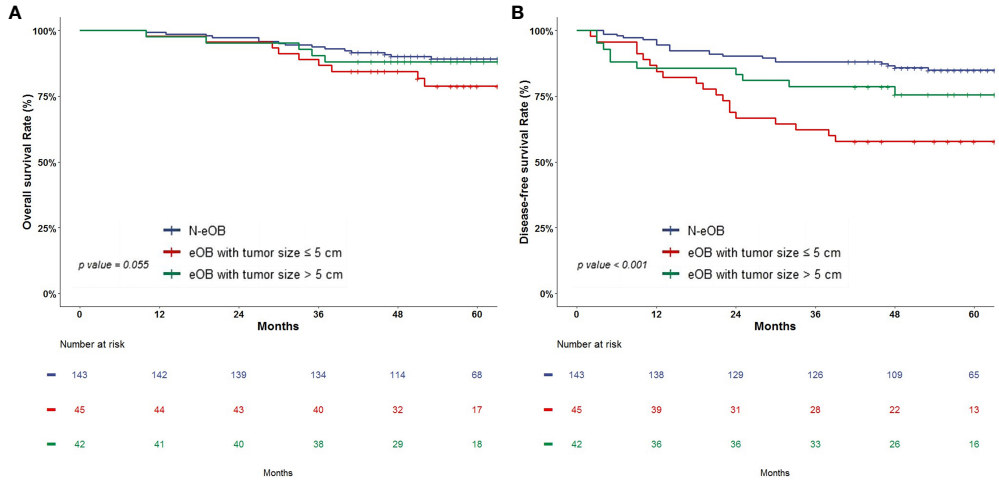

FIGURE 2
Kaplan-Meier survival analysis of overall survival **(A)** and disease-free survival **(B)**.

cancers are generally at a more advanced stage of progression compared to left-side colon cancers. Relatedly, this study reveals a strong correlation between a more advanced pT stage and the emergence of eOB, which appears to support the hypothesis that eOB is more common in right-side colon cancers. In addition to the reasons previously mentioned, the inability of the colonoscope to smoothly navigate through the space-occupying lesions in the right-side colon, as compared to those in left-side colon and rectum, may also be attributed to discomfort experienced during the colonoscopy or to technical challenges encountered.

In this study, we observed that eOB with tumor size ≤ 5 cm exhibited a poorer DFS, compared to N-eOB. This relationship was

TABLE 4 Cox regression analysis of prognostic predictors for disease-free survival.

Variable	Reference		Univariable analysis			Multivariable analysis		
			HR	95%CI	*P* value	HR	95%CI	*P* value
Age, years	<50	≥50	0.97	0.51–1.86	0.931	–	–	–
Sex	Female	Male	1.00	0.57–1.75	0.990	–	–	–
BMI, kg/m²	<18.5	18.5–24.9	0.74	0.26–2.09	0.569	–	–	–
		>24.9	0.91	0.30–2.74	0.864	–	–	–
History of smoking	No	Yes	1.03	0.58–1.83	0.927	–	–	–
History of drinking	No	Yes	1.14	0.55–2.35	0.721	–	–	–
Diabetes mellitus	No	Yes	0.35	0.08–1.44	0.145	–	–	–
Hypertension	No	Yes	1.32	0.69–2.52	0.403	–	–	–
History of abdominal surgery	No	Yes	1.09	0.49–2.42	0.835	–	–	–
Preoperative CEA, ng/ml	≤5	>5	1.76	1.01–3.06	0.047	1.11	0.61–2.01	0.735
Preoperative CA19–9, U/ml	≤37	>37	2.81	1.37–5.79	0.005	2.38	1.11–5.12	0.026
eOB-size group	N-eOB	eOB with tumor size ≤ 5 cm	3.42	1.84–6.37	<0.001	2.32	1.20–4.48	0.012
		eOB with tumor size > 5 cm	1.78	0.84–3.78	0.134	1.89	0.83–4.32	0.130
Differentiation	Well/Moderate	Poor	2.34	1.05–5.19	0.037	1.89	0.80–4.45	0.147
Harvested lymph nodes	≥12	<12	0.23	0.03–1.67	0.147	–	–	–

(Continued)

TABLE 4 Continued

Variable	Reference		Univariable analysis			Multivariable analysis		
			HR	95%CI	*P* value	HR	95%CI	*P* value
Vascular invasion	No	Yes	2.79	1.60–4.86	<0.001	0.99	0.48–2.06	0.986
Perineural invasion	No	Yes	3.51	1.97–6.26	<0.001	1.97	1.03–3.74	0.040
Tumor location	Right-side colon	Left-side colon	0.59	0.28–1.26	0.175	–	–	–
		Rectum	0.75	0.40–1.42	0.380	–	–	–
pTNM stage	stage I	stage II	4.37	0.99–19.36	0.052	2.74	0.54–13.89	0.225
		stage III	10.75	2.58–44.7	0.001	6.06	1.17–31.30	0.032
Surgical technique	Open	Laparoscopy	1.01	0.43–2.38	0.974	–	–	–
Adjuvant chemotherapy	No	Yes	2.36	1.21–4.61	0.012	0.99	0.46–2.13	0.981

present even when controlling for multiple patient-specific prognostic factors, such as preoperative CA19–9, perineural invasion, pTNM stage. Therefore, to give a potential explanation of our findings, we hypothesized that eOB with tumor size ≤ 5 cm may be a surrogate marker for biological aggressiveness resulting in inferior DFS, which indicated that initial biological heterogeneity of colorectal cancers determined their distinct growth pattern and different invasive and metastatic abilities. In this study, eOB with tumor size ≤ 5 cm was associated with higher pT stage, reflecting a vertical growth pattern. Tumors with a vertical growth pattern may have early acquired high metastatic potential which enable them to breach basal membrane, invading the surrounding tissue and finally disseminating to regional lymph nodes and distant metastasis (25). In contrast, tumors with a horizontal growth pattern reflected by eOB with larger tumors underline a biologically indolent disease and a lower metastatic ability. The fact that clinicians are more likely to treat large tumors more aggressively may also explain our results. Multivisceral resection (MVR) is associated with increased tumor size in locally advanced colorectal cancer (26). In addition, the larger tumor size often leads to more complete lymph node resection and evaluation in colorectal cancer (27). These more aggressive treatments may result in better survival rates. Therefore, in the diagnosis and treatment of colorectal cancer, the evaluation of eOB with smaller tumors should not be overlooked. In addition, preoperative CEA was not an independent prognostic biomarker according to our study. One study has shown that patients with elevated preoperative CEA that normalizes after surgery have a similar outcome to patients with normal preoperative CEA (28). This adequately demonstrates the limitations of preoperative CEA in predicting postoperative recurrence.

This study indeed has several limitations. (1) This study was conducted as a single-center retrospective analysis. Consequently, the number of patients was limited, and the selection process adhered to stringent inclusion and exclusion criteria. This approach may have introduced potential selection bias. (2) The stratification by tumor size and eOB led to relatively small subgroups, which reduced the statistical power to discriminate small differences. (3) Neoadjuvant chemotherapy, radiotherapy and laboratory examinations were not included in the present study. Further investigations of multi-center prospective study should be conducted and more baseline characteristics should be enrolled.

Conclusion

Among non-elderly patients, those with eOB were significantly associated with right-side colon cancer as opposed to left-side colon cancer and rectal cancer. The eOB with tumor size ≤ 5 cm was associated with lower DFS, while this association was not observed for eOB with tumor size > 5 cm. The observed shift in the incidence of eOB towards the right-side colon, coupled with the result that eOB with tumor size ≤ 5 cm may denote a more aggressive form of malignancy, highlights the imperative for comprehensive research.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by The Ethical Review Board of the Longyan First Affiliated Hospital of Fujian Medical University (approval number: LYREC2024-k024-01). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this study utilizes medical records obtained from previous research and meets all of the following conditions: Previous research has obtained written consent from the participants, allowing other research projects to use their medical

records or specimens. This research complies with the permission conditions of the original informed consent. The confidentiality of the participants' privacy and identity information is ensured.

Author contributions

NY: Data curation, Methodology, Software, Writing – original draft. SL: Conceptualization, Data curation, Writing – original draft. XW: Writing – review & editing, Conceptualization. GH: Data curation, Methodology, Writing – original draft. RX: Data curation, Writing – original draft. ZQ: Data curation, Writing – original draft. RL: Data curation, Writing – original draft. DX: Conceptualization, Writing – review & editing, Data curation, Methodology.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. (2021) 71:209–49. doi: 10.3322/caac.21660

2. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J Clin*. (2023) 73:233–54. doi: 10.3322/caac.21772

3. Bailey CE, Hu CY, You YN, Bednarski BK, Rodriguez-Bigas MA, Skibber JM, et al. Increasing disparities in the age-related incidences of colon and rectal cancers in the United States, 1975-2010. *JAMA Surg*. (2015) 150:17–22. doi: 10.1001/jamasurg.2014.1756

4. Chalieopanyarwong V, Boonpipattanapong T, Prechawittayakul P, Sangkhathat S. Endoscopic obstruction is associated with higher risk of acute events requiring emergency operation in colorectal cancer patients. *World J Emerg Surg*. (2013) 8:34. doi: 10.1186/1749-7922-8-34

5. Hong KD, Um JW, Ji WB, Jung SY, Kang S, Lee SI, et al. Endoscopic obstruction in rectal cancers: survival and recurrence patterns following curative surgery. *J Laparoendosc Adv Surg Tech A*. (2015) 25:278–84. doi: 10.1089/lap.2014.0566

6. Mo TW, Zhang ZJ, Shi YQ, Xiang HD, Chen YL, Chen ZX, et al. Prognostic implications of endoscopic obstruction in patients with pathological stage II colon cancers: a single-center retrospective cohort study. *J Gastrointest Surg*. (2023) 27:141–51. doi: 10.1007/s11605-022-05516-5

7. Deans GT, Krukowski ZH, Irwin ST. Malignant obstruction of the left colon. *Br J Surg*. (1994) 81:1270–6. doi: 10.1002/bjs.1800810905

8. Smothers L, Hynan L, Fleming J, Turnage R, Simmang C, Anthony T. Emergency surgery for colon carcinoma. *Dis Colon Rectum*. (2003) 46:24–30. doi: 10.1007/s10350-004-6492-6

9. Yang Z, Wang L, Kang L, Xiang J, Peng J, Cui J, et al. Clinicopathologic characteristics and outcomes of patients with obstructive colorectal cancer. *J Gastrointest Surg*. (2011) 15:1213–22. doi: 10.1007/s11605-011-1563-1

10. Saha S, Shaik M, Johnston G, Saha SK, Berbiglia L, Hicks M, et al. Tumor size predicts long-term survival in colon cancer: an analysis of the National Cancer Data Base. *Am J Surg*. (2015) 209:570–4. doi: 10.1016/j.amjsurg.2014.12.008

11. Compton C, Fenoglio-Preiser CM, Pettigrew N, Fielding LP. American Joint Committee on Cancer prognostic factors consensus conference. *Cancer*. (2000) 88:1739–57. doi: 10.1002/(ISSN)1097-0142

12. Crozier JE, McMillan DC, McArdle CS, Angerson WJ, Anderson JH, Horgan PG, et al. Tumor size is associated with the systemic inflammatory response but not survival in patients with primary operable colorectal cancer. *J Gastroenterol Hepatol*. (2007) 22:2288–91. doi: 10.1111/j.1440-1746.2006.04792.x

13. Santullo F, Biondi A, Cananzi FCM, Fico V, Tirelli F, Ricci R, et al. Tumor size as a prognostic factor in patients with stage IIa colon cancer. *Am J Surg*. (2018) 215:71–7. doi: 10.1016/j.amjsurg.2017.03.038

14. Huang B, Feng Y, Zhu L, Xu T, Huang L, Cai G. Smaller tumor size is associated with poor survival in stage II colon cancer: An analysis of 7,719 patients in the SEER database. *Int J Surg*. (2016) 33 Pt A:157–63. doi: 10.1016/j.ijsu.2016.07.073

15. Weiser MR. AJCC 8th edition: colorectal cancer. *Ann Surg Oncol*. (2018) 25:1454–5. doi: 10.1245/s10434-018-6462-1

16. Lv Z, Liang Y, Liu H, Mo D. Association of chemotherapy with survival in stage II colon cancer patients who received radical surgery: a retrospective cohort study. *BMC Cancer*. (2021) 21:306. doi: 10.1186/s12885-021-08057-3

17. Wong SK, Jalaludin BB, Morgan MJ, Berthelsen AS, Morgan A, Gatenby AH, et al. Tumor pathology and long-term survival in emergency colorectal cancer. *Dis Colon Rectum*. (2008) 51:223–30. doi: 10.1007/s10350-007-9094-2

18. Diggs JC, Xu F, Diaz M, Cooper GS, Koroukian SM. Failure to screen: predictors and burden of emergency colorectal cancer resection. *Am J Manag Care*. (2007) 13:157–64.

19. Bass G, Fleming C, Conneely J, Martin Z, Mealy K. Emergency first presentation of colorectal cancer predicts significantly poorer outcomes: a review of 356 consecutive Irish patients. *Dis Colon Rectum*. (2009) 52:678–84. doi: 10.1007/DCR.0b013e3181a1d8c9

20. Lv X, Yu H, Gao P, Song Y, Sun J, Chen X, et al. A nomogram for predicting bowel obstruction in preoperative colorectal cancer patients with clinical characteristics. *World J Surg Oncol*. (2019) 17:21. doi: 10.1186/s12957-019-1562-3

21. Phillips RK, Hittinger R, Fry JS, Fielding LP. Malignant large bowel obstruction. *Br J Surg*. (1985) 72:296–302. doi: 10.1002/bjs.1800720417

22. Kumar A, Dhungana SB, Gupta RK, Sah SP, Khanal B. Clinico-pathological characteristics of obstructing colorectal cancer and its management outcomes at a tertiary referral center of Eastern Nepal. *BMC Gastroenterol*. (2022) 22:303. doi: 10.1186/s12876-022-02380-0

23. Kubisch CH, Crispin A, Mansmann U, Goke B, Kolligs FT. Screening for colorectal cancer is associated with lower disease stage: A population-based study. *Clin Gastroenterol Hepatol*. (2016) 14:1612–8 e3. doi: 10.1016/j.cgh.2016.04.008

24. Moreno CC, Mittal PK, Sullivan PS, Rutherford R, Staley CA, Cardona K, et al. Colorectal cancer initial diagnosis: screening colonoscopy, diagnostic colonoscopy, or emergent surgery, and tumor stage and size at initial presentation. *Clin Colorect Cancer*. (2016) 15:67–73. doi: 10.1016/j.clcc.2015.07.004

25. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science*. (2011) 331:1559–64. doi: 10.1126/science.1203543

26. Nakafusa Y, Tanaka T, Tanaka M, Kitajima Y, Sato S, Miyazaki K. Comparison of multivisceral resection and standard operation for locally advanced colorectal cancer: analysis of prognostic factors for short-term and long-term outcome. *Dis Colon Rectum*. (2004) 47:2055–63. doi: 10.1007/s10350-004-0716-7

27. Chou JF, Row D, Gonen M, Liu YH, Schrag D, Weiser MR. Clinical and pathologic factors that predict lymph node yield from surgical specimens in colorectal cancer: a population-based study. *Cancer*. (2010) 116:2560–70. doi: 10.1002/cncr.25032

28. Konishi T, Shimada Y, Hsu M, Tufts L, Jimenez-Rodriguez R, Cercek A, et al. Association of preoperative and postoperative serum carcinoembryonic antigen and colon cancer outcome. *JAMA Oncol*. (2018) 4:309–15. doi: 10.1001/jamaoncol.2017.4420

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Development and validation of a faecal immunochemical test-based model in the work-up of patients with iron deficiency anaemia

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Objective: In patients with iron deficiency anaemia (IDA), the diagnostic yield of gastroscopy and colonoscopy (bidirectional endoscopy) in detecting neoplastic lesions is low. This study aimed to develop and validate a faecal immunochemical test (FIT)-based model to optimise the work-up of patients with IDA.

Methods: Outpatients with IDA were enrolled in a prospective, multicentre study from April 2016 to October 2019. One FIT was performed before bidirectional endoscopy. Significant gastrointestinal lesions were recorded and a combined model developed with variables that were independently associated with significant colorectal lesions in the multivariate analysis. The model cut-off was selected to provide a sensitivity of at least 95% for colorectal cancer (CRC) detection, and its performance was compared to different FIT cut-offs. The data set was randomly split into two groups (developed and validation cohorts). An online calculator was developed for clinical application.

Results: The development and validation cohorts included 373 and 160 patients, respectively. The developed model included FIT value, age, and sex. In the development and validation cohorts, a model cut-off of 0.1375 provided a negative predictive value of 98.1 and 96.7% for CRC and 90.7 and 88.3% for significant colorectal lesions, respectively. This combined model reduced the rate of missed significant colorectal lesions compared to FIT alone and could have avoided more than one-fourth of colonoscopies.

Conclusion: The FIT-based combined model developed in this study may serve as a useful diagnostic tool to triage IDA patients for early endoscopic referral, resulting in considerable reduction of unnecessary colonoscopies.

KEYWORDS

colorectal cancer, cancer-diagnosis, colonoscopy, diagnostic tests, endoscopy, iron deficiency, anaemia

Introduction

Iron deficiency anaemia (IDA) is a major public health problem with a worldwide prevalence of 4.5 to 18% (1), accounting for up to 13% of referrals from general practitioners to gastroenterologists (2). Gastrointestinal blood loss or malabsorption are the main causes of IDA in postmenopausal women and men (2).

IDA is a predictive factor for gastrointestinal malignancies, especially in males of advanced age (3). The prevalence of gastrointestinal neoplasia in patients with IDA has been reported to be 10–20% (4). Therefore, IDA is considered an indication for urgent endoscopic referral (5). However, the primary care setting is associated with a low positive predictive value (PPV), representing only 5.8 and 1.0% for colorectal cancer (CRC) and stomach cancer, respectively (6).

After excluding celiac disease, clinical guidelines recommend diagnostic colonoscopy and gastroscopy (bidirectional endoscopy), which account for a significant workload in endoscopic units. However, no consensus has yet been reached on whether these two procedures should be carried out simultaneously, if one should take preference over the other, or if the second procedure could be omitted if the cause of IDA is detected by the first procedure (2, 7–9). Moreover, only approximately 10% of patients have significant lesions taking into consideration both endoscopic procedures (2).

Available non-invasive tests for detecting faecal occult blood loss, *Helicobacter pylori (H. pylori)* infection, or autoimmune gastritis may identify patients at risk for clinically significant gastrointestinal lesions and could help guide the work-up of patients with IDA (10, 11). The faecal immunochemical test (FIT) detects the globin from human haemoglobin (Hb) by means of monoclonal or polyclonal antibodies and allows quantification of the faecal Hb concentration. Since globin is degraded in the upper gastrointestinal tract, it cannot be detected by FIT assays. Therefore, FIT mostly detects intact globin coming from lower gastrointestinal blood loss. The 2017 Institute for Health and Care Excellence (NICE) (DG30) guidelines included FIT in the work-up of patients with lower abdominal symptoms for suspected CRC (12). Recently, FIT triage has been suggested to be useful in symptomatic patients with diagnoses other than CRC (13). However, the accuracy of FIT and its optimal cut-offs in specific subgroups of symptomatic patients are unknown. The few studies assessing the accuracy of FIT to guide the work-up of patients with IDA have been inconclusive, mainly due to their retrospective design, small sample size, heterogeneous definition of significant gastrointestinal lesions, or use of the less accurate guaiac-based faecal occult blood test (14–27).

In the current study, we hypothesised that FIT may serve as a decision-making tool in the work-up of patients with IDA, as patients with a negative test are likely to not have a significant colorectal lesion. Therefore, we aimed to build a risk prediction model based on FIT analysis to help in the endoscopic work-up of patients with IDA. The study was registered at ClinicalTrials.gov, identifier: NCT02792023.

Methodology

Study design, setting, and participants

This prospective multicentre cohort study was carried out in five Spanish hospitals and one Uruguayan hospital from April 2016 to October 2019. We included patients referred for bidirectional endoscopy who satisfied the following inclusion criteria: men and women aged ≥18 years with non-investigated IDA, defined as serum Hb ≤11.9 g/dL for men and ≤10.9 g/dL for women, with a serum ferritin concentration ≤30 ng/mL and transferrin saturation index (TSI) ≤16%. We also considered for inclusion patients with chronic kidney disease and/or heart failure with serum ferritin ≤200 ng/mL and TSI ≤25% and patients with an inflammatory disorder with serum ferritin ≤100 ng/mL and TSI ≤16%. These patients were diagnosed with IDA both when they consulted for symptoms of an anemic syndrome and those in whom it was detected incidentally during laboratory exams. Exclusion criteria included: hospitalised patients and those who were not candidates for endoscopic procedures due to a poor performance status; patients with any other source of blood loss, including metrorrhagia or menorrhagia; abdominal or rectal mass on physical exploration; a personal history of inflammatory bowel disease (IBD) or family history of a hereditary CRC syndrome; previous gastrointestinal surgery or having undergone colonoscopy, gastroscopy, or videocapsule endoscopy in the last 5 years; pregnancy; and refusal to participate. Moreover, none of the patients that were included in this study followed a vegan or vegetarian diet. Patients were not included for further analysis if they did not return a FIT sample, did not attend the endoscopic procedures, and/or had an incomplete gastroscopy and/or colonoscopy, unless due to neoplasia.

Colonoscopy was considered incomplete if the bowel cleansing score according to the Boston Bowel Preparation Scale (28) was <2 points at any colonic segment. The protocol was approved by the Local Ethics Committee of Hospital Universitario de Canarias, and all participants provided written informed consent.

Outcomes

The primary outcome of this study was to develop and validate a FIT-based combined predictive model for detecting CRC and other significant colorectal lesions to prioritise patients with IDA for colonoscopy. The secondary outcomes were to compare the

diagnostic accuracy of the FIT-based combined model vs. FIT at cut-offs of 2 μg Hb/g and 10 μg Hb/g in faeces to detect CRC or other significant colorectal lesions.

Study interventions

All patients with IDA referred for bidirectional endoscopy attended an appointment with a gastroenterologist to verify inclusion/exclusion criteria and to perform a physical examination. On the day of the appointment, eligible participants received a single FIT kit (OC-Sensor™, EikenChemical Company, Tokyo, Japan) and were instructed to collect a sample from a spontaneous bowel movement 24 to 48 h before starting bowel cleansing for colonoscopy. Samples were returned the day of the endoscopic procedures and processed at each institution according to the manufacturer's instructions. A blood sample was also obtained to investigate the presence of anti-transglutaminase antibodies.

Bidirectional endoscopy was scheduled within 1 month after inclusion. Both procedures were performed under conscious intravenous sedation following the protocol of each centre. Endoscopists were blind to the FIT result. At colonoscopy, biopsies were taken and therapeutic techniques applied as needed. In patients with incomplete colonoscopy for technical reasons, a colonic videocapsule endoscopy was scheduled. At gastroscopy, biopsies were taken systematically at the gastric body and antrum to rule out *H. pylori* infection and atrophic gastritis, and at the bulb and second portion of the duodenum for celiac disease diagnosis. Oral or intravenous iron therapy was initiated as needed. Patients in whom bidirectional endoscopy did not detect the cause of IDA and had an inadequate response to iron therapy were scheduled for small bowel videocapsule endoscopy.

Definitions

The following significant colorectal lesions were considered as potential sources of IDA: CRC, advanced polyp (defined as an adenoma or serrated lesion ≥10 mm in size, and any polyp with high-grade dysplasia, tubulovillous component or traditional serrated adenoma), angiodysplasia, IBD, colitis, actinic proctitis, and solitary colorectal ulcer. In the upper gastrointestinal tract, gastric, oesophageal, and stomach cancer, angiodysplasia, gastric antral vascular ectasia, peptic ulcer, acute gastric mucosal lesions, IBD, celiac disease, gastric polyp ≥10 mm in size, Los Angeles C and D peptic esophagitis, Cameron lesions, *H. pylori*, and atrophic gastritis were considered significant lesions responsible for IDA. Significant lesions (ulcer or erosion, IBD, angiodysplasia, or neoplasia) detected by small bowel capsule endoscopy were classified as significant upper gastrointestinal lesions.

Statistical analysis, validation, and development of the statistical model

The sample size was calculated following the four-step guide proposed by Riley et al. (29). Briefly, a confidence level of 95%, an absolute margin of error of 0.05, an outcome proportion in the study population of 0.2, a mean absolute prediction error of 0.05, eight candidate predictor parameters, an expected shrinkage factor of 0.9, and a Cox-Snell R-squared statistic anticipating a value of 0.15 were set. Based on this premise, the necessary sample size should be at least 417 individuals. In addition, our final cohort was divided by random selection into a development cohort (70%) and validation cohort (30%).

The characteristics of the two cohorts were considered as frequencies (%), means (±standard deviation [SD]), or medians with interquartile ranges (IQRs) depending on the type of variable and whether it follows a Gaussian distribution. Comparisons between the development and validation cohorts were performed using the chi-squared test, Student *t*-test, U-Mann–Whitney, or Fisher test. p values <0.05 were considered significant. Data were analysed using the Statistical Package for Social Sciences v. 26.0 (IBM SPSS Statistics, Armonk; NY: IBM Corp) and MedCalc v. 11.5 (MedCalc Software, Mariakerke, Belgium).

To assess the risk of presenting a significant colorectal lesion, a simple logistic regression was performed including demographic data (age, sex, and body mass index), clinical data (Charlson's comorbidity index) (30), elapsed time from the IDA diagnosis to the date of study inclusion, treatment with antiplatelet agents, anticoagulants, non-steroidal anti-inflammatory drugs, proton pump inhibitors, and/or corticosteroids and laboratory tests (FIT [μg Hb/g in faeces], serum Hb [g/dL], serum ferritin [ng/mL], and TSI [%]). Serum parameters were measured at study inclusion, 4 weeks, and at 6 months of follow-up. Variables with $p \leq 0.1$ were included in a multiple logistic regression analysis and expressed as odds ratios (ORs) and 95% confidence intervals (CIs). Significant variables ($p < 0.05$) and Wald's forward variable selection method were used to construct the risk score. FIT value was entered on a logarithmic scale (Ln) to stabilise its variability. ROC curves were developed to estimate the negative predictive value (NPV), PPV, sensitivity, and specificity for FIT at cut-offs of 2 μg Hb/g, as it is the manufacturer's defined lower detection limit, and 10 μg Hb/g in faeces according to NICE guideline criteria for symptomatic patients at low risk for CRC (11). Colonoscopy is the gold standard technique for detecting CRC, with a reported sensitivity of 94.7% (95% CI 90.4 to 92.7%) (31). Thus, ROC curves were developed to select the threshold for the positivity of the FIT-based combined model, which could prevent or delay colonoscopies without losing diagnostic value by fulfilling the following criteria: (1) sensitivity of at least 95% and (2) NPV of 98% for CRC detection. Thus, according to this threshold patients were divided into high vs. low risk for having a significant colorectal lesion or CRC. To validate the predictive value of the resultant model, we used the selected cut-off of the development model to calculate its performance by ROC curves in the validation cohort. The study accomplished the STROBE checklist for observational studies.

Results

Of 1,219 consecutive patients with an endoscopic referral for IDA investigation, 611 satisfied the inclusion and exclusion criteria. Among them, 78 patients were excluded after inclusion, mostly due to not attending endoscopic procedures. Overall, 533 patients were included in the study and followed-up during 6 months. They were split up into the development ($n = 373$) and validation ($n = 160$) cohorts (Figure 1).

No significant differences were found between the development and validation cohorts regarding demographics, comorbidities, time to diagnosis, medications, or laboratory findings (Table 1). The mean age was 69.7 ± 12.8 years and females predominated over males. The

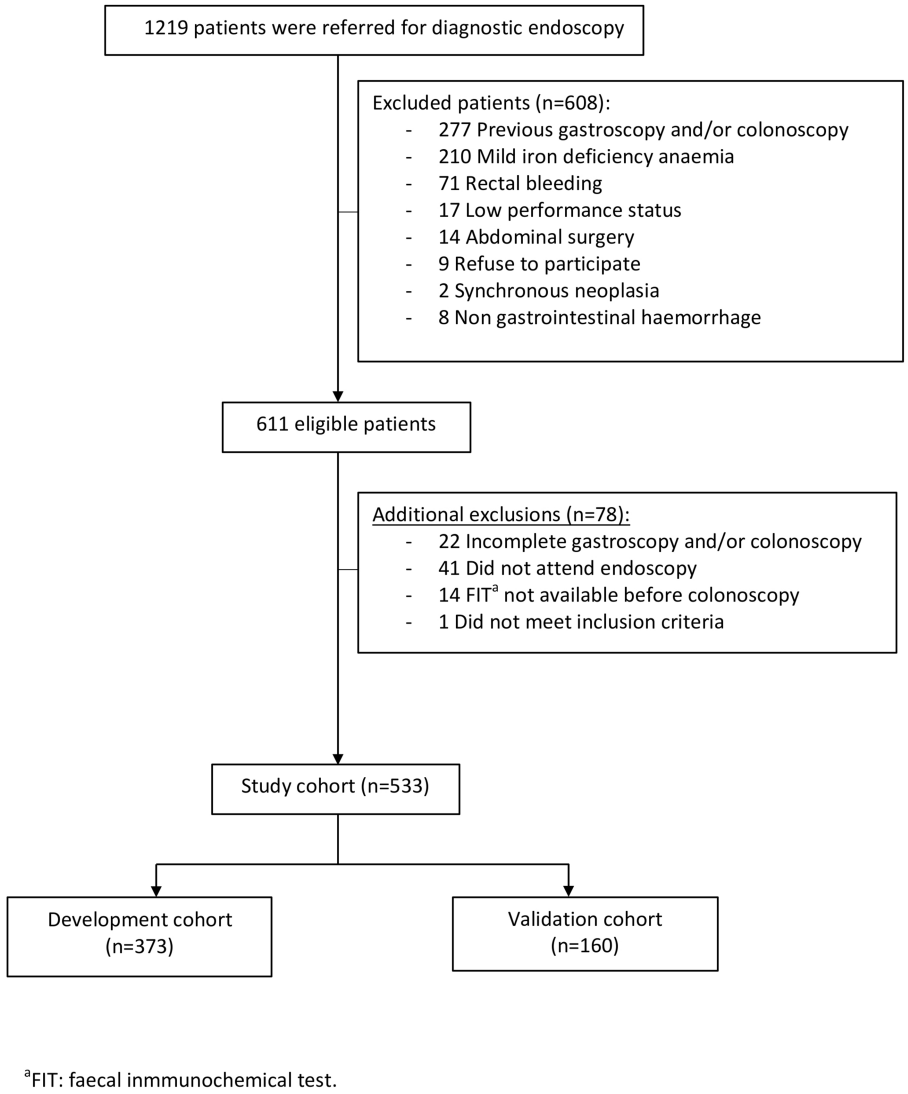


FIGURE 1
Study flow chart.

mean serum Hb and MCV levels were 9.5 ± 1.4 g/dL and 77.2 ± 9.1 fL, respectively. The median serum ferritin level and TSI were 10 ng/mL (IQR 7–15.5 ng/mL) and 5.9% (IQR 4.1–8.6%), respectively. Up to 50% of patients received antiaggregants and/or anticoagulants, and almost 62% of patients were on proton pump inhibitors.

Table 2 shows the lesions found at colonoscopy and gastroscopy. There were no significant differences between the development and validation cohorts regarding endoscopic findings. Most patients (66.6%) had a normal colonoscopy. CRC was found in 68 (12.8%) patients, equally distributed between the development ($n = 48$, 12.9%) and validation cohorts ($n = 20$, 12.5%). Conversely, in the whole cohort, an upper significant lesion was detected in 62.9% at gastroscopy, with a non-neoplastic lesion being the most relevant finding. *H. pylori* infection accounted for up to 28.3% of the upper significant lesions. A gastric neoplasia was found in 17 (3.2%) patients. Concomitant lesions in the upper and lower gastrointestinal tract were found in 66 (17.7%) and 30 (18.8%) of patients in the development and validation cohorts, respectively. However, none of these patients had a synchronous neoplasia in both locations (Supplementary Table S1). Small bowel videocapsule endoscopy was performed in 75 patients, 40 (53.3%) of whom had a significant upper gastrointestinal lesion, with angiodysplasia (21%) being the most prevalent finding (Supplementary Table S2).

Fit-based combined model

Briefly, the model was constructed with the variables that were independently associated with the detection of significant colorectal lesions in the univariate analysis (Supplementary Table S3): age, sex, and FIT value. An imputation method was not applied due to absecense of missing data in these variables. Accordingly, the combined model $p = 1/(1+\exp(-\eta(x)))$ was built, with p representing the probability of suffering from a significant colorectal lesion and $\eta(x)$ representing the linear predictor given by the following equation: $\eta(x) = -3.277 + 0.473 * \text{Ln}(\text{FIT} + 1) - 0.596 * (\text{if Sex} = \text{Female}) - 0.023 * \text{Age}$. The cut-off of $p > 0.1375$ was selected according to ROC curves, as it was the first cut-off that yielded at least

TABLE 1 Baseline characteristics of patients at the time of diagnosis of iron deficiency anaemia.

	Total ($N = 533$)	Development cohort ($N = 373$)	Validation cohort ($N = 160$)	p
Clinical data				
Age (years), mean ± SD	69.7 ± 12.8	69.9 ± 12.7	69.2 ± 13.1	0.560
Female, *n* (%)	340 (63.8)	236 (63.3)	104 (65.0)	0.768
Charlson's score, mean ± SD	3.6 ± 2.2	3.6 ± 2.2	3.7 ± 2.4	0.458
Time to diagnosis (months), median (IQR)	9 (3–23)	9 (3–24)	8 (2–21)	0.168
BMI (kg/m^2), mean ± SD	28.8 ± 5.1	28.8 ± 5.2	28.9 ± 5.0	0.773
Basal laboratory findings				
Hb (g/dL), mean ± SD	9.5 ± 1.4	9.6 ± 1.4	9.4 ± 1.6	0.265
MCV (fL), mean ± SD	77.2 ± 9.1	77.5 ± 9.4	76.6 ± 8.2	0.274
Ferritin (ng/mL), median (IQR)	10 (7–15.5)	10 (7–15)	10 (7–16)	0.991
TSI (%), median (IQR)	5.9 (4.1–8.6)	5.9 (4.1–8.4)	5.7 (4.0–9.0)	0.799
FIT (μg Hb/g of faeces), median (IQR)	6 (0–72.5)	6 (0–69.8)	5.5 (0–78.2)	0.780
Medication, *n* (%)				
Antiaggregant	204 (38.3)	139 (37.3)	65 (40.6)	0.497
NSAIDs	59 (11.1)	42 (11.3)	17 (10.6)	0.881
Oral anticoagulant	64 (11.8)	40 (10.7)	23 (14.4)	0.243
PPI	330 (61.9)	231 (61.9)	99 (61.9)	0.990

SD, standard deviation; IQR, interquartil range; BMI, body mass index; MCV, median corpuscular volume; TSI, transferrin saturation index; FIT, fecal immunochemical test; NSAID, non-steroideal anti-inflammatory drug; PPI, proton pump inhibitor. There were only missing data in BMI in 120 patients and TSI in 20 patients.

a 95% sensitivity (95.8%) for CRC detection; its specificity, NPV, and PPV were 32.31, 98.1, and 17.3%, respectively (Table 3). The corresponding sensitivity, specificity, NPV, and PPV for detecting a significant colorectal lesion were 91.87, 38.8, 90.7, and 42.5%, respectively. The sensitivity and NPV of the FIT-based combined model at the selected cut-off were higher than those achieved at FIT cut-offs of 2 and 10 μg Hb/g in faeces, respectively (Table 4).

According to the FIT-based combined model, 107 colonoscopies (28.7%) would have been prevented or delayed. Consequently, the development cohort was divided according to the FIT-based combined cut-off into groups of patients at high risk (>0.1375) and low risk (≤0.1375) for having a significant colorectal lesion or specifically CRC. As shown in Figures 2A,B, up to 10 significant colorectal lesions had not been identified, even when using the FIT-based combined model, though only two of them were CRC. However, the number of significant colorectal lesions and CRC that would be missed with this model is considerably lower than the number of missed lesions found when the FIT is applied alone, either at 2 or 10 μg Hb/g in faeces (Supplementary Table S4). In addition, there were no significant differences in the demographic and clinicopathological features when CRC patients were classified as high vs. low risk (Supplementary Table S5).

Study validation

A total of 160 patients were included in the validation cohort. The corresponding sensitivity, specificity, NPV, and PPV for detecting significant colorectal lesions or only CRC using the FIT cut-offs of 2 and 10 μg Hb/g faeces are shown in Table 4. Applying the FIT-based combined model developed in the validation cohort at the selected cut-off of 0.1375, the sensitivity and NPV for detecting a significant colorectal lesion were 87.27 and 88.3%, respectively. The corresponding sensitivity and NPV increased for CRC detection, up to 90.0 and 96.7%, respectively (Table 4). Based on this threshold, the validation cohort was divided into high (>0.1375) and low risk (≤0.1375) groups regarding significant colorectal lesions or CRC (Figures 2C,D). Using this model, 60 colonoscopies (37.5%) would be prevented or delayed and 10 significant colorectal lesions, 2 of them CRC, would be missed (Supplementary Table S4). No significant differences were found in the demographic and clinicopathological features of CRC patients classified as high vs. low risk (Supplementary Table S5).

Proposed work-up algorithm in patients with IDA

Considering the higher sensitivity and NPV of the proposed FIT-based combined model, the low rate of concomitant lesions at the upper and lower gastrointestinal tract, and the absence of synchronous neoplasia in both locations, we propose the following algorithm for the work-up of patients with IDA (Figure 3): in patients with a FIT-based combined model score ≤ 0.1375, gastroscopy should be performed first, as the probability of having a significant colorectal lesion is very low. If a significant upper gastrointestinal lesion is detected in this procedure, it should be treated as needed. After that, a safe netting for the patient must be ensured by assessing whether the IDA persists, if there are any additional symptoms, or if clinical concern remains to undergo further colorectal investigation. On the other hand, if the FIT-based combined model score is >0.1375,

TABLE 2 Results of bidirectional endoscopy at the development and validation cohorts.

Findings[†]	Total (*n* = 533)	Development cohort (*n* = 373)	Validation cohort (*n* = 160)	*p*
Colonoscopy, *n* (%)				
No lesions	353 (66.6)	250 (67.0)	105 (65.6)	0.764
CRC[‡]	68 (12.8)	48 (12.9)	20 (12.5)	0.907
Advanced adenoma[‡,§]	57 (10.7)	38 (10.2)	19 (11.9)	0.545
Angiodysplasia	47 (8.8)	33 (8.8)	14 (8.8)	0.971
IBD[‡]	2 (0.4)	2 (0.5)	–	0.877
Other lesions	6 (1.1)	4 (1.1)	2 (1.3)	0.859
Gastroscopy, *n* (%)				
No lesions	198 (37.1)	137 (36.7)	61 (38.1)	0.770
Helicobacter pylori[‡]	151 (28.3)	105 (28.2)	46 (28.7)	0.917
Atrophic gastritis[‡]	92 (17.3)	68 (18.2)	24 (15.0)	0.385
Peptic ulcer	41 (7.7)	27 (7.2)	14 (8.8)	0.595
Polyp ≥10 mm	25 (4.7)	18 (4.8)	7 (4.4)	0.998
Angiodysplasia	23 (4.3)	18 (4.8)	5 (3.1)	0.488
Stomach neoplasia[‡,¶]	17 (3.2)	11 (2.9)	6 (3.8)	0.600
Esophagitis C/D and/or hiatal hernia with bleeding stigmata	16 (3.0)	11 (2.9)	5 (3.1)	0.913
Celiac disease[‡]	5 (0.9)	3 (0.8)	2 (1.3)	0.639

[†]The number of significant lower and upper gastrointestinal lesions may overcome the total number of patients in each cohort because there are patients with several lesions. CRC, colorectal cancer; IBD, inflammatory bowel disease. [‡]Histological confirmation. [§]defined as: size ≥10 mm. Villous histology and/or high grade displasia or in situ adenocarcinoma; [¶](gastric cancer + gastrointestinal stromal tumour).

TABLE 3 Accuracy of FIT-based combined model cut-offs for colorectal cancer detection in the development cohort.

FIT cut-off	Sensitivity % (95% CI)	Specificity % (95% CI)	NPV % (95% CI)	PPV % (95% CI)
>0.1369	95.83 (85.7–99.5)	31.38 (26.4–36.7)	98.1 (93.2–99.8)	17.1 (12.8–22.1)
>0.137	95.83 (85.7–99.5)	31.69 (26.7–37.1)	98.1 (93.3–99.8)	17.2 (12.8–22.2)
>0.1372	95.83 (85.7–99.5)	32.00 (27.0–37.4)	98.1 (93.4–99.8)	17.2 (12.9–22.3)
>0.1375	95.83 (85.7–99.5)	32.31 (27.3–37.7)	98.1 (93.4–99.8)	17.3 (12.9–22.4)
>0.1395	93.75 (82.8–98.7)	32.31 (27.3–37.7)	97.2 (92.1–99.4)	17.0 (12.7–22.1)
>0.1396	93.75 (82.8–98.7)	32.62 (27.5–38.0)	97.2 (92.2–99.4)	17.0 (12.7–22.1)
>0.1408	93.75 (82.8–98.7)	32.92 (27.8–38.3)	97.3 (92.2–99.4)	17.1 (12.8–22.2)

FIT, faecal immunochemical test; NPV, negative predictive value; PPV, positive predictive value. †Selected cut-off with the highest accuracy for detecting colorectal cancer.

bidirectional endoscopy is recommended. Supplementary Figures S1, S2 show how this algorithm was applied for the development and validation cohorts, respectively.

Online calculator

The formula derived from the FIT-based combined model allows the development of an easy-to-use clinical online calculator by entering only three variables (age, sex, and the FIT value). This approach provides the likelihood of having a significant colorectal lesion and/or CRC in patients with IDA. This calculator is freely available online at https://idafit.org/ (Figure 4) and could help guide the work-up of patients with IDA, avoiding unnecessary explorations.

Discussion

The current study suggests that a FIT-based combined model including age, gender, and FIT value could be of great help in the work-up of patients with IDA, avoiding bidirectional endoscopy in about one-third of patients. Interestingly, the high NPV of the model allows CRC to be ruled out with high confidence, preventing a substantial number of unnecessary colonoscopies.

This study has several strengths. First, to the best of our knowledge, this is the first time that a prospective study has specifically assessed a FIT-based combined model in the work-up of patients with IDA. Second, its multicentre nature, strict inclusion criteria involving only patients with mild-severe non-investigated IDA who completed a high-quality bidirectional endoscopy, a follow-up of at least 6 months, and confirmation of results in a

TABLE 4 FIT cut-offs accuracy comparison to detect colorectal cancer or any significant colorectal lesion in the development and validation cohorts.

	Colorectal cancer			Significant colorectal lesion		
	FIT 2 μg/g	FIT 10 μg/g	Combined model 0.1375	FIT 2 μg/g	FIT 10 μg/g	Combined model 0.1375
Development cohort						
Sensitivity (%)	91.7	81.2	95.8	86.2	72.4	91.9
Specificity (%)	44.6	61.2	32.3	52.8	69.6	38.8
NPV (%)	97.3	95.7	98.1	88.6	83.7	90.7
PPV (%)	19.6	23.6	17.3	47.3	53.9	42.5
Validation cohort						
Sensitivity (%)	90.0	90.0	90.0	85.4	78.2	87.3
Specificity (%)	46.3	62.1	41.4	56.2	73.3	50.5
NPV (%)	97.0	97.8	96.7	88.1	86.5	88.3
PPV (%)	19.4	25.4	18	50.5	65.5	48

FIT, faecal immunchemical test; CRC, colorectal cancer; SCL, significant colorectal lesion; NPV, negative predictive value; PPV, positive predictive value.

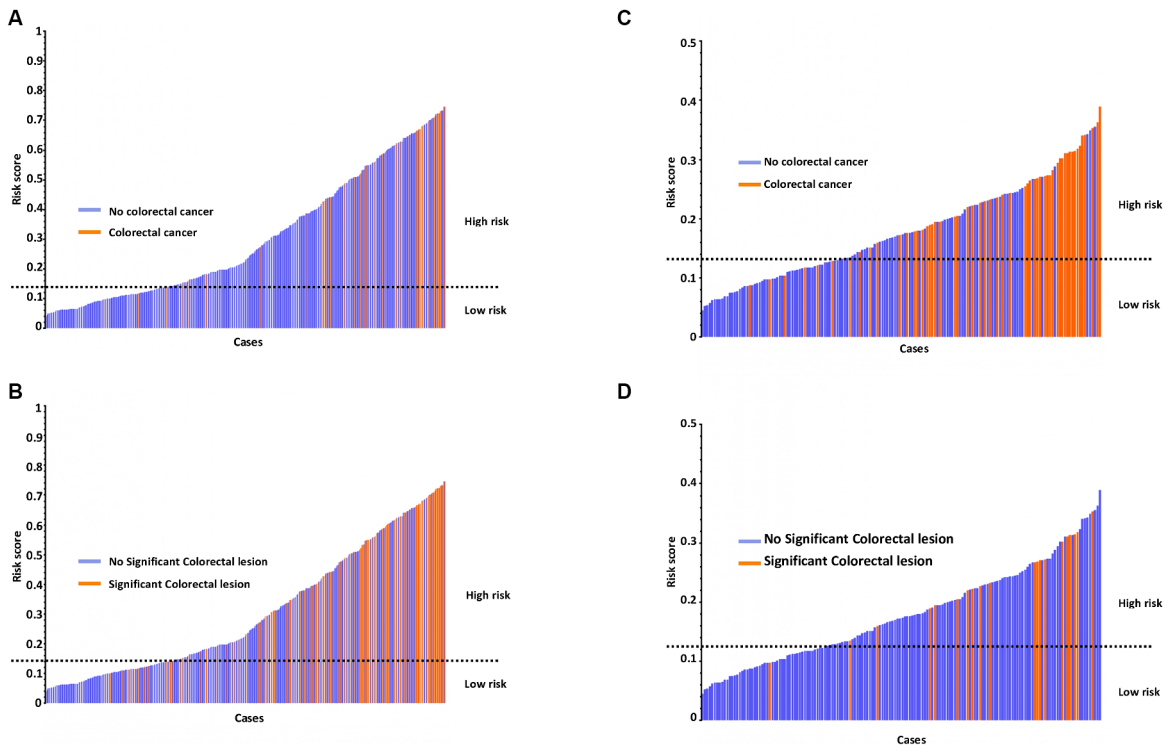
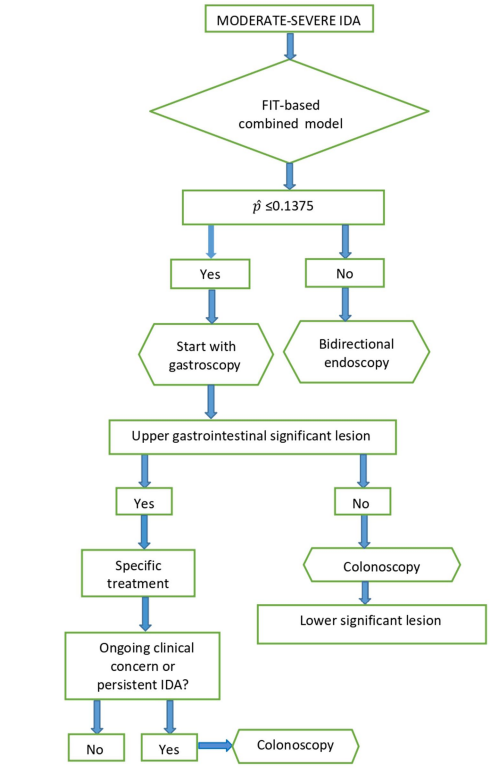


FIGURE 2
Risk score for the detection of CRC or any significant colonic lesion according to the FIT-based combined model in the development cohort **(A,B)** and validation cohort **(C,D)**.

validation cohort reinforce the power of the study. Third, the analysis incorporates a rationale threshold for detecting significant colorectal lesions that resulted from the FIT-based combined model, which improved the diagnostic accuracy of the FIT cut-offs (2 and 10 μg Hb/g faeces) previously recommended for symptomatic patients. Fourth, an online calculator constructed with the variables included in the FIT-based combined model (age, gender, and FIT value) facilitates the decision-making process for initiating the work-up of IDA patients with either bidirectional endoscopy or gastroscopy alone.

We acknowledge that there are some limitations to overcome. First, the low PPV of the developed model for detecting a significant colorectal lesion means that a substantial volume of patients would be misclassified as high risk, resulting in unnecessary referrals for colonoscopy. However, the high NPV observed in this study is more confident than the PPV in ruling out significant colorectal lesions. As a tool for triaging patients, this model could avoid or delay approximately one-third of colonoscopies, with a rate of 5.8% CRC missed in the whole cohort, which is considerably lower than the previously reported



IDA: iron deficiency anaemia, FIT: faecal inmmunochemical test, CRC: colorectal cancer

FIGURE 3
Proposed algorithm for the work-up of patients with moderate-severe IDA based on the FIT-based combined model.

9.12% when a FIT cut-off of 10 µg Hb/g faeces is applied in symptomatic patients (31). Second, the exclusion of 77 (12.6%) patients that fulfilled the inclusion criteria but did not attend endoscopy or had incomplete explorations could introduce a selection bias, although this small percentage of patients is unlikely to alter the results of the study. Third, we did not perform an external validation, which was not possible because of the advent of the Covid-19 pandemic. Nevertheless, the internal validation could mitigate the extent of overfitting in the developed cohort.

IDA is a major health issue that leads to an unaffordable burden of endoscopic procedures in endoscopic units, which has recently worsened due to the COVID-19 pandemic. A recent meta-analysis of 12 retrospective studies suggested that FIT could be useful for prioritising symptomatic patients for colonoscopy (32). However, methodological flaws in most of these studies make this statement questionable. First, several studies included patients with IDA together with others having vague symptoms (abdominal pain, change in bowel habits), in whom the likelihood of having a significant colorectal lesion is very low. Second, this meta-analysis showed that FIT had a low sensitivity (64%) for detecting significant colorectal lesions in patients with IDA. Third, some studies assessed the efficacy of FIT together with the stool guaiac test, which is already considered obsolete for clinical practice. Fourth, among the studies that specifically assessed the diagnostic yield of FIT, only two of them had a prospective design, and both had heterogeneous definitions for IDA (22, 24). In addition, the FIT threshold was not reported in one study (22) and was >10 µg Hb/g in faeces in another (24), though it is the recommended cut-off for symptomatic patients at low risk of having CRC according to NICE guidelines (12).

On the other hand, the NICE guidelines do not include FIT for the assessment of patients with high-risk symptoms for CRC, including those with IDA (12). D'Souza et al. (31), Chapman et al. (33), and Lanas et al. (34), recently evaluated the diagnostic accuracy of FIT in these patients and concluded that FIT could be useful for ruling out CRC in this setting. Interestingly, they suggested that the threshold for FIT positivity should be reduced at the lower limit of detectability provided by the manufacturer (0–2 µg Hb/g faeces for OC-Sensor™) to have a high sensitivity for CRC. The results of the current study are in line with this finding and reinforce the concept that a lower cut-off is needed to rule out CRC with greater confidence. We found that a FIT threshold ≥10 µg Hb/g of faeces was not accurate enough to rule out significant colorectal lesions, including CRC, due to its low sensitivity (81–90%). This limitation was overcome by the FIT-based combined model developed in our study, which allowed us to select a rational threshold with a sensitivity of 95.8% and NPV 98.1% for CRC detection. Moreover, the sensitivity and NPV were also higher than 90% for detecting a significant colorectal lesion. In any case, the choice of the threshold should be a trade-off among the number of CRC cases and significant colorectal cancer lesions missed and the number of patients referred

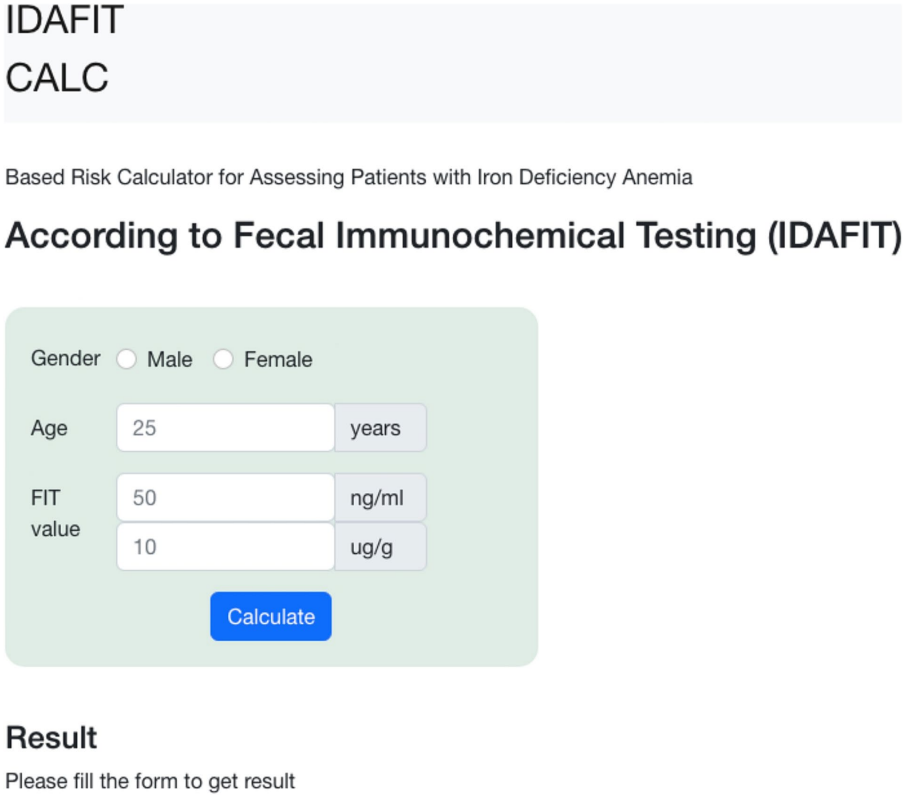


FIGURE 4
Online mobile application-based risk calculator to guide patients with iron deficiency anaemia according to faecal immunochemical testing (IDAFIT score).

for colonoscopy considering the potential complications that could be associated with the endoscopic procedures and the waiting list dealt with by endoscopic units.

An interesting finding of our study was that most significant lesions causing IDA were found in the upper gastrointestinal tract, with *H. pylori* infection being the most prevalent cause, a condition that can be diagnosed with high accuracy by non-invasive tools (35). *H. pylori* eradication therapy solves IDA in most of these patients. Further studies assessing the role *H. pylori* eradication prior to endoscopic referral are needed to evaluate the number of gastroscopies that could be avoided in this setting.

Currently, bidirectional endoscopy is recommended for all patients with IDA (9, 36). This approach is reinforced by the lack of methods capable of discriminating between upper or lower gastrointestinal lesions. The current study suggests that the implementation of a model developed by combining the patient's age, gender, and FIT value substantially improves the diagnostic accuracy for detecting CRC and other significant colorectal lesions. Interestingly, the resultant model threshold of 0.1375 allowed us to categorise patients as high risk (score > 0.1375) or low risk (score ≤ 0.1375) of having significant colorectal lesions. Based on this model, an algorithm can be proposed for the work-up of patients with IDA (Figure 3).

In summary, the current FIT-based combined model increases the diagnostic accuracy of significant colorectal lesions and provides a sensitivity equivalent to colonoscopy for detecting CRC in patients with IDA. This model provides a diagnostic tool by which to triage these patients for urgent endoscopic referral, preventing a substantial number of unnecessary colonoscopies.

Ethics statement

The studies involving humans were approved by Comité de Ética de la Investigación con Medicamentos (CEIm), Complejo Hospitalario Universitario de Canarias. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

GH: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. EQ: Conceptualization, Data curation, Funding acquisition, Investigation, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DM-A: Investigation, Project administration, Validation, Visualization, Writing – review & editing. GR: Investigation, Resources, Validation, Visualization, Writing – review & editing. GM: Investigation, Resources, Validation, Visualization, Writing – review & editing. NF:

Investigation, Resources, Visualization, Writing – review & editing. LC: Investigation, Resources, Validation, Visualization, Writing – review & editing. M-VÁ-S: Investigation, Resources, Validation, Visualization, Writing – review & editing. CO: Investigation, Resources, Validation, Visualization, Writing – review & editing. DR-A: Investigation, Resources, Validation, Visualization, Writing – review & editing. CG: Investigation, Resources, Validation, Visualization, Writing – review & editing. IA: Investigation, Resources, Validation, Visualization, Writing – review & editing. DN-P: Investigation, Methodology, Writing – review & editing. MC-P: Investigation, Resources, Validation, Visualization, Writing – review & editing. EG-D: Data curation, Formal analysis, Software, Writing – review & editing. AG-G: Funding acquisition, Investigation, Methodology, Resources, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1407812/full#supplementary-material

References

1. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. *Lancet*. (2016) 387:907–16. doi: 10.1016/S0140-6736(15)60865-0

2. Goddard AF, James MW, McIntyre AS, Scott BB. British Society of Gastroenterology. Guidelines for the management of iron deficiency anaemia. *Gut*. (2011) 60:1309–16. doi: 10.1136/gut.2010.228874

3. Capurso G, Baccini F, Osborn J, Panzuto F, Di Giulio E, Delle Fave G, et al. Can patient characteristics predict the outcome of endoscopic evaluation of iron deficiency anemia: a multiple logistic regression analysis. *Gastrointest Endosc*. (2004) 59:766–71. doi: 10.1016/s0016-5107(04)00348-7

4. Kawasaki K, Hamamoto Y, Horibe M, Shimura K, Nakamura A, Kanai T, et al. Curative resectability of gastrointestinal cancer identified from iron deficiency anemia. *Oncol Lett*. (2017) 14:4301–4. doi: 10.3892/ol.2017.6650

5. Fireman Z, Kopelman Y. The role of video capsule endoscopy in the evaluation of iron deficiency anaemia. *Dig Liver Dis*. (2004) 36:97–102. doi: 10.1016/j.dld.2003.10.009

6. NG12 suspected cancer: recognition and referral. Colorectal Cancer, Available at: www.nice.org.uk/guidance/ng12. (2017)

7. Jimenez K, Kulnigg-Dabsch S, Gasche C. Management of iron deficiency anemia. *Gastroenterol Hepatol*. (2015) 11:241–50.

8. Short MW, Domagalski JE. Iron deficiency anemia: evaluation and management. *Am Fam Physician*. (2013) 87:98–104.

9. Ko CW, Siddique SM, Patel A, Harris A, Sultan S, Altayar O, et al. AGA clinical practice guidelines on the gastrointestinal evaluation of iron deficiency anemia. *Gastroenterology*. (2020) 159:1085–94. doi: 10.1053/j.gastro.2020.06.046

10. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of *Helicobacter pylori* infection-the Maastricht V/Florence consensus report. *Gut*. (2017) 66:6–30. doi: 10.1136/gutjnl-2016-312288

11. Lahner E, Zagari RM, Zullo A, Di Sabatino A, Meggio A, Cesaro P, et al. Chronic atrophic gastritis: natural history, diagnosis and therapeutic management. A position paper by the Italian Society of Hospital Gastroenterologists and Digestive Endoscopists [AIGO], the Italian Society of Digestive Endoscopy [SIED], the Italian Society of Gastroenterology [SIGE], and the Italian Society of Internal Medicine [SIMI]. *Dig Liver Dis*. (2019) 51:1621–32. doi: 10.1016/j.dld.2019.09.016

12. NICE DG30. Quantitative faecal immunochemical tests to guide referral for colorectal cancer in primary care. Available at: https://www.nice.org.uk/guidance/dg30. (2017), (Accessed 26th July 2017)

13. Pin-Vieito N, Puga M, Fernández-de-Castro D, Cubiella J. Faecal immunochemical test outside colorectal cancer screening? *World J Gastroenterol*. (2021) 27:6415–29. doi: 10.3748/wjg.v27.i38.6415

14. Jung YS, Lee J, Moon CM. Positive fecal immunochemical test results are associated with increased risks of esophageal, stomach, and small intestine cancers. *J Clin Med*. (2020) 9:2172. doi: 10.3390/jcm9072172

15. Rodriguez-Alonso L, Rodriguez-Moranta F, Ruiz-Cerulla A, Arajol C, Serra K, Gilabert P, et al. The use of faecal immunochemical testing in the decision-making process for the endoscopic investigation of iron deficiency anaemia. *Clin Chem Lab Med*. (2020) 58:232–9. doi: 10.1515/cclm-2019-0203

16. Cilona A, Zullo A, Hassan C, Ridola L, Annese M. Is faecal-immunochemical test useful in patients with iron deficiency anaemia and without overt bleeding? *Dig Liver Dis*. (2011) 43:1022–4. doi: 10.1016/j.dld.2011.08.002

17. Green BT, Rockey DC. Gastrointestinal endoscopic evaluation of premenopausal women with iron deficiency anemia. *J Clin Gastroenterol*. (2004) 38:104–9. doi: 10.1097/00004836-200402000-00004

18. Bini EJ, Micale PL, Weinshel EH. Evaluation of the gastrointestinal tract in premenopausal women with iron deficiency anemia. *Am J Med*. (1998) 105:281–6. doi: 10.1016/s0002-9343(98)00260-5

19. Majid S, Salih M, Wasaya R, Jafri W. Predictors of gastrointestinal lesions on endoscopy in iron deficiency anemia without gastrointestinal symptoms. *BMC Gastroenterol*. (2008) 8:52. doi: 10.1186/1471-230X-8-52

20. Nakama H, Zhang B, Fattah AS, Zhang X. Colorectal cancer in iron deficiency anemia with a positive result on immunochemical fecal occult blood. *Int J Color Dis*. (2000) 15:271–4. doi: 10.1007/s003840000255

21. Kim NH, Lee MY, Park JH, Park DI, Sohn CI, Choi K, et al. A combination of fecal immunochemical test results and iron deficiency anemia for detection of advanced colorectal neoplasia in asymptomatic men. *Yonsei Med J*. (2017) 58:910. doi: 10.3349/ymj.2017.58.5.910

22. Bampton PA, Holloway RH. A prospective study of the gastroenterological causes of iron deficiency anaemia in a general hospital. *Aust NZ J Med*. (1996) 26:793–9. doi: 10.1111/j.1445-5994.1996.tb00627.x

23. Fireman Z, Gurevich V, Coscas D, Kopelman Y, Segal A, Sternberg A. Results of gastrointestinal evaluation in 90 hospitalized iron deficiency anemia patients. *Isr Med Assoc J*. (1999) 1:232–5.

24. Ayling RM, Lewis SJ, Cotter F. Potential roles of artificial intelligence learning and faecal immunochemical testing for prioritisation of colonoscopy in anaemia. *Br J Haematol*. (2019) 185:311–6. doi: 10.1111/bjh.15776

25. Chowdhury ATMD, Longcroft-Wheaton G, Davis A, Massey D, Goggin P. Role of faecal occult bloods in the diagnosis of iron deficiency anaemia. *Frontline Gastroenterol*. (2014) 5:231–6. doi: 10.1136/flgastro-2013-100425

26. Rockey DC, Cello JP. Evaluation of the gastrointestinal tract in patients with iron-deficiency anemia. *N Engl J Med*. (1993) 329:1691–5. doi: 10.1056/NEJM199312023292303

27. Kepczyk T, Kadakia SC. Prospective evaluation of gastrointestinal tract in patients with iron-deficiency anemia. *Dig Dis Sci*. (1995) 40:1283–9. doi: 10.1007/bf02065539

28. Parmar R, Martel M, Rostom A, Barkun AN. Validated scales for colon cleansing: a systematic review. *Am J Gastroenterol*. (2016) 111:197–204. doi: 10.1038/ajg.2015.417

29. Riley RD, Ensor J, Snell KIE, Harrell FE, Martin GP, Reitsma JB, et al. Calculating the sample size required for developing a clinical prediction model. *BMJ*. (2020) 368:m441. doi: 10.1136/bmj.m441

30. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. (1987) 40:373–83. doi: 10.1016/0021-9681(87)90171-8

31. D'Souza N, Georgiou Delisle T, Chen M, Benton S, Abulafi MNICE FIT Steering Group. Faecal immunochemical test is superior to symptoms in predicting pathology in patients with suspected colorectal cancer symptoms referred on a 2WW pathway: a diagnostic accuracy study. *Gut*. (2021) 70:1130–8. doi: 10.1136/gutjnl-2020-321956

32. Lee MW, Pourmorady JS, Laine L. Use of fecal occult blood testing as a diagnostic tool for clinical indications: a systematic review and meta-analysis: a systematic review and meta-analysis. *Am J Gastroenterol*. (2020) 115:662–70. doi: 10.14309/ajg.0000000000000495

33. Chapman C, Bunce J, Oliver S, Ng O, Tangri A, Rogers R, et al. Service evaluation of faecal immunochemical testing and anaemia for risk stratification in the 2-week-wait pathway for colorectal cancer: faecal immunochemical testing and anaemia in suspected colorectal cancer. *BJS Open*. (2019) 3:395–402. doi: 10.1002/bjs5.50131

34. Lanas A, Balaguer F, Sánchez-Luengo M. Fecal occult blood and calprotectin testing to prioritize primary care patients for colonoscopy referral: the advantage study. *United European Gastroenterol J*. (2023) 11:692–9. doi: 10.1002/ueg2.12446

35. Delgado S, García-Iglesias J, Titó P. Update on the management of *Helicobacter pylori* infection. Position paper from the Catalan society of. *Gastroenterol Hepatol*. (2018) 41:272–80. doi: 10.1016/j.gastrohep.2017.12.009

36. Snook J, Bhala N, Beales ILP, Cannings D, Kightley C, Logan RP, et al. British Society of Gastroenterology guidelines for the management of iron deficiency anaemia in adults. *Gut*. (2021) 70:2030–51. doi: 10.1136/gutjnl-2021-325210

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Different impacts of adipose tissue dynamics on prognosis in patients with resectable locally advanced rectal cancer treated with and without neoadjuvant treatment

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Background: Body composition is recognized to be associated with clinical outcomes in patients with locally advanced rectal cancer (LARC). This study aimed to determine the prognostic role of regional adipose tissue distribution in patients with resectable LARC treated with or without neoadjuvant chemoradiotherapy (nCRT).

Methods: This retrospective study included 281 consecutive patients who underwent radical surgery for LARC with or without preoperative nCRT between 2013 and 2019. Patients underwent contrast-enhanced CT scans before nCRT and before surgery. Visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (aSAT), and gluteal subcutaneous adipose tissue (gSAT) were quantified on the CT images. The association of adipose tissue distribution with progression-free survival (PFS) was analyzed using Cox proportional hazards analysis.

Results: A total of 102 nCRT-treated and 179 primarily resected patients were included. During a median follow-up period of 24 months, 74 (26.3%) patients experienced local recurrence or metastasis. Multivariable analysis showed that VAT was associated with PFS in all patients (hazard ratio [HR] 1.28, 95% confidence interval [CI] 1.04–1.57; P = 0.021). This association was only maintained in primarily resected patients (HR 1.31, 95% CI 1.02–1.69; P = 0.037). For patients receiving preoperative nCRT, VAT was not significantly associated with PFS, while the dynamic change in gSAT (ΔgSAT) between nCRT and surgery was associated with PFS (HR 0.43, 95%CI 0.27–0.69, P = 0.001).

Conclusion: Visceral obesity is an adverse prognostic factor in patients with resectable LARC treated by primary resection, while increased gluteal subcutaneous adiposity during preoperative nCRT may indicate favorable clinical outcomes.

KEYWORDS

locally advanced rectal cancer, neoadjuvant chemoradiotherapy, adipose tissue, prognosis, computed tomography

1 Introduction

The global incidence of locally advanced rectal cancer (LARC) is on the rise, with a high risk of postoperative recurrence or distant metastasis (1, 2). Surgical excision has been the basis of LARC treatment. In recent years, with the development of multidisciplinary comprehensive therapy concepts and medical technology, preoperative neoadjuvant chemoradiotherapy (nCRT) has been widely used for resectable LARC (3). However, regardless of the treatment methods, 25% to 30% of patients with LARC experience a distant relapse after radical surgery in clinical practice (4). Therefore, identifying the risk predictors of recurrence or distant metastasis for resectable LARC may help to screen high-risk individuals to improve the prognosis by providing active surveillance or early intervention.

Obesity, sarcopenia, and abnormal distribution of adipose tissue have been found to be negative prognostic factors for patients with LARC (5–7). Excess abdominal adipose tissue can cause serial obesity-related metabolic disorders, including insulin resistance, adipokine perturbation, and chronic inflammation, which promote carcinogenesis and cancer progression (8, 9). Moreover, the difference in the intracellular development of the adipocyte population also results in opposite effects of upper and lower-body obesity on the immune and metabolic capacities (10). Previous studies tended to investigate the impact of visceral adipose tissue on the clinical outcomes in patients with resectable LARC at a single time point, revealing that visceral obesity was associated with shorter overall survival, increased risk of postoperative complications and increased length of stay in patients undergoing surgery in LARC treated with nCRT (11–13). However, studies evaluating the association between dynamic changes in regional adipose tissue and prognosis in patients with resectable LARC are still lacking. We hypothesized that the impacts of adipose tissue distribution on prognosis between patients treated with primary resection and those receiving preoperative nCRT differed, and the dynamic changes in regional adipose tissue during nCRT were associated with prognosis.

Therefore, this study evaluated the prognostic role of regional adipose tissue in patients with resectable LARC treated with or without nCRT. Meanwhile, we further investigated the potential impact of nCRT on adipose tissue redistribution.

2 Methods

2.1 Study population

The study was approved by the institutional review board of The Third Xiangya Hospital, Central South University (Changsha, China) and Hunan Cancer Hospital (Changsha, China), and the requirement to obtain informed consent from patients was waived. This retrospective study included 281 consecutive patients who underwent radical surgery for LARC in The Third Xiangya Hospital, Central South University and Hunan Cancer Hospital from July 2013 to July 2019. LARC is defined as T3/T4 primary tumors or node-positive malignancies with no distant metastases (14). The diagnosis of LARC was based on the pathological examination of the tissue taken from the rectum. Patients with LARC included in the study were divided into patients treated with primary resection and those receiving preoperative nCRT by two different treatment methods. Patients were included if they satisfied the following criteria: (a) all patients underwent radical surgery and were confirmed pathologically; (b) patients underwent contrast-enhanced CT scans before nCRT and before surgery; (c) clinical data and pathology results were available. The exclusion criteria were as follows: (a) patients had a history of preoperative treatment other than nCRT; (b) CT image quality was poor; (c) nCRT treatment was incomplete.

Baseline demographic information, laboratory tests, and pathological results were obtained from electronic medical records, which included age, gender, height, weight, body mass index (BMI, weight divided by height squared), neutrophil, lymphocyte, monocyte, albumin, carcinoembryonic antigen (CEA), and TNM tumor stage.

2.2 Adipose tissue quantification

Baseline enhanced CT venous phase images of patients at the level of the third vertebra (L3) and ischial tuberosity were obtained for adipose tissue measurement from the PACS imaging system (15, 16). Each selected CT image was assessed by a single reviewer who was blinded to the clinical, pathological, and outcome data, using

opensource software (NIH ImageJ version 1.51j8, https://imagej.nih.gov/ij/), which has previously been validated to provide reliable measurements (17). Standard radiodensity thresholds measured in Hounsfield units (HU) were used to quantify the visceral adipose tissue area (VAT), abdominal subcutaneous adipose tissue area (aSAT), and gluteal subcutaneous adipose tissue area (gSAT). Thresholds for VAT are between –150 and –50 HU, and thresholds for aSAT and gSAT are between –190 and –30 HU (15). Visceral obesity was defined as the VAT area greater than 100cm^2 (18). Patients treated with preoperative nCRT underwent enhanced CT scans before nCRT and preoperative, and patients undergoing primarily resection underwent preoperative enhanced CT scan. The longitudinal change of adipose tissue in nCRT patients was expressed by the rate of change, which was the change of adipose tissue area (Δ) (the difference between preoperative and pre-nCRT) divided by the time interval (day) (Equation 1).

$$\begin{aligned}&\Delta \text{VAT; aSAT; gSAT}\\&= ((\text{preoperative CT area} - \text{pre}\\&- \text{nCRT CT area}) (\llbracket cm \rrbracket \hat{}2))\\&/((\text{time between preoperative and pre} - \text{nCRT CT}) (\text{day}))\end{aligned} \quad (1)$$

2.3 Treatment procedures

Primarily resected patients were treated by radical surgery using laparoscopic or open routes. Radical surgery included low anterior resection, abdominoperineal resection, and extended Hartmann procedure. nCRT treated patients underwent radical surgery at 5–12 weeks after completing continuous nCRT. nCRT regimens were as follows: patients were treated with long-term radiotherapy/capecitabine or long-term radiotherapy/continuous 5-Fu or long-term radiotherapy/5-Fu/LV. The recommended irradiation dose was 45–50Gy, divided into 25–28 times, and multi-field irradiation (usually 3–4 field technique) was adopted (19).

2.4 Follow-up and outcomes

Patients were followed up every 3–6 months after surgery for surveillance imaging (computed tomography chest imaging as well as abdominal and pelvic imaging on computed tomography, magnetic resonance imaging, or positron emission tomography) until disease progression, the end of the study period, or loss to follow-up. Analyses in this current report are based on updated clinical data and patient follow-up as of July 30, 2022. The primary outcome in this study was progression-free survival (PFS), which was defined as time from surgery to first occurrence of documented disease progression. Patients without an event were censored at their last disease evaluation date.

2.5 Statistical analysis

Statistical analysis was performed using the statistical software R (the R Foundation for Statistical Computing; Version 4.1.1; https://www.r-project.org/). Continuous variables were expressed as means and standard deviations (SDs), and categorical variables were expressed as numbers and percentages. Continuous variables were compared using the independent two samples t-test, and categorical variables were compared using the chi-square test or Fisher exact test. Survival curves were constructed by the Kaplan-Meier method, and the log-rank test was performed to compare the difference between groups. The Cox proportional hazard model was adopted for univariable and multivariable analyses of potential risk factors associated with PFS, and hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. The time-dependent receiver operating characteristic (ROC) curve was used to evaluate the predictive ability of variables for survival outcomes. A $P < 0.05$ was considered statistically significant and all reported P values were two-sided.

3 Results

3.1 Patient characteristics

From July 2013 to July 2019, a total of 281 patients with LARC (median age, 54.65 years; 190 males) were included, consisting of 102 patients who received preoperative nCRT (mean age, 51.87 years; 67 men) and 179 patients who underwent primary resection (mean age 56.23 years; 123 men) (Supplementary Figure 1). The demographic and clinical characteristics are shown in Table 1. The two groups showed significant differences in age ($P = 0.001$), monocyte count ($P = 0.001$), albumin level ($P = 0.008$), CEA level ($P = 0.023$), clinical stage ($P < 0.001$), lymph node stage ($P < 0.001$) and postoperative TNM stage ($P < 0.001$). No differences were found in other characteristics.

3.2 Association between visceral obesity and survival

During a median follow-up of 24 months (IQR, 13.0–34.5 months), 74 (26.3%) patients experienced local recurrence or distant metastases, including 21 patients treated with preoperative nCRT and 53 patients treated with primary resection. Kaplan-Meier curves showed that visceral obesity was associated with an increased risk of local recurrence or metastasis in all patients ($P = 0.048$). However, this association was not maintained in patients treated with primary resection ($P = 0.1$) and in patients treated with preoperative nCRT ($P = 0.13$) (Figure 1). Supplementary Figure 2 shows the CT results of adipose tissue measured at L3 and TI levels in patients with LARC.

TABLE 1 Baseline clinical characteristics of patients.

Characteristics	Total (*n* = 281)	nCRT (*n* = 102)	Primary Resection (*n* = 179)	*P*
Age, yrs, mean (SD)	54.65 (10.38)	51.87 (8.47)	56.23 (11.03)	0.001**
Gender	281	102	179	0.697
Male	190 (67.6%)	67 (65.7%)	123 (68.7%)	
Female	91 (32.4%)	35 (34.3%)	56 (31.3%)	
BMI, kg/m^2, mean (SD)	22.55 (2.74)	22.58 (3.10)	22.54 (2.53)	0.914
NLR, mean (SD), mean (SD)	2.72 (2.28)	2.70 (2.31)	2.72 (2.27)	0.937
Monocytes,10^9/L	0.45 (0.17)	0.49 (0.18)	0.42 (0.16)	0.001**
Albumin, g/L, mean (SD)	41.63 (4.50)	42.57 (3.90)	41.09 (4.74)	0.008**
CEA ng/ml, mean (SD)	10.45 (14.19)	7.91 (11.28)	11.90 (15.45)	0.023 *
Clinical stage				<0.001***
II	92 (32.7%)	15 (14.7%)	77 (43.0%)	
III	189 (67.3%)	87 (85.3%)	102 (57.0%)	
Clinical T stage				0.515
T2	13 (4.6%)	4 (3.9%)	9 (5.0%)	
T3	159 (56.6%)	54 (52.9%)	105 (58.7%)	
T4	109 (38.8%)	44 (43.1%)	65 (36.3%)	
Clinical N stage				<0.001***
N0	92 (32.7%)	15 (14.7%)	77 (43.0%)	
N1	76 (27.1%)	21 (20.6%)	55 (30.7%)	
N2	113 (40.2%)	66 (64.7%)	47 (26.3%)	
ypT/pT stage				<0.001***
T0	15 (5.3%)	15 (14.7%)	–	
T1	4 (1.5%)	4 (3.9%)	–	
T2	51 (18.1%)	42 (41.2%)	9 (5.0%)	
T3	140 (49.8%)	35 (34.3%)	105 (58.7%)	
T4	71 (25.3%)	6 (5.9%)	65 (36.3%)	
ypN/pN stage				<0.001***
N0	150 (53.4%)	73 (71.6%)	77 (43.0%)	
N1	74 (26.3%)	19 (18.6%)	55 (30.7%)	
N2	57 (20.3%)	10 (9.8%)	47 (26.3%)	
VAT, cm^2	83.02 (56.78)	89.19 (67.22)	79.51 (49.72)	0.170
aSAT, cm^2	98.41 (51.98)	106.07 (58.28)	94.04 (47.64)	0.062
gSAT, cm^2	130.64 (49.90)	127.85 (56.80)	132.23 (45.60)	0.480
VSR	0.93 (0.63)	0.90 (0.62)	0.95 (0.64)	0.569

Continuous variables were expressed as mean and standard deviation (SD), categorical variables were expressed as numbers (percentages).
BMI, body mass index; NLR, Neutrophil to Lymphocyte Ratio; SD, standard deviation; VAT, visceral adipose tissue; aSAT, abdominal subcutaneous adipose tissue; gSAT, gluteal subcutaneous adipose tissue; VSR, the ratio of VAT to aSAT. "*" represents a p-value of less than 0.05, "**" represents a p-value of less than 0.01, "***" represents a p-value of less than 0.0001.

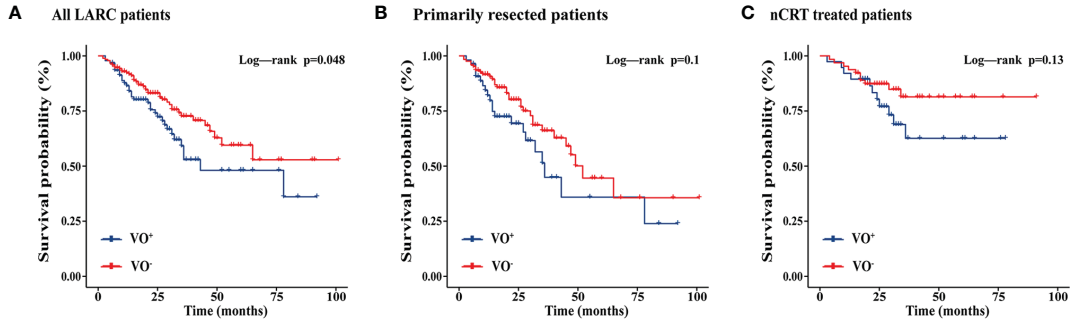

FIGURE 1
Kaplan-Meier curves showing the effects of visceral adipose tissue on progression-free survival of all LARC patients, primarily resected patients, and nCRT treated patients. nCRT, neoadjuvant chemoradiotherapy; LARC, locally advanced rectal cancer; VO, Visceral obesity **(A)** All LARC patients; **(B)** Primarily resected patients; **(C)** nCRT treated patients.

3.3 Association between regional adipose tissue and survival

To further explore the prognostic impact of regional adipose tissue on PFS, we performed a cox regression analysis. Univariable analysis revealed that VAT (HR 1.34, 95% CI 1.11–1.62; $P = 0.002$) and aSAT (HR 1.27, 95% CI 1.05–1.54, $P = 0.016$) were associated with poor prognosis in all patients. Multivariable analysis confirmed that VAT (HR 1.28, 95% CI 1.04–1.57, $P = 0.021$) was an important prognostic factor (Table 2).

Univariable analysis showed that pN staging (HR 2.70, 95% CI 1.39–5.26; $P = 0.003$), VAT (HR 1.36, 95% CI 1.11–1.68; $P = 0.003$) and aSAT (HR 1.50, 95% CI 1.15–1.96, $P = 0.003$) were significantly associated with PFS in patients with primary resection. They remained as predictive factors for poorer prognosis ($P = 0.005$, $P = 0.037$, and $P = 0.042$, respectively) in multivariable analysis (Supplementary Table 1).

However, univariable analysis showed that no statistically significant association between VAT ($P = 0.071$) and patient prognosis in patients treated with preoperative nCRT, while the ΔgSAT stage (HR 0.48, 95% CI 0.31–0.75, $P = 0.001$) was an important predictor of PFS. In multivariable analysis, ΔgSAT (HR 0.43, 95% CI 0.27–0.69, $P = 0.001$) was still a positive prognostic factor (Table 3).

3.4 Prognostic impact of gluteal adipose tissue redistribution in nCRT-treated patients

To further evaluate the relationship between gluteal adipose tissue and prognosis in nCRT patients, we performed Kaplan-Meier curve analysis and time-dependent ROC curve analysis. Kaplan-Meier survival analysis showed (Figure 2) that high ΔgSAT was associated with a reduced risk of local recurrence or metastasis ($P = 0.002$). Patients with ΔgSAT above the corresponding median were classified as high ΔgSAT.

Time-dependent ROC curve analysis in patients treated with preoperative nCRT showed that the area under the 1, 2, and 3-year curves for ΔgSAT were 0.85 (95% CI 0.73–0.96), 0.72 (95% CI: 0.56–0.87) and 0.74 (95% CI: 0.56–0.93) respectively. The area under the 1, 2, and 3-year curves for VAT were 0.58 (95% CI 0.37–0.78), 0.61 (95% CI 0.44–0.78), and 0.58 (95% CI:0.36–0.80), respectively (Figure 3). ΔgSAT showed a better ability to predict early local recurrence and distant metastasis, compared with baseline VAT.

4 Discussion

This study has evaluated the prognostic effect of regional adipose tissue on patients with LARC. Meanwhile, we have done further investigation adipose tissue changes in different groups and the redistribution effect on adipose tissue from nCRT. In our study, visceral obesity was a negative prognostic predictor in patients with LARC, while in patients treated with preoperative nCRT, ΔgSAT was associated with significantly reduction in cancer recurrence and distant metastasis. It indicated that ΔgSAT was a positive prognostic factor, and nCRT might play a role in the redistribution of body adipose tissue. Based on the research results, the accumulation of preoperative gSAT had a protective effect on the prognosis of LARC patients, which would help establish a preoperative nCRT metabolic risk assessment for LARC and improve the prognosis of the patient.

Some studies have investigated the effects of VAT on patients undergoing the surgery for bowel cancer. Basile (20) et al. reported a significant association between high VAT and poor prognosis of metastatic colorectal cancer. Guiu (21) et al. demonstrated that VAT was an independent predictive biomarker ensued from the first-line bevacizumab-based treatment in metastatic colorectal cancer. We found that VAT was a negative prognostic factor in patients with resectable LARC, confirming the survival rate between VAT and LARC in other studies.

In addition, so far only a few studies have evaluated changes in body composition of cancer patients during nCRT. Yip et al. (22) shown that after nCRT for esophageal cancer, differential loss of visceral to subcutaneous adipose tissue ratio associated with the risk of circumferential resection margin positivity. Liu (23) et al. showed that pre-nCRT low muscle density and loss of total abdominal fat area were related to a high incidence of short- and long-term ileus, respectively. Heus (12) et al. found that visceral obesity related with

TABLE 2 Univariable and multivariable Cox regression analysis of predictors associated with PFS in all patients.

Variables	Univariable HR (95% CI)	*P*	Multivariable HR (95% CI)	*P*
Age, yrs	1.00 (0.98-1.03)	0.777		
Male gender	0.86 (0.53-1.40)	0.553		
BMI, kg/m^2	1.08 (1.00-1.17)	0.050		
NLR	1.03 (0.94-1.13)	0.477		
Monocytes,10^9/L	0.29 (0.07-1.25)	0.098		
Albumin, g/L	1.05 (0.99-1.10)	0.110		
CEA ng/ml	1.00 (0.99-1.02)	0.815		
Clinical stage				
II	Ref	Ref		
III	1.18 (0.69-2.02)	0.541		
T stage				
T2	Ref	Ref		
T3	1.31 (0.40-4.30)	0.657		
T4	2.49 (0.76-8.18)	0.133		
N stage				
N0	Ref	Ref		
N1	1.03 (0.54-1.95)	0.928		
N2	1.28 (0.73-2.27)	0.388		
Baseline VAT, cm^2	1.34 (1.11-1.62)	0.002**	1.28 (1.04-1.57)	0.021*
Baseline aSAT, cm^2	1.27 (1.05-1.54)	0.016*	1.17 (0.94-1.46)	0.168
Baseline gSAT, cm^2	1.22 (0.99-1.49)	0.061		
VSR	1.10 (0.87-1.38)	0.427		

BMI, body mass index; NLR, Neutrophil to Lymphocyte Ratio; SD, standard deviation; VAT, visceral adipose tissue; aSAT, abdominal subcutaneous adipose tissue; gSAT, gluteal subcutaneous adipose tissue; VSR, the ratio of VAT to aSAT. "*"represents a p-value of less than 0.05, "**"represents a p-value of less than 0.01, "***" represents a p-value of less than 0.0001.

TABLE 3 Univariable and multivariable Cox regression analysis of predictors associated with PFS in patients receiving preoperative nCRT.

Variables	Univariable HR (95% CI)	*P*	Multivariable HR (95% CI)	*P*
Age, yrs	0.97 (0.92-1.01)	0.176		
Male gender	0.79 (0.33-1.90)	0.597		
BMI, kg/m^2	1.11 (0.97-1.28)	0.123		
NLR	1.13 (0.99-1.28)	0.078		
Monocytes,10^9/L	0.12 (0.01-1.87)	0.129		
Albumin, g/L	1.10 (0.99-1.22)	0.086		
CEA ng/ml	1.00 (0.97-1.04)	0.807		
Clinical TNM stage				
II	Ref	Ref		
III	0.57 (0.19-1.72)	0.323		
ypT stage				
T0、Tis	Ref	Ref	Ref	Ref

(Continued)

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TABLE 3 Continued

Variables	Univariable HR (95% CI)	*P*	Multivariable HR (95% CI)	*P*
ypT stage				
T1	0 (0-Inf)	0.998	0.00 (0.00-Inf)	0.998
T2	1.49 (0.32-7.02)	0.614	1.28 (0.26-6.26)	0.758
T3	1.89 (0.39-9.13)	0.427	1.68 (0.31-9.02)	0.545
T4	12.38 (2.15-71.16)	0.005**	8.27 (1.18-58.17)	0.034*
ypN stage				
N0	Ref	Ref	Ref	Ref
N1	2.98 (1.15-7.70)	0.024*	1.88 (0.63-5.58)	0.257
N2	2.11 (0.59-7.58)	0.251	1.69 (0.41-6.87)	0.464
baseline VAT, cm^2	1.41 (0.97-2.04)	0.071		
baseline aSAT, cm^2	1.26 (0.87-1.83)	0.222		
baseline gSAT, cm^2	1.23 (0.83-1.80)	0.300		
VSR	1.11 (0.74-1.65)	0.613		
ΔVAT, cm^2/d	0.89 (0.62-1.27)	0.519		
ΔaSAT, cm^2/d	0.73 (0.47-1.16)	0.187		
ΔgSAT, cm^2/d	0.48 (0.31-0.75)	0.001***	0.43 (0.27-0.69)	0.001***

BMI, body mass index; NLR, Neutrophil to Lymphocyte Ratio; SD, standard deviation; VAT, visceral adipose tissue; aSAT, abdominal subcutaneous adipose tissue; gSAT, gluteal subcutaneous adipose tissue; VSR, the ratio of VAT to aSAT; ΔVAT, the change rate of VAT; ΔaSAT, the change rate of aSAT; ΔgSAT, the change rate of gSAT. "*" represents a p-value of less than 0.05, "**" represents a p-value of less than 0.01, "***" represents a p-value of less than 0.0001.

more complications and a longer length of stay in rectal cancer surgery, but during nCRT VAT area was not affected by chemoradiotherapy. In our study, the change rate of adipose tissue was used to represent the dynamic change of adipose tissue, which could more intuitively see the change trend of adipose tissue. Meanwhile, considering the developmental and functional differences between the upper body adipose depot and the lower body adipose tissue, we have analyzed the relationship between the lower body adipose tissue and LARC. We focused on the effect of nCRT on adipose tissue distribution, and the relevance of dynamic changes in adipose tissue to the prognosis of LARC. We found that the ratio of VAT to aSAT (VSR), ΔVAT, and ΔaSAT were found to have no significant correlation with the prognosis of nCRT treated patients, but ΔgSAT had an obvious predictive value. We have further analyzed ΔgSAT and classified by the median, to find high ΔgSAT have associated with the lower recurrence and distant metastasis of LACR.

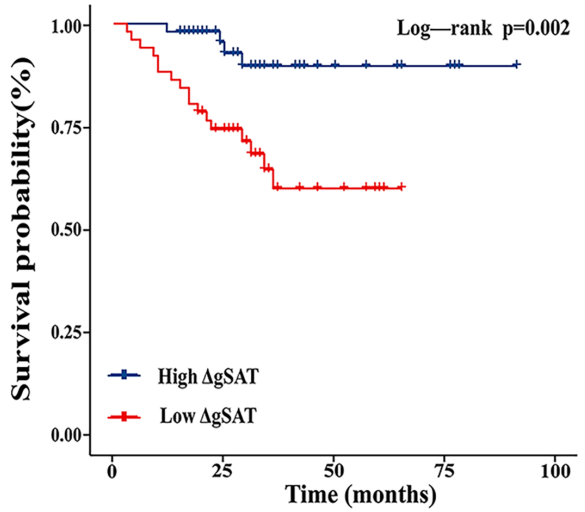

FIGURE 2
Kaplan-Meier curves showing the effect of gluteal subcutaneous adipose tissue change rate (ΔgSAT) on progression-free survival in patients receiving preoperative nCRT.

Compared with VAT, ΔgSAT was a positive prognostic factor for LARC, due to differences in microvascular and metabolic characteristics resulted from different patterns of adipokine secretion and endocrine function between upper and lower body fat (24, 25). The Intra-abdominal adipose depot was related to the viscera. VAT had strong lipolytic activity and could release more free fatty acids, which could induce insulin resistance, inflammation and oxidative stress through lipid mediators such as ceramides, increasing the risk of cancer (26, 27). Furthermore, VAT could produce higher proinflammatory cytokines and immune cells to induce tumor occurrence and diffusion (28). The reduced lipid turnover of lower body adipose storage could accommodate redistributed adipose tissue and show fewer signs of inflammatory

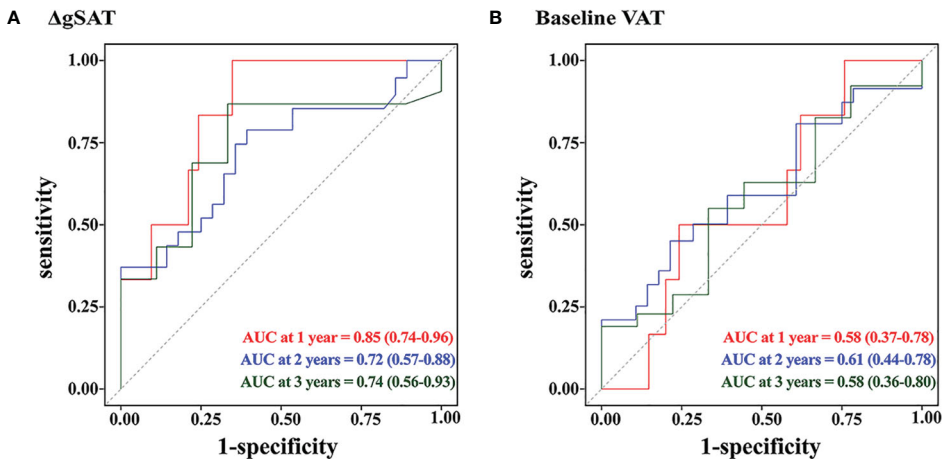

FIGURE 3
Time-dependent ROC analysis in patients with LARC treated by nCRT. **(A)** ΔgSAT; **(B)** Baseline VAT.

damage (25). What's more, the gluteal vascular network was not as rich as the abdomen, the blood flow being low, and the action rate of hormone sensitive lipase being also low, causing a lower overall fatty acid release rate and uptake rate than the abdomen, the energy supply reflex reduced, which were opposite to metabolic effects of the abdominal adipose (29, 30).

We hypothesized that nCRT affected the distribution of adipose tissue in the buttocks and abdomen. For patients with adipose tissue metastases from the abdomen to the gluteal, the overall release of fatty acids and pro-inflammatory cytokines was less than in patients without fat transfer, which caused a decrease in tumor oxidative stress and an increase in the sensitization of tumor cells to radiotherapy and chemotherapy, thereby slowing down the progress of tumor. In view of this, we could help provide quantitative imaging markers to assist patients by monitoring changes in adipose tissue distribution and provide datum for active postoperative monitoring and early intervention in high-risk patients, which could be clinically useful.

During the past years, deep learning (DL) has steadily found its way into the field of medicine and pathology, and tend nowadays to have an expanding role in all fields of medicine. Several studies have found that deep learning advances have the potential to improve the accuracy and validity of CRC detection (31, 32). Deep learning algorithms can accurately predict patients who will have a complete pathological response after nCRT for LARC (33); Deep learning-based body composition can be used to model survival in LARC (34). We will also consider referencing these algorithms in ongoing studies.

Our research has several limitations. First, this study was retrospectively conducted, which might introduce potential selection biases. Second, the results were only applicable to tumors at local clinical progression stage, and could not represent all rectal cancer patients, which needed further verifying in patients with advanced diseases. Third, since the study was a retrospective analysis, other metabolic characteristics related to obesity need to be considered in future prospective studies. However, a large amount of so-called hidden data could be extracted from medical images through radiology, which was helpful in improving diagnostic performance.

In conclusion, visceral obesity is an adverse prognostic factor in patients with resectable LARC treated by primary resection, while increased gluteal subcutaneous adiposity during preoperative nCRT may indicate favorable clinical outcomes. Preoperative nCRT may cause the redistribution of gluteal and abdominal adipose tissue in patients with resectable LARC.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Third Xiangya Hospital, Central South University (Changsha, China) and Hunan Cancer Hospital (Changsha, China). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

WH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. JC: Funding acquisition, Methodology, Supervision, Writing – review & editing. PR: Funding acquisition, Resources, Supervision, Writing – review & editing. ZF:

Formal analysis, Methodology, Software, Writing – original draft. MM: Methodology, Software, Writing – original draft. FLS: Data curation, Writing – original draft. SZ: Conceptualization, Writing – original draft. FS: Conceptualization, Writing – original draft. XY: Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1421651/full#supplementary-material

References

1. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin*. (2020) 70:145–64. doi: 10.3322/caac.21601

2. Liu Z, Meng X, Zhang H, Li Z, Liu J, Sun K, et al. Predicting distant metastasis and chemotherapy benefit in locally advanced rectal cancer. *Nat Commun*. (2020) 11:4308. doi: 10.1038/s41467-020-18162-9

3. Oronsky B, Reid T, Larson C, Knox SJ. Locally advanced rectal cancer: The past, present, and future. *Semin Oncol*. (2020) 47:85–92. doi: 10.1053/j.seminoncol.2020.02.001

4. Body A, Prenen H, Lam M, Davies A, Tipping-Smith S, Lum C, et al. Neoadjuvant therapy for locally advanced rectal cancer: recent advances and ongoing challenges. *Clin Colorectal Cancer*. (2021) 20:29–41. doi: 10.1016/j.clcc.2020.12.005

5. Chern H, Chou J, Donkor C, Shia J, Guillem JG, Nash GM, et al. Effects of obesity in rectal cancer surgery. *J Am Coll Surg*. (2010) 211:55–60. doi: 10.1016/j.jamcollsurg.2010.03.010

6. Choi MH, Oh SN, Lee IK, Oh ST, Won DD. Sarcopenia is negatively associated with long-term outcomes in locally advanced rectal cancer. *J Cachexia Sarcopenia Muscle*. (2018) 9:53–9. doi: 10.1002/jcsm.12234

7. Pacquelet B, Morello R, Pelage JP, Eid Y, Lebreton G, Alves A, et al. Abdominal adipose tissue quantification and distribution with CT: prognostic value for surgical and oncological outcome in patients with rectal cancer. *Eur Radiol*. (2022) 32:6258–69. doi: 10.1007/s00330-022-08697-4

8. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol*. (2006) 6:772–83. doi: 10.1038/nri1937

9. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. *Obes Rev*. (2010) 11:11–8. doi: 10.1111/j.1467-789X.2009.00623.x

10. Pinnick KE, Nicholson G, Manolopoulos KN, McQuaid SE, Valet P, Frayn KN, et al. Distinct developmental profile of lower-body adipose tissue defines resistance against obesity-associated metabolic complications. *Diabetes*. (2014) 63:3785–97. doi: 10.2337/db14-0385

11. Clark W, Siegel EM, Chen YA, Zhao X, Parsons CM, Hernandez JM, et al. Quantitative measures of visceral adiposity and body mass index in predicting rectal cancer outcomes after neoadjuvant chemoradiation. *J Am Coll Surg*. (2013) 216:1070–81. doi: 10.1016/j.jamcollsurg.2013.01.007

12. Heus C, Cakir H, Lak A, Doodeman HJ, Houdijk AP. Visceral obesity, muscle mass and outcome in rectal cancer surgery after neo-adjuvant chemo-radiation. *Int J Surg*. (2016) 29:159–64. doi: 10.1016/j.ijsu.2016.03.066

13. Bocca G, Mastoridis S, Yeung T, James DRC, Cunningham C. Visceral-to-subcutaneous fat ratio exhibits strongest association with early post-operative outcomes in patients undergoing surgery for advanced rectal cancer. *Int J Colorectal Dis*. (2022) 37:1893–900. doi: 10.1007/s00384-022-04221-8

14. Monson JR, Weiser MR, Buie WD, Chang GJ, Rafferty JF, Buie WD, et al. Practice parameters for the management of rectal cancer (revised). *Dis Colon Rectum*. (2013) 56:535–50. doi: 10.1097/DCR.0b013e31828cb66c

15. Montano-Loza AJ, Mazurak VC, Ebadi M, Meza-Junco J, Sawyer MB, Baracos VE, et al. Visceral adiposity increases risk for hepatocellular carcinoma in male patients with cirrhosis and recurrence after liver transplant. *Hepatology*. (2018) 67:914–23. doi: 10.1002/hep.29578

16. Ko Y, Shin Y, Sung YS, Lee J, Lee JH, Kim JK, et al. A reliable and robust method for the upper thigh muscle quantification on computed tomography: toward a quantitative biomarker for sarcopenia. *BMC Musculoskelet Disord*. (2022) 23:93. doi: 10.1186/s12891-022-05032-2

17. Irving BA, Weltman JY, Brock DW, Davis CK, Gaesser GA, Weltman A. NIH ImageJ and Slice-O-Matic computed tomography imaging software to quantify soft tissue. *Obes (Silver Spring)*. (2007) 15:370–6. doi: 10.1038/oby.2007.573

18. Examination Committee of Criteria for 'Obesity Disease' in J, Japan Society for the Study of O. New criteria for 'obesity disease' in Japan. *Circ J*. (2002) 66:987–92. doi: 10.1253/circj.66.987

19. Benson AB, Venook A, Saltz L. NCCN Guidelines version 2.2015(rectal cancer). Available online at: https://www.nccn.org/.

20. Basile D, Bartoletti M, Polano M, Bortot L, Gerratana L, Di Nardo P, et al. Prognostic role of visceral fat for overall survival in metastatic colorectal cancer: A pilot study. *Clin Nutr*. (2021) 40:286–94. doi: 10.1016/j.clnu.2020.05.019

21. Guiu B, Petit JM, Bonnetain F, Ladoire S, Guiu S, Cercueil JP, et al. Visceral fat area is an independent predictive biomarker of outcome after first-line bevacizumab-based treatment in metastatic colorectal cancer. *Gut*. (2010) 59:341–7. doi: 10.1136/gut.2009.188946

22. Yip C, Goh V, Davies A, Gossage J, Mitchell-Hay R, Hynes O, et al. Assessment of sarcopenia and changes in body composition after neoadjuvant chemotherapy and associations with clinical outcomes in oesophageal cancer. *Eur Radiol*. (2014) 24:998–1005. doi: 10.1007/s00330-014-3110-4

23. Liu Z, Lu S, Wang Y, Lin X, Ran P, Zhou X, et al. Impact of body composition during neoadjuvant chemoradiotherapy on complications, survival and tumor response in patients with locally advanced rectal cancer. *Front Nutr*. (2022) 9:796601. doi: 10.3389/fnut.2022.796601

24. Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. *Mol Aspects Med*. (2013) 34:1–11. doi: 10.1016/j.mam.2012.10.001

25. Karpe F, Pinnick KE. Biology of upper-body and lower-body adipose tissue–link to whole-body phenotypes. *Nat Rev Endocrinol*. (2015) 11:90–100. doi: 10.1038/nrendo.2014.185

26. Hsu IR, Kim SP, Kabir M, Bergman RN. Metabolic syndrome, hyperinsulinemia, and cancer. *Am J Clin Nutr*. (2007) 86:s867–871. doi: 10.1093/ajcn/86.3.867S

27. Cai F, Dupertuis YM, Pichard C. Role of polyunsaturated fatty acids and lipid peroxidation on colorectal cancer risk and treatments. *Curr Opin Clin Nutr Metab Care*. (2012) 15:99–106. doi: 10.1097/MCO.0b013e32834feab4

28. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes*. (2007) 56:1010–3. doi: 10.2337/db06-1656

29. Tan GD, Goossens GH, Humphreys SM, Vidal H, Karpe F. Upper and lower body adipose tissue function: a direct comparison of fat mobilization in humans. *Obes Res*. (2004) 12:114–8. doi: 10.1038/oby.2004.15

30. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. *J Clin Endocrinol Metab*. (2008) 93:S57–63. doi: 10.1210/jc.2008-1585

31. Bousis D, Verras G-I, Bouchagier K, Antzoulas A, Panagiotopoulos I, Katinioti A, et al. The role of deep learning in diagnosing colorectal cancer. *Gastroenterol Rev*. (2023) 18:266–73. doi: 10.5114/pg.2023.129494

32. Chlorogiannis DD, Verras G-I, Tzelepi V, Chlorogiannis A, Apostolos A, Kotis K, et al. Tissue classification and diagnosis of colorectal cancer histopathology images using deep learning algorithms. Is the time ripe for clinical practice implementation? *Gastroenterol Rev*. (2023) 18:353–67. doi: 10.5114/pg.2023.130337

33. Bibault J-E, Giraud P, Housset M, Durdux C, Taieb J, Berger A, et al. Deep Learning and Radiomics predict complete response after neo-adjuvant chemoradiation for locally advanced rectal cancer. *Sci Rep*. (2018) 8:12611. doi: 10.1038/s41598-018-30657-6

34. Keyl J, Hosch R, Berger A, Ester O, Greiner T, Bogner S, et al. Deep learning-based assessment of body composition and liver tumour burden for survival modelling in advanced colorectal cancer. *J Cachexia Sarcopenia Muscle*. (2022) 14:545–52. doi: 10.1002/jcsm.13158

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Butyrylcholinesterase levels correlate with surgical site infection risk and severity after colorectal surgery: a prospective single-center study

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Introduction: Surgical site infections (SSIs) after colorectal surgery remain a significant concern, which warrants effective predictive markers for prompt diagnosis and treatment. Butyrylcholinesterase (BChE), a non-specific cholinesterase enzyme, has been correlated with the risk of hepatic dysfunction progression and, more recently, infectious diseases and septic shock with ongoing research into the utility of BChE in multiple systemic inflammatory conditions. Whether these preliminary results can be translated into predicting infection after colorectal surgery remains in remains in question. This prospective study aimed to assess BChE's potential as a predictive marker for surgical site infections and anastomotic leaks after colorectal surgery.

Materials and methods: This single-center prospective study (11/2019–05/2023) enrolled 402 patients who underwent colorectal surgery. BChE levels were measured at four postoperative time points. The primary endpoints focused on BChE's association with complications, particularly surgical site infections (SSIs). Further known predictors of SSI were utilized to construct multivariable models to assess for independent association with SSI development.

Results: During the third and fifth day postsurgery, SSI patients had significantly lower mean BChE levels (3.90 KU/L vs. 4.54 KU/L p-value < 0.05, and 4.14 KU/L vs. 4.73 KU/L, p-value < 0.05; t-test, respectively). However, multivariate analysis revealed that when adjusted for other factors, low BChE levels on the first postoperative day were associated with 2.6 times higher odds of developing SSI (OR: 2.6, 95%CI: 1.3–3.9, p-value < 0.05). Similar results were found for low BChE levels on the third postoperative day as they were associated with a. 2.53 times higher odds for developing SSI (OR: 2.5, 95%CI: 1.27–3.87, p-value < 0.05) when adjusted for other factors.

Conclusion: In conclusion, in this prospective observational study, low levels in the first and third postsurgery were associated with an increased risk for the development of SSIs but not sepsis.

KEYWORDS

butyrylcholinesterase, inflammation, prediction, surgical site infection, colorectal surgery

1 Introduction

Colonic resections during colorectal surgery are generally associated with high rates of infectious complications, notably surgical site infections (SSIs) (1–11). SSIs constitute approximately one-quarter of all hospital-acquired infections, affecting up to 5% of all surgical patients, with one-fourth of those cases reported after colorectal surgery (1–11). SSI post-colorectal surgery is associated with poor prognosis, increased mortality rates, lengthier hospitalization, and up to threefold increase in hospital costs making it a major healthcare challenge (5, 7, 12–16).

To confront this, both the American College of Surgeons and Surgical Infection Society and the World Health Guidelines recommend prophylactic antibiotic therapy for SSI prevention in high-risk patients (1, 2, 12, 17, 18). Butyrylcholinesterase (BChE) is an alpha-glycoprotein present in most tissues, particularly in the liver. Lower BChE levels have been linked with increased mortality in liver transplant surgery. In addition, terminal ill cancer is accompanied by mild to moderate inflammation and various degrees of protein–energy malnutrition (PEM), resulting in reduced plasma BChE levels and increased mortality risk (19). Moreover, contemporary data from retrospective observational studies report that low BChE levels are independent predictors of severe systemic inflammation with this phenomenon occurring early in the inflammation cascade. This phenomenon raises the possibility of minimizing the time delays between the clinical assessment and treatment of the underlying inflammatory process factors such as SSI. However, to date, there is paucity of data concerning the translation of these data to colorectal cancer surgery patients.

This study aimed to evaluate BChE as a potential marker for the risk of developing SSI and septic complications in patients undergoing colorectal surgery.

2 Materials and methods

2.1 Study design

This prospective single-center study was conducted according to the STROBE statement and recruited consecutive patients undergoing colorectal surgery from November 2019 to May 2023 in an academic tertiary hospital in Greece. Patients were enrolled in the study after providing informed consent. The study protocol was approved by the hospital's Ethical and Scientific Review Board (Approval No. 42687/0519) and was registered in an open-access database available on the Internet (www.clinicaltrials.gov: NCT04748744).

2.2 Inclusion and exclusion criteria

In this study, data were evaluated from 403 consecutive patients who underwent colorectal surgery at the Surgical Clinic of the General University Hospital of Patras (GUHP). Patients were included in the study, provided they had completed the necessary informed consent documents after a counseling session with members of the research team. In these sessions, the purposes of the study, the research perspective, the interventions to which they were to be subjected, as well as the fact that their participation is voluntary and they retain the right to withdraw from it at any time, even after its end, until the publication of its results, were analyzed to the patients. Patients were included in this study if they met the following inclusion criteria: (1) age 18 years and older, (2) ability to undergo surgical intervention on an urgent or an elective basis, and (3) requiring colorectal surgery for any surgical pathology. We included both elective and urgent/emergency operations, as they will be further analyzed as separate subgroups in the analysis. Due to the effects of systematic inflammatory states and neurological degenerative disorders in BChE levels, we decided to exclude patients who exhibited signs of metastatic disease, either localized or with extended metastatic disease burden.

In total, 489 patients were screened for inclusion in the present study. Of them, 67 were deemed unfit for surgery, or their management plan was switched to conservative management before undergoing surgery, and 19 patients were not able to provide consent at the time of operation or in the early postoperative period.

2.3 Serum BChE measurement

During hospitalization, patient serum samples were obtained at four time points: (1) on the day of surgery, preoperatively, and a second sample immediately postoperatively, (2) first postoperative day, (3) on the third postoperative day, and (4) on the fifth postoperative day.

The reasoning behind this measurement protocol was to incorporate preoperative (baseline) measurements of BChE within our modeling process. For the measurement of BChE, materials and resources of the GUHP were used, which had been approved at the time of study submission. The quantitative *in vitro* determination of BchE in the serum was done using a colorimetric method. For the determination of BChE levels, a spectrophotometric method with a Randox RX Imola Autoanalyzer was used. The values were expressed in IU/L (international units per liter). The values of BChE levels, as well as all the patient data concerning their hospitalization, were recorded in a special postoperative monitoring software of the clinic, transferred to separate databases, and anonymized before the analysis phase. As part of the project's protocol, the biological patient samples were kept until the end of the analysis phase (September 2023), after which they were appropriately disposed of and destroyed without storing any tissue samples.

2.4 Study endpoints and outcome measures

Our primary endpoint was the development of surgical site infections (SSIs), as a subcategory of septic complications. SSIs

were defined using the CDC criteria for diagnosis and classified in accordance with the CDC/NSQIP classifications of SSIs. SSIs were classified as superficial incisional SSIs, deep incisional SSIs, and organ/space SSIs (20, 21).

As a secondary endpoint of our study, we set the development of any postoperative septic complication, with a subset analysis on patients whose septic profile was secondary to an anastomotic leak.

For the definition of septic syndromes, the latest definition according to Sepsis-3 was used: sepsis should be defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. For clinical operability, organ dysfunction can be represented by an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 or more points, which is associated with in-hospital mortality greater than 10%. For this composite measure, all septic complications were considered, including but not limited to anastomotic leaks, surgical site infections (of all grades), hospital-associated pneumonia, UTIs, and more.

As a special patient subpopulation of interest, patients with septic syndrome secondary to anastomotic leaks were studied separately. The diagnosis of the leak was made using clinical indicators and was radiologically confirmed in all cases as per local workup protocol. These three outcomes were studied in relation to age, gender, preoperative diagnosis of the patient, the degree of urgency of the surgery, the presence of malignancy, the duration of the operation, and the number of pRBC units transfused.

As a final secondary outcome of the study, we set the development of any postoperative complication, as defined by the ESA-ESICM joint task force on perioperative outcome measures. The existence or not of a postoperative complication, and its categorization, was made after the evaluation of the patient by at least two doctors at the time of diagnosis, and any disagreements as to the definition and identification of it were resolved by a senior, third specialized colorectal surgeon.

All patients received a bundle of SSI prophylaxis based on the 2019 NICE guidelines for the prevention of SSIs. Patients in our institution received preoperative antibiotics within 30–60 min from the first incision. Appropriate warming, hair removal, and glycemic control were ensured throughout the procedure. Skin preparation included alcohol-based chlorhexidine solution for all patients. Elective colorectal patients received intravenous cefuroxime and metronidazole. Emergency cases routinely received beta-lactamase plus metronidazole; however, ciprofloxacin was also utilized in some cases. In our institution, mechanical bowel preparation is used routinely in elective cases. Oral antibiotics in elective colorectal cases are not part of the institution's protocol and therefore were not administered (20).

2.5 Statistical analysis

For the statistical analysis of the results of the work, the statistical data processing packages SPSS (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY, USA: IBM Corp), jamovi [The jamovi project (2021). jamovi (Version 1.6)], and the R programming language were used. The variables of interest were expressed as binomial (binomial variables) and concerned the development or not of SSI, the development or not of postoperative sepsis, and the presence of anastomotic leakage, as a cause of postoperative sepsis. The presence or absence of malignancy, gender, operative approach, urgency on which the patient was operated, smoking, and the ASA score greater than 2 were also expressed as binomial. Preoperative diagnosis, type of surgery, TNM staging, and the number of RBC units administered were expressed as categorical variables. The days of hospitalization, age, BChE levels in the blood, BMI, and surgical time in minutes were the continuous variables. At the same time, some continuous variables were deemed necessary to be transformed either logarithmically or to the root of the variable to meet the requirements of the regression models.

The initial approach to the data was made using univariate analysis techniques for pairs of variables to establish the existence of any associations between them. For the comparison of continuous variables, the means for each category and the standard deviations were checked for statistical significance using either *t*-tests (Student's) or Mann–Whitney *U*-test when the use of parametric tests was not feasible. The normality of data was evaluated by using visualization of the variable distribution (histograms) and with the utilization of the Shapiro–Wilk test. For the comparison of categorical variable values, Fischer's exact test and the Chi-squared test of statistical significance were used as appropriate. For all tests of statistical significance, $p < 0.05$ was considered the threshold of significance, while all tests were two-tailed.

As the final step in the statistical analysis of the study data, the construction of a predictive model for the outcomes of interest was defined. For the creation of the predictive models, we relied on the principles of logistic regression modeling. The stepwise selection regression technique was used for the selection of parameters and manual parameter selection to achieve optimal model fit.

To assess the weighed and independent utility of BChE in assessing SSI patients, multivariate logistic regression analysis using backward variable selection techniques was used. The optimal logistic regression model resulted from both manual extraction and insertion of certain variables, determined by the reported AIC. After constructing the original model, we utilized the omnibus likelihood ratio test to assess for each model whether the variance explained by the model in the observed data is statistically more significant than the unexplained variance. We selected the variables that proved to be independently associated with SSI, to construct the final predictive model. To assess the final model fit, McFadden's pseudo-R^2 test was employed.

For the evaluation of the goodness of fit of the predictive models, on our data, the Akaike information criterion and the R^2 indices according to McFadden's pseudo-R^2 test were employed.

Finally, after construction of the optimal model, we evaluated its ability to predict the occurrence of SSIs by using bootstrapping sample drawing and plotting the corresponding ROC curve.

3 Results

The study included a total of 403 patients of which 226 were males (56.2%). The presence of malignancy constituted the most

TABLE 1 Baseline patient characteristics.

Factors	No. of patients	Total percentage (%)	*p*-value
Presence of malignancy			0.405
Malignancy	288	71.6	
No malignancy	114	28.4	
Gender			0.064
Male	226	56.2	
Female	176	43.8	
Operative approach			<0.01
Open	353	87.8	
Laparoscopic	49	12.2	
Urgency of operation			0.041
Elective	279	69.4	
Urgent/emergency	123	30.6	
ASA score			<0.01
ASA score <2	287	71.4	
ASA score >2	115	28.6	
Smoking			0.12
Smoking	73	18.1	
Not smoking	330	81.9	
Diabetes mellitus			0.002
Diabetes	89	22.08	
No diabetes	314	87.92	
BMI			0.004
Less than 30	129	32.0	
More than 30	274	68.0	
pT			0.284
T1	19	4.71	
T2	193	47.8	
T3	162	40.1	
T4	29	7.19	
pN			0.175
N0	271	67.25	
N1–N3	132	32.75	
AJCC eighth stage			0.231
I	182	45.16	
II	72	17.86	
III	149	36.97	

significant category of preoperative diagnosis, with a prevalence of 71.6% among the patients. Sigmoid colon malignancy accounted for 16.2% of the preoperative diagnoses. The surgeries performed were (in descending order) right hemicolectomy (39.3%), anterior resection (17.9%), low anterior resection (11.2%), and Hartmann's sigmoidectomy (10.2%). Most surgeries were performed with an open approach (87.8%). Of note, this was attributed to the percentage of patients urgently directed to surgery, constituting 30.6% of cases. Table 1 outlines all demographic patient characteristics. Figure 1 illustrates the preoperative diagnoses of enrolled patients, and Supplementary Table S1 outlines them in detail.

3.1 Complications and surgical site infection rates

Among the remaining patients, 15.2% (61 patients) experienced surgical site infection (SSI) as the predominant postoperative complication. In addition, 6.5% (26 patients) developed any form of postoperative infection, while 4.2% (17 patients) developed septic complications, primarily due to postoperative leakage from a newly formed anastomosis. Most of the enrolled patients (69.7%) had a complication-free early postoperative period. The development of any complication affected a total of 122 patients, which corresponds to 30.3% of the participants. SSI as a complication affected 61 patients (15.2%).

3.2 Univariate analysis—surgical site infections

Operative approach, urgency of operation, ASA grading, preoperative BMI, and diabetes mellitus were all found to be significantly associated with the occurrence of SSI in the univariate analysis (Table 1). These are the parameters that multivariable analysis and model building will be based on; however, crucial parameters such as sex and age will also be included based on previous larger studies that have documented a strong relationship between them and SSI.

3.3 Serum butyrylcholinesterase levels at different time points between patients with SSI and uncomplicated patients

Looking at Table 2, we can observe that patients with surgical site infection (SSI) tend to differ from uncomplicated patients in terms of the mean BChE levels in their blood serum. On the day of surgery and the first postoperative day, patients with SSI had higher BChE levels on average (5.41 KU/L vs. 5.16 KU/L, $p = 0.164$ and 4.69 KU/L, vs. 4.61 KU/L, $p = 0.658$, respectively). This trend reverses on the third and fifth postoperative days, where patients who developed SSI had significantly lower serum BChE levels on average (3.90 KU/L vs. 4.54 KU/L, $p < .001$ and 4.14 KU/L vs. 4.73 KU/L, $p < .001$, respectively). Preoperative (baseline) serum BChe levels did not differ significantly between patients.

3.4 Multivariate analysis

Multivariate analysis revealed that when adjusted for other factors, lower BChE levels on the first postoperative day were associated with 2.6 times higher odds of developing SSI (OR: 2.6, 95%CI: 1.3–3.9, p-value < 0.05). Similar results were found for low BChE levels on the third postoperative day as they were associated with 2.53 times higher odds for developing SSI (OR: 2.5, 95%CI: 1.27–3.87, p-value < 0.05) when adjusted for other factors. Lastly, when adjusting for other factors, BChE levels on the fifth day were not independent risk factors for SSI development (OR: 0.38, 95%CI: 0.02–1.23, p-value > 0.05). All the above results were obtained, using baseline BChE measurements on the operative day, as the reference level (Table 3).

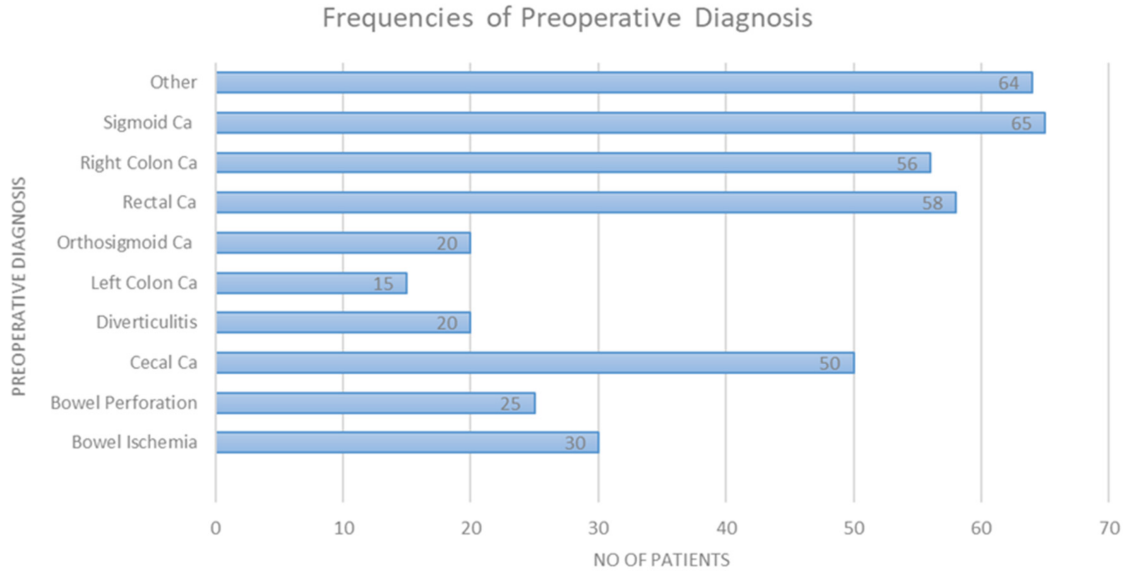

FIGURE 1
Preoperative patient diagnoses.

TABLE 2 Univariate analysis of serum BuChE levels in SSI.

BuChE value	Group	Mean	SD	*p*-value
BuChE (day of operation)	SSI	5.41	1.32	0.194
	Uncomplicated	5.16	1.28	
BuChE (first postoperative day)	SSI	4.69	1.27	0.658
	Uncomplicated	4.61	1.25	
BuChE (third postoperative day)	SSI	3.90	1.06	<0.001
	Uncomplicated	4.54	1.20	
BuChE (fifth postoperative day)	SSI	4.14	1.06	<0.001
	Uncomplicated	4.73	1.22	

An ASA score of less than 2 was an independent negative predictor of SSI occurrence (Table 3, with an OR of 0.138). Malignancy status was also an independent predictor of SSI, with an OR of 0.190 (no malignancy vs. malignancy).

Gender, age higher than 65 years, and length of hospitalization were not independently, significantly associated with the development of postoperative SSI. Operative approach, urgency of operation, smoking, diabetes mellitus, BMI, and TNM levels did not prove to have statistically significant association in the initial, cumulative model, and therefore were not included in the final model. The final ROC curve and AUC can be seen in Supplementary Figure S1. The multivariable logistic regression model was able to predict the occurrence of postoperative surgical site infection with satisfying accuracy following the bootstrapping process. The pseudo-R^2 (McFadden's) confirmed a relatively good fit of our model to the data, and the final model was the one with the optimal AIC value (Table 3), further confirming that this is the optimal parameter selection (from the available) to predict SSI utilizing BChE levels. The overall accuracy of the final model is 0.952 with a sensitivity of 0.978 and a specificity of 0.852. The model's AUC (area under the curve) was calculated as 0.981 (Table 3), indicating that it has good predictive capability for the outcome of interest.

3.5 Butyrylcholinesterase levels at different time points between patients with any form of sepsis including secondary to anastomotic leak

When examining the relationship between BChE and the development of any type of infectious/septic complication, the differences do not appear to be statistically significant in favor of any patient group (see Table 4). Therefore, based on the univariate analysis, BChE does not seem to correlate with septic complications when they are grouped together.

The same pattern is observed when one examines the mean BChE levels in the serum of patients with postoperative anastomotic leakage (see Table 5). We observe that the BChE levels in patients with leakage did not significantly differ from those of uncomplicated patients.

The association between BChE levels and the development of any form of sepsis on the first, third,' and fifth day after surgery were also evaluated. BChE levels in patients with sepsis did not show statistically significant differences compared to uncomplicated patients during any time point postsurgery between patients with any form of sepsis and the cohort that did not (4.94 vs. 4.6, $p = 0.903$ 4.81 vs. 4.54, $p = 0.818$ and 4.88 vs. 4.73, $p = 0.433$, respectively; tested with Mann–Whitney *U*-test and Welch's *t*-test).

TABLE 3 Multivariate analysis and predictive model metrics for the development of SSI.

Predictor	Estimate	SE	Z	p	Odds ratio
Intercept	1.8451	1.6092	1.1466	0.252	6.329
BuChE (first postoperative day)	−11.3132	1.8003	−6.2840	**<.001**	**2.6**
BuChE (third postoperative day)	11.4673	2.1995	5.2135	**<.001**	**2.53**
BuChE (fifth postoperative day)	1.0813	0.9687	1.1162	0.264	0.38
Gender					
M—F	0.1302	0.6167	0.2111	0.833	1.139
Age categorical					
Under 65–65 or older	−0.0377	0.6323	−0.0596	0.952	0.963
ASA score					
<2 ->2 (reference)	−1.9801	0.6512	−3.0407	**0.002**	**0.138**
Malignancy/no malignancy					
No malignancy—malignancy (reference)	−1.6631	0.6568	−2.5319	**0.011**	**0.190**
Hospital stay (days)	−0.0296	0.0466	−0.6366	0.524	0.971
Model fit measures					
Model	**Deviance**		**AIC**		**R^2_{McF}**
1	79.1		97.1		0.735
Predictive measures					
Accuracy	**Specificity**		**Sensitivity**		**AUC**
0.952	0.852		0.978		0.981

Entries in bold indicate statistical significance.

TABLE 4 Univariate analysis of serum BuChE levels in septic complications.

BuChE value	Group	Mean	SD	p-value
BuChE (day of operation)	Septic complications	5.23	1.39	0.808
	Uncomplicated	5.16	1.28	
BuChE (first postoperative day)	Septic complications	4.73	1.27	0.649
	Uncomplicated	4.61	1.25	
BuChE (third postoperative day)	Septic complications	4.65	1.23	0.672
	Uncomplicated	4.54	1.20	
BuChE (fifth postoperative day)	Septic complications	4.82	1.21	0.725
	Uncomplicated	4.73	1.22	

TABLE 5 Univariate analysis of serum BuChE levels in anastomotic leak patients.

BuChE value	Group	Mean	SD	p-value
BuChE (day of operation)	Leak	5.36	1.39	0.584
	Uncomplicated	5.16	1.28	
BuChE (first postoperative day)	Leak	4.94	1.31	0.903
	Uncomplicated	4.61	1.25	
BuChE (third postoperative day)	Leak	4.81	1.16	0.818
	Uncomplicated	4.54	1.20	
BuChE (fifth postoperative day)	Leak	4.88	1.20	0.433
	Uncomplicated	4.73	1.22	

3.6 Butyrylcholinesterase levels at different time points between patients with complications and uncomplicated patients

On the day of surgery and the first postoperative day, patients with any complication exhibited higher mean BChE levels although this difference was not statistically significant (5.31 KU/L vs. 5.16 KU/L, p-value = 0.438; and 4.68 KU/L vs. 4.61 KU/L; p-value = 0.640, respectively). During the third and fifth day postsurgery, a statistically significant difference was found in the mean serum BChE levels between patients with any complication and those without. Patients had significantly lower mean BChE levels (4.22 KU/L vs. 4.54 KU/L p-value = 0.015, and 4.45 KU/L vs. 4.73 KU/L, p-value = 0.029, respectively).

4 Discussion

To the best of our knowledge, this study is the first to try to evaluate the association between BChE levels and SSI after colorectal surgery in a prospective manner. The present study reveals that when adjusting for established risk factors for the development of SSI, low and decreasing BChE levels on the first and third day after colorectal surgery are correlated with an increased risk for SSI.

Various biomarkers for inflammation have been proposed, yet none have proven sufficient for early, specific, and accurate diagnosis of systemic inflammation (22). In this domain, BChE has recently been proposed as a diagnostic marker for low-grade systemic inflammation (23–25). Rapid changes in cholinesterase activity usually occur in patients following trauma, infections, burns, and critical illness (26–29). Both enzymes may act as systemic inflammation indicators and have potential prognostic value for mortality in critically ill patients. Zivkovic et al. (30) demonstrated that reduced BChE activity indicates severe systemic inflammation in critically ill patients. In this domain, a recent study indicated a prolonged reduction in serum cholinesterase activity predicts patient outcomes after sepsis (31).

The added benefit of the evaluation of BChE serves as a cost-effective, readily available laboratory indicator routinely measured. BChE activity is considered a surrogate parameter for

the general clinical conditions of patients (32, 33). A study with 4,077 patients confirmed the role of BChE as an indicator of nutritional status and hepatic function (34, 35). To add to the increasing wealth of evidence, other studies have also supported low preoperative plasma cholinesterase activity as a risk factor for postoperative complications in the elderly population (31). However, it has been demonstrated that lower BChE levels correlate with complications and inflammatory conditions (36, 37). In a study of 453 patients, BChE was negatively correlated with complications, sepsis, and changes in nutritional status (38). BChE was directly correlated with leukocyte count and inversely correlated with bilirubin and sepsis (p-value < 0.01). Postoperatively, BChE decreased to 60% of preoperative values, remaining directly connected and decreasing further with sepsis. A study on patients with septic shock revealed a significant reduction in BChE levels compared to healthy controls (p-value < 0.01) (39–41). Survival rates were higher in patients with higher BChE levels. These results were also translated in the present study featuring colorectal surgery patients since low BChE levels on the first and third day after surgery were independent risk factors and correlated with increased odds for SSI.

Postoperative complications are not the only domain in which cholinesterase activity and levels should be studied in the surgical patient. A recent study looking into the BioCog patients concluded that a decrease in BchE activity was noticed more prominently in patients with postoperative delirium and complications, as opposed to uncomplicated patients (42). This is in line with the findings of our study that a decrease in BChE levels is strongly associated with postoperative septic complications. One hypothesis would be that the team's observations regarding postoperative delirium could be partially attributed to an underlying increase in the systemic inflammatory response, as heralded by a septic complication. Cholinesterase levels were lower in adults admitted to the ITU who exhibited signs of brain dysfunction and delirium, as seen in several studies (43–45). Lower BChE plasma levels were also successfully associated with worse cancer-specific prognosis, in a cohort of pancreatic cancer patients (46). In this 2020 study, the authors managed to associate the BChE plasma levels independently with pancreatic carcinoma survival rates in a single—institution study. Additional studies have also indicated that BChE is negatively associated with survival in various other cancers, such as renal cell, urothelial, and cervical carcinoma.

Another prime example of the utilization of BChE in alimentary tract carcinomas comes from the study of Gensthaler et al. (47), looking at baseline BChE levels in patients with resectable adenocarcinoma of the gastroesophageal junction. In this study, the authors also utilized multivariable regression modeling, in which BChE levels were negatively associated with overall survival and disease-free survival in patients. Although not being used in conjunction with postoperative outcomes, the authors in this study also commented on the possible association of lower BChE serum levels and an increase in systemic inflammation, as is our hypothesis. Furthermore, in both aforementioned studies, it would be interesting to see the possible differentiation of patients with postoperative complications, as they are both expected to have lower survival rates and possibly lower BChE plasma levels as well, therefore driving the initial observation of a strong correlation between BChE levels and overall survival in general. A smaller 2021 cohort study investigated the correlation between BChE and postoperative complications, only this time in patients after transcatheter aortic valve replacement (TAVI) surgery (38). Utilizing point-of-care measurements, the researchers were able to prove and present a strong association between BChE levels and complications after TAVI operations. This is indicative that the decrease in plasma BChE levels can be successfully used as an acute phase reactant biomarker, in response to a variety of postoperative complications, including in extra-abdominal procedures. In addition to septic postoperative complications, and postoperative delirium, the authors identified a strong correlation with the development of heart rhythm disturbances. Therefore, the results of the investigators are in line with our observations that an early decrease in BChE levels can be a herald of systemic inflammation, strongly correlated with postoperative complications. These observations can be extended beyond the qualitative approach and into quantitative observations. A recent study indicated that a higher decrease in plasma BChE levels independently correlated with worse patient outcomes, in burn patients (48), with the authors hypothesizing that the decrease in plasma BChE levels is directly proportional to the magnitude of the systemic inflammatory response.

The present study is not without limitations. Firstly, due to its observational nature, it carries the inherent patient allocation bias, and not every confounder has been accounted for, as would be expected if it was randomized. Similarly, there was a lack of a control group to also aid the adjustment for extra confounders. Future study designs by our research group intend to conduct prospective studies that will factor in more postoperative outcomes in colorectal patients, such as stroke and postoperative delirium since cholinesterase levels seem to be closely related to the neurological function of postoperative patients. In addition, the present study's unique research question makes it difficult to assume the correct sample size for the necessary sample size and power that it would need to reject the null research hypotheses and in other time points, though a positive correlation was indeed found and represents real-world data. To evaluate the predictive capabilities of the final model built as a result of this study, we intend to run a second, validation study with an independent cohort of postoperative colorectal patients. It is with this validation study that we intend to establish a cutoff value that could potentially be used in future clinical practice, as an evaluator of early postoperative complications in colorectal surgery. Future research efforts should explore a stronger correlation of BChE with specific inflammatory conditions in postoperative patients.

In conclusion, in this prospective observational study, low BChE levels in the first and third day postsurgery were associated

with an increased risk for the development of SSIs but not sepsis. Further prospective studies are still needed and should be conducted to confirm these findings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the University of Patras Ethics Committee (Approval No. 42687/0519). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

G-IV: Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing, Funding acquisition, Resources. FM: Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing, Supervision, Validation, Visualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2024.1379410/full#supplementary-material

References

1. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR, Committee THICPA. Guideline for prevention of surgical site infection, 1999. *Infect Control Hosp Epidemiol.* (1999) 20:247–80. doi: 10.1086/501620

2. Smyth ETM, McIlvenny G, Enstone JE, Emmerson AM, Humphreys H, Fitzpatrick F, et al. Four country healthcare associated infection prevalence survey 2006: overview of the results. *J Hosp Infect.* (2008) 69:230–48. doi: 10.1016/j.jhin.2008.04.020

3. Smyth ETM, McIlvenny G, Enstone JE, Emmerson AM, Humphreys H, Fitzpatrick F, et al. Four country healthcare associated infection prevalence survey 2006: overview of the results. *J Hosp Infect.* (2008) 69:230–48. doi: 10.1016/J.JHIN.2008.04.020

4. Leaper DJ, van Goor H, Reilly J, Petrosillo N, Geiss HK, Torres AJ, et al. Surgical site infection—a European perspective of incidence and economic burden. *Int Wound J.* (2004) 1:247–73. doi: 10.1111/J.1742-4801.2004.00067.X

5. Romy S, Eisenring MC, Bettschart V, Petignat C, Francioli P, Troillet N. Laparoscope use and surgical site infections in digestive surgery. *Ann Surg.* (2008) 247:627–32. doi: 10.1097/SLA.0B013E3181638609

6. Smith RL, Bohl JK, McElearney ST, Friel CM, Barclay MM, Sawyer RG, et al. Wound infection after elective colorectal resection. *Ann Surg.* (2004) 239:599–607. doi: 10.1097/01.SLA.0000124292.21605.99

7. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Grace Emori T. CDC Definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. *Am J Infect Control.* (1992) 20:271–4. doi: 10.1016/S0196-6553(05)80201-9

8. Konishi T, Watanabe T, Kishimoto J, Nagawa H. Elective colon and rectal surgery differ in risk factors for wound infection: results of prospective surveillance. *Ann Surg.* (2006) 244:758–63. doi: 10.1097/01.SLA.0000219017.78611.49

9. Kobayashi M, Mohri Y, Inoue Y, Okita Y, Miki C, Kusunoki M. Continuous follow-up of surgical site infections for 30 days after colorectal surgery. *World J Surg* (2008) 32:1142. doi: 10.1007/S00268-008-9536-6

10. Hawkins AT, Samuels LR, Rothman RL, Geiger TM, Penson DF, Resnick MJ. National variation in elective colon resection for diverticular disease. *Ann Surg.* (2022) 275:363–70. doi: 10.1097/SLA.0000000000004236

11. Zhang X, Yang Y, Liu P, Wang P, Li X, Zhu J, et al. Identification of risk factors and phenotypes of surgical site infection in patients after abdominal surgery. *Ann Surg.* (2023) 278:E988–94. doi: 10.1097/SLA.0000000000005939

12. Pastor C, Baek JH, Varma MG, Kim E, Indorf LA, Garcia-Aguilar J. Validation of the risk index category as a predictor of surgical site infection in elective colorectal surgery. *Dis Colon Rectum.* (2010) 53:721–7. doi: 10.1007/DCR.0B013E3181CC573B

13. Plowman R, Graves N, Griffin MAS, Roberts JA, Swan A V, Cookson B, et al. The rate and cost of hospital-acquired infections occurring in patients admitted to selected specialties of a district general hospital in England and the national burden imposed. *J Hosp Infect.* (2001) 47:198–209. doi: 10.1053/jhin.2000.0881

14. Coello R, Charlett A, Wilson J, Ward V, Pearson A, Borriello P. Adverse impact of surgical site infections in English hospitals. *J Hosp Infect.* (2005) 60:93–103. doi: 10.1016/j.jhin.2004.10.019

15. Markell KW, Hunt BM, Charron PD, Kratz RJ, Nelson J, Isler JT, et al. Prophylaxis and management of wound infections after elective colorectal surgery: a survey of the American Society of Colon and Rectal Surgeons membership. *J Gastrointest Surg.* (2010) 14:1090–8. doi: 10.1007/S11605-010-1218-7

16. Weber WP, Zwahlen M, Reck S, Feder-Mengus C, Misteli H, Rosenthal R, et al. Economic burden of surgical site infections at a European University Hospital. *Infect Control Hosp Epidemiol.* (2008) 29:623–9. doi: 10.1086/589331

17. Astagneau P, Rioux C, Golliot F, Brücker G. Morbidity and mortality associated with surgical site infections: results from the 1997–1999 INCISO surveillance. *J Hosp Infect.* (2001) 48:267–74. doi: 10.1053/jhin.2001.1003

18. Leaper D, Burman-Roy S, Palanca A, Cullen K, Worster D, Gautam-Aitken E, et al. Prevention and treatment of surgical site infection: summary of NICE guidance. *Br Med J.* (2008) 337:1049–51. doi: 10.1136/BMJ.A1924

19. Neumayer L, Hosokawa P, Itani K, El-Tamer M, Henderson WG, Khuri SF. Multivariable predictors of postoperative surgical site infection after general and vascular surgery: results from the patient safety in surgery study. *J Am Coll Surg.* (2007) 204:1178–87. doi: 10.1016/J.JAMCOLLSURG.2007.03.022

20. Overview|surgical site infections: prevention and treatment|Guidance|NICE. Available online at: https://www.nice.org.uk/guidance/ng125 (Accessed November 11, 2023).

21. Seidelman JL, Mantyh CR, Anderson DJ. Surgical site infection prevention: a review. *JAMA*. (2023) 329:244–52. doi: 10.1001/JAMA.2022.24075

22. Montgomery RD. The relation of oedema to serum protein and pseudocholinesterase levels in the malnourished infant. *Arch Dis Child*. (1963) 38:343–8. doi: 10.1136/ADC.38.200.343

23. Otero RM, Nguyen B, Huang DT, Gaieski DF, Goyal M, Gunnerson KJ, et al. Early goal-directed therapy in severe sepsis and septic shock revisited: concepts, controversies, and contemporary findings. *Chest*. (2006) 130:1579–95. doi: 10.1378/CHEST.130.5.1579

24. Sergi G, Coin A, Enzi G, Volpato S, Inelmen EM, Buttarello M, et al. Role of visceral proteins in detecting malnutrition in the elderly. *Eur J Clin Nutr* (2006) 60:203–9. doi: 10.1038/sj.ejcn.1602289

25. Brooke-Wavell K, Perrett LK, Howarth PA, Haslam RA, Hubbard R, Smeeth L, et al. Relationship between age and plasma esterases. *Age Ageing*. (2001) 30:41–5. doi: 10.1093/AGEING/30.1.41

26. Gu SZ, Zhao XH, Ping Q, Bin LS, Pan BR. Alterations of serum cholinesterase in patients with gastric cancer. *World J Gastroenterol*. (2005) 11:4604–6. doi: 10.3748/wjg.v11.i29.4604

27. Chougule A, Hussain S, Agarwal D. Prognostic and diagnostic value of serum pseudocholinesterase, serum aspartate transaminase, and serum alinine transaminase in malignancies treated by radiotherapy. *J Cancer Res Ther*. (2008) 4:21–5. doi: 10.4103/0973-1482.39601

28. Giovannini I, Chiarla C, Giuliante F, Vellone M, Ardito F, Nuzzo G. The relationship between albumin, other plasma proteins and variables, and age in the acute phase response after liver resection in man. *Amino Acids*. (2006) 31:463–9. doi: 10.1007/S00726-005-0287-5/METRICS

29. Vijayakumar E. Profile of serum cholinesterase in systemic sepsis syndrome (septic shock) in intensive care unit patients. *Clin Chem Lab Med*. (1995) 33:11–4. doi: 10.1515/CCLM.1995.33.1.11/MACHINEREADABLECITATION/RIS

30. Rao AA, Sridhar GR, Das UN. Elevated butyrylcholinesterase and acetylcholinesterase may predict the development of type 2 diabetes mellitus and Alzheimer's disease. *Med Hypotheses*. (2007) 69:1272–6. doi: 10.1016/J.MEHY.2007.03.032

31. Zivkovic AR, Bender J, Brenner T, Hofer S, Schmidt K. Reduced butyrylcholinesterase activity is an early indicator of trauma-induced acute systemic inflammatory response. *J Inflamm Res*. (2016) 9:221–30. doi: 10.2147/JIR.S117590

32. Kamolz LP, Andel H, Greher M, Andel D, Meiss G, Frey M. Serum cholinesterase activity in patients with burns. *Clin Chem Lab Med*. (2002) 40:60–4. doi: 10.1515/CCLM.2002.012/MACHINEREADABLECITATION/RIS

33. Zivkovic AR, Decker SO, Zirnstein AC, Sigl A, Schmidt K, Weigand MA, et al. A sustained reduction in serum cholinesterase enzyme activity predicts patient outcome following sepsis. *Mediators Inflamm* (2018) 2018:1942193. doi: 10.1155/2018/1942193

34. Cerejeira J, Batista P, Nogueira V, Firmino H, Vaz-Serra A, Mukaetova-Ladinska EB. Low preoperative plasma cholinesterase activity as a risk marker of postoperative delirium in elderly patients. *Age Ageing*. (2011) 40:621–6. doi: 10.1093/AGEING/AFR053

35. John M, Ely EW, Halfkann D, Schoen J, Sedemund-Adib B, Klotz S, et al. Acetylcholinesterase and butyrylcholinesterase in cardiosurgical patients with postoperative delirium. *J Intensive Care*. (2017) 5:1–10. doi: 10.1186/S40560-017-0224-1/FIGURES/3

36. Zivkovic AR, Schmidt K, Sigl A, Decker SO, Brenner T, Hofer S. Reduced serum butyrylcholinesterase activity indicates severe systemic inflammation in critically ill patients. *Mediators Inflamm* (2015) 2015:274607. doi: 10.1155/2015/274607

37. Chiarla C, Giovannini I, Giuliante F, Vellone M, Ardito F, Nuzzo G. Plasma cholinesterase correlations in acute surgical and critical illness. *Minerva Chir*. (2011) 66(4):323–7. Available online at: https://www.minervamedica.it/en/journals/minerva-surgery/article.php?cod=R06Y2011N04A0323 (Accessed March 10, 2024).

38. Michels B, Holzamer A, Graf BM, Bredthauer A, Petermichl W, Müller A, et al. Butyrylcholinesterase as a perioperative complication marker in patients after transcatheter aortic valve implantation: a prospective observational study. *BMJ Open*. (2021) 11:e042857. doi: 10.1136/BMJOPEN-2020-042857

39. Distelmaier K, Winter MP, Rützler K, Heinz G, Lang IM, Maurer G, et al. Serum butyrylcholinesterase predicts survival after extracorporeal membrane oxygenation after cardiovascular surgery. *Crit Care*. (2014) 18:1–7. doi: 10.1186/CC13711/FIGURES/1

40. Combes A, Leprince P, Luyt CE, Bonnet N, Trouillet JL, Léger P, et al. Outcomes and long-term quality-of-life of patients supported by extracorporeal membrane oxygenation for refractory cardiogenic shock. *Crit Care Med*. (2008) 36:1404–11. doi: 10.1097/CCM.0B013E31816F7CF7

41. Bratzler DW, Houck PM, Richards C, Steele L, Dellinger EP, Fry DE, et al. Use of antimicrobial prophylaxis for major surgery: baseline results from the national surgical infection prevention project. *Arch Surg*. (2005) 140:174–82. doi: 10.1001/ARCHSURG.140.2.174

42. Zajonz TS, Kunzemann C, Schreiner AL, Beckert F, Schneck E, Boening A, et al. Potentials of acetylcholinesterase and butyrylcholinesterase alterations in on-pump coronary artery bypass surgery in postoperative delirium: an observational trial. *J Clin Med*. (2023) 12:5245. doi: 10.3390/JCM12165245

43. Hughes CG, Boncyk CS, Fedeles B, Pandharipande PP, Chen W, Patel MB, et al. Association between cholinesterase activity and critical illness brain dysfunction. *Crit Care*. (2022) 26:1–12. doi: 10.1186/S13054-022-04260-1/FIGURES/3

44. Rump K, Holtkamp C, Bergmann L, Nowak H, Unterberg M, Orlowski J, et al. Midazolam impacts acetyl—and butyrylcholinesterase genes: an epigenetic explanation for postoperative delirium? *PLoS One* (2022) 17:e0271119. doi: 10.1371/JOURNAL.PONE.0271119

45. Bosancic Z, Spies CD, Müller A, Winterer G, Piper SK, Heinrich M. Association of cholinesterase activities and POD in older adult abdominal surgical patients. *BMC Anesthesiol*. (2022) 22:1–10. doi: 10.1186/S12871-022-01826-Y/TABLES/2

46. Klocker EV, Barth DA, Riedl JM, Prinz F, Szkandera J, Schlick K, et al. Decreased activity of circulating butyrylcholinesterase in blood is an independent prognostic marker in pancreatic cancer patients. *Cancers (Basel)* (2020) 12:1154. doi: 10.3390/CANCERS12051154

47. Gensthaler L, Jomrich G, Brugger J, Kollmann D, Paireder M, Bologheanu M, et al. Preoperative BChE serves as a prognostic marker in patients with resectable AEG after neoadjuvant chemotherapy. *Langenbecks Arch Surg*. (2023) 408:1–10. doi: 10.1007/S00423-023-02938-W/FIGURES/2

48. Schmidt K, Zivkovic AR, Thiele M, Horter J, Brenner T, Weigand MA, et al. Point-of-care measured serum cholinesterase activity predicts patient outcome following severe burns. *Burns*. (2021) 47:863–72. doi: 10.1016/J.BURNS.2020.10.027

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