Prognostic Value of Lymphovascular Invasion in Stage Ⅱ Colorectal Cancer Patients with an Inadequate Examination of Lymph Nodes.

Zhenyan Gao
Third Affiliated Hospital of Soochow University: Changzhou First People's Hospital
Huihua Cao
Traditional Chinese Medicine Hospital of Kunshan
Xiang Xu
Third Affiliated Hospital of Soochow University: Changzhou First People's Hospital
Qing Wang
Third Affiliated Hospital of Soochow University: Changzhou First People's Hospital
Yugang Wu (czyywyg89@163.com)
Third Affiliated Hospital of Soochow University: Changzhou First People's Hospital
https://orcid.org/0000-0001-8447-8252
Qicheng Lu
Third Affiliated Hospital of Soochow University: Changzhou First People's Hospital

Research

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Abstract

Background

Lymphovascular invasion (LVI) is defined as the existence of cancer cells in lymphatics or blood vessels. This study aimed to evaluate the prognostic value of LVI in stage Ⅱ colorectal cancer (CRC) patients with inadequate examination of lymph nodes (ELNs) and further combined LVI with the TNM staging system to determine the predictive efficacy for CRC prognosis. Adjuvant chemotherapy (ACT) was then evaluated for stage Ⅱ CRC patients with LVI positivity (LVI +).

Methods

The clinicopathologic records of 1420 CRC patients treated at the Third Affiliated Hospital of Soochow University between February 2007 and February 2013 were retrospectively reviewed. LVI was examined by hematoxylin-eosin (HE) staining. Kaplan-Meier analysis followed by a log-rank test was used to analyze survival rates. Univariate and multivariate analyses were performed using a Cox proportional hazards model. The Harrell's concordance index (C-index) was used to evaluate the accuracy of different systems in predicting prognosis.

Results

The LVI status was significantly associated with pT stage, degree of differentiation, tumor stage, serum CEA and CA19-9 levels, perineural invasion (PNI) and KRAS status. The 5-year overall survival (OS) rate of stage Ⅱ patients with < 12 ELNs and LVI + was less than stage ⅡA. Multivariate analyses showed that LVI, pT-stage, serum CEA and CA19-9 levels, PNI and KRAS status were significant prognostic factors for stage Ⅱ patients with < 12 ELNs. The 8th TNM staging system combined with LVI showed a higher C-index than the 8th TNM staging system alone (C-index, 0.895 vs. 0.833). Among patients with LVI + the ACT group had a significantly higher 5-year OS and 5-year disease-free survival (DFS) than the surgery alone (SA) group (5-year OS, 66.7% vs. 40.9%, $P = 0.004$; 5-year DFS, 64.1% vs. 36.3%, $P = 0.002$).

Conclusions

LVI is an independent prognostic risk factor for stage Ⅱ CRC patients. Combining LVI with the 8th TNM staging system improved the predictive accuracy for CRC prognosis. ACT in stage Ⅱ CRC patients with LVI + is beneficial for survival.

Introduction

Colorectal cancer (CRC) is the third most common malignancy and the fourth leading cause of tumor-related deaths worldwide [1]. Although advances have been achieved in early detection and effective treatment, the survival rate of CRC is still poor [2]. Among all CRC patients, approximately one-third are diagnosed as stage Ⅱ [3]. The National Comprehensive Cancer Network (NCCN) guidelines recommend

adjuvant chemotherapy (ACT) for stage Ⅱ and Ⅲ CRC [4]. For stage Ⅱ CRC, the current guidelines recommend that ACT should be considered for patients at high risk for recurrence [5].

In addition, the current guidelines recommend that at least 12 lymph nodes (LNs) should be examined for nodal evolution [6]. Adequate LN retrieval from the specimen is essential to ensure accuracy in nodal staging [7]. An inadequate examination of lymph nodes (ELNs) may cause a false-negative result or a lower pN stage [8].

Lymphovascular invasion (LVI) is defined as the existence of cancer cells in lymphatics or blood vessels, and is considered to be an early step in lymph node metastasis (Fig. 1a) [1]. Many studies have reported that LVI positivity (LVI +) is a critical prognostic indicator in some cancers, including breast, bladder, and gastric cancers [9-11]. It has been reported that the presence of LVI in CRC varies from 4.1%-89.5% [12]. Currently, few studies have focused on LVI in stage Ⅱ CRC with inadequate ELNs. Moreover, no study involving the combination of LVI and the TNM staging system in CRC patients has been published.

Perineural invasion (PNI) is the process of nerve tumor infiltration, including tumor cells located in the three layers of the peripheral nerve sheath or adjacent to the nerve, and involving at least one third of its surroundings (Fig. 1b) [13, 14]. PNI has become a key pathological feature of many malignant tumors, including malignant tumors of the stomach, colon and rectum, pancreas and biliary tract [15-18]. At present, there is no consensus regarding the inclusion of PNI in staging, although PNI has been proven to be a sign of poor survival in colorectal cancer.

We conducted the current study to evaluate the prognostic value of LVI in stage Ⅱ CRC patients with inadequate ELNs and combined LVI with the tumor-node-metastasis (TNM) staging system to determine the predictive efficacy for CRC prognosis. ACT was then evaluated for stage Ⅱ CRC patients with LVI +.

Materials And Methods

Patients

We retrospectively examined the clinicopathologic records of CRC patients who were treated at the Third Affiliated Hospital of Soochow University between February 2007 and February 2013. The inclusion criteria were as follows: (1) adenocarcinoma confirmed by histopathology; (2) curative resection with lymphadenectomy; (3) no neoadjuvant chemoradiation; (4) complete clinicopathologic records; and (5) no evidence of distant metastases. The exclusion criteria were as follows: (1) received neoadjuvant chemoradiation; (2) incomplete clinicopathologic records; (3) lost to follow-up; and (4) distant metastases. CRC stage was classified according to the 8th edition of the American Joint Committee on Cancer (AJCC) TNM staging system. Follow-up was carried out by telephone calls, emails, and on-site visits. Informed written consent was obtained from all CRC patients. The study was approved by the Ethics Committee of the Third Affiliated Hospital of Soochow University.

ACT regimens for stage Ⅱ CRC patients

After curative resection, some stage □ CRC patients chose to receive ACT for further treatment. The ACT regimen was established by our clinicians based on the patient's general performance, clinicopathologic features, and operative factors. A 6-month oxaliplatin-based regimen (FOLFOX [5-fluorouracil with oxaliplatin] or CapeOx [capecitabine with oxaliplatin]) was recommended for stage □ CRC patients. For those patients with a contraindication to oxaliplatin, a 6-month fluoropyrimidine-based regimen (5-FU/LV [5- fluorouracil/leucovorin] or 5-FU [5- fluorouracil]) was an acceptable alternative.

Data collection and LVI examination

Patient medical records were reviewed to obtain clinicopathologic data. Age, sex, tumor size, tumor location, LVI, TNM stage, degree of differentiation, ELNs, serum CEA and CA19-9 levels, perineural invasion and KRAS status were recorded. Specimens were fixed in formalin, then cut into multiple slices. Slices were then embedded in paraffin and stained with hematoxylin-eosin (HE). All H&E slides (one in each case) were evaluated by at least two experienced pathologists, who independently assessed small and large vessel invasion.

Statistical analysis

All analyses were performed using SPSS (version 19.0 software; IBM, Chicago, IL, USA) and R software (version 3.0.0; www.r-project.org). Statistical significance was tested using a Student's t-test and chi-squared test. Univariate and multivariate analyses were performed using a Cox proportional hazards model. Kaplan-Meier analysis followed by a log-rank test was used to analyze survival rates. The Harrell's concordance index (C-index) was used to evaluate the accuracy of different systems in predicting prognosis. All statistical analyses were two-sided and a $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

One thousand four hundred and twenty CRC patients from February 2007 to February 2013 who met the inclusion criteria were evaluated in this study. The clinicopathologic characteristics of 1420 CRC patients are listed in table 1. Of all CRC patients, there were 822 males (57.9%) and 598 females (42.1%); 47.1% of patients were ≤ 60 years of age and 52.9% of patients were > 60 years of age. The number of stages □, □, and □ patients were 173, 409, and 838, respectively. Of the 409 stage □ patients, 144 patients did not receive ACT, 121 patients received FOLFOX regimen, 56 patients received CapeOx regimen, 48 patients received 5-FU/LV regimen and 40 received 5-FU regimen.

In order to avoid the effects of different ACT regimens, among 409 stage □ patients we chose 121 patients who received FOLFOX regimen and the 144 patients who did not receive ACT as the object of study. Finally, these 265 stage □ patients were analyzed in this study whose clinicopathologic characteristics were listed in table 1. 100 patients (37.7%) were LVI + and 165 (62.3%) were LVI -. We divided these stage □ patients into ACT and surgery alone (SA) groups.

Occurrence of LVI in stage Ⅱ CRC patients

As shown in table 2, the incidence of LVI in 265 stage Ⅱ CRC patients is listed based on clinicopathologic characteristics. The LVI status was significantly associated with pT stage, degree of differentiation, tumor stage, serum CEA and CA19-9 levels, perineural invasion and KRAS status. The incidence of LVI in pT3, pT4a, and pT4b stage was 22.9%, 47.7%, and 64.3%, respectively. There was a statistically significant difference between LVI and pT stage. No significant difference existed with respect to sex, age, tumor size, tumor site, and ELNs. We also found that the LVI + rate in the ACT group was significantly higher than the SA group (64.5% vs. 15.3%, $P < 0.001$).

Overall survival of CRC patients

We divided the 265 stage Ⅱ patients into ELNs < 12 and ELNs ≥ 12 groups. The 5-year OS rate of the ELNs ≥ 12 group (75.2%) was greater than the ELNs < 12 group (68.5%); however, there was no statistically significant difference (Fig. 2). The 5-year OS rate of the LVI - group was greater than the LVI + group (79.4% *vs.* 61.0%; $P < 0.001$; Fig. 3a). The 5-year DFS rate of the LVI - group was greater than the LVI + group (78.2% *vs.* 58.0%; $P < 0.001$; Fig. 3b). We further compared the OS rates among stage Ⅱ patients with ≥ 12 ELNs, stage Ⅱ LVI - patients with < 12 ELNs, stage Ⅱ LVI + patients with < 12 ELNs, and stages ⅡA, ⅡB, and ⅡC patients (Fig. 4). The 5-year OS rate of stage Ⅱ LVI + patients with < 12 ELNs was 60.4%, which is significantly less than stage Ⅱ LVI - patients with < 12 ELNs and stage Ⅱ patients with ≥ 12 ELNs, respectively (60.4% *vs.* 75%, $P < 0.001$; 60.4% *vs.* 75.2%, $P < 0.001$); however, the 5-year OS rate of stage Ⅱ LVI + patients with < 12 ELNs was even lower than stage ⅡA. No significant differences existed between stage Ⅱ LVI + patients with < 12 ELNs and stages ⅡA and ⅡB patients (60.4% *vs.* 65.7% *vs.* 54.3 %, $P = 0.052$). The 5-year OS rate of the PNI - group was greater than the PNI + group (74.2% *vs.* 42.1%; $P = 0.003$; Fig. 5).

Univariate and multivariate analyses for the prognosis of stage Ⅱ patients with < 12 ELNs

Owing to the specific characteristics of stage Ⅱ patients with < 12 ELNs, the prognostic factors were further analyzed. Univariate analyses showed that LVI, pT-stage, degree of differentiation, and CEA and CA19-9 levels were significant prognostic factors for stage II patients with < 12 ELNs; Further multivariate analysis identified that LVI, pT-stage, degree of differentiation, CEA and CA19-9 levels PNI and KRAS status were significant prognostic factors for stage II patients with < 12 ELNs (all P<0.05) (table 3).

Improvement of the 8th TNM staging system

Because of the similarity in 5-year OS rates between stage Ⅱ LVI + patients with < 12 ELNs and stages ⅡA and ⅡB patients, we combined LVI with the 8th TNM staging system. A comparison was made to estimate the prognostic value between the new system and the 8th TNM staging system (table 4). Stage Ⅱ LVI + patients with < 12 ELNs were upgraded to stage Ⅲ, while stage Ⅱ LVI – patients with < 12 ELNs remained stage Ⅱ. The 8th TNM staging system combined with LVI had a higher C-index than the 8th TNM staging system alone (C-index, 0.895 vs. 0.833), which indicates a better prognostic value for CRC patients.

Relationship between LVI and ACT in stage Ⅱ CRC patients

In addition to analyzing OS, we also analyzed disease-free survival (DFS), especially in stage Ⅱ CRC patients. The 5-year DFS rate of the LVI - group was greater than the LVI + group (78.2% *vs.* 58.0%; $P < 0.001$; Fig. 3b). We further divided the stage Ⅱ CRC patients into ACT and SA groups. There was no significant difference in the 5-year OS and DFS between stage II CRC patients in the ACT and SA groups (5-year OS, 81.4% *vs.* 78.7%, $P = 0.738$; Fig. 6a and 5-year DFS, 79.1% *vs.* 77.9%, $P = 0.896$; Fig. 6b). When LVI + patients were analyzed, however, ACT group patients had significantly higher 5-year OS and DFS rates than the SA group (5-year OS, 66.7% *vs.* 40.9%, $P = 0.004$; Fig. 6c and 5-year DFS, 64.1% *vs.* 36.3%, $P = 0.002$; Fig. 6d).

Discussion

CRC has become a major public health issue worldwide, with 1.4 million new cases and 0.7 million deaths each year [19]. Curative surgery with or without chemotherapy and radiotherapy is the mainstay of treatment for CRC [20]. ACT is recommended for stages Ⅲ and Ⅱ CRC patients [4]. It has been reported that ACT improves OS in stage Ⅲ patients [21]; however, the benefit of ACT in stage II CRC patients is is controversial.

The 8th TNM staging system remains the most important prognostic indicator for CRC patients [8]. For pN stage patients, at least 12 ELNs are recommended to avoid false-negative prognostication; however, it is unavoidable that some cases have < 12 ELNs, which may interfere with the nodal classification and even influence prognosis. LVI has been reported to occur in 10%-89.5% of CRC patients [22], which is also considered to increase the risk for micrometastases in localized cancer [23]. Thus, in this study we focused on stage Ⅱ LVI + patients with < 12 ELNs.

As a common histopathologic finding, LVI serves as a prognostic risk factor in many carcinomas [22, 24, 25]. In this study, the LVI + rate was 34.2% among all CRC patients and 37.7% of stage Ⅱ CRC patients, which is in agreement with previous studies [19]. Differences in the LVI + rate might reflect the diagnostic technique used and the number of patients in various studies [26]. In our 100 stage Ⅱ LVI + patients, LVI was significantly correlated with pT stage, degree of differentiation, tumor stage, serum CEA and CA19-9 levels, KRAS status and PNI. Similar to our results, Lim et al. [22] reported an association between LVI and more advanced T and N categories, higher pre-CEA levels, and worse tumor grade. Al-Sukhni et al. [27] also concluded that LVI is related to several factors in patients with advanced CRC, including larger size, more advanced T stage, LN involvement, and distant metastasis. Zhong et al. [1] also showed that LVI is significantly associated with an increased CEA level, increased tumor differentiation, and advanced tumor stage. These studies all support our results. Thus, it has been suggested that the presence of LVI should serve as an indicator of extending the resection area [28].

Survival analyses were conducted in this study. The 5-year OS and DFS rates in stage Ⅱ LVI + patients were significantly less than LVI - patients. We even found that stage Ⅱ LVI + patients with < 12 ELNs had a poor 5-year OS rate that was similar to stage Ⅲ CRC patients. Multivariate analysis showed that LVI, KRAS

status and PNI were significant prognostic factors for stage Ⅱ CRC patients. Similar to our conclusion, it has been shown that LVI is an independent poor prognostic factor for survival among CRC patients [29]. Huh et al. [30] also reported that N0 stage CRC patients, especially stage Ⅱ, may benefit most from the presence of LVI because these patients may have a superior response to ACT. The current meta-analysis shows that mutations in the KRAS gene appear to be associated with OS in CRC patients [31]. However, another study found that KRAS and BRAF mutations are independent poor prognostic factors for the OS of stage IV tumors rather than stage I-III tumors [32]. Jang et al. [33] concluded that KRAS mutations are significantly associated with high-grade Tumor budding; furthermore, tumors with KRAS mutations in exons 3 and 4 tended to have LVI and PNI. Marx et al. [34] concluded that higher Tumor budding status is related to higher tumor grade and stage, positive lymph nodes and LVI. Al-Sukhni et al. [27] reported an association between LVI, PNI and advanced CRC, and found that PNI is an independent poor prognostic marker for survival in CRC. Skancke et al. [35] also showed that LVI and PNI have an adverse effect on the survival of patients with stage II colon cancer. When LVI and PNI are present, ACT may have a protective effect.

To explore the benefit from ACT, we focused on the survival of ACT patients with or without LVI. Our results showed that ACT improved the 5-year OS and DFS rates in LVI + patients. Several studies have reported that ACT is beneficial for stage Ⅱ CRC patients [36, 37]. Similar conclusions were reported by Skancke et al. [4], who demonstrated that CRC patients with high-risk factors, including LVI, can benefit from ACT. Arakawa et al. [38] reported that a significant prognostic benefit is achieved after ACT for stage Ⅱb/c CRC patients. Lin et al. [39] enrolled 1039 stage Ⅱ CRC patients and concluded that ACT improves the DFS rate in high-risk stage Ⅱ CRC patients.

It has been reported that the improvement in OS and DFS rates with ACT did not differ significantly between high- and low-risk stage Ⅱ CRC patients [21]. Fu et al.[3] suggested that the value of ACT in stage Ⅱ colon cancer is much less than previously thought; de-escalating chemotherapy for these patients is necessary. Booth et al. [40] reported that ACT is not related to improved survival for stage Ⅱ CRC patients with high-risk factors. Although there exist some differences in opinions, we still believe ACT is beneficial for LVI + patients. The current therapy strategies for N0 stage CRC patients do not directly account for LVI.

To further explain the prognostic value of LVI in CRC patients, we combined LVI with the 8th TNM staging system, which had a better predictive efficacy than the 8th TNM staging system alone. Incorporating the negative impact of LVI into the staging system of CRC may predict the prognosis with greater precision and further establish a more reasonable therapeutic strategy for stage Ⅱ CRC patients.

This study had some limitations. This was a retrospective study from a single center and the sample size was not sufficiently large, which may have led to selection bias. A multicenter collaborative study with a large sample size may overcome this issue. In addition, we only focused on the phenomenon and the consequences resulting from LVI, thus it is necessary for us to explore the genetic mechanism underlying LVI, which may provide novel biomarkers and establish new tumor therapeutic strategies for CRC.

Conclusions

LVI and PNI are independent prognostic risk factors for stage Ⅱ CRC patients. Stage II CRC with < 12 LNM and LVI+ could benefit from adjuvant chemotherapy. The inclusion of LVI can improve the predictive accuracy of the 8th TNM staging system for CRC prognosis. ACT in stage Ⅱ CRC patients with LVI + is beneficial for survival.

Abbreviations

LVI: Lymphovascular invasion

CRC: colorectal cancer

ELNs: examination of lymph nodes

ACT: Adjuvant chemotherapy

PNI: perineural invasion

OS: overall survival

LNs: lymph nodes

TNM: tumor-node-metastasis

Declarations

Acknowledgments

Not applicable.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Zhenyan Gao and Huihua Cao wrote the manuscript and analyzed data. Qing Wang and Xiang Xu collected the data of patients. Yugang Wu and Qicheng Lu assisted Zhenyan Gao and Huihua Cao to

complete the work. Yugang Wu funded the study.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Third Affiliated Hospital of Soochow University.

Consent for publication

Informed consent was obtained from all patients.

Competing interests

The authors have no conflict of competing interests.

Statement

All methods in this article are implemented in accordance with relevant guidelines and regulations.

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Tables

Table 1. Clinicopathologic characteristics of CRC patients. One thousand four hundred and twenty CRC patients from February 2007 to February 2013 who met the inclusion criteria were analyzed in this study. The clinicopathologic characteristics of 1420 CRC patients and the 265 studied stage patients, are listed in Table 1. Of the 265 stage patients, 100 patients (37.7%) were LVI + and 165 (62.3%) were LVI -.

Parameters	Overall patients	Stage II patients
	n (%)	n (%)
Sex		
Female	598 (42.1)	108 (40.8)
Male	822 (57.9)	157 (59.2)
Age (years)		
≤65	669 (47.1)	126 (47.5)
>65	751 (52.9)	139 (52.5)
Tumor site		
Colon	846 (59.6)	145 (54.7)
Rectum	574 (40.4)	120 (45.3)
Tumor Size (cm)		
≤4	688 (48.5)	141 (53.2)
>4	732 (51.5)	124 (46.8)
Lymphovascular invasion		
Positive	486 (34.2)	100 (37.7)
Negative	934 (65.8)	165 (62.3)
T-stage		
T1	233 (16.4)	
T2	393 (27.7)	
T3	457 (32.2)	144 (54.3)
T4	337 (23.7)	121 (45.7)
N-stage		
N0	582 (41.0)	265 (100.0)
N1	498 (35.1)	
N2	340 (23.9)	
Differentiation degree		
Well	666 (46.9)	135 (51.0)
Moderate	582 (41.0)	118 (44.5)

Poor	172 (12.1)	12 (4.5)
CEA		
≤5ng/ml	836 (58.9)	161 (60.8)
>5ng/ml	584 (41.1)	104 (39.2)
CA19-9		
≤37U/ml	1106 (77.9)	212 (80.0)
>37U/ml	314 (22.1)	53 (20.0)
Retrieved LN		
<12	564 (39.7)	108 (40.8)
≥12	856 (60.3)	157 (59.2)
Treatment		
ACT	960 (67.6)	121 (45.7)
SA	460 (32.4)	144 (54.3)
TNM stage		
I	173 (12.2)	
II	409 (28.8)	
IIA	174 (12.3)	
IIB	121 (8.5)	
IIC	114 (8.0)	
III	838 (59.0)	
IIIA	324 (22.8)	
IIIB	287 (20.2)	
IIIC	227 (16.0)	
KRAS status		
Wild type	975(68.7)	160(60.4)
Mutant type	445(31.3)	105(39.6)
PNI		
Positive	276(19.4)	58(21.9)
Negative	1144(80.6)	207(78.1)

CRC, colorectal cancer; LN, lymph nodes; PNI, perineural invasion;

Table 2. Occurrence of LVI in 265 stage II CRC patients. The incidence of LVI in stage Ⅱ CRC patients is listed in Table 2 according to clinicopathologic characteristics. The LVI status was significantly associated with pT stage, degree of differentiation, tumor stage, serum CEA and CA19-9 levels, perineural invasion and KRAS status. No significance existed in sex, age, tumor size, tumor site, and ELNs.

Parameters	LVI (+)	LVI (-)	*P* value	LVI (+) rate (%)
	n	n		
Sex			0.563	
Female	43	65		39.8
Male	57	100		36.3
Age (years)			0.102	
≤65	54	72		42.9
>65	46	93		33.1
Tumor site			0.276	
Colon	59	86		40.7
Rectum	41	79		34.2
T-stage			< 0.001	
T3	33	111		22.9
T4a	31	34		47.7
T4b	36	20		64.3
Tumor Size (cm)			0.186	
≤4	48	93		34.0
>4	52	72		41.9
Differentiation degree			< 0.001	
Well	38	97		28.1
Moderate	52	66		44.1
Poor	10	2		83.3
CEA			< 0.001	
≤5ng/ml	28	133		17.4
>5ng/ml	72	32		69.2
CA19-9			< 0.001	
≤37U/ml	61	151		28.8
>37U/ml	39	14		73.6
Retrieved LN			0.062	

<12	48	60		44.4
≥12	52	105		33.1
Treatment			< 0.001	
ACT	78	43		64.5
SA	22	122		15.3
II			0.023	
IIA	37	76		32.7
IIB	34	44		43.6
IIC	39	35		52.7
KRAS status			< 0.001	
Wild type	39	121		24.4
Mutant type	61	44		58.1
PNI			< 0.001	
Negative	66	141		31.9
Positive	34	24		58.6

CRC, colorectal cancer; LN, lymph nodes; LVI, Lymphovascular invasion; PNI, perineural invasion;

Table 3. Univariate and multivariate analyses of prognostic factors for stage Ⅱ patients with <12 ELNs. We analyzed the prognostic factors for those patients. As listed in Table 3, univariate analysis showed that LVI, pT-stage, degree of differentiation, serum CEA and CA19-9 levels, perineural invasion and KRAS status. were significant prognostic factors for those patients.

Parameters	Patients	5-year OS (%)	Univariate analysis	Multivariate analysis		
	n (%)		*P*	HR	95% CI	*P*
Sex			0.675			
Female	53	67.9				
Male	55	69.1				
Age (years)			0.255			
≤65	54	64.8				
>65	54	72.2				
Tumor site			0.384			
Colon	57	63.2				
Rectum	51	68.6				
Tumor Size (cm)			0.078			
≤4	60	61.7				
>4	48	77.1				
Lymphovascular invasion			0.016	2.313	1.897-4.562	0.033
Positive	48	60.4				
Negative	60	75.0				
T-stage			<0.001	2.358	1.767-3.897	0.001
T3	59	81.4				
T4	49	53.1				
Differentiation degree			0.002	1.879	1.223-4.563	0.044
Well	53	77.4				
Moderate	53	62.3				
Poor	2	0				
CEA			<0.001	3.011	1.997-4.967	<0.001
≤5ng/ml	61	78.7				
>5ng/ml	47	55.3				

CA19-9			0.001	1.935	1.156-3.768	0.015
≤37U/ml	69	75.4				
>37U/ml	39	56.4				
KRAS status			<0.001	2.277	1.115-4.653	0.013
Wild type	68	76.5				
Mutant type	40	55.0				
PNI			<0.001	2.837	1.090-5.385	0.003
Positive	19	42.1				
Negative	89	74.2				

CRC, colorectal cancer; LN, lymph nodes; ELNs, examined lymph nodes; OS, overall survival; PNI, perineural invasion;

Table 4. Comparison of the performance of the 8th edition of the TNM staging system alone and the 8th edition of the TNM staging system combined with LVI. We combined the LVI with the 8th TNM staging system. A comparison was made to estimate the prognostic value between the new system and the 8th TNM staging system. As listed in Table 4, the 8th TNM staging system combined with LVI had a higher C-index than the 8th TNM staging system alone (C-index, 0.895 vs. 0.833), which indicates a better prognostic value for CRC patients.

Classification	Stage	n	5-year OS (%)	C-Index	95% CI
	I	173	90.2		
8th TNM	II	409	72.5	0.833	0.785-0.889
	III	838	56.2		
	I	173	90.2		
8th TNM+LVI	II	361	74.2	0.895	0.812-0.924
	III	886	56.9		

C-Index, The Harrell's concordance index; LVI, Lymphovascular invasion; OS, overall survival; CI, confidence interval

Figures

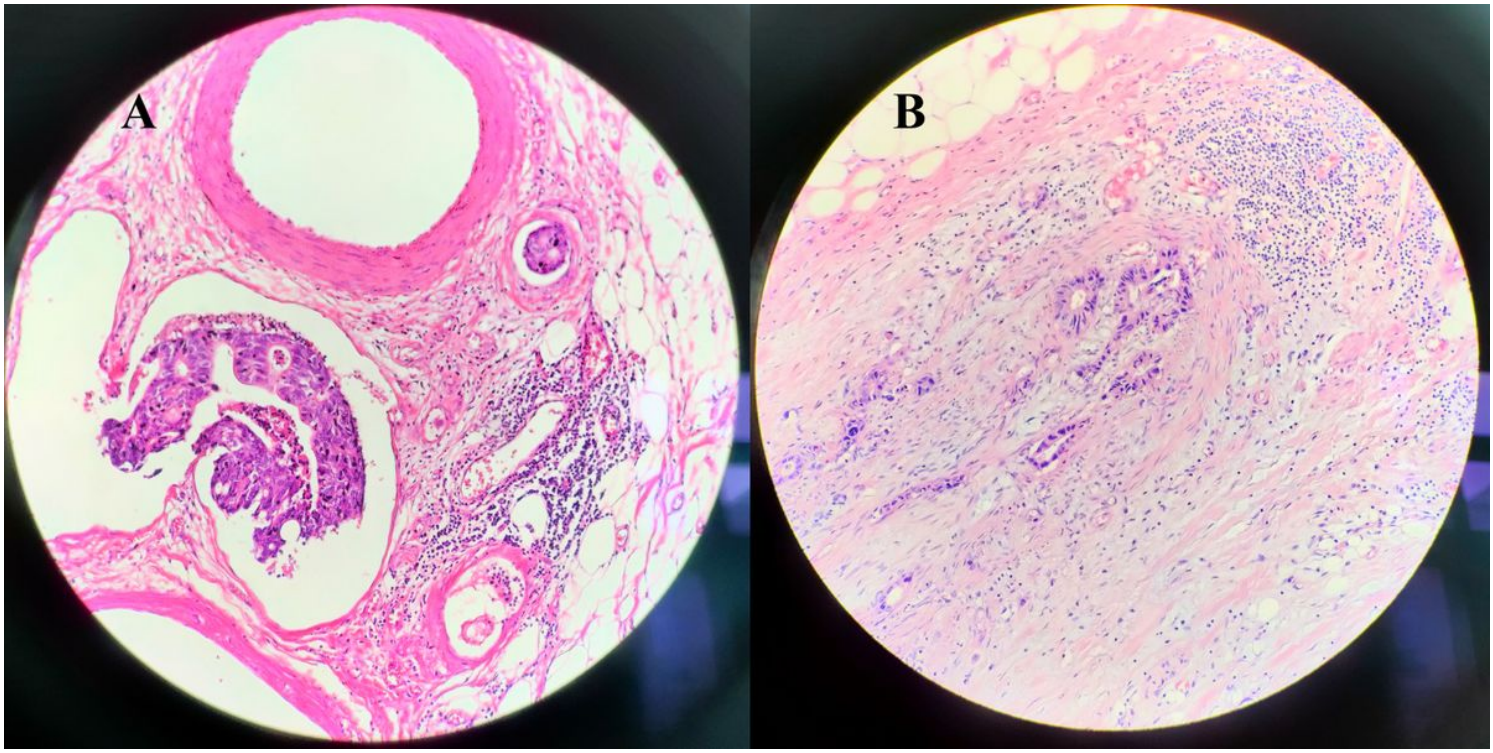
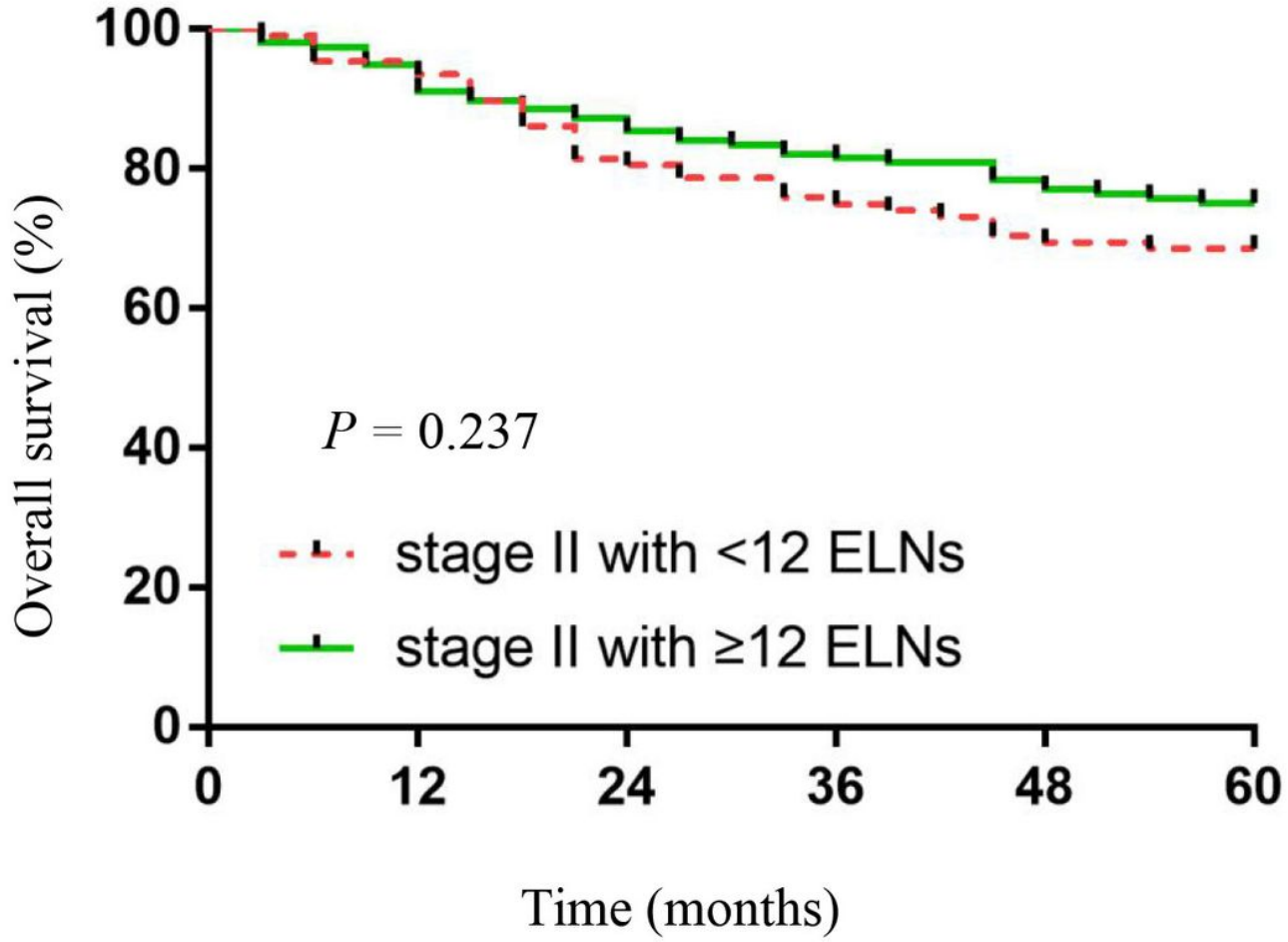


Figure 1

An example of positive lymphovascular invasion (LVI), diagnosed by at least two experienced pathologists on H&E examination. H&E, 200× (Fig. 1a); an example of positive perineural invasion (PNI), H&E, 200× (Fig. 1b)



No. at risk

< 12	108	103	88	82	76	74
≥ 12	157	149	137	129	123	118

Figure 2

The 5-year overall survival (OS) rate among stage Ⅱ CRC patients according to examined lymph nodes (ELNs). We divided the stage Ⅱ patients into ELNs < 12 and ELNs ≥ 12 groups. The 5-year OS rate of the ELNs ≥ 12 group (75.2%) was greater than the ELNs < 12 group (68.5%); however, there was no statistical significance.

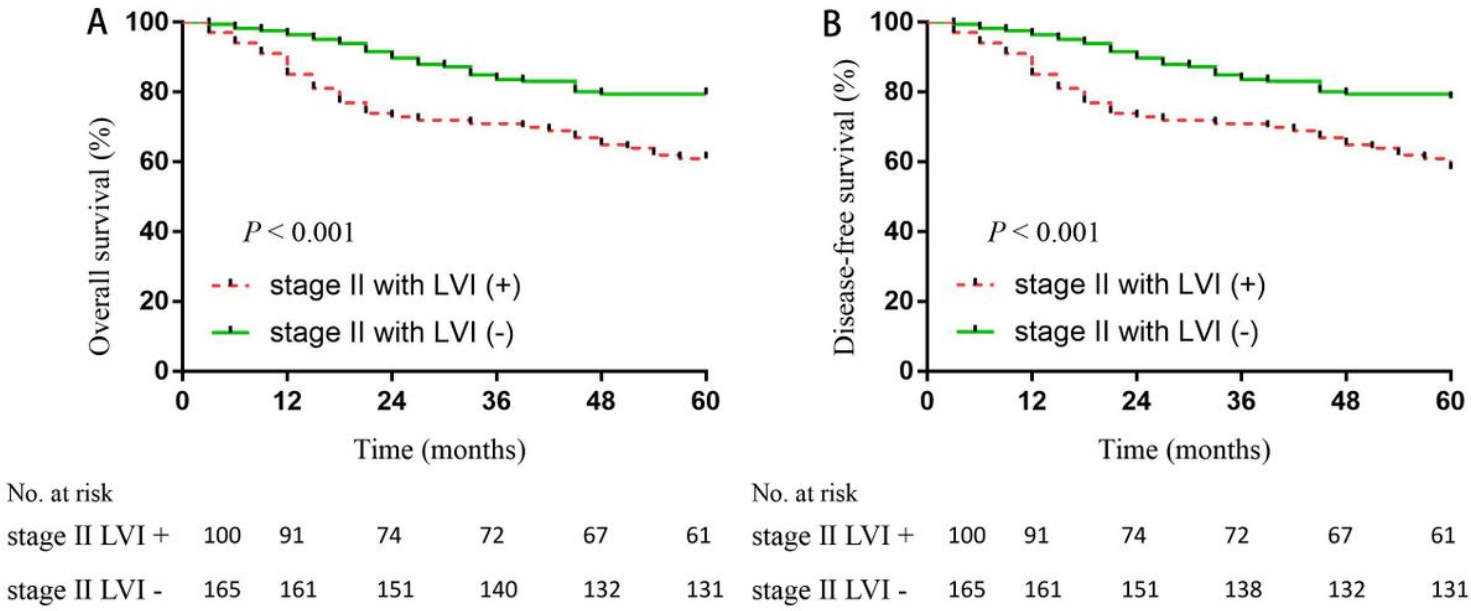
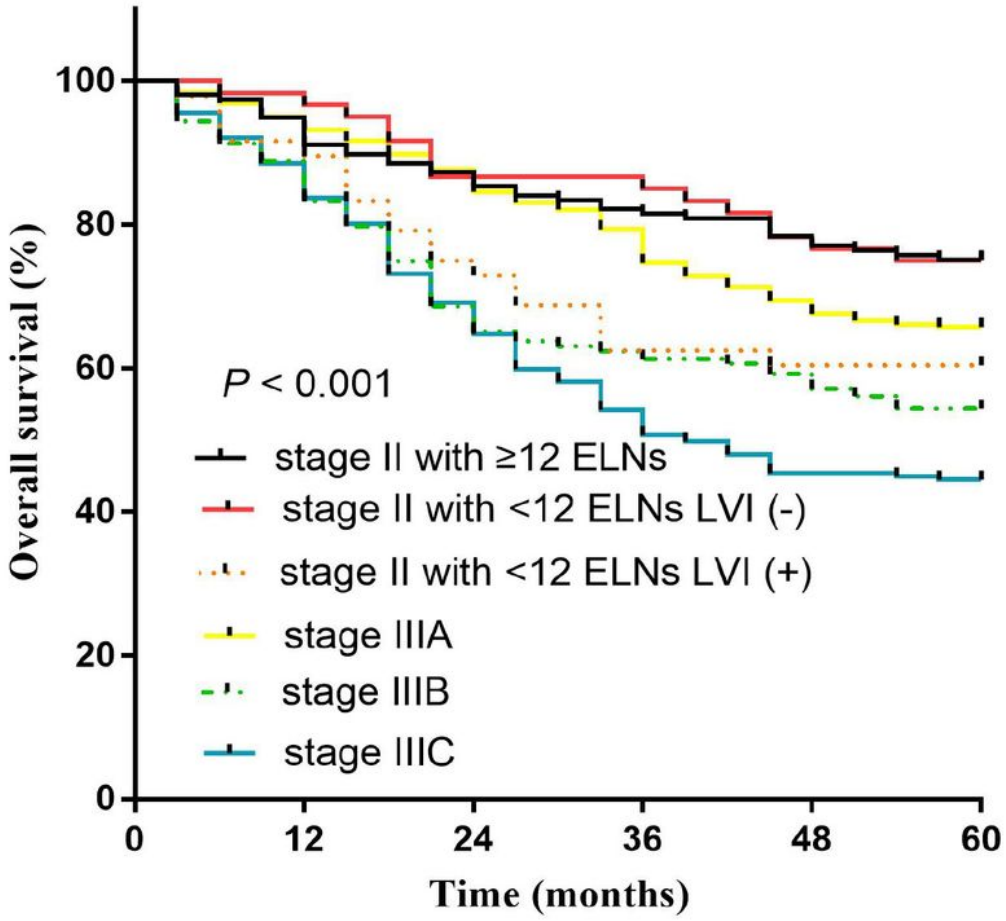


Figure 3

The 5-year overall survival (OS) and disease-free survival (DFS) rates among stage CRC patients according to lymphovascular invasion (LVI). When dividing the stage patients into LVI + and LVI - groups, the 5-year OS rate of the LVI + group was greater than LVI – (79.4% vs. 61.0%, P < 0.001; Fig. 3a); The 5-year DFS rate of the LVI - group was greater than the LVI + group (78.2% vs. 58.0%; P < 0.001; Fig. 3b).

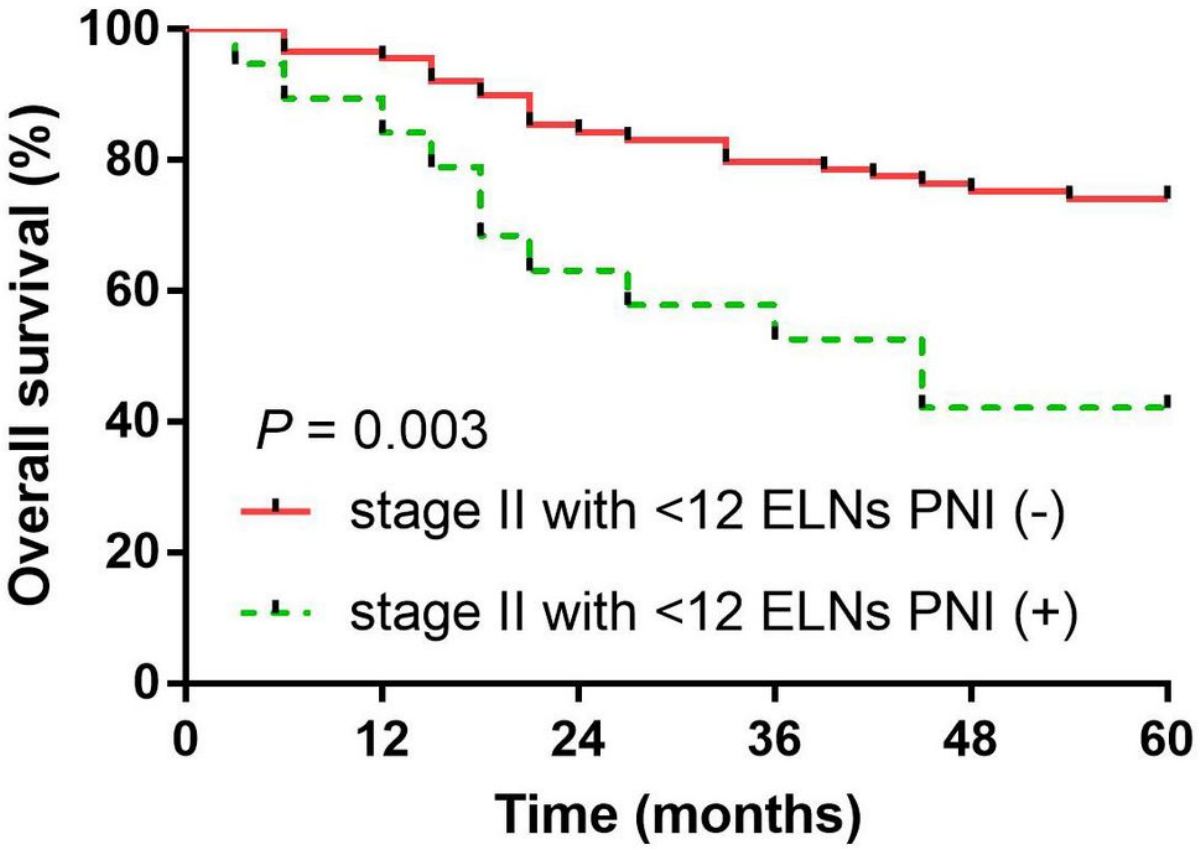


No. at risk						
stage II with ≥ 12 ELNs	157	149	137	129	123	118
stage II with < 12 ELNs LVI -	60	59	55	52	47	45
stage II with < 12 ELNs LVI +	48	44	36	33	30	29
stages IIIA	324	308	284	257	225	213
stages IIIB	287	255	197	179	170	156
stages IIIC	227	201	157	123	109	101

Figure 4

The 5-year overall survival (OS) rates among the different groups. We compared the OS rate among stage Ⅱ patients with ≥ 12 ELNs, stage Ⅱ LVI - patients with < 12 ELNs, stage Ⅱ LVI + patients with < 12 ELNs, and stages ⅢA, ⅢB, and ⅢC patients. The 5-year OS rate of stage Ⅱ LVI + patients with < 12 ELNs was 60.4%, which is significantly lower than stage Ⅱ LVI - patients with < 12 ELNs and stage Ⅱ patients with ≥ 12 ELNs, respectively, (60.4% vs. 75%, P < 0.001; 60.4% vs. 75.2%, P < 0.001); however, the 5-year OS rate of

stage Ⅱ LVI + patients with < 12 ELNs was even lower than stage ⅡA; no significance existed among stage Ⅱ LVI + patients with < 12 ELNs, and stages ⅡA and ⅡB (60.4% vs. 65.7% vs. 54.3 %, P = 0.052).



No. at risk

stage II with <12 ELNs (-) PNI (-)	89	86	76	74	68	66
stage II with <12 ELNs (-) PNI (+)	19	17	13	11	10	8

Figure 5

The 5-year OS rate of the PNI - group was greater than the PNI + group (74.2% vs. 42.1%; P = 0.003).

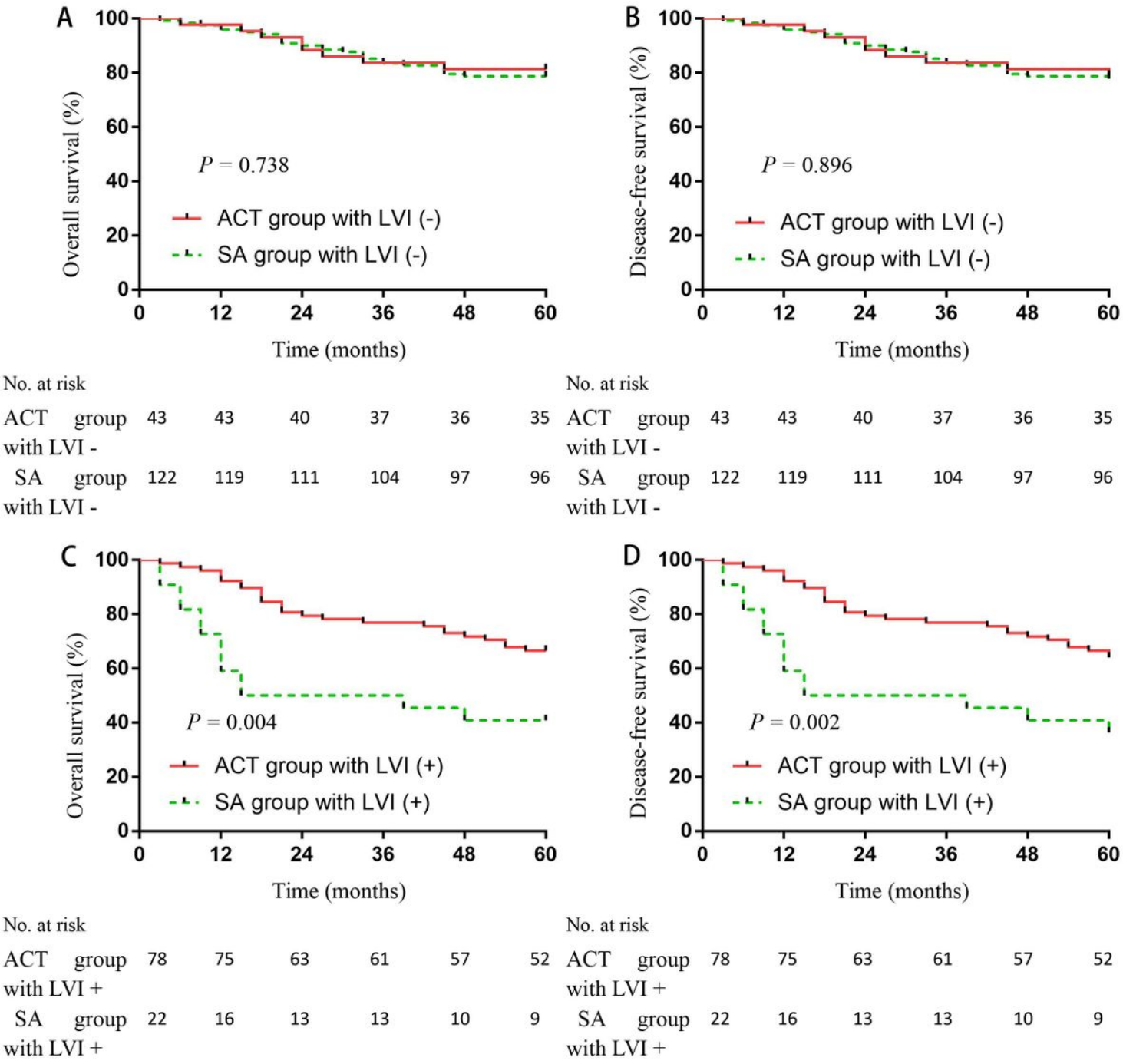


Figure 6

The 5-year overall survival (OS) and disease-free survival (DFS) rates among stage Ⅲ CRC patients according to adjuvant chemotherapy (ACT). We divided the stage Ⅲ CRC patients into ACT and surgery alone (SA) groups. In stage Ⅲ LVI - patients, no significant difference existed in the 5-year OS and DFS rates between the ACT and SA groups (5-year OS, 81.4% vs. 78.7%; P = 0.738, Fig. 6a and 5-year DFS, 79.1% vs. 77.9%, P = 0.896; Fig. 6b). When LVI + patients were analyzed, the ACT group had significantly higher 5-year OS and DFS rates than the SA group (5-year OS, 66.7% vs. 40.9%, P = 0.004, Fig. 6c and 5-year DFS, 64.1% vs. 36.3%, P = 0.002; Fig. 6d).