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Prognostic Value of Lymphovascular Invasion in Patients with Stage III Colorectal Cancer: A Retrospective Study

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Background: Lymph node metastasis and tumor progression depend on lymphovascular invasion (LVI). This study aimed to investigate the prognostic role of LVI in patients with stage III colorectal cancer (CRC) and to develop a prognostic nomogram.





Material/Methods: A retrospective study included 437 patients with stage III CRC. The impact of LVI on overall survival (OS) was analyzed with the Kaplan-Meier method and Cox regression model. A nomogram was constructed, and its predictive accuracy was evaluated using the concordance index (C-index) and the calibration plot.

Results: LVI was found in 19.7% of cases of stage III CRCs and was significantly correlated with high tumor grade (poor differentiation) and advanced tumor stage (all $P < 0.05$). Patients age, a family history of cancer in a first-degree relative, pre-treatment levels of carcinoembryonic antigen (CEA), prognostic nutritional index (PNI), histological tumor grade, tumor-node-metastasis (TNM) stage, and LVI were independent prognostic indicators (all $P < 0.05$). Compared with the LVI(-) group, patients in the LVI(+) group showed a 1.748-fold increased risk of death ($P = 0.004$) and a significantly reduced OS rate ($P < 0.001$). In the prognostic nomogram, the C-index was significantly increased with LVI compared with the TNM stage alone (0.742 vs. 0.593; $P < 0.001$). Calibration plots showed good fitness of the nomogram for prediction of survival. Comparison of the nomograms with and without LVI showed that inclusion of LVI improved the C-index from 0.715 to 0.742.

Conclusions: LVI was an indicator of more aggressive biological behavior and poor prognosis in patients with stage III CRC.

MeSH Keywords: **Colorectal Neoplasms • Lymphatic Vessels • Nomograms • Risk Factors**

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Background

Worldwide, colorectal cancer (CRC) is the third most common malignancy and ranks fourth as a cause of cancer-related death, accounting for about 1.4 million new cases and 0.7 million deaths in 2012 [1]. Despite the advances in medical management, postoperative recurrence and tumor metastasis continue to reduce patient survival [2,3]. The tumor-node-metastasis (TNM) staging system is currently used to predict patient prognosis and to plan treatment in patients with CRC [4]. However, the predictive accuracy of the TNM system is limited, especially in patients with locally advanced disease [5]. The outcome for patients with stage III CRC varies, and the 5-year survival rates have been reported to range from 80% and 30% for stage IIIA and IIIC CRC, respectively [6]. Therefore, there remains a need to identify other prognostic indicators that will enable improved and individualized prediction of prognosis for patients with stage III CRC.

The lymphatic system is the primary pathway for cancer metastasis, due to loose epithelial junctions and the absence of a basement membrane [7]. Lymphovascular invasion (LVI) is the presence of cancer cells in lymphatic or blood vessels and is considered to be an early and pivotal step in lymph node metastasis and cancer dissemination [8,9]. Previous studies have shown that LVI correlated with more aggressive tumor behavior and increased mortality rates in some cancers, including breast [10], bladder [11], and gastric cancers [12]. The presence of LVI in CRC has been reported to vary from 4.1–89.5%, due to the different size of the study population and different diagnostic techniques used [13–15]. LVI is useful to identify patients with high-risk stage II CRC who might benefit from adjuvant chemotherapy [16].

Currently, there have been few studies that have focused on the role of LVI in stage III CRC. Therefore, this retrospective study aimed to investigate the prognostic role of LVI in 437 patients with stage III colorectal cancer (CRC), and to develop a prognostic nomogram that might be a useful guide in patient management.

Material and Methods

Study population

A total of 437 patients with histologically confirmed stage III colorectal carcinoma (CRC) were enrolled in this retrospective study between July 2012 and June 2014 with follow-up to June 2019. There were 328 patients recruited from The First Affiliated Hospital of Wenzhou Medical University and 109 patients were recruited from The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University.

All patients underwent surgical resection and adjuvant chemotherapy with or without subsequent radiotherapy. Patients with recurrent disease or current treatment for other malignancy or those whose clinical status could not be determined were excluded from this study. Informed written consent was obtained from all study participants. The Ethics Committee of Wenzhou Medical University (LCKY2019-168) approved the study protocol.

Data collection

Patient medical records were reviewed to obtain demographic and clinical information. Data retrieved included the date of surgery, age, gender, a history of cancer in a first-degree relative, tumor location, histological tumor grade, tumor stage, and lymphovascular invasion (LVI). Tumor location was categorized as the proximal colon (from the cecum to the distal transverse colon), distal colon (from the splenic flexure to the sigmoid colon), and rectum (rectosigmoid junction and rectal ampulla). The tumor was staged according to the 8th edition of the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) system and histologically graded based on the 2010 World Health Organization (WHO) classification. LVI was histologically defined by the presence of tumor cells within endothelial cell lined lymphatic or vascular channels on light microscopy using tissue sections stained with hematoxylin and eosin (H&E) [8]. Also, three hematological parameters were recorded before surgery, including the lymphocyte count, serum albumin, and carcinoembryonic antigen (CEA) levels.

The prognostic nutritional index (PNI) was calculated as: $10 \times \text{serum albumin value (g/dl)} + 0.005 \times \text{lymphocyte count in peripheral blood (per mm}^3\text{)}$ [17].

Age of 65 years, CEA of 5 ng/ml, and PNI of 45 were the cut-off points that allowed classification of the patients into two groups. Follow-up information was obtained by telephone or physician interview.

Statistical analysis

Data were analyzed using R version 3.3.3 software (R Foundation for Statistical Computing, Vienna, Austria). The endpoint of this study was overall survival (OS), which was defined as the time from surgery to the date of last follow-up or death from any cause. Continuous variables were reported as medians and ranges, and categorical variables were described as frequencies and percentages. Comparison between groups was performed by the chi-squared test or Fisher's exact test, as appropriate. The survival rates were assessed using the Kaplan-Meier method and compared with the log-rank test. The Cox proportional hazard model was used to calculate the hazard ratio (HR) and 95% confidence interval (CI). Variables with $P < 0.1$

in the univariate analysis were entered into the multivariate Cox analysis with stepwise backward selection. A nomogram was then constructed based on these independent prognostic factors, and the predictive accuracy was determined using the concordance index (C-index) and calibration plot. Calibration was evaluated by the Hosmer-Lemeshow test, where $P > 0.05$ indicated good agreement. Bootstrap analysis with 1000 resamples were applied. A two-sided $P < 0.05$ was taken as statistically significant.

Results

Patient characteristics and the presence of lymphovascular invasion (LVI)

There were 437 patients with colorectal cancer (CRC) who were enrolled in this study, with 43.2% women and 56.8% men. The average age was 62 years (range, 21–91 years). There were 88 (20.1%) proximal colon cancers, 104 (23.8%) distal colon cancers, and 245 (56.1%) rectal cancers. All patients underwent radical surgery following adjuvant therapy. Postoperative staging showed that 11.9% of the cases were stage IIIA, 71.6% were stage IIIB, and 16.5% were stage IIIC. Lymphovascular invasion (LVI) was present in 19.7% (86/437) of the study population, which increased from 9.6% in stage IIIA CRC to 13.4% in stage IIIB CRC and 54.2% in stage IIIC CRC.

Table 1 shows that the presence of LVI was positively correlated with increased tumor grade ($P < 0.001$) and advanced tumor stage, including a higher T stage ($P = 0.001$), N stage ($P < 0.001$) and TNM stage ($P < 0.001$). Patients who were LVI(+) had significantly increased serum levels of carcinoembryonic antigen (CEA) ($P = 0.016$) and a significantly lower prognostic nutritional index (PNI) value ($P = 0.008$). No significant association was observed with age, gender, a history of cancer in a first-degree relative, or tumor location (all $P > 0.05$).

Association of LVI with survival in patients with CRC

Within the observation period, there were 197 (45.1%) deaths with a median follow-up time of 64 months. The 5-year overall survival (OS) rate for all patients were 58.1%. Univariate analysis showed that nine of the 11 clinical variables were associated with patient survival. Further multivariate analysis identified that age > 65 years, a negative first-degree family history of cancer, the pre-treatment CEA > 5 ng/ml, pre-treatment PNI ≤ 45 , high histological grade, advanced TNM stage and LVI(+) were independent poor prognostic factors (all $P < 0.05$) (Table 2).

Compared with LVI(-) group, the LVI(+) group showed a 1.748-fold increased risk of death (95% CI, 1.197–2.553; $P = 0.004$) and a significantly lower 5-year OS rate (67.5% vs. 41.0%

(log-rank $P < 0.001$) (Figure 1). Also, the 5-year OS rates of patients with stage IIIA, stage IIIB, and stage IIIC CRC were 74.6%, 64.8%, and 41.0%, respectively, with a significant difference between the groups (log-rank $P < 0.001$). Subgroup analysis showed that the 5-year OS rate of patients with IIIA-B/LVI(+) was significantly lower than that of patients with IIIA-B/LVI(-) (44.9% vs. 70.5%) (log-rank $P = 0.002$). No significant difference was found between the IIIA-B/LVI(+) and IIIC subgroups (log-rank $P = 0.352$) (Figure 2).

Nomogram based on LVI

A prognostic nomogram was constructed based on the seven independent prognostic indicators from the multivariate Cox analysis, including LVI. The total scores were used to calculate the 5-year survival rate (Figure 3). The calibration plot showed that the nomogram was well-calibrated, with no significant difference between the predicted probability and actual observation, using the Hosmer-Lemeshow test ($P = 0.990$) (Figure 4). Comparison of the predictive accuracy for 5-year OS was made between the nomogram and TNM staging classification. After internal validation using 1000 bootstrap resamples, the C-index was 0.742 (95% CI, 0.699–0.784) and 0.593 (95% CI, 0.558–0.629), respectively, which showed a significant difference ($P < 0.001$). We also compared the discriminatory ability between the nomograms with and without LVI and found the C-index increased from 0.715 to 0.742 when LVI was included.

Discussion

Tumor-node-metastasis (TNM) staging remains the most important prognostic indicator in patients with colorectal cancer (CRC). However, there is prognostic heterogeneity within patients with stage III CRC, which results in unsatisfactory predictive accuracy [6,18]. The main factor that contributes to cancer recurrence and prognosis is the systemic dissemination of tumor cells by metastasis [19]. Lymphovascular invasion (LVI) is a common histopathological finding in malignant tumors, including CRC. LVI is characterized by the ability of malignant cells to detach from the primary tumor mass and then penetrate the lymphatic or other vascular channels [20,21]. Therefore, the addition of the assessment of LVI to the current TNM staging system may be a more accurate indicator of patient prognosis in stage III CRC, which may allow planning for more effective use of adjuvant therapy and patient follow up.

In the present study, LVI was assessed by light microscopy and detected in 19.7% of the 437 patients with stage III CRC, which was comparable with previously reported findings [14,22]. Also, our data showed that the presence of LVI were significantly correlated with increased serum levels of carcinoembryonic antigen (CEA), lower prognostic nutritional index (PNI), increased

Table 1. Comparison of clinicopathological features of patients with colorectal cancer (CRC) with and without lymphovascular invasion (LVI).

Variable	Total (n=437)	LVI(+) (n=86)	LVI(-) (n=351)	P-value
Age (years)				
≤65	238 (54.5%)	47 (54.7%)	191 (54.4%)	1.000
>65	199 (45.5%)	39 (45.3%)	160 (45.6%)	
Gender				
Female	189 (43.2%)	36 (41.9%)	153 (43.6%)	0.809
Male	248 (56.8%)	50 (58.1%)	198 (56.4%)	
First-degree relative cancer history				
Negative	386 (88.3%)	71 (82.6%)	315 (89.7%)	0.089
Positive	51 (11.7%)	15 (17.4%)	36 (10.3%)	
Pre-treatment CEA level (ng/ml)				
≤5	210 (48.1%)	31 (36.0%)	179 (51.0%)	0.016
>5	227 (51.9%)	55 (64.0%)	172 (49.0%)	
Pre-treatment PNI value				
≤45	238 (54.5%)	58 (67.4%)	180 (51.3%)	0.008
>45	199 (45.5%)	28 (32.6%)	171 (48.7%)	
Tumor location				
Rectum	245 (56.1%)	49 (57.0%)	196 (55.8%)	0.519
Proximal colon	88 (20.1%)	20 (23.3%)	68 (19.4%)	
Distal colon	104 (23.8%)	17 (19.8%)	87 (24.8%)	
Histological grade				
Low/moderate	299 (68.4%)	38 (44.2%)	261 (74.4%)	<0.001
High/mucinous differentiation	138 (31.6%)	48 (55.8%)	90 (25.6%)	
T stage				
T1–T2	64 (14.6%)	9 (10.5%)	55 (15.7%)	0.001
T3	200 (45.8%)	28 (32.5%)	172 (49.0%)	
T4	173 (39.6%)	49 (57.0%)	124 (35.3%)	
N stage				
N1	323 (73.9%)	38 (44.2%)	285 (81.2%)	<0.001
N2	114 (26.1%)	48 (55.8%)	66 (18.8%)	
TNM stage				
IIIA	52 (11.9%)	5 (5.8%)	47 (13.4%)	<0.001
IIIB	313 (71.6%)	42 (48.8%)	271 (77.2%)	
IIIC	72 (16.5%)	39 (45.4%)	33 (9.4%)	

LVI – lymphovascular invasion; CEA – carcinoembryonic antigen; PNI – prognostic nutritional index; TNM – tumor-node-metastasis.

Table 2. Univariate and multivariate Cox regression analysis for overall survival (OS).

Variable*	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (years)				
≤65	1 (Reference)		1 (Reference)	
>65	1.939 (1.425–2.637)	<0.001	1.969 (1.437–2.699)	<0.001
Gender				
Female	1 (Reference)			
Male	1.239 (0.909–1.689)	0.174		
First-degree relative cancer history				
Negative	1 (Reference)		1 (Reference)	
Positive	0.487 (0.271–0.877)	0.016	0.482 (0.265–0.878)	0.017
Pre-treatment CEA level (ng/ml)				
≤5	1 (Reference)		1 (Reference)	
>5	1.517 (1.116–2.062)	0.008	1.425 (1.044–1.945)	0.026
Pre-treatment PNI value				
≤45	1 (Reference)		1 (Reference)	
>45	0.492 (0.356–0.678)	<0.001	0.568 (0.409–0.790)	0.001
Tumor location				
Rectum	1 (Reference)			
Proximal colon	1.105 (0.752–1.623)	0.611		
Distal colon	0.978 (0.670–1.426)	0.907		
Histological grade				
Low/moderate	1 (Reference)		1 (Reference)	
High/mucinous differentiation	1.724 (1.265–2.348)	0.001	1.446 (1.042–2.007)	0.027
T stage				
T1–T2	1 (Reference)			
T3	1.231 (0.739–2.052)	0.424		
T4	2.075 (1.259–3.419)	0.004		
N stage				
N1	1 (Reference)			
N2	1.579 (1.136–2.196)	0.007		
TNM stage				
IIIA	1 (Reference)		1 (Reference)	
IIIB	1.611 (1.015–2.708)	0.049	1.076 (0.612–1.894)	0.799
IIIC	3.477 (1.886–6.411)	<0.001	2.059 (1.085–3.908)	0.027
LVI				
Negative	1 (Reference)		1 (Reference)	
Positive	2.286 (1.627–3.210)	<0.001	1.748 (1.197–2.553)	0.004

LVI – lymphovascular invasion; CEA – carcinoembryonic antigen; PNI – prognostic nutritional index; TNM – tumor-node-metastasis; HR – hazard ratio; CI – confidence interval.

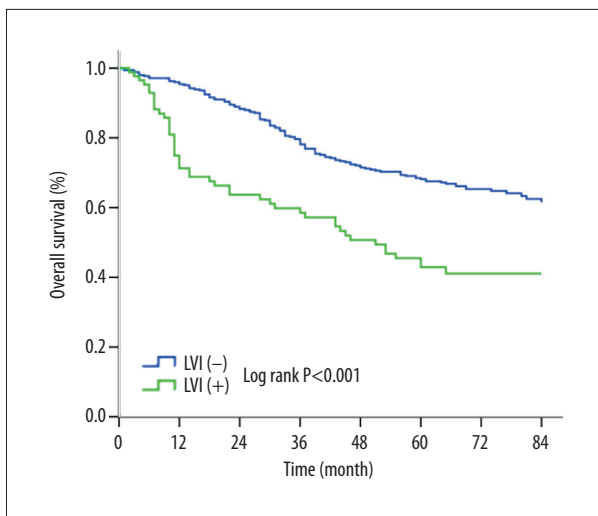


Figure 1. Overall survival (OS) of patients with and without lymphovascular invasion (LVI).

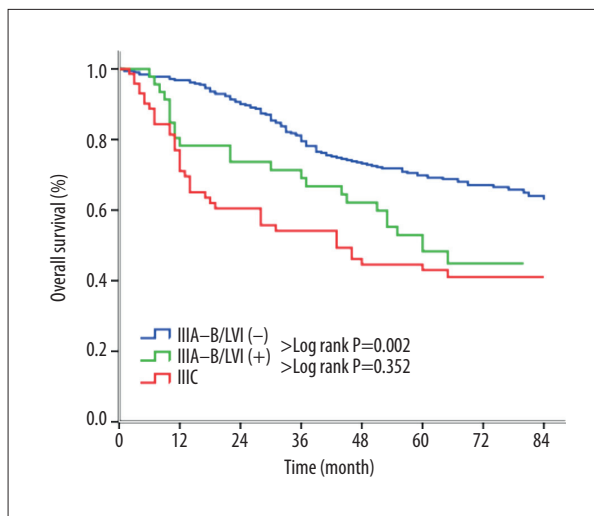


Figure 2. Overall survival (OS) of patients with lymphovascular invasion (LVI), stage IIIA-B/LVI(-), stage IIIA-B/LVI(+), and stage IIIC.

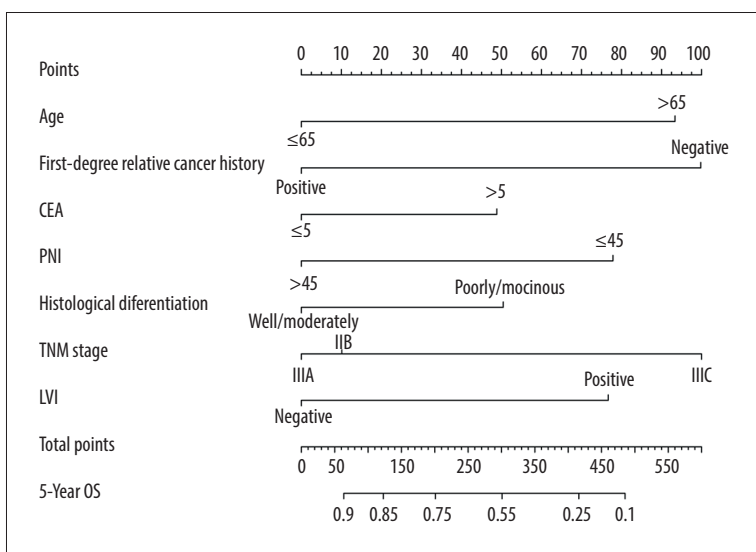


Figure 3. Nomogram for predicting 5-year overall survival (OS) of patients with stage III colorectal cancer (CRC). OS – overall survival; CEA – carcinoembryonic antigen; PNI – prognostic nutritional index; TNM – tumor-node-metastasis; LVI – lymphovascular invasion.

histological tumor grade, and advanced tumor stage, including both the depth of invasion and spread to regional lymph nodes, supporting its important role in more aggressive biological behavior in CRC [23,24]. Recent studies have shown that the process of LVI can be regulated and promoted by lymphangiogenic growth factors. The growth factors include the glycoproteins vascular endothelial growth factor-C (VEGF-C) and VEGF-D, and chemokines may also contribute by attracting tumor cells towards lymphovascular vessels [25]. Lymphangiogenic factors can be released not only by the tumor cells but also by activated stromal and immune cells associated with tumors [26]. Increased understanding of these mechanisms might improve future therapeutic strategies to inhibit metastatic spread of cancer and prolong the survival of patients with CRC. Further studies are required on the mechanisms of LVI in CRC.

Consistent with previous studies in other cancers [10–12], our results showed that LVI was an independent negative prognostic factor in stage III CRC, with a hazard ratio (HR) of 1.748. Also, patients with stage IIIC CRC had a similar prognosis as patients with stage IIIA-B CRC if they had LVI, which indicated that TNM staging lost its prognostic significance in patients with stage IIIA-B/LVI(+) or stage IIIC CRC. A previously published study by Lee et al. [12] also reported that no significant difference in overall survival (OS) or recurrence-free survival (RFS) between patients with gastric cancer with T1-3N0/LVI(+) and T1-3N1. Given the sample size in our study, further large-scale studies are required to confirm this finding.

In the present study, we constructed a prognostic nomogram based on the results of multivariate Cox analysis. In addition

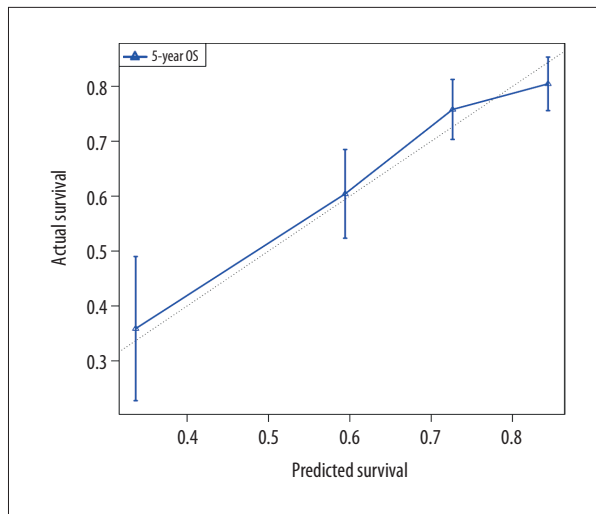


Figure 4. Calibration plot of the nomogram for predicting the 5-year overall survival (OS). Hosmer-Lemeshow test, $P=0.990$. OS – overall survival.

to LVI, six common clinical variables were incorporated into the prognostic nomogram, including age, a history of cancer in a first-degree relative, serum CEA, the PNI, histological grade, and TNM stage, which have all been previously reported to be associated with cancer prognosis [17,27,28]. Compared with TNM staging alone, this nomogram showed a significantly higher predictive accuracy. The nomograms with and without LVI were compared to demonstrate the improvement of incorporating LVI on the prediction of patient survival, which showed that inclusion of LVI improved the C-index from 0.715 to 0.742. Highly accurate, well-calibrated, and discriminatory

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nomograms, may provide clinicians with a more precise method of patient prognosis in CRC to improve treatment decisions and treatment planning for each patient [29].

To the best of our knowledge, this is the first study that has focused specifically on the prognostic value of LVI in patients with stage III CRC. However, the results should be interpreted in the context of several study limitations. This was a retrospective study, and patient selection bias was inevitable. We attempted to minimize selection bias by enrolling consecutive patients who met the pre-specified inclusion criteria. Also, other potential prognostic indicators were not included in this study, such as molecular markers, and could not be adjusted as confounding effects. This study was performed at two hospitals at a single center, which might have introduced reporting bias and limits the findings to a specific study population. Therefore, validation of the study findings using multicenter studies with larger sample size is needed.

Conclusions

The findings from this study showed that the presence of lymphovascular invasion (LVI) was a significant prognostic indicator in patients with stage III colorectal cancer (CRC). A prognostic nomogram was developed that might provide an individualized prediction of prognosis as a guide to patient management.

Conflict of interest

None.

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