

## Lymphovascular invasion in rectal cancer following neoadjuvant radiotherapy: A retrospective cohort study

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### Abstract

**AIM:** To investigate the meaning of lymphovascular invasion (LVI) in rectal cancer after neoadjuvant radiotherapy.

**METHODS:** A total of 325 patients who underwent radical resection using total mesorectal excision (TME) from January 2000 to January 2005 in Beijing cancer hospital were included retrospectively, divided into a preoperative radiotherapy (PRT) group and a control group, according to whether or not they underwent preoperative radiation. Histological assessments of tumor specimens were made and the correlation of LVI and prognosis were evaluated by univariate and multivariate analysis.

**RESULTS:** The occurrence of LVI in the PRT and control groups was 21.4% and 26.1% respectively. In the control group, LVI was significantly associated with histological differentiation and pathologic TNM stage, whereas these associations were not observed in the PRT group. LVI was closely correlated to disease progression and 5-year overall survival (OS) in both groups. Among the patients with disease progression, LVI positive patients in the PRT group had a significantly longer median disease-free period (22.5 mo vs 11.5 mo,  $P = 0.023$ ) and overall survival time

(42.5 mo vs 26.5 mo,  $P = 0.035$ ) compared to those in the control group, despite the fact that no significant difference in 5-year OS rate was observed (54.4% vs 48.3%,  $P = 0.137$ ). Multivariate analysis showed the distance of tumor from the anal verge, pretreatment serum carcinoembryonic antigen level, pathologic TNM stage and LVI were the major factors affecting OS.

**CONCLUSION:** Neoadjuvant radiotherapy does not reduce LVI significantly; however, the prognostic meaning of LVI has changed. Patients with LVI may benefit from neoadjuvant radiotherapy.

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**Key words:** Lymphovascular invasion; Rectal cancer; Neoadjuvant radiotherapy; Total mesorectal excision; Pathology; Prognosis

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### INTRODUCTION

Currently, the treatment of rectal cancer has stepped into a new era of multimodality therapy<sup>[1]</sup>. Neoadjuvant therapy, including preoperative radiotherapy (RT) or radiochemotherapy (RCT), has become a standard regimen for locally advanced rectal cancer<sup>[2]</sup>. There have been growing concerns in recent years about the pathologic evaluation of rectal cancer after neoadjuvant therapy, since the pathologic stage (ypTNM) is now significantly different from its original meaning<sup>[3,4]</sup>. Due to neoadjuvant therapy, a considerable number of patients experience tumor regression or downstaging, and a minority of patients experience a complete pathologic response (CPR)<sup>[5]</sup>. However, the problem is that there are still few favorable pathological indicators to reflect and predict the clinical consequence of rectal cancer after neoadjuvant therapy.

Lymphovascular invasion (LVI) has been widely acknowledged as a useful independent pathological indicator for predicting prognosis, as well as a good index to guide postoperative therapy in colorectal cancer. Patients with LVI usually have a higher chance of disease progression and poorer prognosis<sup>[6-8]</sup>. The NCCN (National Comprehensive Cancer Network) guideline recommended LVI as a high risk factor of disease advance for colon cancer after surgery<sup>[9]</sup>. For rectal cancer, LVI is also a crucial high risk factor for recurrence post transanal local resection<sup>[10]</sup>. However, it has still to be established whether LVI has the same predictive meaning and clinical significance for patients with rectal cancer undergoing preoperative radiation. Does the biological behavior of the cancer cells involved in the blood or lymphatic vessels change after radiation? This is the issue we focus on.

## MATERIALS AND METHODS

### Clinical data

Data from all consecutive patients with resectable rectal carcinoma treated in our hospital from January 2000 to January 2005 were collected retrospectively. Among them, we selected eligible patients according to the following criteria: (1) resectable rectal cancer 12 cm or less from the anal verge; (2) evaluated by endorectal ultrasound (ERUS) or magnetic resonance imaging (MRI) before treatment; (3) histologically identified primary carcinoma of the rectum; (4) no clinical evidence of distant metastases; (5) transabdominal radical resection based on the principle of total mesorectal excision (TME); (6) R0 resection.

Exclusion criteria were: (1) patients who underwent concurrent RCT; (2) patients with CPR after neoadjuvant radiotherapy; (3) patients with synchronous tumors or history of other malignant tumors within 5 years; (4) familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal carcinoma (HNPCC); (5) died of complications or other non-cancer related reasons.

In total 325 patients were included (Table 1). All included patients were divided into a preoperative radiotherapy (PRT) group ( $n = 103$ ) and a control group ( $n = 222$ ), according to whether or not they underwent neoadjuvant radiation. There was no statistically significant difference in the gender, age, tumor location, preoperative serum carcinoembryonic antigen (CEA) level, pathologic stage and LVI between the two groups (Table 1). The conditions of histological differentiation and pretreatment stage (by imaging) were better in the control group, which implied a potentially better prognosis of patients in the control group. But the following multivariate analysis demonstrated that these two factors were not the major factors affecting the clinical consequence, so we considered the patients in the two groups to be comparable. Furthermore, we believe it more reasonable to investigate the influence of LVI on clinical consequence under the same pathologic stage rather than the same pretreatment stage in the

Table 1 Demographic details of patients and tumor characteristics

	PRT <i>n</i> = 103	Control <i>n</i> = 222	<i>P</i> value
Sex			
Male	62	130	0.875
Female	41	92	
Age (yr) <sup>1</sup>	56 (52-61)	57 (53-64)	0.232
Distance of tumor from anal verge			
< 5 cm	35	53	0.061
5-12 cm	68	169	
Surgery			
APR	27	55	0.564
LAR	71	161	
CR	5	6	
Preoperative serum CEA level			
Normal	52	121	0.691
Abnormal	35	65	
Unknown	16	36	
Pretreatment TNM stage (%)			
I (T1-2 N0)	0 (0)	43 (19.4)	< 0.01
II A (T3 N0)	25 (24.3)	63 (28.4)	
II B (T4 N0)	4 (3.9)	5 (2.3)	
III A (T1-2 N1)	3 (2.9)	8 (3.6)	
III B (T3-4 N1)	33 (32.0)	43 (19.4)	
III C (AnyT N2)	38 (36.9)	60 (27.0)	
Pathologic TNM stage (%)			
I (T1-2 N0)	35 (34.0)	55 (24.8)	0.377
II A (T3 N0)	27 (26.2)	62 (27.9)	
II B (T4 N0)	1 (1.0)	1 (5.0)	
III A (T1-2 N1)	6 (5.8)	7 (3.2)	
III B (T3-4 N1)	18 (17.5)	45 (20.3)	
III C (AnyT N2)	16 (15.5)	52 (23.4)	
Histological differentiation (%)			
High	4 (3.9)	29 (13.1)	< 0.01
Moderate	70 (68.0)	156 (70.3)	
Poor	24 (23.3)	27 (12.2)	
Mucinous and signet	5 (4.9)	10 (4.5)	
LVI (%)			
Present	22 (21.4)	58 (26.1)	0.353
Absent	81 (78.6)	164 (73.9)	
Disease progression (%)			
Local Recurrence	6 (5.8)	32 (14.4)	0.025
Distant Metastasis	22 (21.4)	47 (21.2)	0.969
Death	24 (23.3)	66 (29.7)	0.228

<sup>1</sup>Values are medians (interquartile ranges). PRT: Preoperative radiotherapy.

two groups, so it was inevitable that the pretreatment stage of the PRT group was later because of tumor-downstaging after neoadjuvant radiotherapy.

### Pretreatment evaluation

All included patients underwent ERUS or MRI to evaluate the tumor size, invasion depth and extent, and the involvement of pararectal lymph nodes. In total 280 patients (86.2%) were evaluated by ERUS and 45 patients (13.8%) by MRI. Serum CEA was measured and abdominal CT and chest radiography were also routinely performed before treatment.

### Neoadjuvant therapy

We adopted neoadjuvant radiation with a total dose of 30 Gy (30 Gy/10 fractions), recommended by the

Chinese Anti-Cancer Association (CACA)<sup>[11]</sup>, based on some high-level clinical evidence<sup>[12,13]</sup>. Surgery was performed 2-3 weeks after full dose radiation.

### **Surgery**

All included patients underwent radical resection strictly according to the principles of TME<sup>[14]</sup>, regardless of abdominoperineal resection (APR) or low anterior resection (LAR). In addition, 11 patients underwent combined resection (CR) involving partial or total resections of some pelvic organs; all resection margins were identified as negative by pathologic examination.

### **Pathologic evaluation**

All slides of postoperative specimens were stained with hematoxylin and eosin (HE) and were reviewed by one senior pathologist who was blind to the prognosis of patients. The available criteria for the histologic diagnosis of LVI included<sup>[15]</sup>: presence of tumor cells within lymphatic or vascular space; identification of endothelial cells lining the space; the presence of an elastic lamina surrounding the tumor; and attachment of tumor cells to the vascular wall.

Tumor regression was mostly in the form of fibro-inflammatory changes or necrosis replacing neoplastic glands. Mucin pools were also seen sometimes, as another type of degeneration post radiotherapy. Comparison between the pathologic T stage and clinical T stage (by imaging) was made to identify tumor-downstaging in the PRT group<sup>[16]</sup>.

### **Postoperative therapy**

All patients in the PRT group were given postoperative chemotherapy for 6-8 cycles, using the standard regimens based on 5-FU or capecitabine, such as FOLFOX, CapeOX or capecitabine alone. In the control group, only patients with lymph node involvement or with the pathologic T3 or T4 stage were given adjuvant chemotherapy, with the same regimens as were used in the PRT group. Notably, 95% (76/80) of LVI positive patients underwent postoperative adjuvant chemotherapy, while only 4/58 LVI positive patients in the control group were not given postoperative chemotherapy due to the early TNM stage. Therefore, our results concerning prognosis involved the influence of postoperative chemotherapy.

For the patients with disease progression, three patients with resectable liver metastasis underwent partial liver resection and one patient with solitary lung metastasis underwent lung wedge resection. Two patients with resectable local recurrence underwent APR. Other patients with disease progression underwent systematic chemotherapy or support therapy.

### **Follow up**

Patients were followed at 3 mo intervals for the first two years and then at 6 mo intervals for the next three years. Evaluations consisted of physical examination, serum CEA, a complete blood count, and blood chemical analysis. Proctoscopy, abdominal ultrasonography, CT of

the abdomen and pelvis, and chest radiography were also routinely used every 6-12 mo, according to the NCCN guideline<sup>[9]</sup>. Follow up time ranged from 3 to 96 mo, and the median follow up time was 72 mo. We chose 5 years as a time terminal for evaluation of outcomes. The follow up rate was 86.5% (281/325), with 44 inconclusive patients.

### **Statistical analysis**

Statistical analyses were performed using SPSS 16.0 statistical software. The categorical variables were analyzed with the Pearson chi-squared or Fisher's exact test, as appropriate. The Kaplan-Meier survival curve was used to estimate the proportion of patients surviving or remaining disease-free at each time interval. Disease free survival (DFS) and overall survival (OS) curves were compared between groups using the Wilcoxon's test for time-to-event parameters. Disease-free periods were compared using a log-rank test. Multivariate Cox proportional hazards regression was used to analyze the major factors affecting overall survival. All statistical tests were 2-sided, and the level of significance set at 5%.

## **RESULTS**

### **The distribution and related factors of LVI**

The overall positive rate of LVI was 24.6% (80/325), with no statistically significant difference in distribution between the PRT and control groups (21.4% and 26.1% respectively,  $P = 0.353$ ) (Table 1). Within the PRT group, LVI was not significantly reduced in patients with tumor-downstaging (Table 2). In the control group, LVI strongly correlated with histological differentiation and pathologic T and N stages whereas these associations were not observed in the PRT group (Table 2).

### **The influence of LVI to clinical consequence**

To get credible statistical results, we merged the local recurrence and distant metastasis to disease progression data because of the limited number of local recurrences in the PRT group. LVI was significantly associated with disease progression in both groups (Table 3): 38.5%-43.2% of LVI positive patients developed recurrence or metastasis, whereas 15.6%-17.6% of LVI negative patients progressed finally ( $P < 0.05$ ). LVI was also strongly correlated with DFS and OS: patients with LVI had lower rates of 5 year DFS and OS in both groups (Table 3, Figure 1A and B).

However, the influence of LVI on prognosis was not same in the two groups (Figure 2A and B). Among the patients with disease progression, LVI positive patients in the PRT group had a longer disease-free period and survival time than those in the control group (the median DFS time was 22.5 and 11.5 mo respectively,  $P = 0.023$ ; the median OS time was 42.5 and 26.5 mo respectively,  $P = 0.035$ ). There were no statistically significant differences in the 5 year DFS rate (54.4% *vs* 44.8%,  $P = 0.099$ ) and OS rate (54.4% *vs* 48.3%,  $P = 0.137$ ). For LVI negative patients, neither DFS nor OS showed significant differences between the two groups

Table 2 The relevance of LVI and pathologic factors					
		LVI		Positive rate (%)	P value
		Present	Absent		
PRT ypT	T1	1	5	16.7	0.592
	T2	6	31	16.2	
	T3	14	44	24.1	
	T4	1	1	50.0	
ypN	N0	9	54	14.3	0.057
	N1	9	15	37.5	
	N2	4	12	25.0	
Histological differentiation	High	1	3	25.0	0.998
	Moderate	15	55	21.4	
	Poor	5	19	20.8	
	Mucinous and signet	1	4	21.0	
Downstaging	Yes	7	34	17.1	0.388
	No	15	47	24.2	
Control pT	T1	0	11	0	< 0.01
	T2	8	50	13.8	
	T3	49	98	33.3	
	T4	1	5	16.7	
pN	N0	15	103	12.7	< 0.01
	N1	15	37	28.8	
	N2	28	24	53.8	
Histological differentiation	High	7	22	24.1	0.015
	Moderate	34	122	21.8	
	Poor	11	16	40.7	
	Mucinous and signet	6	4	60.0	

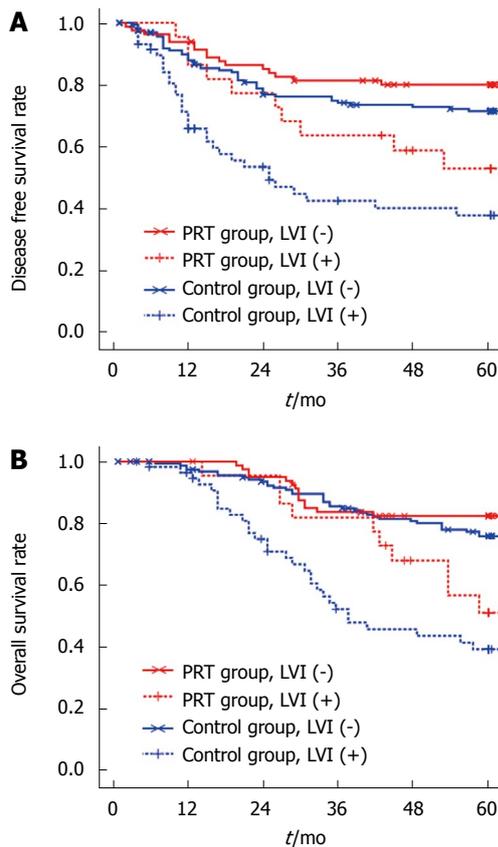


Figure 1 K-M plot of DFS (A) and OS (B) for LVI between the two groups. The LVI negative patients had a significantly higher 5-year DFS rate ( $P < 0.05$ ) and OS rate ( $P < 0.01$ ) than LVI positive patients in both groups ( $P < 0.05$ ).

(the median DFS time was 14.0 and 15.0 mo respectively,  $P = 0.980$ ; the median OS time was 29.8 and 34.0 mo respectively,  $P = 0.247$ ).

**The factors influencing prognosis**

Multivariate analysis demonstrated that the distance of the tumor from the anal verge, pretreatment serum CEA level, pathologic TNM stage and LVI were the major factors affecting the 5 year OS (Table 4) whereas gender, age, neoadjuvant radiotherapy, tumor-downstaging, histological differentiation and pretreatment stage were not significantly associated with long-term survival.

**DISCUSSION**

Strictly speaking, lymphovascular invasion implies involvement of vascular and lymphatic vessels. However, histological distinction between larger lymphatic and smaller venous channels may not always be possible. Therefore, the term lymphovascular is used to refer to any or all of these structures<sup>[17]</sup>. LVI could be recognized clearly on HE-stained slides, despite the fact that some reports indicate using immunohistochemical stains with CD31 and D2-40 may improve the diagnosis<sup>[18,19]</sup>.

LVI has long been recognized as a favorable independent pathological indicator for predicting the prognosis of patients with colorectal cancer, which is usually associated with a poor consequence<sup>[6-8]</sup>. However, most studies concerning LVI were made in patients not given neoadjuvant therapy. Although several authors mentioned

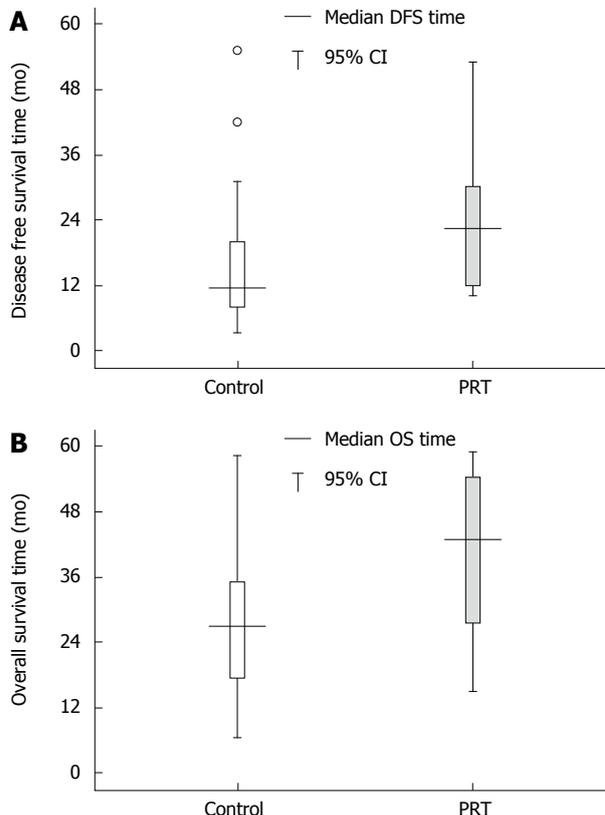


Figure 2 Stem and Leaf plot for comparison of DFS time (A) and OS time (B) for LVI positive patients between the two groups. Among the 42 (A) and 40 (B) LVI positive patients with disease progression, the patients in the PRT group had a longer median DFS time ( $P < 0.05$ ).

**Table 3 The association of LVI and prognosis**

		PRT		P	Control		P
		Present	Absent		Present	Absent	
Disease progression (%)	Yes	10 (38.5)	16 (61.5)	0.014	32 (43.2)	42 (56.8)	< 0.01
	No	12 (15.6)	65 (84.4)		26 (17.6)	122 (82.4)	
5-year DFS rate	%	54.5 (12/22)	80.2 (65/81)	0.020	44.8 (26/58)	73.2 (120/164)	< 0.01
5-year OS rate	%	54.5 (12/22)	82.7 (67/81)	< 0.010	48.3 (28/58)	78.0 (128/164)	< 0.01

**Table 4 Multivariate analysis of OS by COX model (backward elimination)**

Variable	Hazard ratio	95% CI	P value
Distance from anal verge	0.540	0.346-0.845	< 0.01
Serum CEA level	1.334	1.027-1.732	0.031
pTNM stage	1.347	1.198-1.514	< 0.01
LVI	0.450	0.292-0.692	< 0.01

in their studies that LVI correlated with awful prognosis in patients undergoing neoadjuvant therapy<sup>[20,21]</sup>, there have been very few studies that specifically investigated the difference in LVI after neoadjuvant therapy compared to LVI after surgery directly. Our study was undertaken to illuminate such differences.

Despite the fact that preoperative radiation may lead to some histological changes, such as tumor regression or even a complete response<sup>[3,22,23]</sup>, our study found that preoperative radiotherapy alone did not significantly reduce the occurrence of LVI: at the same pathologic stage, the positive rates of LVI in the two groups were not significantly different (21.4% *vs* 26.1%, *P* = 0.353). Even for patients with tumor-downstaging, who could be considered sensitive to radiation, the LVI positive rate was not significantly reduced which implied the killing effect of X-rays to cancer cells involved in the vessels may be limited. Perhaps the addition of concurrent chemotherapy may work on LVI more effectively, which will be our continuing work based on the results from the current study. However, the influence of radiochemotherapy on LVI is more complicated, so we chose radiotherapy alone as the first step.

However, the behavior of tumor cells involved in the vessels somewhat changed after radiation. Our results demonstrated that the disease progression of patients with LVI in the PRT group was significantly delayed, which suggested that the aggression of those tumor cells in the blood or lymphatic vessels may have been significantly weakened by X-ray, though they were not completely eliminated. Currently, it is generally believed that radiation can cause DNA damage and chromosome aberrations, leading to an abortion of cell mitosis and proliferation, as well as inducing cell apoptosis<sup>[24,25]</sup>. Therefore, we inferred that the tumor cells involved in the vessels may be partly killed or inhibited by neoadjuvant radiotherapy so that disease progression was delayed and the survival time was prolonged.

At present, the issue about who would benefit from

neoadjuvant therapy is still being debated. Some authors believe that only patients with a good response to radiation could benefit from neoadjuvant therapy<sup>[3,16,26,27]</sup>. Our study demonstrated patients with LVI could gain a prolonged disease-free period and survival time from neoadjuvant radiotherapy. Thus, LVI positive patients may also benefit from neoadjuvant radiotherapy in a sense.

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## COMMENTS

### Background

Currently, neoadjuvant therapy has become a standard protocol for locally advanced rectal cancer. Although some studies have demonstrated that lymphovascular invasion (LVI) after neoadjuvant therapy is a high risk factor for recurrence, there have been very few studies that specially addressed the meaning of LVI after neoadjuvant therapy. The study is designed to compare the difference in LVI between patients undergoing neoadjuvant radiotherapy and those undergoing surgery directly.

### Research frontiers

The pathologic evaluation of the rectal cancer after neoadjuvant therapy is the hotspot most oncologists and scholars focus on.

### Innovations and breakthroughs

The study specifically addresses the meaning of LVI in rectal cancer after neoadjuvant radiotherapy; furthermore, they are first to report the difference in LVI after neoadjuvant radiotherapy.

### Applications

They demonstrated that patients with LVI could gain a prolonged disease-free period and survival time from neoadjuvant radiotherapy which provides significant evidence and reference for the application of neoadjuvant radiotherapy.

### Terminology

Lymphovascular invasion: a morphological concept which refers to the presence of tumor cells within lymphatic or vascular vessels, representing strong aggression of tumor cells.

### Peer review

The manuscript, reported by Du *et al.*, investigates, in a retrospective cohort study, the difference in LVI after neoadjuvant therapy compared to LVI after surgery directly. This manuscript is well presented and gives us an interesting result. The patient's cohort is important.

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