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Prognostic Value of Lymph Node Ratio in Locally Advanced Rectal Cancer Patients After Preoperative Chemoradiotherapy Followed by Total Mesorectal Excision

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Abstract: Although the absolute number of positive lymph nodes (LNs) has been established as 1 of the most important prognostic factors in rectal cancers, many researchers have proposed that the lymph node ratio (LNR) may have better predicted outcomes. We conducted a retrospective study to compare the predictive ability of LNR and ypN category in rectal cancer.

A total of 264 locally advanced rectal cancer (LARC) patients who underwent preoperative chemoradiotherapy (CRT) followed by total mesorectal excision (TME) between 2005 and 2012 were reviewed. All patients were categorized into 3 groups or patients with metastatic LNs were categorized into 2 groups according to the LNR. The prognostic effect on overall survival (OS) and disease-free survival (DFS) was evaluated.

With a median follow-up of 45 months, the OS and DFS were 68.4% and 59.3% for the entire cohort, respectively. The respective 5-year OS and DFS rates for the 3 groups (LNR = 0, $0 < \text{LNR} \leq 0.20$, and $0.20 < \text{LNR} \leq 1.0$) were as follows: 83.2%, 72.6%, and 49.4% ($P < 0.001$) and 79.5%, 57.3%, and 33.5% ($P < 0.001$), respectively. Multivariate analysis revealed that LNR and differentiation, but not the number of positive LNs, had independent prognostic value for OS (hazard ratio [HR] = 2.328, 95% confidence interval [CI]: 1.850–4.526, $P < 0.001$) and DFS (HR = 3.004, 95% CI: 1.616–5.980, $P < 0.001$). As for patients with positive LNs, the respective 5-year OS and DFS rates for the 2 groups ($0 < \text{LNR} \leq 0.20$, and $0.20 < \text{LNR} \leq 1.0$) were 72.6% and 49.4% ($P < 0.001$) and 57.3% and 33.5% ($P < 0.001$), respectively. Multivariate analysis revealed that only LNR was an independent factor for OS (HR = 3.214, 95% CI: 1.726–5.986, $P < 0.001$) and DFS (HR = 4.230, 95% CI: 1.825–6.458, $P < 0.001$). Subgroups analysis demonstrated that the ypN category had no impact on survival whereas increased LNR was a significantly prognostic indicator for worse survival in the LNs < 12 subgroup.

LNR is an independent prognostic factor in LARC patients treated with preoperative CRT followed by TME. It may be a better independent staging method than the number of metastatic LNs when <12 LNs are harvested after preoperative CRT.

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Abbreviations: AJCC = American Joint Committee on Cancer, APR = abdominoperineal resection, CA 19-9 = cancer antigen 19-9, CEA = carcinoembryonic antigen, CI = confidence interval, CRT = chemoradiotherapy, CT = computed tomography, DFS = disease-free survival, HR = hazard ratio, ISR = intersphincteric, LAR = low anterior resection, LARC = locally advanced rectal cancer, LN = lymph node, LNR = lymph node ratio, OS = overall survival, TME = total mesorectal excision, TNM = tumor-node-metastasis, ypN = number of positive lymph nodes, ypT = depth of invasion.

INTRODUCTION

In the past 3 decades, the incidence of colorectal cancer is increasing rapidly with economic development and improvement in the average life span in China. Epidemiological statistics in 2012 showed that the number of new cases increased from the 6th most numerous to the 2nd most numerous since the 1970s and the average annual growth rate was >4%.[1] Although the percentage of rectal cancer in colorectal cancer has decreased in recent years, rectal cancers still occur much more frequently in China than in the Western world. Moreover, more than half of the rectal cancer patients have been diagnosed with locally advanced rectal cancer (LARC, T3 or T4 tumors and/or positive lymph nodes [LNs]) at the time of diagnosis, which poses many challenges to the colorectal surgeons in China.

Preoperative chemoradiotherapy (CRT) followed by total mesorectal excision (TME) and postoperative chemotherapy are now considered the standard strategy for treating patients with LARC.[2,3] The number of positive LNs is 1 of the most important factors in determining the prognosis of patients with colorectal cancer,[4] and is also a major determinant of the need for adjuvant therapy.[5,6] The number of positive LNs is not only significantly associated with the severity of disease but also relies on the total number of retrieved LNs.[4] This method

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disregards the number of harvested LNs, which was identified to be an important prognostic factor in colorectal cancer.[7–9] Many researchers found that preoperative CRT decreases the number of harvested LNs in rectal cancer patients[10–12]; <12 retrieved LNs were frequently found in patients with LARC treated with preoperative CRT, as based on the American Joint Committee on Cancer (AJCC) recommendations.[4] For this reason, lymph node ratio (LNR = number of metastatic LNs/number of harvested LNs), which incorporates both the number of positive LNs and of total harvested LNs in 1 value has been regarded as a key prognostic factor in rectal cancer and may serve as a better prognostic indicator in patients with LARC who received preoperative CRT.[4]

In fact, some studies have shown that LNR is a more accurate stratification system than the current staging system based on the number of metastatic LNs for colorectal cancer.[13–15] However, few studies have focused on the prognostic value of LNR in patients with LARC who received preoperative CRT followed by TME and postoperative chemotherapy. Therefore, the purpose of this study is to evaluate the impact of LNR on prognosis in patients with LARC treated with preoperative CRT followed by TME and postoperative chemotherapy, and compare its applicable value with ypN category (the absolute number of LNs) in patients with different total number of harvested LNs.

PATIENTS AND METHODS

Patients

The records of all patients who underwent preoperative CRT followed by TME and postoperative chemotherapy were collected from the colorectal cancer database of the First Affiliated Hospital of Wenzhou Medical University, the Third People's Hospital of Hangzhou City, and Changhai Hospital of the Second Military Medical University from January 2005 to December 2012. This study was approved by the institutional review board of the First Affiliated Hospital of Wenzhou Medical University, the Third People's Hospital of Hangzhou City, and Changhai Hospital of the Second Military Medical University.

The study inclusion criteria were the histological examination confirmed adenocarcinoma of the middle-third and lower-third rectum (12 cm above the anal verge), the location of the tumor was defined as the distance between the distal margin of the tumor and the anal verge, measured by digital examination and rigid proctoscopy; patients were diagnosed with T3 or T4 tumor with/or stages N1 or N2 and M0 according to preoperative evaluation; patients received preoperative long-course CRT and postoperative chemotherapy.

The study exclusion criteria were synchronous distant metastases to lung, liver, bones, or other organs (85 patients); patients who refused to receive preoperative CRT or postoperative chemotherapy (67 patients); patients treated with a short-radiation course (38 patients); circumferential resection margin (CRM) of rectal specimen was positive.

Based on the including and excluding criterion, a total of 264 patients were eligible for this study (including 121 patients with metastatic LNs). Parameters such as age, gender, tumor location, type of surgery, preoperative carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA 19-9) levels, ypTNM, depth of invasion, number of metastatic LNs, number of harvested LNs, perineural invasion, and lymphovascular invasion were evaluated. Recurrences and distant metastases were also documented.

Preoperative Evaluation and Chemoradiotherapy

All patients received digital rectal examination, colonoscopy, computed tomography (CT) of the abdominal and pelvic, and chest radiography. In 85 (32.2%) patients transrectal ultrasonography was done, and pelvic magnetic resonance imaging was performed in 176 (66.7%) patients for preoperative staging of rectal cancer. The clinical stages were scored according to AJCC stage classification system (7th edition).

The patients with T3/T4 stage and/or metastatic LNs were received preoperative CRT. A total dose of 50 Gy was delivered to the whole pelvic in daily fractions of 2.0 Gy. During the period of radiotherapy, all patients received concomitant chemotherapy. Preoperative chemotherapy began on the 1st day of pelvic radiotherapy. Capecitabine at 1600 mg/m^2 per day was given concurrently with radiotherapy for 25 days.

Surgery and Postoperative Chemotherapy

All patients received curative resection followed by preoperative CRT, and colorectal specialists performed TME for all the rectal cancers. High ligation of the inferior mesenteric artery proximal to the origin of the left colonic artery was performed in all patients as a routine procedure at the Department of Colorectal Surgery. The interval between preoperative CRT and surgery ranged from 4 to 8 weeks. Surgical procedures included abdominoperineal resection (APR), Hartmann operation, low anterior resection (LAR), and intersphincteric (ISR) resection with or without proximal diverting ileostomy. All had clear longitudinal and CRMs.

About 2 to 4 weeks after surgery, all patients received postoperative adjuvant chemotherapy. Two different chemotherapy regimens were used: mFOLFOX6 and Capox.

Pathologic Analysis

After gross examination by the surgeon in the operating room, each specimen was sent to pathology. The samples were fixed in 10% formalin solution for 24 h, and LNs were dissected through manual palpation. All rectal specimens were examined by at least 2 pathologists specializing in colorectal cancer. The pathologic stage of the tumor was determined according to the AJCC (7th edition) staging system and marked as ypTNM because of the preoperative CRT. Tumors were assessed for histological type, ypN stage, ypT stage, tumor differentiation, vascular invasion, lymphatic invasion, and perineural invasion. The number of examined LNs, the number of determined positive LNs, lymphovascular invasion, and the LNR were calculated for each patient.

Follow-Up

Patients were evaluated at the hospital or contacted through telephone or mail, every 3 months within the first 2 years after operation, every 6 months for the next 3 years, and annually thereafter in according with the guidelines issued by the National Health and Family Planning Commission of the People's Republic of China.

Patients received a series of follow-up evaluations that included complete blood count, liver function test, serum CEA and CA-199 measurements, and digital rectal examination. Abdominal and pelvic CT and chest X-ray were performed every 6 months after surgery. An annual colonoscopy was also performed.

The median follow-up time was 45 months (range 6–89). Overall survival (OS) was defined as time between date of surgery and date of death or last follow-up. Disease-free

TABLE 1. Clinicopathological Characteristics of 264 Patients Enrolled in This Study

Variables	Characteristics	No. of Cases (%)
Sex	Male	152 (57.6)
	Female	112 (42.4)
Age, y	<40	25 (9.5)
	40–60	125 (47.5)
	≥60	114 (43.0)
Tumor location, cm	0–5	102 (38.6)
	5–12	162 (61.4)
Preoperative CEA, ng/mL	≤5	158 (60.0)
	>5	106 (40.0)
Type of operation	Sphincter-preserving operation	192 (72.7)
	APR	60 (22.7)
	Hartmann operation	12 (4.5)
Stage (ypTNM)	yp0–I	44 (16.8)
	ypII	99 (37.5)
	ypIII	121 (45.7)
Depth of invasion (ypT)	ypT0	28 (10.6)
	ypT1–pT2	112 (42.4)
	ypT3–pT4	124 (47.0)
Number of metastatic LN (ypN)	ypN0	113 (42.8)
	ypN1	97 (36.7)
	ypN2	54 (20.5)
Number of harvested LN	<12	157 (59.4)
	≥12	107 (40.6)
LNR	0	113 (42.8)
	0.00–0.20	102 (38.6)
	0.21–1.00	49 (18.6)
Lymphovascular invasion	No	201 (76.1)
	Yes	63 (23.9)
Perineural invasion	No	231 (90.6)
	Yes	33 (12.5)
Differentiation	Well	33 (12.5)
	Moderate	205 (77.7)
	Poor	26 (9.8)

APR = abdominoperineal resection, CEA = carcinoembryonic antigen, LN = lymph node, LNR = lymph node ratio, TNM = tumor-node-metastasis, ypN = number of positive lymph nodes, ypT = depth of invasion.

survival (DFS) was defined as time between date of surgery and date of local recurrence or metastasis.

Statistical Analysis

Continuous variables are expressed as mean (±standard deviation). Data showed a normal distribution and were therefore analyzed using the Student *t* test. Chi-squared tests or Fisher exact tests were used to compare proportions when appropriate. Multivariate Cox proportional hazards' models were used to calculate adjusted hazard ratios (HRs) and 95% confidence intervals (CIs). Multivariate analyses were performed with Cox regression model using the forward conditional method, and the HRs were calculated. A multivariate model was performed using forward selection with the selected covariates ($P < 0.10$ on univariate analysis). P values <0.05 are considered statistically significant.

Patients were stratified into 3 groups: LNR = 0, 0 < LNR ≤ 0.20, and 0.20 < LNR ≤ 1.0. LNR in the whole population include ypTNM I and II patients with an LNR of 0. Because the median LNR for the entire cohort is 0, we refer to other literatures[4,7,16,17] and use the mean LNR (0.20) of stage ypIII patients as a cutoff. Survival analyses were performed using the Kaplan–Meier method, and the log-rank test was used to compare variables.

RESULTS

Patient Population and Clinicopathological Characteristics

The study enrolled 264 patients with local rectal cancer who underwent preoperative CRT followed by TME and postoperative chemotherapy. The detailed clinicopathological data of all patients are listed in Table 1. The median age of patients was 56 years (range 26–70). One hundred ninety-two (72.7%) patients underwent sphincter-preserving operation (including LAR and ISR), 60 (22.7%) patients underwent APR, and 12 (4.5%) patients underwent Hartmann operation. The median LNR was 0.2 (range 0.0–1.0), and the median follow-up was 45 months (range 6–121). The patients were divided into 2 groups according to LNR of patient (LNR ≤ 0.2 and >0.2). The age, sex, tumor location, preoperative CEA, type of operation, lymphovascular invasion, perineural invasion, and differentiation of the 2 groups were no significant difference.

TABLE 2. Univariate Analysis of Prognostic Factors for Overall Survival and Disease-Free Survival in Whole Population

		OS, mo		DFS, mo	
Variables	**Characteristics**	**Mean ± SD**	***P* Value**	**Mean ± SD**	***P* Value**
Preoperative CEA	≤5	76.7 ± 2.4	0.038	74.5 ± 5.6	0.042
	>5	57.9 ± 3.9		50.9 ± 1.9	
yp stage	yp0–I	82.3 ± 2.4	0.004	81.5 ± 2.6	0.001
	ypII	65.7 ± 4.0		61.7 ± 3.1	
	ypIII	52.2 ± 3.1		49.2 ± 4.1	
ypT stage	ypT0–pT2	79.4 ± 2.7	0.025	73.6 ± 4.7	0.034
	ypT3–pT4	61.9 ± 3.1		58.3 ± 4.6	
ypN stage	ypN0	80.6 ± 3.5	<0.001	78.3 ± 3.2	<0.001
	ypN1	62.5 ± 3.4		57.1 ± 3.1	
	ypN2	57.2 ± 5.9		52.3 ± 3.6	
LNR	0	81.0 ± 3.1	<0.001	79.4 ± 2.5	<0.001
	0.00–0.20	65.9 ± 3.6		64.3 ± 2.6	
	0.20–1.00	52.4 ± 5.0		51.2 ± 4.3	
Differentiation	Well	81.6 ± 3.8	<0.001	80.1 ± 1.8	<0.001
	Moderate	72.3 ± 3.1		68.3 ± 1.5	
	Poor	51.0 ± 5.4		48.1 ± 3.4	

CEA = carcinoembryonic antigen, DFS = disease-free survival, LNR = lymph node ratio, OS = overall survival, SD = standard deviation, ypN = number of positive lymph nodes, ypT = depth of invasion.

Prognostic Factors for OS and DFS in Whole Population With Preoperative CRT

After a median follow-up of 45 months, a total of 78 (29.5%) patients died, which included 45 stage ypIII patients. The 5-year OS and DFS rates for the entire population were 68.4% and 59.3%, respectively. The OS and DFS rates were 83.2% (median OS: 82.3 ± 2.4 months), 62.1% (median OS: 65.7 ± 4.0 months), 50.6% (median OS: 52.2 ± 3.1 months; $P < 0.001$) and 79.5% (median DFS: 81.5 ± 2.6 months), 54.3% (median DFS: 61.7 ± 3.1 months), 45.5% (median DFS: 49.2 ± 4.1 months; $P < 0.001$) for stages yp0–I, ypII, and ypIII, respectively (Table 2). For the whole population, the clinicopathological variables, including sex, age, ypTNM stage, tumor location, preoperative CEA, type of operation, depth of invasion (ypT), number of positive LNs (pN), number of harvested LNs, LNR, lymphovascular invasion, and perineural invasion and differentiation were tested by using the univariate analysis and Cox proportional hazard model.

Univariate analysis showed that preoperative CEA, ypTNM stage, depth of invasion, number of positive LNs, LNR, and differentiation were significantly related to OS and DFS (Table 2). The 5-year OS and DFS rates were 83.2% (median OS: 81.0 ± 3.1 months), 72.6% (median OS: 65.9 ± 3.6 months), 49.4% (median OS: 52.4 ± 5 months; $P < 0.001$) and 79.5% (median DFS: 79.4 ± 2.5 months), 57.3% median DFS: 64.3 ± 2.6 months), 33.5% (median DFS: 51.2 ± 4.3 months; $P < 0.001$) for the LNR = 0, 0 < LNR ≤ 0.20, and 0.20 < LNR ≤ 1.0 group in the whole population (Table 2; Figure 1A and B), respectively. According to the univariate analysis, preoperative CEA, yp stage, ypT, pN, LNR, and differentiation were selected for the multivariate analysis, and as potential risk factors, number of harvested LNs was also included. Multivariate analysis revealed that LNR and differentiation were independent risk factors for OS and DFS: the HR was 2.328 (95% CI: 1.850–4.526, $P < 0.001$), 3.004 (95% CI: 1.616–5.986, $P < 0.001$) and 1.865 (95% CI: 1.462–2.816, $P = 0.028$), 1.986 (95% CI: 1.701–5.597, $P = 0.034$), respectively, for LNR and differentiation (Table 3).

Prognostic Factors for OS and DFS in Node-Positive Rectal Cancer Patients

Patients with node-positive rectal cancer were stratified into 2 groups: 0 < LNR ≤ 0.20 and 0.20 < LNR ≤ 1.0, and the

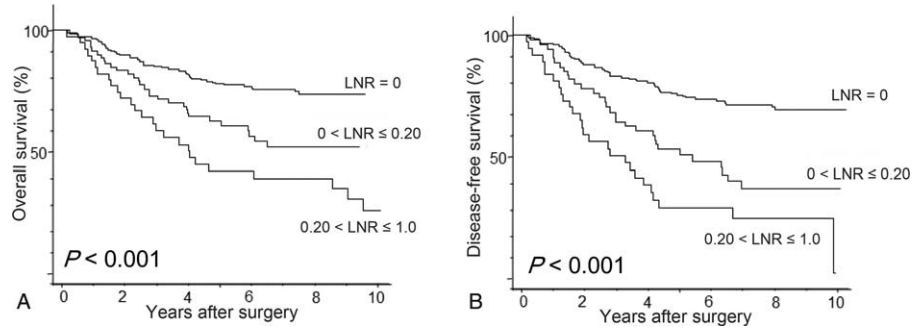

FIGURE 1. Kaplan–Meier curves for OS (A) and DFS (B) according to LNR. DFS = disease-free survival, LNR = lymph node ratio, OS = overall survival.

TABLE 3. Multivariate Analysis of Prognostic Factors for Overall Survival and Disease-Free Survival in Whole Population

	OS		DFS	
Variables	**HR (95% CI)**	***P* Value**	**HR (95% CI)**	***P* Value**
LNR	2.328 (1.850–4.526)	<0.001	3.004 (1.616–5.986)	<0.001
Differentiation	1.865 (1.462–2.816)	0.028	1.986 (1.701–5.597)	0.034

CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, LNR = lymph node ratio, OS = overall survival.

OS and DFS rates were 72.6% (median OS: 65.9 ± 3.6 months), 49.4% (median OS: 52.4 ± 5.0 months; $P < 0.001$) and 57.3% (median DFS: 64.3 ± 2.6 months), 33.5% (median DFS: 51.2 ± 4.3 months; $P < 0.001$) for the 2 groups, respectively (Table 4). All clinicopathological variables used for the univariate analysis of the whole population were also applied for the node-positive rectal cancer patients using the same method. ypT stage, ypN stage, LNR, and differentiation had prognostic significance for OS and DFS (Table 4). According to the results from the univariate analysis, depth of invasion, number of positive LNs, LNR, and differentiation were applied for the multivariate analysis. Results demonstrated that only LNR had prognostic significance for OS and DFS in patients with node-positive rectal cancer and the HR was 3.214 (95% CI: 1.726–5.986, $P < 0.001$) and 4.230 (95% CI: 1.825–6.458, $P < 0.001$; Table 5).

Subgroup Analysis for OS and DFS According to Number of Retrieved LNs

In the whole population, the median number of LNs retrieved was 11 (range 1–32) and median metastatic LN numbers were 2 (range 1–10). Less than 12 LNs were harvested in 157 (59.5%) patients. The whole population was stratified into 2 groups according to the number of retrieved LNs: LNs < 12 and LNs ≥ 12. OS was 69.5% and 74.2% ($P = 0.62$) and DFS was 64.6% and 67.8% ($P = 0.87$) in LNs < 12 versus LNs ≥ 12 as shown in Figure 2A and B, respectively. No difference was found in 5-year OS and DFS rates when cases are stratified into 2 groups by the number of harvested LNs (<12 and ≥12; Figure 2).

As LNR was an independent prognostic factor for OS and DFS in whole population with preoperative CRT and node-positive rectal cancer patients as found above, we further evaluated the prognostic value of LNR in different subgroups according to the number of harvested LNs and compared its applicable value with ypN category according to the number of harvested LNs in the same subgroups (Table 6).

Increased LNR was a significantly prognostic indicator for worse OS and DFS in patients with <12 harvested LNs (Figure 3), and as well as in those ≥12 harvested LNs ($P < 0.001$; Table 6). In order to find out whether ypN had the same prognostic impact in different groups according to the number of harvested LNs, we performed a subgroup analysis of ypN in patients with LNs < 12 and LNs ≥ 12, respectively. In the LNs ≥ 12 subgroup, increased ypN was significantly associated with reduced OS and DFS rates ($P = 0.002$ and 0.006, respectively). In the LNs < 12 subgroup, increased ypN also associated with worse OS and DFS, but the difference did not reach statistically significant (Figure 4; Table 6; OS was 65.4% and 46.3%, $P = 0.138$, and DFS was 40.5% and 34.5%, $P = 0.455$). In other words, OS and DFS differed according to LNR when <12 and ≥12 LNs were harvested. In contrast, the ypN category did not significantly impact OS and DFS when the number of harvested LN was <12 (Figure 4).

DISCUSSION

The primary value of a staging system for cancer is its capability to provide an accurate prediction of outcome and to

TABLE 4. Univariate Analysis of Prognostic Factors for Overall Survival and Disease-Free Survival in Node-Positive Rectal Cancer

		OS, mo		DFS, mo	
Variables	**Characteristics**	**Mean ± SD**	***P* Value**	**Mean ± SD**	***P* Value**
Preoperative CEA	≤5	71.5 ± 4.7	0.076	64.5 ± 5.6	0.052
	>5	64.4 ± 5.0		50.9 ± 1.9	
ypT stage	ypT0–pT2	71.4 ± 2.8	0.035	63.6 ± 4.7	0.017
	ypT3–pT4	60.5 ± 6.1		49.3 ± 4.6	
ypN stage	ypN1	62.5 ± 3.4	0.045	57.1 ± 3.1	0.045
	ypN2	57.2 ± 5.9		52.3 ± 3.6	
LNR	≤0.20	65.9 ± 3.6	<0.001	64.3 ± 2.6	<0.001
	>0.20	52.4 ± 5.0		51.2 ± 4.3	
Differentiation	Well	75.2 ± 3.7	<0.001	66.1 ± 1.1	<0.001
	Moderate	65.3 ± 5.4		56.3 ± 8.2	
	Poor	36.9 ± 3.1		30.4 ± 1.6	

CEA = carcinoembryonic antigen, DFS = disease-free survival, LNR = lymph node ratio, OS = overall survival, SD = standard deviation, ypN = number of positive lymph nodes, ypT = depth of invasion.

TABLE 5. Multivariate Analysis of Prognostic Factors for Overall Survival and Disease-Free Survival in Node-Positive Rectal Cancer

	OS		DFS	
Variables	**HR (95% CI)**	***P* Value**	**HR (95% CI)**	***P* Value**
LNR	3.214 (1.726–5.986)	<0.001	4.230 (1.825–6.458)	<0.001
Differentiation	1.635 (0.659–4.059)	0.289	1.286 (0.581–2.830)	0.541

CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, LNR = lymph node ratio, OS = overall survival.

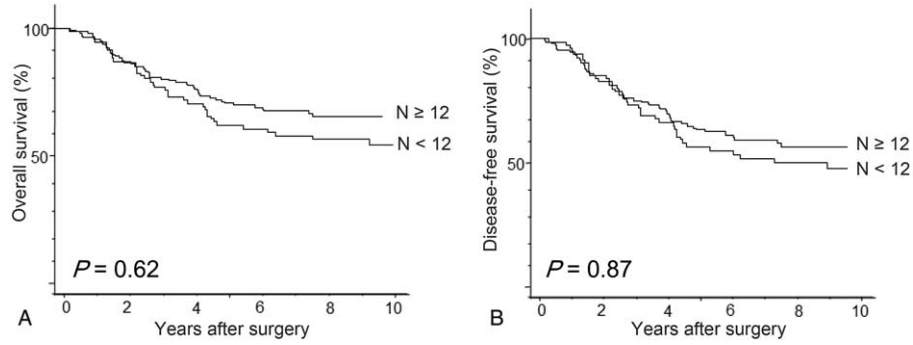

FIGURE 2. Kaplan–Meier curves of OS (A) and DFS (B) with respect to <12 versus ≥12 lymph nodes retrieved. DFS = disease-free survival, OS = overall survival.

guide postoperative treatment decisions and follow-up.[18–20] Currently, the tumor-node-metastasis (TNM) classification system is the most popularly used staging system for colorectal cancer.[19,20] This system divides patients into different prognostic groups based on primary tumor thickness, the presence of LN spread, and distant metastasis.[19,20] Increase in the number of positive LNs retrieved and the higher the stage indicate a worse prognosis. Therefore, the number of dissected LNs plays a key role in determining the pN category and adjuvant chemotherapy.[21] The current guidelines established by AJCC recommend that a minimum of 12 LNs should be harvested and examined in the resected specimen for accurate staging.[22] However, not all resected specimens of colorectal carcinoma meet the recommendation as the number of LNs retrieved depend on many factors including patient's age, gender, other diseases, tumor size and localization, differentiation grade, lymphoid reaction, and preoperative CRT.[23,24] In order to overcome the limitations of the current nodal staging system

TABLE 6. Subgroup Analysis of ypN and LNR for Overall Survival and Disease-Free Survival According to Number of LNs

		OS			DFS	
Variables	**n**	**%**	**HR (95% CI)**	***P* Value**	**%**	**HR (95% CI)**	***P* Value**
LNs < 12	69			<0.001			<0.001
LNR ≤ 0.2	41	84.6	1 (reference)		76.5	1 (reference)	
LNR > 0.2	28	40.5	4.286 (2.181–12.250)		31.8	4.886 (2.081–8.842)	
LNs ≥ 12	82			<0.001			<0.001
LNR ≤ 0.2	61	88.4	1 (reference)		73.2	1 (reference)	
LNR > 0.2	21	45.6	10.068 (3.256–38.704)		32.4	12.226 (4.381–32.325)	
LNs < 12	69			0.138			0.455
ypN1 (1–3)	45	65.4	1 (reference)		40.5	1 (reference)	
ypN2 (>4)	24	46.3	1.905 (0.759–4.129)		34.5	1.542 (0.785–3.830)	
LNs ≥ 12	82			0.002			0.006
ypN1 (1–3)	52	73,6	1 (reference)		65.5	1 (reference)	
ypN2 (>4)	30	45.3	9.686 (1.581–70.330)		30.8	16.286 (2.588–103.53)	

CI = confidence interval, DFS = disease-free survival, HR = hazard ratio, LN = lymph node, LNR = lymph node ratio, OS = overall survival, ypN = number of positive lymph nodes.

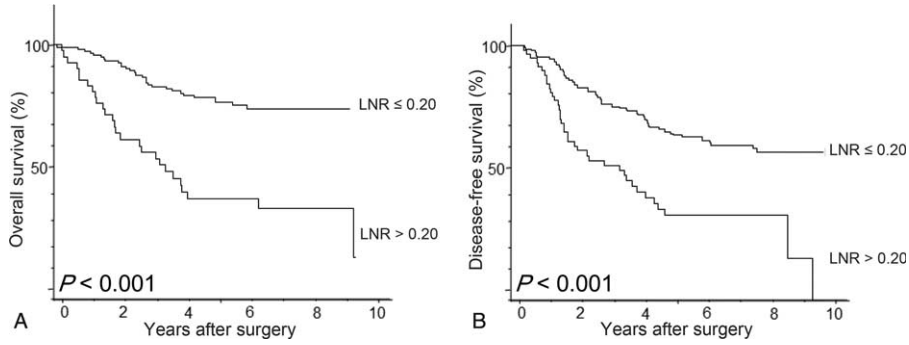

FIGURE 3. Kaplan–Meier curves of OS (A) and DFS (B) with respect to LNR ≤ 0.2 versus >0.2 in LNs < 12 subgroup. DFS = disease-free survival, LN = lymph node, LNR = lymph node ratio, OS = overall survival.

and provide a more accurate prediction of patient prognosis and proper staging, LNR was considered as a better prognostic indicator and an alternative staging method in patients with colorectal cancer.[20,25,26] LNR as a prognostic factor in colon cancer was first reported by Berger et al in 2005.[27] The authors analyzed data of stages II and III colon cancer patients with adjuvant chemotherapy from the Intergroup trial 0089 and suggested that LNR is an important prognostic factor for colon cancer.[27]

In 2008, Peng et al[28] was the first to report the prognostic significance of LNR in node positive rectal cancer. On quartiles, LNR was stratified into 3 groups in their study: <0.14, 0.14–0.49, and 0.5–1.0. The 5-year DFS rate was 72.57%, 58.54%, and 34.75% ($P = 0.0001$) and the 5-year OS rate was 72.19%, 61.92%, and 38.47% ($P = 0.002$) in the 3 groups, respectively. Till now, the prognostic value of LNR has been proven in previous studies of patients with rectal cancer treated with postoperative CRT.[29,30] The status of LNs, however, is absolutely different in rectal cancer patients with or without preoperative CRT. Preoperative CRT followed by TME and postoperative chemotherapy is the current standard treatment for the patients with T3 or T4 tumors and/or positive LNs according to the guidelines issued by the National Health and Family Planning Commission of the People's Republic of China. However, the preoperative CRT may affect LNs harvest in resected specimens. Some studies have shown that the total number of retrieved LNs is often fewer than 12 in patients who undergo preoperative CRT despite of vigorous pathological and surgical standards because of LN atrophy, fibrosis, and lymphocyte depletion caused by radiotherapy and/or chemotherapy. In this cohort study, only 40.5% (107/264) patients had more than 12 LNs retrieved, which was consistent with the results reported in other studies.[10–12,31,32]

In 2011, Klos et al[8] was the first to study the prognostic value of LNR after preoperative chemoradiation and rectal cancer surgery, which demonstrated LNR could provide a better independent staging method than absolute positive LN counts. However, the evidence is still limited in rectal cancer with preoperative CRT. In our cohort study, a series of 264 LARC patients were enrolled (121 stage node-positive patients included), the 5-year OS and DFS rates were 68.4% and 59.3%, respectively. For the entire population, multivariate analysis revealed that LNR was the most important prognostic factor (HR = 2.328) superior to differentiation (HR = 1.865) for OS. However, for node-positive patients, LNR was the only independent prognostic factor for OS in multivariate Cox proportional hazards models, with the HR 3.214. In the current widely used TNM stage system, the number or positive LNs was thought to be an important prognostic factor. In our study, the number of metastatic LNs did affect the 5-year OS and DFS rates in the univariate analysis (Table 2). However, it lost its efficacy after adjusting for preoperative CEA, depth of invasion, LNR, and differentiation both for the entire population and for node-positive patients. We compare staging by LNR with staging method by absolute number of positive LNs in different groups according to total number of harvested LNs. The results

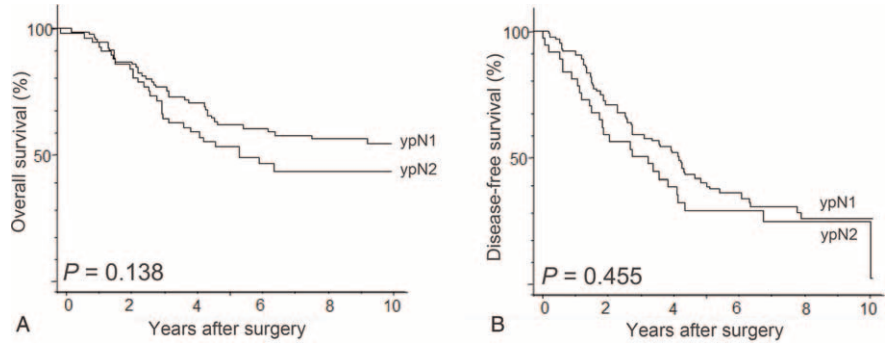

FIGURE 4. Kaplan–Meier curves of OS (A) and DFS (B) with respect to ypN1 versus ypN2 in LNs < 12 subgroup. DFS = disease-free survival, LN = lymph node, OS = overall survival, ypN = number of positive lymph nodes.

showed that LNR could stratify patient prognosis more accurately than the number of metastatic LNs when <12 LNs are harvested after preoperative CRT, which was seldom illustrated in the previous studies.

Although the results of this study have shown the potential value of LNR in patients with LARC who underwent preoperative CRT followed by TME, there are several obvious flaws with this retrospective analysis. First, many patients refused to undergo the preoperative CRT because of poor compliance and the sample size from a single center was small, so the clinical data collected from 3 different centers may have introduced a selection bias. Second, patients were operated on by different surgeons from different hospitals, thus substantially influenced the ypN and the results. To decrease the influence of surgeons, we excluded patients with positive CRM from the study. Next, pathologic analysis was not standardized and performed by a variety of pathologists. At last, although the prognostic value of LNR was widely proven, the lack of best cutoffs restricted its clinical use severely. Previous studies used different methodologies to determine the best cutoffs, some based on quartiles, some based on mean or median of LNR. In the present study, we refer to other literatures[4,16] and used the mean LNR (0.20) of stage ypIII patients as a cutoff because the median LNR for the entire cohort is 0. Currently, different studies proposed different cutoff values. It is obvious that further larger-scale comprehensive studies are warranted to determine the LNR cut-off values for rectal cancer with preoperative CRT. In addition, besides the clinical/pathological features, tumor-infiltrating T-cell subsets and molecular alterations including microsatellite instability (MSI), CpG island methylator phenotype, BRAF mutation, PIK3CA mutation, and tumor LINE-1 hypomethylation in colorectal cancer were also reported to be associated with clinical outcome.[33] MSI is associated with several characteristic clinicopathological features, including increased number of LNs in the specimen.[34,35] Lymphocytic infiltration has been associated with many of these molecular variables and a favorable prognosis in colorectal cancer.[33,36] Therefore, the correlations between the clinical/pathological and molecular features and their impact on the survivals of colorectal cancer patients must be evaluated more clearly in the future study.

In conclusion, we showed that LNR, other than the number of positive nodes or number of harvested LNs, was an independent prognostic factor for locally rectal cancer patients who underwent preoperative CRT. The total number of LNs retrieved did not affect the prognostic value of the LNR, even if the total was <12. However, large-scale studies are needed to determine the best cutoffs for LNR.

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