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Research article

Examined lymph node numbers influence prognosis in rectal cancer treated with neoadjuvant therapy

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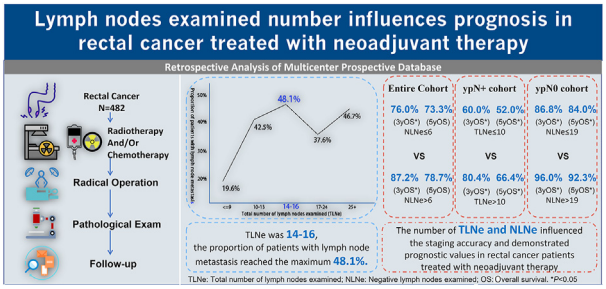
HIGHLIGHTS

- Many lymph nodes should be examined in patients with rectal cancer treated with neoadjuvant therapy.
- The number of lymph nodes examined might have prognostic value.
- Study conclusions should be drawn based on a sufficient or entire number of lymph nodes examined.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The number of lymph nodes examined (LNe) is often insufficient in patients with rectal cancer (RC) treated with neoadjuvant therapy; however, its prognostic value remains controversial. Thus, we retrospectively explored whether LNe had an influence on staging and prognosis and investigated whether there was a cut-off value for better prognosis in patients with RC treated with neoadjuvant therapy.
Methods: Data were collected from seven prospective hospital databases in China from July 2002 to May 2018. Binary logistic regression models were used to predict lymph node metastasis. The cut-off value for LNe was

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determined using X-tile 3.6.1. Survival outcomes and risk factors were analyzed using the log-rank test and Cox regression model.
Results: A total of 482 patients were included, of whom 459 had complete overall survival (OS) information. Using the percentile method, the total number of lymph nodes examined (TLNe) was 14–16 (40th–60th percentile), and the proportion of patients with lymph node metastasis reached a maximum of 48.1%. Cox multivariate analysis showed that the odds ratio (OR) remained the highest when TLNe was 14–16 (OR = 3.379, $P = 0.003$). The 3-year and 5-year OS were 85.4% and 77.8%, respectively. Negative lymph nodes examined (NLNe) of ≤6 was an independent risk factor for 3-year and 5-year OS (3-year OS 71.1% *vs.* 85.9%, $P = 0.004$; 5-year OS 66.3% *vs.* 74.3%, $P = 0.035$). Subgroup analysis for patients with ypN + showed that higher 3-year and 5-year OS were achieved when the TLNe was >10, 78.8% *vs.* 54.0% ($P = 0.005$), and 60.8% *vs.* 36.0% ($P = 0.012$), respectively. Patients with ypN_0M_0 had a higher 5-year OS when the TLNe was >19 ($P = 0.055$).
Conclusion: The TLNe and NLNe influenced the staging accuracy and demonstrated prognostic value in patients with RC treated with neoadjuvant therapy.

Introduction

Colorectal cancer is one of the most common malignancies in China, leading to approximately 180,000 deaths in 2014.[1] The number of metastatic lymph nodes is regarded as an essential factor in staging and is based on a sufficient number of lymph nodes examined (LNe).[2,3] According to the Eighth American Joint Committee on Cancer (AJCC) guideline, at least 12 lymph nodes should be examined for colorectal cancer.[4] Neoadjuvant therapy has become the standard treatment for locally advanced rectal cancer (RC), but only about 20% of these patients have sufficient LNe.[2,3,5] Both radiation and chemotherapy decrease the examined number of lymph nodes, which might influence staging accuracy, as positive lymph nodes might be missed.[6–8] Moreover, the postoperative chemotherapy decision was made based on staging, and whether ypN0 disease requires further treatment remains controversial. Therefore, the number of LNe might have an impact on prognosis, but the results of existing studies differed significantly.[9–12] Moreover, all the lymph nodes in the specimens should be examined, but it is quite difficult in patients with RC who underwent treatment with neoadjuvant therapy, and there is no unified standard with regard to the minimum number of LNe in these patients; therefore, the prognostic value of the number of LNe has been unclear to date.[8,13–19]

Hence, this study aimed to explore whether the number of LNe had an influence on staging and prognosis and to investigate whether there was a cut-off value for better prognosis in patients with RC treated with neoadjuvant therapy.

Methods

Patients

A total of 482 patients with RC treated with neoadjuvant therapy from seven prospective hospital databases in China from July 2002 to May 2018 were retrospectively reviewed. Perioperative evaluation was performed by the respective colorectal cancer multiple-disciplinary team (MDT) in each hospital. All patients underwent curative surgery after neoadjuvant therapy. The patients received treatments based on the recommendations of the National Comprehensive Cancer Network (NCCN) guidelines, and the final decision was made based on the patient's choice. Patients with a history of other types of cancers, those who required emergency surgical procedures, and those who had distant metastasis during the first clinical visit were excluded from the study.

Data collection

Data regarding patient demographics, clinicopathological characteristics, and treatment were retrospectively collected from the prospective databases. Follow-up evaluation assessments were conducted every 3–6 months until the third postoperative year and every year thereafter for at least 5 years. Overall survival (OS) was calculated from the time of surgery until the last follow-up visit or date of death.

Statistical analysis

Statistical analyses were performed using the SPSS 24.0 software (IBM Corp., Armonk, NY, USA). Categorical variables were presented as frequencies with percentages and assessed using the chi-square or Fisher's exact test, as appropriate. Multivariable binary logistic regression models were used to predict metastatic lymph nodes. The optimal cut-off points of total number of lymph nodes examined (TLNe), negative lymph nodes examined (NLNe), and positive lymph nodes examined (PLNe) were calculated and determined using X-tile 3.6.1 (*https://medicine.yale.edu/lab/rimm/research/software.aspx*). This identified the cut-off with minimum P values from log-rank χ^2 statistics for categorical lymph nodes in terms of survival.[20] Survival outcomes were analyzed using Kaplan–Meier method and compared by log-rank test. A Cox regression model was utilized to determine the risk factors for OS. Survival analyses were performed using R version 3.5.1 software (The R Foundation for Statistical Computing, Vienna, Austria) with "survival" and "survminer" packages. P values of <0.05 were considered to be significant.

Results

Patient characteristics

A total of 482 patients met the selection criteria, including 287 males and 195 females, with a median age of 58 (22–88) years. The median distance from the distal margin of the tumor to the anal verge was 5.0 cm (1.0–12.0). There were 24 patients with cT1-2 stage (5.0%), 406 patients with cT3 (84.2%), 51 patients with cT4 (10.6%), and one missing data. There were 89 patients with cN0 stage (18.5%), 392 patients with cN + stage (81.3%), and one missing data. Of all patients, 382 (79.2%) received 30 Gy (Gy)/10f preoperative radiation, 77 (16%) received ≥45 Gy radiation, and 23 (4.8%) received 25 Gy/5 f radiation. The downstaging rate was 4.8% during clinical evaluation and 55.2% during pathological evaluation.

A total of 324 patients (67.2%) underwent low anterior resection (LAR), 152 patients (31.5%) underwent abdominoperineal resection (APR), and six patients (1.3%) underwent the Hartmann procedure. There were 35 (7.3%), 24 (5.0%), 134 (27.8%), 261 (54.1%), and 28 (5.8%) patients with ypT stages 0, 1, 2, 3, and 4, respectively. There were 291 ypN0 patients (60.4%), 115 patients (23.9%) with 1–3 lymph node metastases (ypN1a-1b), and 69 patients (14.3%) with ≥4 lymph node metastases. The median number of metastatic lymph nodes was 2.0 (1–24). A total of 16 (3.3%) patients had tumor deposits, and seven of these had tumor deposits without lymph node metastasis (ypN_{1c}). There were 35 patients (7.3%) with positive circumferential resection margin (CRM). No complete clinical response (cCR) was observed in any patient, and a partial clinical response (pCR) ratio was observed in 6.4% (31 patients). There were 123 (25.5%) patients with stage ypI, 137 (28.4%) with stage ypII, and 184 (38.2%) with stage ypIII. Seven (1.5%) patients had distant metastases during neoadjuvant treatment,

two of whom had no lymph node metastasis, and five patients had lymph node metastasis.

Among the 392 cN + patients, 42.3% (166 patients) and 56.4% (221 patients) were with and without lymph node metastasis during pathological evaluation, respectively. Among 291 ypN0 patients, 75.6% (220) had clinical lymph node metastases. Among 191 patients who had lymph node metastases or tumor deposits, 9.9% (19 patients) did not have lymph node metastases during the initial clinical evaluation. The median number of TLNe was 14 (0–61) in the entire cohort and 15 (5–61) in patients with lymph node metastases, and the median number of metastatic lymph nodes was 2 (range, 1–24). The other clinicopathological characteristics are shown in Table 1.

There were 459 patients with complete OS information, including 374 cN+, 174ypN + M_0, and 278 ypN$_0$M$_0$ patients. The median follow-up time was 64.3 months, and the 3-year and 5-year OS rates were 85.4% and 77.8%, respectively.

Correlation between lymph node metastasis and total number of lymph nodes examined

The median numbers of TLNe in ypN0 and ypN + patients were 13 and 15, respectively, showing significant differences (Mann–Whitney U test, $U = 22{,}680$, $Z = -3.420$, $P = 0.001$). Spearman's correlation analysis showed a slightly positive linear correlation between the number of metastatic lymph nodes and TLNe (rs = 0.173, $P < 0.001$).

Using the percentile method(20th, 40th, 60th, and 80th percentiles), TLNe was divided into five groups according to the percentiles (≤9, 10–13, 14–16, 17–24, and ≥25). The proportions of patients with metastatic lymph nodes were 19.6%, 42.5%, 48.1%, 37.6%, and 46.7%, respectively. In addition, the proportion was significantly increased in the group with 10–13 ($\chi^2 = 13.332$, $P < 0.001$) and 14–16 ($\chi^2 = 0.576$, $P = 0.448$) LNe.

Univariate and multivariate analysis of risk factors for lymph node metastasis

There were 191 ypN+ and 291 ypN0 patients. Univariate analysis showed that the carcinoembryonic antigen (CEA) level ($\chi^2 = 6.171$, $P = 0.013$), cN status ($\chi^2 = 15.377$, $P < 0.001$), radiotherapy method ($\chi^2 = 5.794$, $P = 0.055$), ycN status ($\chi^2 = 24.860$, $P < 0.001$), ypT stage ($\chi^2 = 58.332$, $P < 0.001$), TLNe ($\chi^2 = 22.680$, $P < 0.001$), tumor differentiation ($\chi^2 = 18.596$, $P < 0.001$), lymphovascular invasion (LVI) status ($\chi^2 = 25.251$, $P < 0.001$), and CRM status ($\chi^2 = 4.368$, $P = 0.037$) showed significant differences between the two groups [Table 1]. Binary logistic regression showed that 414 patients were finally included in the multivariate analysis (with no ypT0 patient), and ypT stage, TLNe, LVI status, and tumor differentiation were considered risk factors [Table 2]. When the TLNe was 14–16, then the OR was maximum (3.379 [95% CI 1.533–7.448, $P = 0.003$]).

Cut-off value calculated by X-tile

As shown in Table 3 and Figure 1, X-tile plots were constructed and identified six as the cut-off value for NLNe to divide the entire cohort; the cN + cohort was divided into low (>6) and high (≤6) risk subsets in terms of OS, and five as the cut-off value for NLNe to divide the ypN + cohort into low (>5) and high (≤5) risk subsets in terms of OS. The results showed significant differences between the low- and high-risk subsets in the three cohorts in 3-year OS (87.2% *vs.* 76.0%, 88.3% *vs.* 74.6%, 81.5% *vs.* 41.2%, $P = 0.003$, 0.001, and <0.001, respectively), and the cN+ and ypN + M_0 cohorts in 5-year OS (79.4% *vs.* 71.2%, 67.6% *vs.* 35.3%, $P = 0.040$, <0.001, respectively). A cut-off value of 10 for TLNe was identified to divide the ypN + M_0 cohort into low (>10) and high (≤10) risk subsets in terms of OS, in which the 3-year OS were 80.4% and 60.0% ($P = 0.007$), and the 5-year OS were 66.4% and 52.0% ($P = 0.030$), respectively. A cut-off value of 19 for TLNe was identified to

Table 1
Patient characteristics and univariate analysis of risk factors for lymph node metastasis.

Parameters	Entire cohort (n = 482)	ypN0 (n = 291)	ypN+ (n = 191)	χ^2 value	P value
Gender				1.181	0.277
Male	287 (59.5)	179 (61.5)	108 (56.5)		
Female	195 (40.5)	112 (38.5)	83 (43.5)		
Age (years)				1.868	0.172
<65	333 (69.1)	194 (66.7)	139 (72.8)		
≥65	148 (30.7)	96 (33.0)	52 (27.2)		
Missing	1 (0.2)	1 (0.3)	0		
CEA				6.171	0.013
Normal	312 (64.7)	201 (69.1)	111 (58.1)		
Abnormal	165 (34.2)	87 (29.9)	78 (40.8)		
Missing	5 (1.1)	3 (1.0)	2 (1.1)		
Dis to AV (cm)				1.048	0.653
≤5	284 (58.9)	176 (60.6)	108 (56.5)		
>5 and ≤10	194 (40.3)	113 (38.8)	81 (42.5)		
>10 and ≤15	2 (0.4)	1 (0.3)	1 (0.5)		
Missing	2 (0.4)	1 (0.3)	1 (0.5)		
cT stage				0.429	0.513
cT1–2	24 (5.0)	16 (5.5)	8 (4.2)		
cT3–4	457 (94.8)	274 (94.2)	183 (95.8)		
Missing	1 (0.2)	1 (0.3)	0		
cN stage				15.377	<0.001
cN0	89 (18.5)	70 (24.1)	19 (9.9)		
cN+	392 (81.3)	220 (75.6)	172 (90.1)		
Missing	1 (0.2)	1 (0.3)	0		
Radiation (Gy)				5.794	0.055
≥45	77 (16.0)	55 (18.9)	22 (11.5)		
30	382 (79.2)	225 (77.3)	157 (82.2)		
25	23 (4.8)	11 (3.8)	12 (6.3)		
ycT stage				2.644	0.104
ycT0–2	28 (5.8)	21 (7.3)	7 (3.7)		
ycT3–4	452 (93.8)	269 (92.4)	183 (95.8)		
Missing	2 (0.4)	1 (0.3)	1 (0.5)		
ycN stage				24.860	<0.001
ycN0	110 (22.8)	89 (30.6)	21 (11.0)		
ycN+	371 (77.0)	202 (69.4)	169 (88.5)		
Missing	1 (0.2)	0	1 (0.5)		
Surgery				0.200	0.950
LAR	324 (67.2)	194 (66.7)	130 (68.1)		
APR	152 (31.5)	93 (31.9)	59 (30.9)		
Hartmann	6 (1.3)	4 (1.4)	2 (1.0)		
ypT stage				58.332	<0.001
ypT0	35 (7.3)	31 (10.7)	4 (2.1)		
ypT1–2	158 (32.8)	127 (43.6)	31 (16.2)		
ypT3–4	289 (59.9)	133 (45.7)	156 (81.7)		
TLNe				22.680	<0.001
≤9	107 (22.2)	85 (29.2)	22 (11.5)		
10–13	113 (23.4)	62 (21.3)	51 (26.7)		
14–16	77 (16.0)	39 (13.4)	38 (19.9)		
17–24	93 (19.3)	56 (19.3)	37 (19.4)		
≥25	92 (19.1)	49 (16.8)	43 (22.5)		
M stage				2.943	0.119
M0	475 (98.5)	289 (99.3)	186 (97.4)		
M1	7 (1.5)	2 (0.7)	5 (2.6)		
ypTNM stage				–	–
0	31 (6.4)	31 (10.6)	0		
I–II	260 (53.9)	258 (88.7)	2 (1.1)		
III	184 (38.2)	0	184 (96.3)		
IV	7 (1.5)	2 (0.7)	5 (2.6)		
Differentiation				18.596	<0.001
High	18 (3.7)	13 (4.5)	5 (2.6)		
Moderate	318 (66.0)	200 (68.7)	118 (61.8)		
Low	103 (21.4)	41 (14.1)	62 (32.5)		
Missing	43 (8.9)	37 (12.7)	6 (3.1)		
Tumor deposit				–	–
Negative	466 (96.7)	291 (100)	175 (91.6)		
Positive	16 (3.3)	0	16 (8.4)		
LVI				25.251	<0.001
Negative	426 (88.4)	274 (94.2)	152 (79.6)		
Positive	55 (11.4)	16 (5.5)	39 (30.4)		
Missing	1 (0.2)	1 (0.3)	0		
CRM				4.368	0.037
Negative	417 (86.5)	254 (87.3)	163 (85.3)		
Positive	35 (7.3)	15 (5.1)	20 (10.5)		
Missing	30 (6.2)	22 (7.6)	8 (4.2)		

Data were presented as *n* (%). APR: Abdominoperineal resection; AV: Anal verge; CEA: Carcinoembryonic antigen; CRM: Circumferential resection margin; Dis: Distance; LAR: Low anterior resection; LVI: Lymphovascular invasion; TLNe: Total number of lymph nodes examined.

divide the ypN_0M_0 cohort into low- (>19) and high-risk (≤19) subsets in terms of OS, in which the 3-year OS were 96.0% and 86.8% ($P = 0.031$), and the 5-year OS were 92.3% and 84.0% ($P = 0.030$), respectively.

Prognostic value of lymph nodes examined

Cox regression was performed to identify the prognostic value of the number of LNe, and any variable achieving a P value < 0.10 in the univariate analysis or clinically considered to be statistically significant, was proposed [Tables 4 and 5]. In the entire cohort, when the NLNe was >6, better 3-year and 5-year OS were achieved (hazard ratio [HR] 0.432, 95% CI 0.242–0.770, $P = 0.004$; HR 0.564, 95% confidence interval [CI] 0.331–0.961, $P = 0.035$, respectively) [Figure 2A and B]. Similar results were also observed in the cN + cohort [Figure 2C and D]. In the ypN + M_0 cohort, better results were obtained when the TLNe was >10, achieving better 3-year and 5-year OS (HR 0.333, 95% CI 0.154–0.718, $P = 0.005$; HR 0.424, 95% CI 0.218–0.826, $P = 0.012$, respectively) [Figure 2E and F]. In the ypN_0M_0 cohort, a trend of better 5-year OS was shown when the TLNe was >19 (HR 0.420, 95% CI 0.173–1.020, $P = 0.055$) [Figure 2G and H].

Discussion

In our study, the number of lymph node metastases demonstrated a positive correlation with TLNe in patients with RC treated with neoadjuvant therapy, regardless of CRM status. When the TLNe was 14–16, then the proportion of patients with lymph node metastasis remained the highest. In these patients, TLNe may be influenced by the experience of the pathologist, tumor aggressiveness, and tumor staging. The increase in TLNe levels may also be related to the immune response. The results from the studies conducted by Parsons et al. and Van Erning et al. showed that an increase in TLNe in colon cancer did not increase the proportion of patients with positive lymph nodes.[21,22] However, Kim et al. showed that an increase in TLNe would increase the positive rate of lymph nodes, and at least 10 LNe could avoid staging shifts in colorectal cancer.[15] In 2014, Bhangu et al. showed that the proportion of patients with lymph node metastasis gradually increased with increasing TLNe in patients with RC treated with neoadjuvant therapy, and the proportion did not increase beyond this when it reached 16.[8] In 2018, Gao et al. showed that when the TLNe reached 10, the rate of proportion increase tended to be gentle, achieving accurate staging.[16] They recommended examination of at least 10 lymph nodes in patients with RC treated with neoadjuvant therapy.[16] In our study, multivariate analysis after adjusting for related clinicopathological factors showed that TLNe was still an independent, influential factor. When examination of 14–16 lymph nodes was achieved, the OR was the highest (OR = 3.379, 95% CI 1.533–7.448, $P = 0.003$). Therefore, we believe that the TLNe in patients with RC treated with neoadjuvant therapy should be at least 14 for accurate staging.

In addition, multivariate analysis showed that positive LVI, ypT3–4, and tumor differentiation were independent risk factors for lymph node metastasis. In our study, 11.4% of patients were LVI-positive, and 21.2% were LVI-positive among patients with lymph node metastases, but only 5.4% were LVI-positive in patients without lymph node metastases. Moreover, among LVI-positive patients, 70.9% had lymph node metastases. ypT stage represents the depth of tumor invasion and increases the area between tumor tissue and lymphatic vessels with increasing depth,

Table 3
Details of cut-off values for the entire cN+, ypN+, and ypN0 cohort, 3-year and 5-year OS calculated by X-tile.

Patients	Factors	Cut-off value	Overall Survival (%)	*P* value
Entire Cohort	TLNe	≤14	3-year: 83.8	0.129
		>14	3-year: 87.0	
	TLNe	≤6	5-year: 87.2	0.237
		>6	5-year: 76.7	
	NLNe	≤6	3-year: 76.0	0.003
		>6	3-year: 87.2	
	NLNe	≤6	5-year: 73.3	0.078
		>6	5-year: 78.7	
cN + Cohort	TLNe	≤14	3-year: 85.1	0.317
		>14	3-year: 86.9	
	TLNe	≤20	5-year: 81.4	0.192
		>20	5-year: 70.3	
	NLNe	≤6	3-year: 74.6	0.001
		>6	3-year: 88.3	
	NLNe	≤6	5-year: 71.2	0.040
		>6	5-year: 79.4	
ypN + M0 Cohort	TLNe	≤10	3-year: 60.0	0.007
		>10	3-year: 80.4	
	TLNe	≤10	5-year: 52.0	0.030
		>10	5-year: 66.4	
	NLNe	≤5	3-year: 41.2	<0.001
		>5	3-year: 81.5	
	NLNe	≤5	5-year: 35.3	<0.001
		>5	5-year: 67.6	
ypN0M0 Cohort	TLNe	≤19	3-year: 86.8	0.031
		>19	3-year: 96.0	
	TLNe	≤19	5-year: 84.0	0.030
		>19	5-year: 92.3	

NLNe: Negative lymph nodes examined; TLNe: Total number of lymph nodes examined.

Table 2
Multivariate analysis of risk factors for lymph node metastasis.

Parameters	B	SE	Wald	*P*	OR	95% CI
ypT stage						
I–II					1.000	
III–IV	1.331	0.268	24.624	<0.001	3.787	2.238–6.407
TLNe			11.808	0.019		
≤9					1.000	
10–13	1.042	0.372	7.855	0.005	2.836	1.368–5.879
14–16	1.218	0.403	9.119	0.003	3.379	1.533–7.448
17–24	0.702	0.385	3.324	0.068	2.017	0.949–4.287
≥25	1.030	0.380	7.357	0.007	2.801	1.331–5.897
LVI						
Negative					1.000	
Positive	1.241	0.373	11.097	0.001	3.459	1.667–7.180
Differentiation			5.848	0.054		
High					1.000	
Moderate	0.548	0.688	0.634	0.426	1.730	0.449–6.665
Low	1.133	0.713	2.529	0.112	3.106	0.768–12.554
Constant	−3.636	0.868	17.531	<0.001	0.026	

LVI: Lymphovascular invasion; TLNe: Total number of lymph nodes examined.

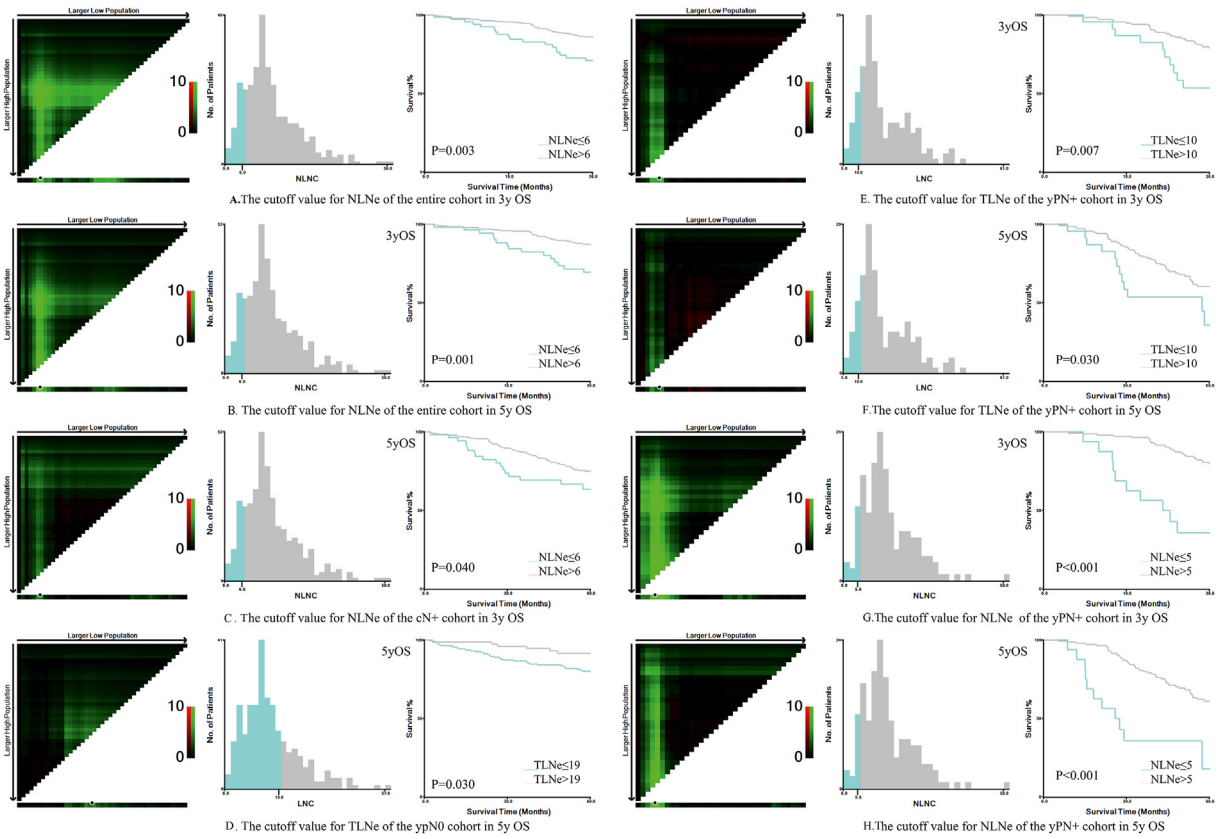

Figure 1. Cut-off points for TLNe and NLNe counts determined by the X-tile program. X-tile analysis divided the entire cohort into the training sets and matched validation sets based on patient surgical data. The cohort was divided into low and high-count groups based on the optimal cut-points, as was shown on a histogram of the cohort, and a Kaplan–Meier curve. The cut-off value for NLNe of the entire cohort in 3-year OS (A) and 5-year OS (B), NLNe of the cN + cohort in 3-year OS (C), TLNe of the ypN0 cohort in 5-year OS (D), TLNe of the yPN + cohort in 3-year OS (E) and 5-year OS (F), NLNe of the yPN + cohort in 3-year OS (G) and 5-year OS (H). NLNe: Negative lymph nodes; OS: Overall survival; TLNe: Total number of lymph nodes examined.

resulting in an increased probability of lymph node metastases. Among the ypT0, T1, T2, T3, and T4 patients, the proportions of lymph node metastases were 11.4%, 12.5%, 20.1%, 52.5%, and 46.4%, respectively.

cN status and ycN status were not considered independent risk factors for lymph node metastases. Among the cN+ and ycN + patients, 43.9% and 45.6% of patients had lymph node metastases, respectively. The consistency of the preoperative evaluation of lymph node metastases included in this study was not high, and the assessment of lymph node metastatic status by preoperative imaging should be further strengthened.

When the prognostic value of the number of LNe was taken into account, X-tile was used to find the cut-off value, followed by Cox regression to validate the prognostic value with clinicopathological factors together. Most previous studies focused on the relationship between TLNe and prognosis, and the log-rank test was used to compare prognosis.[8,17] The cut-off value was mostly 12, which was determined according to the AJCC guidelines, while some studies used the median number of LNe in their series.[18,23] Currently, an increasing number of studies have used an enumeration method to determine the cut-off values. In our study, the results showed that TLNe was not a prognostic factor in the entire cN + cohort, but the 3-year and 5-year OS were better when NLNe was >6.

Li et al. included 6068 patients with RC treated with neoadjuvant therapy from the SEER database from 2004 to 2010 and used X-tile to identify the cut-off value of nine for NLNe in terms of tumor-specific survival, and a better tumor-specific survival was observed when NLNe was >9.[24] However, in our study, subgroup analysis showed NLNe as an unstable factor, whereas TLNe was an important factor in terms of 3-year and 5-year OS. In the ypN + M_0 cohort, when TLNe was >10, then the 3-year and 5-year OS increased significantly, but when CRM was positive, no significant difference in terms of 5-year OS was observed. Sun et al. analyzed 158 ypN + patients and found that NLNe significantly influenced the 3-year disease-free survival (DFS) rate.[25] They identified 4 and 16 as cut-off values to divide the patients into high-, middle-, and low-risk subsets, and the 3-year DFS was 15.2%, 55.5%, and 73.1%, respectively ($P = 0.017$). However, the 3-year OS were 62.1%, 79.8%, and 83.2%, respectively ($P = 0.076$), with no significant differences.[25] While the results in our study showed that when NLNe was >6, a better 5-year OS was observed, NLNe did not influence the 3-year OS.

For ypN + patients, regardless of TLNe, current opinions mostly support that adjuvant chemotherapy is essential.[9,10] However, whether adjuvant chemotherapy should be administered to patients with ypN0 status remains controversial. Loree et al. conducted a retrospective study, and the results after short-term radiation therapy revealed that patients with ypII stage RC did not benefit from adjuvant chemotherapy after multivariate analysis.[26] Subgroup analysis showed that adjuvant chemotherapy improved only disease-specific survival and recurrence-free survival in patients with at least two risk factors: pT4, poor differentiation, less than 12 lymph nodes, LVI, perineural invasion (PNI), and obstructed or perforated condition. However, previous studies did not consider TLNe and did not verify the effect of adjuvant chemotherapy on prognosis when TLNe reached a specific number. The method of predicting prognosis based on the number of LNe is somewhat controversial, but it is important to accurately distinguish ypN0 patients to determine postoperative treatment.[18]

In our study, subgroup analysis in the ypN_0M_0 cohort showed, regardless of the CRM status, an increasing trend in 5-year OS when the lymph node count (LNC) was >19. Unfortunately, we failed to collect information on adjuvant chemotherapy; therefore, it was not possible to

Table 4
Univariate analysis for OS in the entire cohort and cN + cohort.

Factors	Entire cohort							cN + cohort						
	N	3-year OS (%)	χ^2 value	*P* value	5-year OS (%)	χ^2 value	*P* value	*N*	3-year OS (%)	χ^2 value	*P* value	5-year OS (%)	χ^2 value	*P* value
Gender			1.056	0.304		0.148	0.701			1.049	0.306		0.069	0.792
Male	272	82.2			72.6			225	82.9			73.3		
Female	187	85.7			73.8			149	76.6			73.5		
Age (years)			0.190	0.663		0.649	0.420			0.227	0.634		0.011	0.917
<65	315	84.2			74.8			263	83.6			73.9		
≥65	143	82.9			69.9			111	86.0			71.8		
CEA			8.230	0.004		15.929	<0.001			5.144	0.023		12.173	<0.001
Normal	296	87.1			79.1			242	87.4			79.2		
Abnormal	159	76.2			60.8			128	77.9			61.3		
Dis to AV (cm)			0.757	0.685		0.233	0.890			0.785	0.675		0.378	0.828
≤5	268	82.6			72.9			208	82.9			72.5		
>5 and ≤10	188	85.6			73.9			164	86.4			74.4		
>10 and ≤15	1	100			100			1	100			100		
cT stage			0.072	0.788		0.507	0.476			0.037	0.848		0.504	0.477
cT1–2	23	86.1			79.5			23	86.1			79.5		
cT3–4	435	83.7			72.9			351	84.3			72.8		
cN stage			0.567	0.451		0.035	0.851	–	–	–	–	–	–	–
cN0	84	81.4			73.1									
cN+	374	84.4			73.3									
Radiation (Gy)			5.142	0.076		6.090	0.048			7.219	0.027		8.667	0.013
≥45	72	91.0			91.0			60	95.8			95.8		
30	382	83.5			72.5			309	84.0			72.4		
25	5	50.0			50.0			5	50.0			50.0		
ycT stage			1.025	0.311		1.795	0.180			0.780	0.377		1.658	0.198
ycT0–2	28	91.0			84.5			27	90.9			84.4		
ycT3–4	430	83.2			72.4			346	83.9			72.5		
ycN stage			0.003	0.960		0.330	0.566			1.870	0.171		2.872	0.090
ycN0	105	83.7			76.1			22	–			–		
ycN+	353	83.6			72.3			351	83.9			72.5		
Surgery			16.647	<0.001		7.612	0.022			6.520	0.038		2.787	0.248
LAR	311	86.4			74.4			268	87.2			75.0		
APR	143	79.3			72.2			104	78.3			70.4		
Hartmann	5	40.0			40.0			2	50.0			50.0		
ypT stage			9.898	0.007	84.3	16.446	<0.001			12.407	0.002		18.893	<0.001
ypT0	34	89.6			84.5			26	95.5			95.5		
ypT1–2	156	91.1			64.8			114	93.3			86.1		
ypT3–4	269	78.4						234	78.9			64.8		
ypN stage			28.834	<0.001		41.478	<0.001			34.630	<0.001		41.602	<0.001
ypN0	280	89.2			83.4			213	92.6			86.2		
ypN1a–1b	109	84.1			64.1			97	82.1			61.7		
ypN1c	6	83.3			83.3			5	80.0			80.0		
ypN2	64	62.0			45.2			59	62.1			48.0		
NLNe			9.038	0.003		3.073	0.080			10.588	0.001		4.257	0.039
≤6	75	71.1			66.3			59	69.4			63.4		
>6	384	85.9			74.3			315	86.9			75.0		
M stage			5.860	0.015		13.065	<0.001			6.931	0.009		14.515	0.001
M0	452	84.0			73.7			367	84.8			74.0		
M1	7	42.9			0			7	42.9			0		
ypTNM stage			18.154	<0.001		39.180	<0.001			28.079	<0.001		46.885	<0.001
0	31	92.1			86.4			23	100			100		
I–II	247	89.3			83.5			188	92.4			85.2		
III	174	75.5			57.7			156	74.2			57.5		
IV	7	42.9			0			7	42.9			0		
Differentiation			5.144	0.076		10.806	0.005			5.875	0.053		11.521	0.003
High	12	83.3			83.3			8	87.5			87.5		
Moderate	307	85.9			77.0			249	86.6			77.2		
Low	98	75.7			58.6			85	75.1			57.0		
Tumor deposit			8.409	0.004		10.148	0.001			9.990	0.002		11.336	<0.001
Negative	445	84.3			73.9			361	85.2			74.3		
Positive	14	50.5			33.7			13	50.1			33.4		
LVI			0.519	0.471		4.375	0.036			0.333	0.564		3.793	0.051
Negative	405	84.0			74.8			324	84.8			75.3		
Positive	53	80.5			59.5			50	81.7			60.4		
CRM			6.956	0.008		7.799	0.005			10.737	0.001		9.968	0.002
Negative	394	84.7			74.5			320	85.9			75.0		
Positive	35	66.8			53.0			28	62.1			48.9		

APR: Abdominoperineal resection; AV: Anal verge; CEA: Carcinoembryonic antigen; CRM: Circumferential resection margin; Dis: Distance; LAR: Low anterior resection; LVI: Lymphovascular invasion; NLNe: Negative lymph nodes examined; OS: Overall survival.

Table 5
Univariate analysis for OS in ypN + cohort and ypN0 cohort.

Factors	ypN + cohort							ypN0 cohort						
	N	3-year OS (%)	χ^2 value	*P* value	5-year OS (%)	χ^2 value	*P* value	*N*	3-year OS (%)	χ^2 value	*P* value	5-year OS (%)	χ^2 value	*P* value
Gender			1.469	0.225		1.032	0.310			0.243	0.622		0	0.990
Male	97	71.6			54.4			171	88.9			83.8		
Female	77	80.3			61.9			107	90.5			83.7		
Age (years)			0.001	0.977		0.122	0.727			0.145	0.704		1.013	0.314
<65	125	75.9			59.3			186	90.2			86.1		
≥65	49	73.5			53.4			91	89.1			79.9		
CEA			0.705	0.401		2.916	0.088			9.715	0.002		9.216	0.002
Normal	100	77.5			63.7			192	93.3			87.7		
Abnormal	72	72.4			49.9			84	79.7			73.3		
Dis to AV (cm)			0.059	0.809		0.105	0.746			1.173	0.556		0.246	0.884
≤5	98	74.5			56.5			165	88.3			83.6		
>5 and ≤10	75	76.6			59.3			111	92.4			84.8		
>10 and ≤15								1	100			100		
cT stage			1.691	0.193		0.133	0.716			1.654	0.198		0.870	0.351
cT1–2	7	57.1			57.1			16	100			90.0		
cT3–4	167	76.3			57.6			261	89.3			83.7		
cN stage			1.410	0.235		0.518	0.472			9.300	0.002		4.973	0.026
cN0	18	87.1			59.4			66	79.8			75.7		
cN+	156	74.2			57.5			211	93.1			86.7		
Radiation (Gy)			0.375	0.829		1.284	0.527			6.640	0.036		6.565	0.038
≥45	18	93.3			93.3			50	89.8			89.8		
30	155	74.9			56.5			224	90.1			83.9		
25	1	100			100			4	50.0			50.0		
ycT stage			0.171	0.680		0.100	0.752			2.046	0.153		1.257	0.262
ycT0–2	7	68.6			68.6			21	100			90.9		
ycT3–4	167	75.8			57.2			257	88.8			83.2		
ycN stage			1.455	0.228		0.543	0.461			4.488	0.034		1.784	0.182
ycN0	20	87.1			59.4			85	82.9			79.3		
ycN+	154	74.2			57.5			193	92.2			85.5		
Surgery			11.912	0.003		8.852	0.012			12.420	0.002		6.838	0.033
LAR	117	81.4			61.2			187	90.8			84.4		
APR	56	64.3			52.8			87	88.9			84.1		
Hartmann	1	0			0			4	50.0			50.0		
ypT stage			4.450	0.107		4.033	0.133			1.375	0.503		1.953	0.377
ypT0	3	66.7			66.7			31	92.1			86.4		
ypT1–2	28	92.0			76.4			125	91.7			86.7		
ypT3–4	143	72.4			54.1			122	86.5			79.7		
ypN stage			10.288	0.005		7.345	0.025	–	–	–	–	–	–	–
ypN0	106	84.1			64.1									
ypN1a–1b	6	83.3			83.3									
ypN1c	62	62.3			46.9									
ypN2														
TLNe			7.025	0.008		4.604	0.032	–	–	–	–	–	–	–
≤10	27	54.0			36.0									
>10	147	78.8			60.8									
TLNe	–	–	–	–	–	–	–			4.311	0.038		4.384	0.036
≤19								201	86.9			80.4		
>19								77	95.9			91.5		
Differentiation			6.715	0.035		8.049	0.018			0.238	0.888		0.057	0.972
High	3	66.7			66.7			9	88.9			88.9		
Moderate	109	81.7			64.9			194	89.2			84.0		
Low	57	65.5			45.6			39	91.5			81.5		
Tumor deposit			4.947	0.026		4.830	0.028	–	–	–	–	–	–	–
Negative	161	76.8			59.0									
Positive	13	50.1			33.4									
LVI			0.067	0.793		0.547	0.460			0.094	0.759		0.004	0.947
Negative	140	75.6			58.6			261	89.4			83.8		
Positive	34	75.0			53.9			16	92.9			81.2		
CRM			3.873	0.049		4.242	0.039			1.580	0.209		1.589	0.207
Negative	147	77.6			60.1			241	89.8			84.1		
Positive	20	58.2			37.7			15	78.6			71.4		

APR: Abdominoperineal resection; AV: Anal verge; CEA: Carcinoembryonic antigen; CRM: Circumferential resection margin; Dis: Distance; LAR: Low anterior resection; LVI: Lymphovascular invasion; TLNe: Total number of lymph nodes examined; -: No available; OS: Overall survival.

assess whether patients with TLNe of more than 19 needed adjuvant chemotherapy. Moreover, an increase in TLNe might reflect a stronger immune response to neoadjuvant therapy, resulting in a better prognosis. In addition, the tumor regression grade (TRG) was also considered an indicator of the effect of neoadjuvant treatment, and the relationship between TRG and TLNe remains unclear. However, existing evidence also could not prove that a decreased number of LNe in patients has better TRG.[27] Patients who received neoadjuvant therapy showed greater heterogeneity. Different data sources and statistical methods might have caused differences in the results, and the results of our study support that TLNe is an independent prognostic factor of OS in patients with RC treated with neoadjuvant therapy.

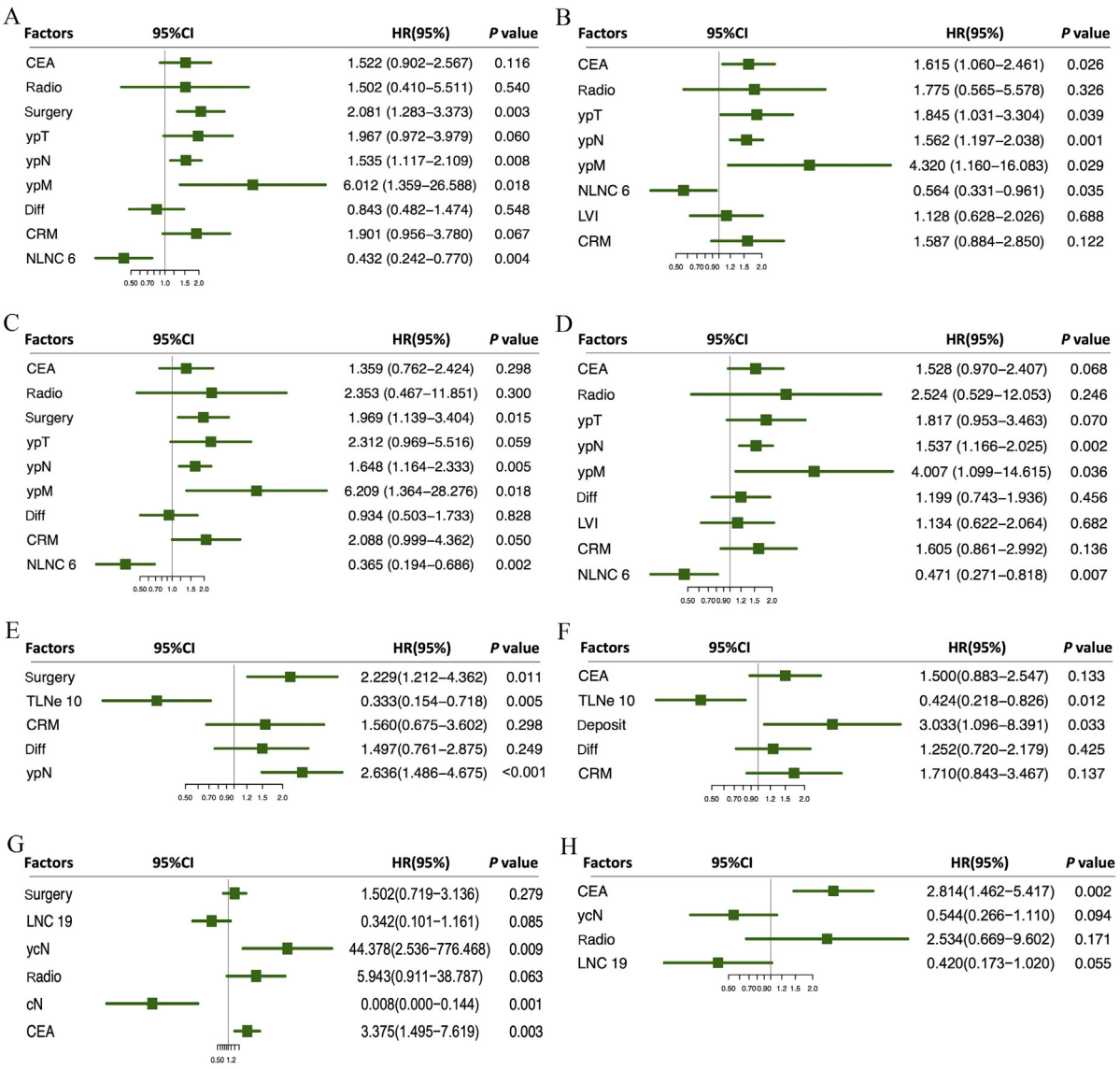

Figure 2. Cox regression results of the entire cohort, the cN+, ypN+, and ypN0 cohorts, showed with forest plots. (A) Multivariate analysis of 3-year OS in the entire cohort; (B) multivariate analysis of 5-year OS in the entire cohort; (C) multivariate analysis of 3-year OS in the cN + cohort; (D) multivariate analysis of 5-year OS in the cN + cohort; (E) multivariate analysis of 3-year OS in the ypN + cohort; (F) multivariate analysis of 5-year OS in the ypN + cohort; (G) multivariate analysis of 3-year OS in the ypN0 cohort; (H) multivariate analysis of 5-year OS in the ypN0 cohort. CEA: Carcinoma embryonic antigen; CRM: Circumferential resection margin; Diff: Differentiation; LNC: Lymph node count; LVI: Lymphovascular invasion; NLNC: Negative lymph nodes count; TLNe: Total number of lymph nodes examined.

However, our study has some limitations. First, this was a retrospective study and lacked information on adjuvant chemotherapy, which might have impacted the accuracy of the conclusions. Second, approximately 80% of the patients included in this study received 30 Gy radiotherapy, which was more than that in other studies, and the heterogeneity of patients and treatment methods might lead to differences in conclusions. Finally, all continuous variables, such as CEA level, age, and LNe, were converted into categorical variables, and a reduction in statistical power might have occurred.

In conclusion,the numbers of TLNe and NLNe influenced the staging accuracy and had prognostic value in patients with RC treated with neoadjuvant therapy.

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Author contributions

Contributions to conception and design, or acquisition of data, or analysis and interpretation of data: Liyu Zhu, Lin Wang, Yujian Zeng, Kaixiong Tao, Quan Wang, Xinming Li, Huanhu Zhang, Zhanlong Shen, Kai Shen, Zhidong Gao, Yingjiang Ye, and Aiwen Wu. Drafting the article or revising it critically for important intellectual content: Liyu Zhu, Lin Wang, Zhidong Gao, Yingjiang Ye, and Aiwen Wu. Final approval of the version to be published: Aiwen Wu, Zhidong Gao, and Yingjiang Ye.

Ethics statement

The study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Institutional Review Board of the Peking

University People's Hospital, Beijing, China (No. 2021PHD010-001). Written informed consent was obtained from all patients prior to their enrollment in the study.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

None.

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