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ORIGINAL ARTICLE

Prognostic Implication of Negative Lymph Node Count in ypN+ Rectal Cancer after Neoadjuvant Chemoradiotherapy and Construction of a Prediction Nomogram

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Abstract

Purpose This study aimed to investigate the prognostic significance of negative lymph nodes (NLNs) for ypN+ rectal cancer after neoadjuvant chemoradiotherapy (nCRT) and radical surgery and to construct a nomogram predicting disease-free survival (DFS).

Method One hundred fifty-eight eligible patients were included. X-tile analysis was performed to determine cutoff values of NLNs. Clinicopathological and survival outcomes were compared. A Cox regression analysis was performed to identify prognostic factors of DFS. A nomogram was constructed and validated internally.

Results X-tile analysis identified cutoff values of 4 and 16 in terms of DFS ($\chi^2 = 8.129$, $p = 0.017$). The 3-year DFS rates for low (≤ 4), middle (5–16), and high (≥ 17) NLNs group was 15.2, 55.5, and 73.1%, respectively ($P = 0.017$). NLN count (NLNs ≥ 17, HR = 0.400, $P = 0.022$), IMA nodal metastasis (HR = 1.944, $P = 0.025$), tumor differentiation (poor/anaplastic, HR = 1.805, $P = 0.021$), and ypT4 stage (HR = 7.787, $P = 0.047$) were independent prognostic factors of DFS. A predicting nomogram incorporating the four significant predictors was developed with a C-index of 0.64.

Conclusion NLN count was an independent prognostic factor of DFS in patients with ypN+ rectal cancer following nCRT. A nomogram incorporating NLN count, IMA nodal metastasis, tumor differentiation, and ypT stage could stratify rectal cancer patients with different DFS and might be helpful during clinical decision-making.

Keywords Rectal cancer · Chemoradiotherapy · Negative lymph nodes · Prognosis · Nomogram

Introduction

Neoadjuvant chemoradiotherapy (nCRT) following total mesorectal excision (TME) has become the gold standard for the treatment of locally advanced rectal cancer (LARC), and is associated with improved tumor downstaging and better local disease control.[1–3] Nevertheless, LARC patients exhibit a wide range of responses to neoadjuvant treatment, and thus great survival heterogeneity.[4] In addition, individualized prognostic assessment should be taken into account during clinical decision-making, including adjuvant chemotherapy and postoperative surveillance.[5]

Pathologic lymph node status is considered as a powerful prognostic factor for patients with rectal cancer receiving nCRT.[6] NCRT exerts effects on tumor downstaging and sterilization of lymph nodes (LNs) and induces reduction of the size and number of LNs retrieved.[7] The current AJCC/TNM staging system for rectal cancer[8] defines LN involvement by taking into account the number of positive lymph nodes (PLNs) and tumor deposits. However, PLNs is often affected by many factors, such as neoadjuvant therapy and the number of LNs removed, which is often reduced by nCRT. Additionally, it does not account for tumor-free LNs retrieved.[9] More importantly, node-positive rectal cancers are a heterogeneous group of patients, and the prognosis of these patients cannot be stratified by N stage only.[10] Recently, the concept of negative lymph nodes (NLNs)[11] has attracted a lot of attention as a prognostic factor in colorectal cancer. Additionally, NLN count has been proven to improve the prognostic prediction of TNM

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classification various malignancies, including colorectal,[12] gastric,[13] esophageal,[14] and cervical[15] cancers.

The present study was aimed to evaluate the prognostic significance of NLNs in ypN+ rectal cancer patients following nCRT and TME and to construct a predictive nomogram.

Patients and Methods

Patients

Based on our prospective database, LARC patients treated with nCRT and radical surgery between 2008 and 2014 in the Department of Colorectal Surgery of Fujian Medical University Union Hospital (FMUUH, Fuzhou, China) were included. The inclusion criteria were as follows: (1) histologically proven rectal adenocarcinomas, (2) rectal tumors located < 12 cm from the anal verge, and (3) ypN+ rectal tumor tumors. Exclusion criteria included the following: (1) patients with previous or simultaneous malignancy, (2) patients treated with emergency surgery or palliative surgical resection, and (3) patients treated with local excision or a wait-and-see strategy.

Treatment

The pre- and post-nCRT assessment and staging strategies included digital rectal examination, serum carcinoembryonic antigen (CEA) test, serum carbohydrate antigen 19–9 (CA19–9) test, chest X-ray or computed tomography (CT) scan, abdominopelvic magnetic resonance imaging (MRI), and transrectal ultrasonography (ERUS). Long-course preoperative radiotherapy was comprised of a dose of 45 Gy followed by a primary tumor boost of 5.4 Gy (1.8 Gy/fraction, 5.5 weeks). Concurrent chemotherapy was administered using two therapeutic regimens, including capecitabine plus oxaliplatin (CapeOX) or 5-fluorouracil/folinic acid plus oxaliplatin (FOLFOX). Surgery was performed by the same group of surgeons at 6 to 8 weeks after completing radiotherapy. All patients underwent surgery according to the principle of TME and high ligation of the inferior mesenteric artery were routinely performed. About 4 weeks after surgery, patients received adjuvant chemotherapy (FOLFOX or CapeOX) for 6 months.

Pathologic Examination

Before formalin fixation, LNs were identified and separated from the specimen by surgeons in the operating room. All resected specimens were assessed by at least two experienced pathologists according to a standardized protocol. In case of retrieval of less than 12 LNs, a second-look LN search was indicated as quality control to ensure adequate staging for rectal cancer. Fat clearance technique was not performed in the present study. Lymph node ratio (LNR) was defined as the ratio of PLNs divided by the total number of TLNs. Pathological tumor regression grade (TRG) was assessed according to the four-tier AJCC TRG classification.[16] TRG definitions were as follows: TRG 0, no residual tumor cells; TRG 1, single cells or small groups of cells; TRG 2, residual cancer with desmoplastic response; and TRG 3, minimal evidence of tumor response.

Follow-Up

Postoperative follow-up was performed every 3 months for the first 3 years, then every 6 months for the next 2 years, and annually thereafter. Most patients routinely underwent physical examination, serum CEA and CA19-9 test, chest X-ray or CT scan, abdominopelvic MRI or CT scan, and an annual colonoscopy. If necessary, positron emission tomography (PET) scan was used to better clarify recurrence and/or metastasis. Disease-free survival (DFS) was defined as time from surgery to evidence of local or distant recurrence.

Statistical Analysis

Statistical analyses were performed by SPSS 20.0 software (SPSS INC., Chicago, USA) and graphs were created by GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). Categorical variables were presented as frequencies with percentages and assessed using Chi-square or Fisher's exact test, when appropriate. Continuous variables were described as mean ± standard deviation or median with interquartile range (IQR) and analyzed using one-way analysis of variance, or Kruskal–Wallis test, where appropriate. The optimal cutoff points of NLN count were calculated and determined by using X-tile program (http://www.tissuearray.org/rimmlab/), a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization, which identified the cutoff with the minimum p values from log-rank χ^2 statistics in terms of DFS.[17] Survival outcomes were analyzed using the Kaplan-Meier method and compared by the log-rank test. A Cox regression model was utilized to determine risk factors for DFS. Then, a nomogram was constructed based on the final predictive model by using R 2.12.1 program (http://www.r-project.org) with the survival and rms package. The nomogram was validated internally (1000 bootstrap resamples) to correct for overfitting. A bootstrapping method is a nonparametric data generating method in which new datasets are repeatedly generated from an original dataset and created by random drawing from the sample with replacement.[18] The predictive performance of the nomogram was assessed by calculating Harrell's concordance index (c-index). Nomogram calibration for 3- and 5-year DFS was performed by comparing the predicted and actual probability after bias correction. $p < 0.05$ was considered to be statistically significant.

Results

Patient Characteristics

A total of 158 eligible patients were enrolled in the present study, including 98 males and 60 females. The median age of patients was 54 years old (IQR 44–62). The mean tumor distance from the anal verge was 5.9 cm with a majority of patients being adenocarcinoma (79.7%). The median number of TLNs, PLNs, and NLNs was 14 (IQR 9–20), 2 (IQR 1–4), and 10 (IQR 7–16), respectively. With a median follow-up period of 38 months (range from 20 to 103 months), 47 (29.7%) patients died, and 76 (48.1%) patients developed tumor recurrence. The 3- and 5-year OS rates for the patient cohort were 77.6 and 65.3%, and the 3- and 5-year DFS rates were 57.7 and 46.2%.

Identification of Cutoff Value for NLN Count

Given that NLN counts were continuous variables, X-tile program was utilized to identify the optimal cutoff points for NLN count that determined the greatest actuarial survival difference. As shown in Fig. 1, X-tile plots were constructed and identified 4 and 16 as cutoff values for NLN count to divide the entire cohort into low (≤ 4), middle (5–16), and high (≥ 17) subgroups in terms of DFS ($\chi^2 = 8.129$, $P = 0.017$).

Association Between NLN Count with Clinicopathological Characteristics

The median number of TLNs in low, middle, and high NLN count group was 8 (IQR: 5–18), 13 (IQR 9–15), and 23 (IQR 21–27), respectively ($P < 0.001$). The median number of NLNs in three groups was 4 (IQR 3–4), 9 (IQR 7–12), and 21 (IQR 19–23), respectively ($P < 0.001$). Noticeably, NLN counts showed no correlation with PLN count; the median number of PLNs in three NLN count groups was 2 (IQR 1–8), 2 (IQR 1–4), and 2 (IQR 1–3), respectively ($P = 0.265$).

We further evaluated the association between NLN count and clinical and tumor characteristics in patients with ypN+ rectal cancer. The results demonstrated that NLN count was not significantly associated with sex, age, tumor distance from the anal verge, tumor histopathology, tumor differentiation, preoperative CEA level, ypT stage, and TRG (all $P > 0.05$), as demonstrated in Table 1.

Prognostic Value of NLN Count

Higher NLN count was associated with a higher probability of OS; that is, the 3-year OS rates for low (≤ 4), middle (5–16), and high (≥ 17) NLN group were 62.1, 79.8, and 83.2%, respectively ($P = 0.076$), but the difference was not significant (Fig. 2a). Noticeably, higher NLN count was correlated with improved DFS, and the 3-year DFS rates for low, intermediate, and high NLN groups were 15.2, 55.5, and 73.1%, respectively ($P = 0.017$), as shown in Fig. 2b.

To explore the prognostic impact of NLN count on DFS in ypN+ rectal cancer, we performed a COX regression model analysis. On univariate analysis, tumor differentiation ($P = 0.003$), NLN count ($P = 0.007$), circumferential resection margin (CRM) involvement ($P = 0.037$), and IMA nodal

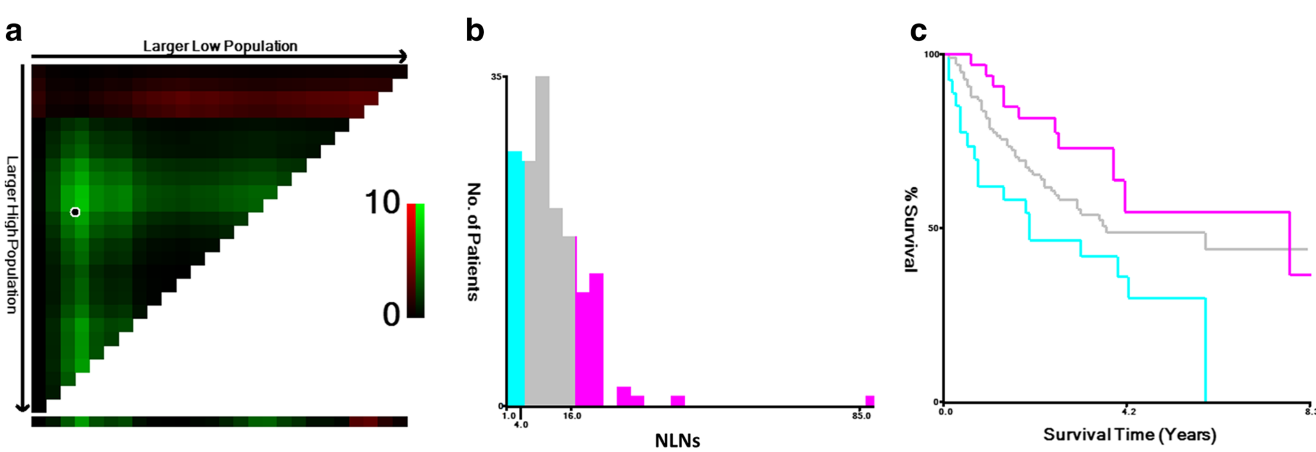

Fig. 1 Cutoff points for NLN counts determined by X-tile program. X-tile analysis divided the entire cohort into the training sets (shown in the upper-left quartile of Fig. 1a) and matched validation sets (shown in the bottom X-axis of Fig. 1a) based on patient survival data. The black dot in the validation set represents the exact cutoff values for the NLN count. The entire cohort was divided into low (blue), middle (gray), and (pink) NLN count groups based on the optimal cut-points (4 and 16), as was shown on a histogram of the entire cohort (Fig. 1b), and a Kaplan-Meier curve (Fig. 1c, $\chi^2 = 8.129$, $P = 0.017$)

Table 1 Clinical and tumor characteristics of ypN+ rectal cancer patients after nCRT according to NLN count

Characteristics	NLN count			*P* value
	Low (≤ 4) (*n* = 27)	Middle (5–16) (*n* = 98)	High (≥ 17) (*n* = 33)	
Sex				0.367
Male	17 (63)	64 (65.3)	17 (51.5)	
Female	10 (37)	34 (34.7)	16 (48.5)	
Age (years)	54.2 ± 14.2	53.9 ± 11.7	52.6 ± 16.3	0.858[a]
Distance from the anal verge (cm)	5.9 ± 2.1	6.0 ± 2.2	5.7 ± 2.1	0.820[a]
Histopathology				0.198
Adenocarcinoma	21 (77.8)	75 (76.5)	30 (90.9)	
Mucinous/signet ring adenocarcinoma	6 (22.2)	23 (23.5)	3 (9.1)	
Tumor differentiation				0.154
Well/moderate	17 (63)	72 (83.5)	28 (84.8)	
Poor/anaplastic	10 (37)	26 (26.5)	5 (15.2)	
Preoperative CEA (ng/ml)				0.827
≤ 5	12 (44.4)	50 (51)	16 (48.5)	
> 5	15 (55.6)	48 (49)	17 (51.5)	
ypT stage				0.530
0	2 (7.4)	4 (4.1)	1 (3.0)	
1	0	4 (4.1)	0	
2	4 (14.8)	9 (9.2)	4 (12.1)	
3	12 (44.4)	62 (63.3)	21 (63.6)	
4	9 (33.3)	19 (19.4)	7 (21.3)	
TRG				0.655
1	6 (22.2)	28 (28.6)	7 (21.2)	
2	13 (48.1)	47 (48.0)	14 (42.4)	
3	8 (29.6)	23 (23.4)	12 (36.4)	
No. of TLNs harvested	8 (5–18)	13 (9–15)	23 (21–27)	< 0.001[b]
PLN count	2 (1–8)	2 (1–4)	2 (1–3)	0.265[b]
NLN count	4 (3–4)	9 (7–12)	21 (19–23)	< 0.001[b]

Data are expressed as number (%), mean ± standard deviation, or median (interquartile range), when appropriate

nCRT neoadjuvant chemoradiotherapy, *NLN* negative lymph node, *CEA* carcinoembryonic antigen, *TRG* tumor regression grade, *TLNs* total lymph nodes, *PLN* positive lymph node

[a] One-way analysis of variance

[b] Kruskal–Wallis test

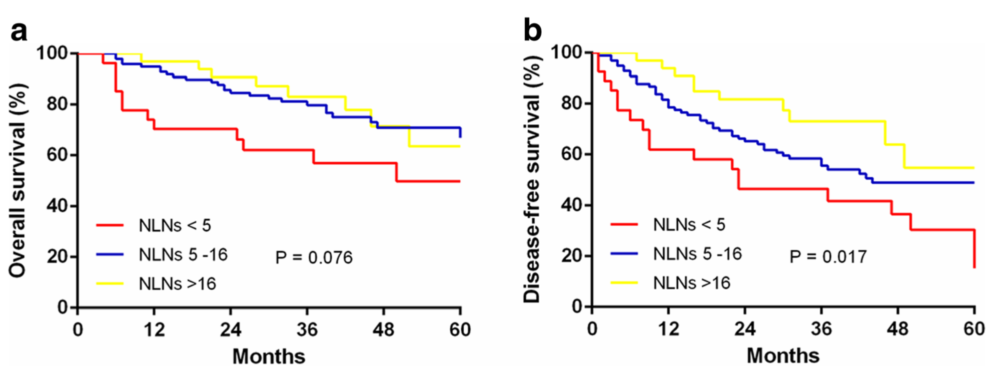

Fig. 2 Overall survival (**a**) and disease-free survival (**b**) between three NLN count groups in ypN+ rectal cancers after nCRT. *NLNs* negative lymph nodes; *nCRT* neoadjuvant chemoradiotherapy

metastasis ($P = 0.002$) were independently associated with DFS in patients with ypN+ rectal cancer following nCRT and TME (Table 2). All significant parameters ($P < 0.10$), including ypT stage (ypT4 stage, $P = 0.070$), in the univariate analysis were entered into a Cox regression model. The results demonstrated that IMA nodal metastasis (HR = 1.944, 95%CI 1.088–3.473, $P = 0.025$), NLN count (NLNs ≥ 17, HR = 0.400, 95%CI 0.182–0.877, $P = 0.022$), tumor differentiation (poor/anaplastic, HR = 1.805, 95%CI 1.092–2.984, $P = 0.021$), and ypT stage (ypT4 stage, HR = 7.787, 95%CI 1.030–58.851, $P = 0.047$) remained to be independent predictors of DFS following nCRT, as shown in Table 2.

Construction of a Nomogram for DFS

To quantitatively predict DFS of ypN+ rectal cancer after nCRT, a nomogram incorporating the four significant predictors was constructed based on the Cox regression model, as shown in Fig. 3a. To use the nomogram, each variable was assigned a score on the top point scale, and the sum of these scores could be converted into probabilities of 3- and 5-year DFS on the bottom two scales. The prognostic nomogram was internally validated with 1000 bootstrap resamples. The C-index of the nomogram was 0.64 (95%CI 0.58–0.70). As shown in Fig. 3b, c, the calibration curve presented good statistical performance between predicted and actual 3- and 5-year DFS.

Discussion

LN metastasis is one of the most potent factors for recurrence and survival in rectal cancer after nCRT.[6] NCRT reduces the number and size of LNs retrieved and induces sterilization of metastatic LNs,[7] and there is an ongoing controversy regarding the prognostic value of LN retrieval on patient survival. Many studies have reported that fewer LN retrieval following

Table 2 Cox regression analysis of factors for disease-free survival in ypN+ rectal cancer patients after nCRT

Factors	Univariate analysis			Cox regression analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Sex (male vs. female)	1.418	0.874–2.301	0.157			
Age	0.995	0.978–1.012	0.551			
Distance from the anal verge	1.040	0.933–1.159	0.483			
Histopathology (mucinous or signet ring adenocarcinoma vs. adenocarcinoma)	0.725	0.405–1.297	0.279			
Tumor differentiation (poor and anaplastic vs. well and moderate)	2.008	1.262–3.196	0.003	1.805	1.092–2.984	0.021
Preoperative chemotherapy regimen (oxaliplatin based vs. fluoropyrimidine only)	0.982	0.742–1.300	0.900			
CRM involvement	2.445	1.056–5.661	0.037	1.754	0.743–4.143	0.200
IMA nodal metastasis	2.285	1.357–3.848	0.002	1.944	1.088–3.473	0.025
NLN count						
Low (≤ 4)	Reference		0.023	Reference		0.068
Middle (5–16)	0.590	0.342–1.018	0.058	0.632	0.354–1.127	0.120
High (≥ 17)	0.353	0.165–0.756	0.007	0.400	0.182–0.877	0.022
ypT stage						
ypT0	Reference		0.313	Reference		0.367
ypT1	3.769	0.338–42.006	0.281	4.492	0.378–53.344	0.234
ypT2	4.054	0.505–32.565	0.188	6.690	0.805–55.625	0.079
ypT3	4.493	0.617–32.693	0.138	7.024	0.941–52.458	0.057
ypT4	6.423	0.861–47.904	0.070	7.787	1.030–58.851	0.047
ypN stage (ypN2 vs. ypN1)	0.854	0.508–1.437	0.553			
LNR	2.221	0.783–6.299	0.133			
TRG						
1	Reference		0.505			
2	1.067	0.598–1.903	0.826			
3	1.381	0.752–2.538	0.298			

NLN negative lymph node, *nCRT* neoadjuvant chemoradiotherapy, *HR* hazard ratio, *CI* confidential interval, *CRM* circumferential resection margin, *IMA* inferior mesenteric artery, *LNR* lymph node ratio, *TRG* tumor regression grade

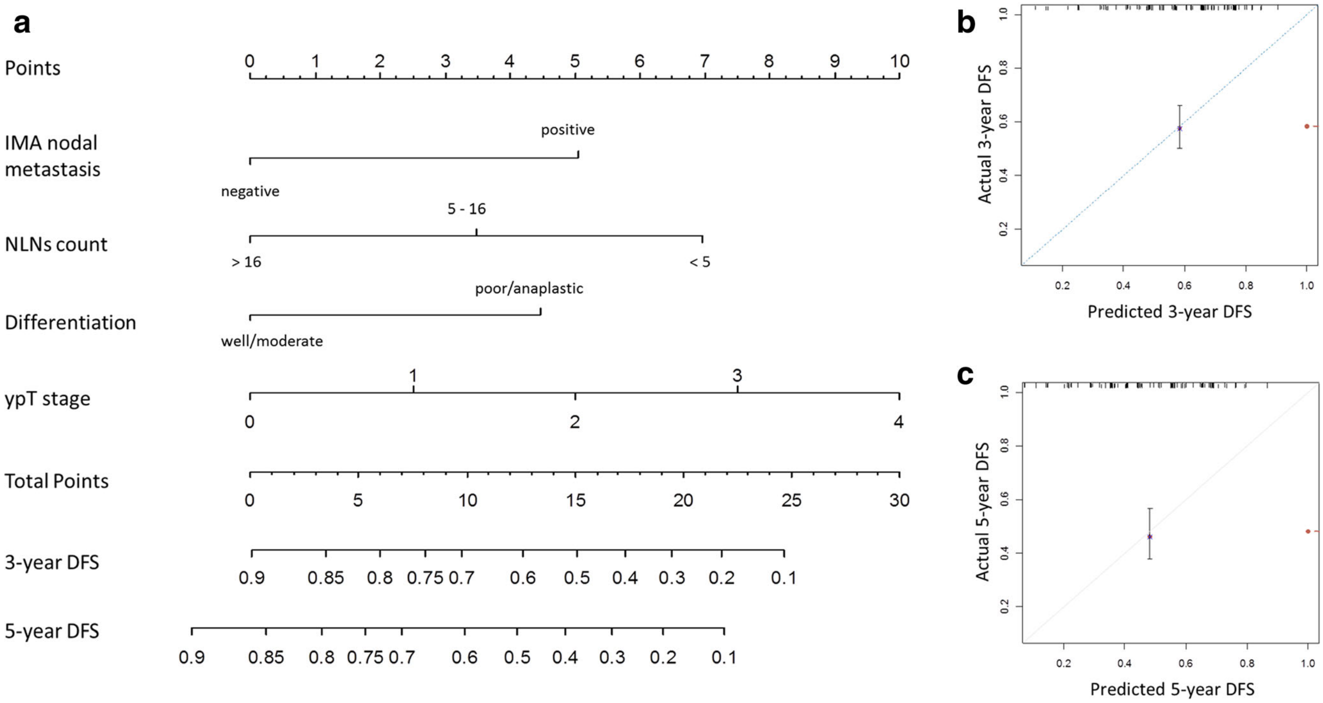

Fig. 3 **a** A nomogram for predicting the probability of 3- and 5-year DFS in patients with ypN+ rectal cancer after nCRT. **b**, **c** Calibration curves for 3- and 5-year DFS in ypN+ rectal cancer patients after nCRT with internal validation. *nCRT* neoadjuvant chemoradiotherapy; *IMA* inferior mesenteric artery; *NLNs* negative lymph nodes; *DFS* disease-free survival

nCRT does not imply an inadequate resection and is associated with good tumor response and superior oncological outcome.[19–21] Whereas, others insisted that inadequate LNs retrieved would result in impaired oncological outcome.[22–24] Additionally, current LN staging strategy takes into account only the number of PLNs, which depends on adequate assessments of TLNs, yet the number of TLNs tends to decrease after nCRT.[25] Therefore, effective assessment of LNs on survival should be investigated for accurate prognostic prediction. The prognostic value of the number of NLNs has been proven in colorectal,[12] gastric,[13] esophageal,[14] and cervical[15] cancers. NLNs might be a good supplement for the current LN staging system evaluating the prognosis of rectal cancer following nCRT.

In our patient cohort, we found that increased NLN count was associated with improved DFS, and COX regression analysis demonstrated that NLN count was an independent prognostic factor of DFS in patients with ypN+ rectal cancer treated with nCRT. By evaluating the relevance between NLN count and clinicopathological parameters, we found NLNs had a negative correlation with PLN counts, indicating that it was a prognostic factor independent of current PLN count-based LN staging. Additionally, NLNs showed no association with tumor regression, suggesting that it is a prognostic factor independent of TRG scores.

There are several potential mechanisms responsible for the favorable effect of NLNs on survival outcome. First, sufficient LN dissection could reflect adequate surgical resection.[26] High numbers of LNs retrieved may decrease stage-migration phenomenon; the more LNs examined, the lower probability of underestimation of nodal stage.[27] Second, metastatic lymph nodes are commonly detected by hematoxylin-eosin staining, while occult lymph node metastases should be evaluated with immunohistochemical staining[28] or molecular detection.[29] By increasing the number of NLNs, the likelihood of leaving behind micrometastases within LNs may decrease. Third, the number of NLNs may be more intrinsically related to the host lymphocytic reaction to the tumor.[30,31] Greater lymphocytic reaction has been reported to be associated with higher microsatellite instability (MSI) proportion and prolonged survival.[32,33] In contrast, Shuji Ogino et al.[34] reported that the prognostic value of NLN count for colorectal cancer patients was independent of lymphocytic reaction and tumoral molecular alterations, such as CpG island methylator phenotype (CIMP), long interspersed element 1 (LINE-1) hypomethylation, MSI, and BRAF mutation. Therefore, further research is warranted to investigate the number of NLNs and the lymphocytic response against tumors for rectal cancer.

Other lymph node-related parameters, such as PLNs, LNR, and distribution of metastatic lymph nodes, have been proven to be associated with survival of patients with colorectal cancer. Since X-tile analysis could not identify optimal cutoff value for LNR in terms of DFS (data not shown), we included LNR as a continuous variable in COX regression analysis. However, univariate analysis did not show LNR as an independent predictive factor of DFS. Similarly, ypN stage was not an independent predictive factor of DFS in univariate analysis. Nevertheless, our COX regression model identified other parameters as independent predictors for DFS, including IMA nodal metastasis, tumor differentiation, and ypT stage. Previous studies have reported that patients with IMA nodal metastasis showed a worse prognosis than other stage III rectal cancer patients.[35,36] Our previous study[37] and other literature[35] have demonstrated that IMA nodal metastasis was a major determinant of DFS, which was in good accordance with the current study. It has been reported that patients with poorly differentiated tumors are associated with unresponsiveness to nCRT and a poor prognosis.[38,39] ypT stage indicates the invasion depth of the primary tumor after nCRT, and its prognostic significance for LARC patients after nCRT has been proved in previous studies.[40,41] Herein, we found that both poor differentiation and ypT4 stage were independent predictors of poor DFS in ypN+ rectal cancer, confirming results from previous studies.

To provide a better prognostication in ypN+ rectal cancer patients, we developed a nomogram based on NLN count and other significant predictors in COX regression model. The present nomogram stratifies the patients with different risk of disease recurrence and offers a more individualized prediction of DFS. Additionally, it can be used to improve decision-making regarding adjuvant therapy and postoperative follow-up. Intensified adjuvant chemotherapy and surveillance strategies are indicated for patients at high risk of tumor recurrence.[42,43] Nevertheless, future research is needed to address these issues.

Nonetheless, the present study has several limitations. First, this study was a retrospective analysis of a single center; it may be subject to selection bias and other confounding factors. Nevertheless, staging workups, therapeutic and follow-up strategies of patients were consistent in our series, which reduced data variations to some extent. Another potential limitation was the lack of inclusion of several clinicopathological parameters in the nomogram, such as lymphovascular invasion (LVI), perineural invasion (PNI), and extramural venous invasion (EMVI). Finally, although the nomogram was validated internally by 1000 bootstrap resampling, further external validation on an independent dataset is warranted before its public application. Given these limitations, our study still provides a prediction model to help clinicians identify aggressive subgroups of ypN+ rectal cancer after nCRT.

In conclusion, the present study demonstrated that NLN count was an independent prognostic factor of DFS in ypN+ rectal cancer patients. A predictive nomogram incorporating NLN count, IMA nodal metastasis, tumor differentiation, and ypT stage could be helpful during clinical decision-making.

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Author Contribution Yanwu Sun and Pan Chi designed the study. Yanwu Sun, Yiyi Zhang, and Zhekun Huang collected the data and were major contributors in writing the manuscript. All the authors read and approved the final manuscript.

Compliance with Ethical Standards

This study was performed according to the ethical principles described in the Declaration of Helsinki. The institutional review board (IRB) of our hospital approved this study.

Conflict of Interest The authors declare that they have no conflict of interest.

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