Retrospective Cohort Study

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Finding the minimum number of retrieved lymph nodes in node-negative colorectal cancer using Real-world Data and the SEER database

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Background: Current clinical guidelines recommend the removal of at least 12 lymph nodes (LNs) in resectable colorectal cancer (CRC). With advancements in lymphadenectomy technologies, the number of retrieved lymph nodes (rLNs) has markedly increased. This study aimed to investigate the lowest number of rLNs in node-negative patients.

Materials and Methods: A total of 1103 N0 and 208 N1a stage patients were enrolled in our cohort, while 8503 N0 and 1276 N1a patients from the Surveillance, Epidemiology, and End Results CRC database were included. Propensity score matching and multivariate Cox regression analyses were performed to mitigate the influence of selection bias and control for potential confounding variables.

Results: The median number of rLNs in N0 patients increased from 13.5 (interquartile range [IQR]: 9–18) in 2013 to 17 (IQR: 15–20) in 2019. The restrictive cubic spline illustrated a nonlinear relationship between rLNs and prognosis (nonlinearity, $P = 0.009$), with a threshold ($N = 16$) influencing clinical outcomes. Patients at either N0 or N1a stage with sufficient rLNs (≥ 16) demonstrated superior prognoses to those with a limited rLNs (< 16). After adjusting for clinical confounders, similar prognoses were observed in N0 limited and N1a adequate populations. Furthermore, Kaplan–Meier curves revealed that N0 limited patients who received chemotherapy exhibited better outcomes than those who did not.

Conclusions: Among patients with node-negative CRC, it is crucial to remove 16 or more LNs effectively. Fewer than 16 rLNs should be regarded as an independent risk factor, implying the need for adjuvant chemotherapy.

Keywords: adjuvant chemotherapy, colorectal cancer, propensity score matching, restrictive cubic spline, retrieved lymph nodes

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, causing 900 000 deaths annually[1]. For patients who have undergone curative resection, the accurate identification of lymph node metastasis (LNM) is crucial for prognostic evaluation and treatment decision-making[2]. An adequate number of resected lymph nodes (LNs) is needed to properly assess the regional LN status.

Currently, clinical guidelines recommend at least 12 negative LNs to confirm the absence of nodal spread[3]. This can be derived from the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) staging manuals from 1997[4] and the National Cancer Institute (NCI) from 2001[5]. This recommendation was quickly adopted as a measure of high-quality AJCC and the National Quality Forum. However, the minimum number of retrieved LNs (rLNs) needed to reliably define LNM status remains controversial, with reported threshold values of

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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13[6], 14[7,8], 17[9], and 18[10]. These recommendations are only suitable for certain populations, and most recommendations have been developed before 2010 and therefore need updating.

Importantly, comprehensive harvesting of LNs in CRC cases enhances the precision of clinical staging, ultimately improving prognosis[10–15]. Advances in lymphadenectomy have increased the LN yield[16,17]. A recent study by Wang *et al*.[18] demonstrated that the median rLNs were 17 for their cohort and 18 for the Surveillance, Epidemiology, and End Results CRC (SEER) cohort. Additionally, for patients with node-negative disease (N0 or TNM stage I–II), the incidence of LNM mainly determines the N stage and informs the decision regarding the necessity for further adjuvant chemotherapy (ACT).

Therefore, the minimum number of rLNs should be further investigated and updated, particularly in the node-negative populations. This study aimed to explore the minimum number of rLNs and to investigate whether limited rLNs could be an independent factor for the N0 patients.

HIGHLIGHTS

- Median retrieved lymph nodes (rLNs) have been greatly increasing in recent years.
- We defined the least rLNs is 16 for node-negative colorectal cancer.
- Patients with adequate rLNs had superior prognoses than patients with limited.
- Similar prognoses were observed in the N0 limited and N1a adequate populations.
- N0 limited patients could benefit from the adjuvant chemotherapy.

Methods

Ethics

This study was approved by the Medical Ethics Committee of First Affiliated Hospital of Air Force Medical University (Xijing Hospital) (No. KY20112170-C-1) in 2021. The study was approved by the Research Registry platform (https://www.researchregistry.com/browse-the-registry, registration UIN: researchregistry9398) and the ChiCTR platform (https://www.chictr.org.cn/searchprojEN.html, registration number: ChiCTR2300070629). This study was performed in line with the STROCSS criteria[19] (Supplemental Digital Content 1, http://links.lww.com/JS9/B105).

Study design and patients

This retrospective cohort study was performed using data from the Xijing Hospital of Digestive Diseases, Air Force Medical University (XJCRC), and the SEER cohort (https://seer.cancer.gov). To identify the minimum number of rLNs in N0 patients and compare the prognostic difference between N0 and N1a patients, we enrolled N0-1a CRC patients in the XJCRC cohort between December 2013 and December 2019 and N0-1a patients in the SEER cohort between 2010 and 2015 who had detailed information on the 7th Derived AJCC TNM stage. The inclusion criteria were as follows: 1) diagnosis of N0 or N1a CRC, 2) available rLNs and more than one node, 3) T1-4 stage and M0 stage, and 4) complete follow-up information. The exclusion criteria were as follows: 1) age less than 18 years; 2) less than 1 month of follow-up; 3) incomplete clinical and histological factors such as microsatellite instability (MSI), tumor biomarkers, and tumor size; 4) diagnosis of other cancers; 5) positive circumference of the resection margin, bowel margin, or anal margin; 6) multiple primary tumors; 7) unavailable ACT or neoadjuvant chemotherapy (neo-ACT); 8) unavailable tumor site; and 9) preoperative or postoperative radiation.

Next, a restrictive cubic spline (RCS) function was applied to present linear or nonlinear prognostic profiles of rLNs and to identify the optimal cutoff for the number of LN in N0 patients. We then validated the prognostic value of the optimal cutoff in N0 and N1a patients. Patients whose rLNs were higher than the optimal cutoff were considered to have an adequate number or, in the opposite circumstance, limited. Finally, we compared the survival difference between N0 with limited rLNs (N0 limited) and N1a with adequate rLNs (N1a adequate) patients in the two independent cohorts and performed propensity score matching (PSM) adjustment to minimize potential confounders. The study flowchart is shown in Figure 1.

Data collection and follow-up

Clinical variables, including sex, age, TNM stage, tumor size, tumor site, neo-ACT, ACT, and MSI status, were collected from electronic medical records, as previously reported[20,21]. Peripheral venous blood was obtained from the patients at 6 AM, before any treatment was initiated. Serum levels of tumor biomarkers, such as carcinoembryonic antigen (CEA), cancer antigen (CA)125, CA199, and alpha-fetoprotein (AFP), were detected using a Cobas 8000 Analyzer (Roche Diagnostics). Tumor site and operation type are strongly associated with rLNs[22]. In our cohort, the tumor sites were divided into three categories: right colon, left colon, rectal, and sigmoid colon, and the operation types were divided into right-sided colectomy, left-sided colectomy, and rectal resection.

In the SEER cohort, the primary tumor sites were divided into three: the right colon (C18.0, C18.1, C18.2, C18.3, and C18.4), the left colon (C18.5, C18.6, and C18.7), and the rectum (C19.9 and C20.9)[18]. The pathological tumor stage of the SEER cohort was characterized according to the 7th edition of the AJCC on Cancer TNM staging system. Regarding ACT information, patients at high-risk of stage II and N1a disease were administered standard chemotherapy regimens, including combination oxaliplatin and capecitabine; combination 5-fluorouracil, leucovorin, and oxaliplatin; and other regimens. Patients with lower rectal cancer accompanied with advanced T stage or a strong desire for anal preservation preferred preoperative ACT, including the folinic acid, fluorouracil, and oxaliplatin/oxaliplatin and capecitabine regimen, spanning a period of 2–3 months.

The primary endpoint was overall survival (OS), calculated from CRC diagnosis to all-cause mortality. Follow-up information was derived from a specialist in our team and updated every 6 months after surgery. Patients who survived until the last follow-up date (December 2021) or were lost to follow-up were excluded. The OS of the SEER cohort was defined using the SEER vital status recode and survival time in the SEER registry[23].

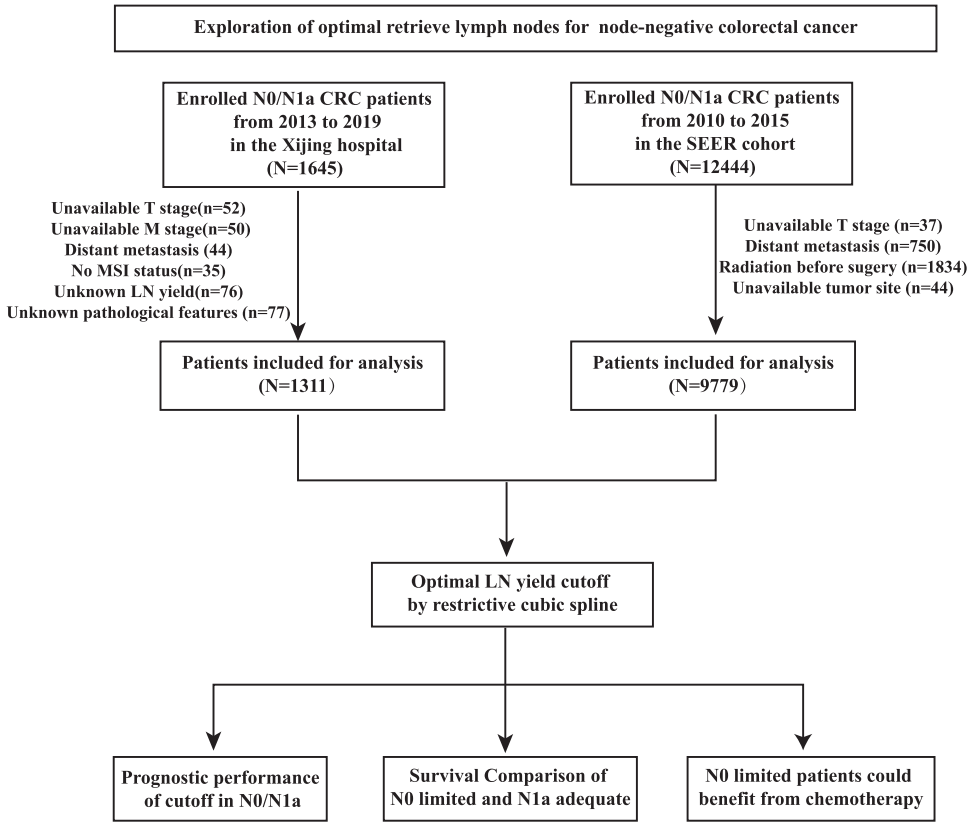

Figure 1. Flowchart of the study. SEER, Surveillance, Epidemiology, and End Results.

Propensity score matching

The PSM method was used to reduce the effect of selection bias and adjust for potential confounding factors such as clinical stage, tumor site, neo-ACT[24], and tumor size[25]. Propensity scores were derived by fitting a logistic regression model based on age, sex, clinical T stage, clinical M stage, tumor size, neo-ACT, ACT, tumor site, and MSI status. The two groups were matched with a caliper width of 0.02, and a ratio of 1:1 nearest neighbor matching without replacement was used with the 'MatchIt' package in R software.

Statistical analysis

All statistical analyses were conducted using R software (R version 3.63, https://www.r-project.org/). Categorical variables were expressed as frequencies and percentages and compared using the Fisher's exact test or χ^2 test. Normally distributed continuous variables were presented as means with a SD and validated using Student's *t*-test, while non-normally distributed variables were expressed as medians with an interquartile range (IQR) and compared using the nonparametric Mann–Whitney *U* test. Survival curves were generated using the Kaplan–Meier (K-M) method and compared using the log-rank test. Multivariate Cox proportional hazards regression analysis was used to mitigate confounding biases and adjust the parameters. R-related packages (survival, rms, survminer, compareGroups, dplyr, and ggplot2) were used to conduct statistical analyses and plot figures. Differences were considered statistically significant at a two-sided *P*-value of <0.05.

Results

Clinicodemographic patient characteristics

Among the 1645 patients diagnosed with N0 or N1a disease between December 2013 and December 2019 in the XJCRC cohort, 1311 patients, including 1103 N0 patients and 208 N1a patients, were eligible for analysis (Fig. 1). The proportions of males in the N0 and N1a groups were 60.0 and 61.5%, respectively. The median age at diagnosis was 61 years for both patients with N0 and with N1a disease. As expected, there were significant disparities in T stage ($P = 0.001$) between N0 and N1a patients, among which advanced T (T3–T4) was observed in patients with N1a disease.

Additionally, N1a patients had a lower prevalence of MSI-high (MSI-H) status (6.7 vs. 15.0%, $P = 0.002$) and a higher prevalence of microvascular invasion (CD34, 60.6 vs. 20.4%, $P < 0.001$) and lymphatic vessel invasion (D240, 59.6 vs. 19.9%, $P < 0.001$) than patients with N0 disease. There were no significant differences in sex, age, largest tumor size, tumor site, perineural invasion (S100), or tumor biomarkers (CEA, CA199, CA125, and AFP) between the two groups. For adjuvant therapy, all N1a patients underwent ACT, whereas only 65.5% of patients underwent ACT alone ($P < 0.001$). Regarding neo-ACT, a higher percentage of N1a patients underwent neo-ACT than N0 patients (16.3 vs. 12.5%). The other basic clinical characteristics of the N0 and N1a cohorts are shown in Table 1.

The SEER cohort involved 9779 patients, including 8503 N0 patients and 1276 N1a patients (Fig. 1 and Table 2). Consistent

Table 1

Basic clinical parameters of CRC patients in the Xijing hospital.

Characteristics	N0 cohort $N=1103$	N1a cohort $N=208$	P
Sex			0.739
Female	441 (40.0%)	80 (38.5%)	
Male	662 (60.0%)	128 (61.5%)	
Age (years)	61.0 [52.0;70.0]	61.0 [53.0;69.0]	0.672
T stage			0.001
T1	72 (6.53%)	6 (2.9%)	
T2	258 (23.4%)	31 (14.9%)	
T3	684 (62.0%)	142 (68.3%)	
T4	89 (8.07%)	29 (13.9%)	
Largest tumor size (cm)	4.00 [3.00;5.00]	4.00 [3.00;5.00]	0.349
ACT			< 0.001
No	380 (34.5%)	0 (0.00%)	
Yes	723 (65.5%)	208 (100%)	
neo-ACT			0.164
No	965 (87.5%)	174 (83.7%)	
Yes	138 (12.5%)	34 (16.3%)	
Low rectal			0.385
No	925 (83.9%)	180 (86.5%)	
Yes	178 (16.1%)	28 (13.5%)	
Tumor site			0.879
Left colon	60 (5.5%)	12 (5.7%)	
Rectal or sigmoid	798 (72.3%)	153 (73.6%)	
Right colon	245 (22.2%)	43 (20.7%)	
CEA (ng/ml)	2.28 [1.49;3.46]	2.33 [1.58;3.92]	0.123
CA199 (U/ml)	11.2 [7.34;19.3]	11.7 [8.22;21.2]	0.106
CA125 (U/ml)	10.7 [7.77;14.8]	10.6 [7.82;16.4]	0.454
AFP (ng/ml)	2.58 [1.94;3.67]	2.77 [2.07;3.79]	0.102
MSI			0.002
MSI-L/MSS	938 (85.0%)	194 (93.3%)	
MSI-H	165 (15.0%)	14 (6.7%)	
S100			0.065
Negative	264 (23.9%)	37 (17.8%)	
Positive	839 (76.1%)	171 (82.2%)	
CD34			< 0.001
Negative	878 (79.6%)	82 (39.4%)	
Positive	225 (20.4%)	126 (60.6%)	
D240			< 0.001
Negative	883 (80.1%)	84 (40.4%)	
Positive	220 (19.9%)	124 (59.6%)	

ACT, adjuvant chemotherapy; CRC, colorectal cancer; MSI, microsatellite instability; neo-ACT, neoadjuvant chemotherapy.

Table 2

Basic clinical parameters of CRC patients in the SEER cohort.

	N0 $N=8503$	N1a $N=1276$	P
Age	69.0 [59.0;79.0]	69.0 [58.0;79.0]	0.64
Race			0.016
White	6669 (78.4%)	964 (75.5%)	
Black	958 (11.3%)	158 (12.4%)	
Other	833 (9.80%)	152 (11.9%)	
Unknown	43 (0.51%)	2 (0.16%)	
Sex			0.094
Female	4081 (48.0%)	645 (50.5%)	
Male	4422 (52.0%)	631 (49.5%)	
Diagnosis year			0.023
2010	1773 (20.9%)	250 (19.6%)	
2011	1597 (18.8%)	219 (17.2%)	
2012	1428 (16.8%)	216 (16.9%)	
2013	1268 (14.9%)	194 (15.2%)	
2014	1283 (15.1%)	179 (14.0%)	
2015	1154 (13.6%)	218 (17.1%)	
Tumor site			0.007
Left colon	3127 (36.8%)	519 (40.7%)	
Rectal	1297 (15.3%)	162 (12.7%)	
Right colon	4079 (48.0%)	595 (46.6%)	
Grade			< 0.001
Grade I	1094 (12.9%)	62 (4.9%)	
Grade II	5813 (68.4%)	960 (75.2%)	
Grade III	782 (9.2%)	180 (14.1%)	
Grade IV	157 (1.9%)	41 (3.2%)	
Unknown	657 (7.6%)	33 (2.6%)	
T stage			< 0.001
T1	2846 (33.5%)	168 (13.2%)	
T2	1696 (19.9%)	202 (15.8%)	
T3	3379 (39.7%)	727 (57.0%)	
T4	582 (6.9%)	179 (14.0%)	
ACT			< 0.001
No	7778 (91.5%)	605 (47.4%)	
Yes	725 (8.5%)	671 (52.6%)	

ACT, adjuvant chemotherapy; SEER, Surveillance, Epidemiology, and End Results.

with the XJCRC cohort, N1a patients in the SEER cohort had more advanced tumor clinical characteristics, such as a higher proportion of T3–4 stage (T3: 57.0 vs. 39.7%; T4: 14.0 vs. 6.9%) and worse tumor differentiation (grade III: 14.1 vs. 9.2%; grade IV: 3.2 vs. 1.9%) than N0 patients. In the context of adjuvant therapy, the proportion of patients who underwent ACT was higher in those with N1a than in those with N0 disease (52.6 vs. 8.5%, $P<0.001$). The detailed clinical features of the SEER cohort are presented in Table 2.

Increased rLNs in routine clinical settings

The median number of rLNs was 17 (IQR: 14–20) for N0 patients and 17 (IQR: 14–19) for N1a patients. The median rLNs of N0 patients had increased in recent years, from 13.5 (IQR: 9–18) in 2013 to 17 (IQR: 15–20) in 2019. Similarly, the median rLNs of N1a patients also increased from 14 (IQR: 12–19) in 2014 to 17 (IQR: 15–19) in 2019. A scatterplot of the rLNs in both the N0 and N1a populations is shown in Figure 2. The median rLNs for the right colon, left colon, and rectum were 19, 16.5, and 16, respectively ($P<0.001$), demonstrating that tumor site was an important confounder for rLNs. Neo-ACT has been reported to affect rLNs during surgery[24]. In our cohort, patients who received neo-ACT had a significantly lower median number of rLNs compared to those who did not (15 vs. 17, $P<0.001$), suggesting that neo-ACT is an inevitable confounder. Therefore, PSM and Cox models were used to balance the differences in tumor site and neo-ACT.

Identification of the optimal cutoff value of LN yield

The K–M survival curve of N0-1a patients is shown in Figure 3A, B. The 1-year, 3-year, and 5-year OS rates were 98.9, 93.7, and 90.3% for N0 patients and 93.1, 82.1, and 75.1% for N1a patients, respectively. In the SEER cohort, the 1-year, 3-year, and 5-year OS rates were 96.1, 95.3, and 93.6% for N0 patients and 94.0, 93.0, and 91.0% for N1a patients, respectively. The K–M curves demonstrated that patients with N0 disease had a more favorable prognosis than the patients with N1a disease before and after PSM (Fig. 3A, B). We found similar prognostic differences between N0 and N1a patients in the SEER cohort

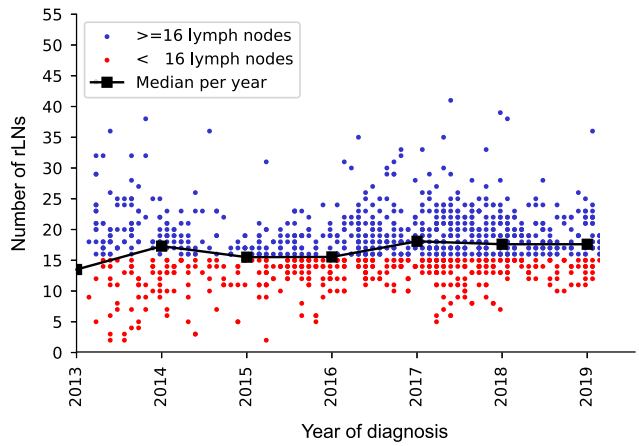

Figure 2. Scatterplot of rLNs in N0 and N1a patients over time. Each point represents one patient from 2013 to 2019; blue dots represent patients with greater than or equal to 16 rLNs, while red dots indicate patients with less than 16 rLNs. rLNs, retrieved Lymph nodes.

(Figure S1A, B, Supplemental Digital Content 2, http://links.lww.com/JS9/B106).

With the advancement of lymphadenectomy technologies and a deeper understanding of the anatomy[26], more rLNs of CRC are required to estimate a more precise N stage and aid clinical decisions[16,26]. Thus, RCS functions were applied to identify the relationship between the LN number and OS. The RCS showed that the LN number presented a nonlinear profile (nonlinearity $P = 0.009$) for the prognosis of CRC, and that there was a cutoff value ($N = 16$) for affecting clinical outcomes, where the hazard ratio (HR) was = 1 (Fig. 3C). Similarly, we investigated the optimal cutoff value in different subgroups, including the neo-ACT and non-neo-ACT subgroups and the left colon, rectal, and right colon subgroups (Figure S2, Supplemental Digital Content 2, http://links.lww.com/JS9/B106). RCS analyses demonstrated 15, 16, 16, and 19 could be optimal rLNs in these four subgroups, respectively (Figure S2A–D, Supplemental Digital Content 2, http://links.lww.com/JS9/B106). The above results suggest that neo-ACT and tumor site are important confounders of LNs. Moreover, these RCSs were similar, and the results demonstrated

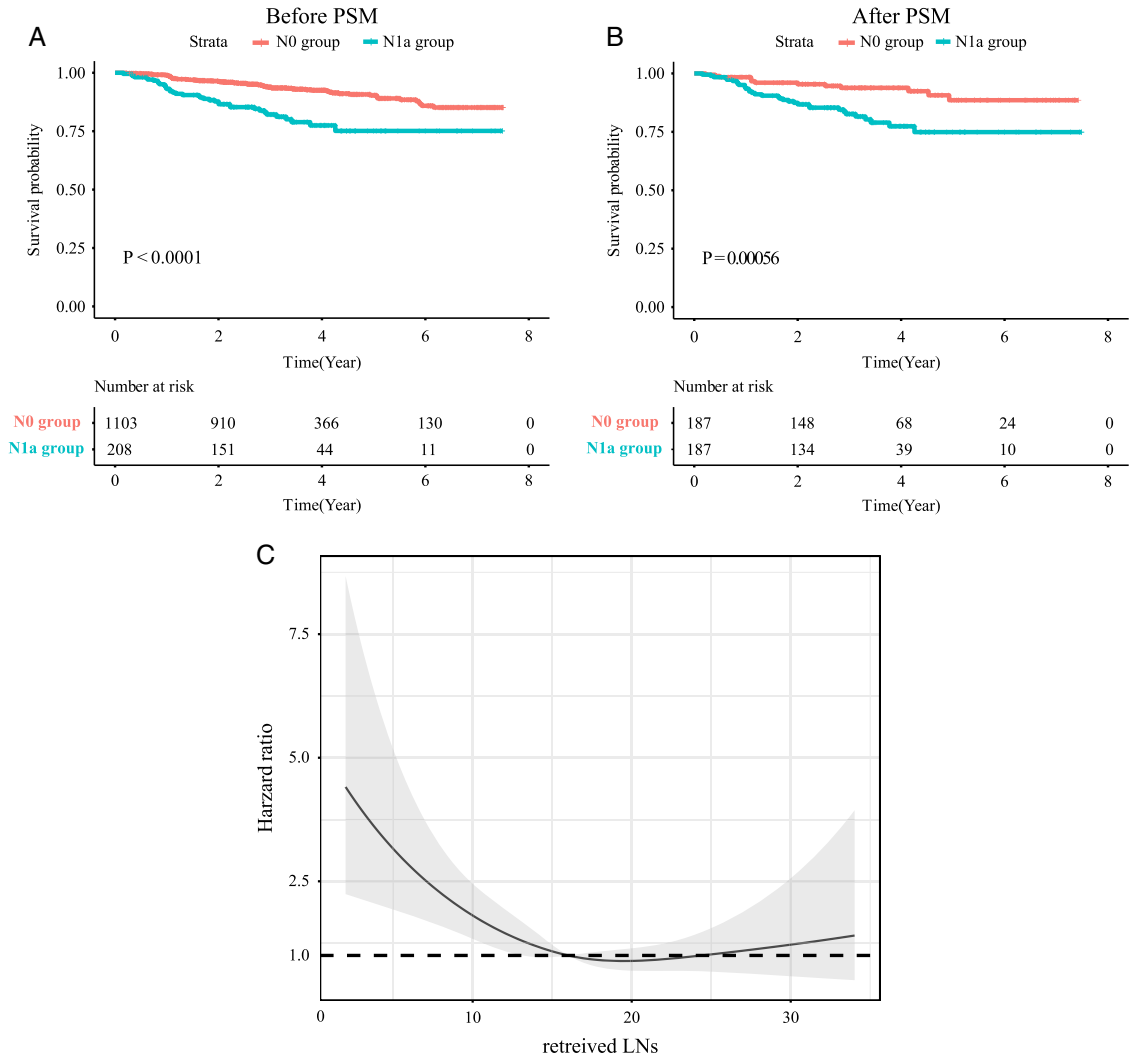

Figure 3. K–M curve and RCS curve associated with rLNs. (A) K–M curve of patients with N0 and N1a before PSM; (B) K–M curve of N0/N1a patients after PSM; (C) Relationship of HR with rLNs. The rLNs approach 16 when HR is 1. K–M, Kaplan–Meier; rLNs, retrieved Lymph nodes; PSM, propensity score matching.

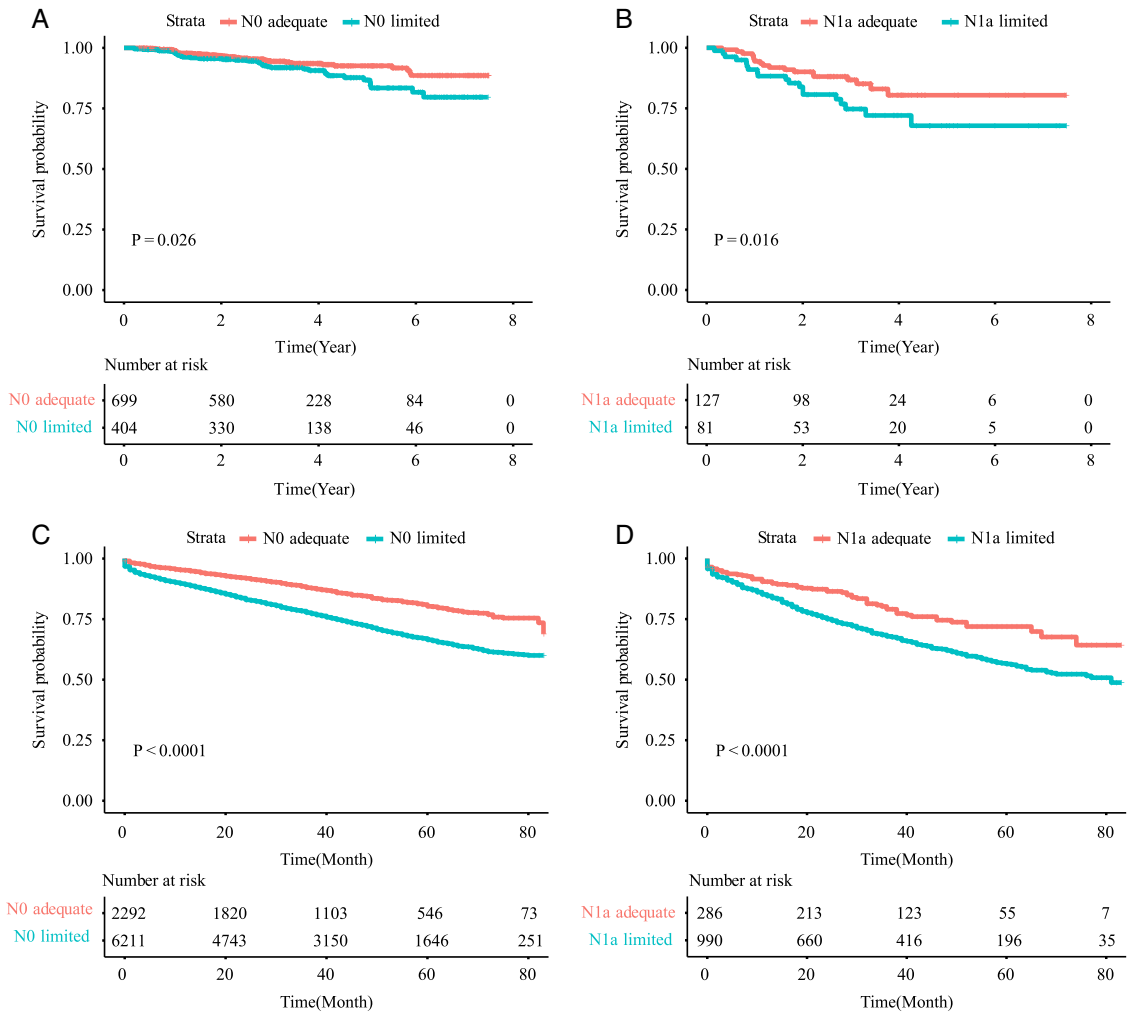

Figure 4. LN yield and prognosis in N0/N1 patients in the XJCRC and SEER cohorts. LN yield (adequate vs. limited) and prognosis in N0 patients (A) and N1a patients (B) in XJCRC cohorts. Prognostic differences of adequate and limited rLNs in N0 patients (C) and N1a patients (D) in the SEER cohort. Adequate population indicates rLNs greater than or equal to 16 while limited patients means rLNs less than 16. rLNs, retrieved Lymph nodes; SEER, Surveillance, Epidemiology, and End Results.

that there could be prognostic differences between populations with rLNs less than 16 (≤ 15) and less than or equal to 16.

LN yield and prognosis in N0/N1a patients

In the XJCRC cohort, among 1103 N0 patients, 404 patients (36.7%) were N0 limited (i.e. <16 rLNs), while 699 patients (63.3%) were N0 adequate (i.e. with ≥ 16 rLNs). Compared with N0 limited patients, N0 adequate patients involved a higher proportion of females (43.5 vs. 33.9%), a greater prevalence of MSI-H (18.2 vs. 9.4%), a higher prevalence of advanced T stage (T3,68.0 vs. 51.7%; T4,8.6 vs. 7.2%), and a lower ratio of positive CD34 (17.3 vs. 25.7%) and D240 (16.7 vs. 25.5%; Table S1, Supplemental Digital Content 2, http://links.lww.com/JS9/B106). As expected, more N0 limited patients underwent neo-ACT (18.3 vs. 9.2%) and ACT (72.5 vs. 61.5%) than N0 adequate patients. The K–M survival curve and log-rank test showed significant differences in clinical outcomes between N0 limited and N0 adequate populations ($P = 0.026$; Fig. 4A). After adjustment for clinical confounders (neo-ACT, ACT, sex, age, T stage, largest tumor size, MSI, CD34, and D240 status; Table S1, Supplemental Digital Content 2, http://links.lww.com/JS9/B106), rLNs less than or equal to 16 remained associated with a better OS than rLNs less than 16 ($P = 0.019$; Figure S3A, Supplemental Digital Content 2, http://links.lww.com/JS9/B106). These findings demonstrate that among N0 patients, those with rLNs less than 16 had a less satisfactory prognosis than those with rLNs greater than or equal to 16.

Of the 208 N1a patients, 127 (61.1%) had N1a limited (i.e. <16 rLNs), while 81 (38.9%) had N1a adequate (i.e. ≥ 16 rLNs). Compared to N1a limited patients, N1a adequate patients had an earlier T stage (T1–T2:23.4 vs. 14.1%), more right colon involvement (13.6 vs. 25.2%), and smaller tumor size [3.50 (2.50–4.50) cm vs. 4.00 (3.00–5.00) cm; Table S2, Supplemental Digital Content 2, http://links.lww.com/JS9/B106]. Survival analysis demonstrated that N1a adequate patients had better clinical outcomes than N1a limited patients when 16 rLNs was used as the cutoff point ($P = 0.016$; Fig. 4B). Additionally, after adjusting for clinical confounders (neo-ACT, T stage, tumor size, and tumor site; Table S2, Supplemental Digital Content 2, http://

links.lww.com/JS9/B106), the N1a limited group still had a worse prognosis than the N1a adequate group ($P = 0.011$; Figure S3B, Supplemental Digital Content 2, http://links.lww.com/JS9/B106).

In the SEER cohort, 2292 (27.0%) patients had N0 adequate while 6211 (73.0%) patients had N0 limited (Table S3, Supplemental Digital Content 2, http://links.lww.com/JS9/B106). Compared to the N0 limited group, the N0 adequate group was younger at diagnosis (median age at diagnosis: 65 years vs. 70 years) and involved more female patients (51.1 vs. 46.8%). Additionally, this group had a higher proportion of patients with tumors located in the right colon (65.8 vs. 41.4%) and an advanced T stage (T3–4:67 vs. 39%). Survival analysis revealed that N0 adequate patients had a better prognosis than N0 limited patients ($P < 0.001$; Fig. 4C). After adjustment for clinical confounders (sex, grade, T stage, tumor site, and ACT), the prognostic differences between the N0 limited and adequate populations were similar (Table S3, Supplemental Digital Content 2, http://links.lww.com/JS9/B106; Figure S3C, Supplemental Digital Content 2, http://links.lww.com/JS9/B106).

Among the N1a patients, 286 (22.4%) were in the N1a adequate group, while 990 (77.6%) were in the N1a limited group. The N1a limited group exhibited a propensity for older age and tumor localization in the left colon or rectum than the N1a adequate group (Table S4, Supplemental Digital Content 2, http://links.lww.com/JS9/B106). The survival curve demonstrated that the prognosis of the N1a limited group was worse than that of the N1a adequate group (Fig. 4D). After adjusting for clinical confounders (tumor site, T stage, and ACT) using PSM, the K–M curve revealed similar results ($P = 0.0018$; Figure S3D, Supplemental Digital Content 2, http://links.lww.com/JS9/B106).

In addition to the PSM method and survival curve, we also conducted a multivariate Cox analysis to adjust for well-established factors that could affect prognosis or rLNs. We created five types of models to adjust for confounders: nonadjusted, T stage-adjusted, neo-ACT-adjusted, tumor site-adjusted, and fully adjusted. In the XJCRC cohort, N0 limited patients had worse clinical outcomes than N0 adequate patients after adjusting for T stage, neo-ACT, and tumor site (all HRs > 1). Similarly, N0 limited was identified as a risk factor in the SEER cohort after adjustment for T stage and tumor site adjustment (all HRs > 1). Among the N1a patients in both the XJCRC and SEER cohorts, N1a limited was an independent prognostic factor after adjusting for T stage and tumor site (all HRs > 1; Table 3). Similar results were obtained after adjusting for all confounders: N0/N1a limited patients experienced worse adverse clinical outcomes than N0/N1a adequate patients.

In summary, regardless of the N0 or N1a classification, 16 rLNs classified the patients into distinct risk categories. Thus, it is a favorable threshold for determining the N stage in clinical settings.

Similar prognosis of N0 limited with N1a adequate

Inspired by a new strategy for N stage advancement and a modified nodal classification and staging system[27], we investigated the survival difference between the N0 limited and N1a adequate populations. Compared with the N0 limited population, the N1a adequate population had more advanced T stages (T3: 76.4 vs. 51.7%, T4: 9.4 vs. 7.2%), larger tumor size [4.00 (3.00–5.00) vs. 3.50 (2.50–4.50) cm], a greater proportion of positive CD34 (56.7 vs. 25.7%) and positive D240 (55.9 vs. 25.5%), and more right-sided colon cancer (25.2 vs. 9.2%) (Table S5, Supplemental Digital Content 2, http://links.lww.com/JS9/B106), and OS was only slightly different ($P = 0.043$, Fig. 5A). This phenomenon could by the higher number of N0 limited patients who accepted neo-ACT than N1 adequate patients (18.3 vs. 10.2%).

Table 3

Association of limited rLNs with OS in the two cohorts.

Group	Adjustment	HR (95% CI)	P
N0 limited for XJCRC cohort[a]	Nonadjusted	1.63 (1.06–2.52)	0.03
	T stage-adjusted	1.84 (1.18–2.86)	0.01
	neo-ACT-adjusted	1.66 (1.07–2.57)	0.02
	Tumor site-adjusted	1.75 (1.11–2.77)	0.02
	Fully-adjusted[b]	3.38 (1.80–6.34)	0.00
N1a limited for XJCRC cohort[c]	Nonadjusted	1.85 (1.01–3.40)	0.04
	T stage-adjusted	1.85 (0.97–3.54)	0.06
	neo-ACT-adjusted	1.67 (0.86–3.22)	0.13
	Tumor site-adjusted	1.69 (0.87–3.27)	0.12
	Fully-adjusted[b]	1.98 (1.00–3.95)	0.05
N0 limited for SEER cohort[a]	Nonadjusted	1.92 (1.71–2.16)	0.00
	T stage-adjusted	2.46 (2.18–2.77)	0.00
	Tumor site-adjusted	2.04 (1.82–2.30)	0.00
	Fully-adjusted[d]	1.86 (1.65–2.10)	0.00
N1a limited for SEER cohort[c]	Nonadjusted	1.69 (1.29–2.20)	0.00
	T stage-adjusted	1.86 (1.42–2.43)	0.00
	Tumor site-adjusted	1.72 (1.31–2.26)	0.00
	Fully-adjusted[d]	1.58 (1.20–2.09)	0.00

[a]N0 adequate as reference.
[b]Fully adjusted by T stage + largest tumor size + ACT + CEA + CA199 + AFP + MSI + tumor site.
[c]N1a adequate as reference.
[d]Fully adjusted by chemotherapy + T stage + Grade + race + tumor site.
ACT, adjuvant chemotherapy; HR, hazard ratio; MSI, microsatellite instability; neo-ACT, neoadjuvant chemotherapy; OS, overall survival; rLNs, retrieved lymph nodes.

After adjusting for clinical confounders (neo-ACT, ACT, T stage, tumor size, CD34, and D240) using PSM, we found favorable homogenization of survival curves between N0 limited and N1a adequate patients, as illustrated in Figure 5B ($P = 0.39$). For the SEER cohort, similar results were observed in the prognosis between N0 limited and N1a adequate groups ($P = 0.23$; Fig. 5C). N1a adequate patients were younger (median age: 67 years vs. 70 years), had more advanced T (T3–4:78 vs. 39%) and worse tumor grade (Grade 3–4: 21.7 vs. 8.8%), and were more inclined to undergo ACT (61.5 vs. 7.3%; Table S6, Supplemental Digital Content 2, http://links.lww.com/JS9/B106). After adjusting for clinical confounders (age, ACT, T stage, grade, race, tumor site, and sex) using PSM (Table S6, Supplemental Digital Content 2, http://links.lww.com/JS9/B106), similar survival outcomes were observed between the N0 limited and N1a adequate groups ($P = 0.4$, Fig. 5D).

Additionally, we conducted multivariate Cox analysis to adjust for potential confounders between the N0 adequate and N1a limited groups (Table 4). In the model adjusted for T-stage and tumor site and in the fully adjusted model, the prognosis of the N1a adequate groups was not significantly different with that of the N0 limited group as the reference ($P > 0.05$). However, in the nonadjusted and ACT-adjusted models, N1a adequate patients had slightly worse clinical outcomes than N0 limited patients (HR > 1, $P < 0.05$). In the SEER cohort, only the T stage-adjusted model indicated that N0 limited patients had worse outcomes than N1a adequate

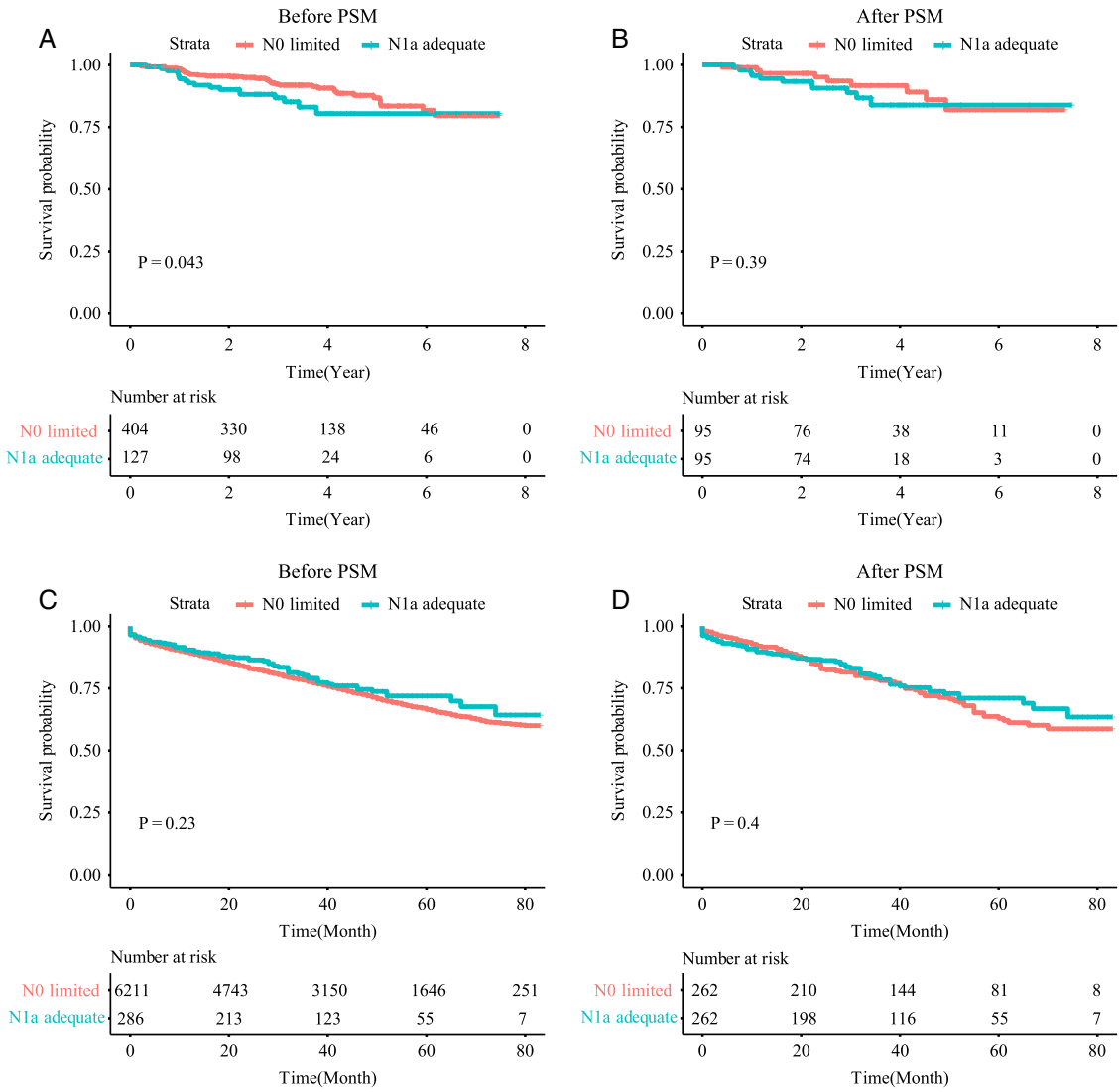

Figure 5. Similar prognosis between N0 limited and N1a adequate in the XJCRC and SEER cohorts. K–M curves between N0 limited and N1a adequate patients before PSM (A) and after PSM (B) in the XJCRC cohort, and before PSM (C) and after PSM (D) in the SEER cohort. SEER, Surveillance, Epidemiology, and End Results; K–M: Kaplan–Meier; PSM, propensity score matching.

patients. Meanwhile, the nonadjusted model, model adjusted for ACT and tumor site, and fully adjusted model demonstrated no significant differences between the two groups ($P > 0.05$). These results indicate that N0 limited patients have a similar prognosis to N1a adequate patients.

Necessity of ACT for N0 limited patients

The latest clinical guidelines indicate that N1a patients are recommended for systematic or combined ACT, whereas N0 patients, barring the presence of high-risk factors, may not require ACT in their treatment regimens[2]. Based on these findings, we investigated the necessity of administering ACT to N0 limited patients. In the XJCRC cohort, 293 patients underwent ACT (ACT group) and 111 did not (non-ACT group). The T stage, neo-ACT, and tumor size differed between the ACT and non-ACT groups. After adjusting for these factors (T stage, neo-ACT, and tumor size) using PSM, no significant differences were observed between the two groups (Table S7, Supplemental Digital Content 2, http://links.lww.com/JS9/B106). The survival curves indicated that the ACT group had more satisfactory outcomes than the non-ACT group, before and after adjustment (Fig. 6A, B). In the SEER cohort, the disparity in survival outcomes between the two groups was notably pronounced. Before PSM, the ACT group had more malignant biological behavior, including advanced T stage (T3–4 patients: 89.2 vs. 35.1%) and poorer levels of cellular differentiation (Grade 3–4: 13.5 vs. 8.4%; Table S8, Supplemental Digital Content 2, http://links.lww.com/JS9/B106). Although there was considerable divergence between the ACT and non-ACT groups, the survival curves showed that the ACT group had a longer survival time than the non-ACT group (Fig. 6C). After adjustment for T stage and tumor grade (Table S8, Supplemental Digital Content 2, http://links.lww.com/JS9/B106), the prognostic disparities

Table 4

Prognostic differences of N0 limited and N1a adequate in the two cohorts.

Group	Adjustment	HR (95% CI)	*P*
N1a adequate for XJCRC cohort[a]	Nonadjusted	1.77 (1.01–3.12)	0.04
	T stage-adjusted	1.37 (0.77–2.43)	0.28
	ACT-adjusted	2.37 (1.26–4.46)	0.01
	neo-ACT-adjusted	1.81 (1.03–3.20)	0.04
	Tumor site-adjusted	1.64 (0.91–2.96)	0.10
	Fully-adjusted[b]	1.86 (0.95–3.64)	0.07
N1a adequate for SEER Cohort[a]	Nonadjusted	0.86 (0.66–1.12)	0.23
	T stage-adjusted	0.60 (0.46–0.77)	0.00
	ACT-adjusted	1.05 (0.80–1.37)	0.74
	Tumor site-adjusted	0.80 (0.62–1.02)	0.08
	Fully-adjusted[c]	0.80 (0.62–1.04)	0.10

[a]N0 limited as reference.
[b]fully adjusted by T stage + largest tumor size + ACT + CEA + CA199 + AFP + MSI + tumor site + neoACT.
[c]fully adjusted by ACT + T stage + Grade + race + tumor site.
ACT, adjuvant chemotherapy; HR, hazard ratio; neo-ACT, neoadjuvant chemotherapy.

between the two groups became more pronounced, as evidenced by the K–M curves (Fig. 6D).

Multivariate Cox analysis was performed to adjust for potential confounders, including T stage, neo-ACT score, tumor site, and grade. For the XJCRC cohort, the HR of the ACT group was less than 1 after adjusting for T stage, neo-ACT, tumor site, and grade, and all confounders in the non-ACT group were regarded as references ($P < 0.05$, Table 5). Similar results were observed in the SEER cohort, with N0 limited patients benefiting from ACT (all HRs <1, $P < 0.05$; Table 5).

Discussion

Similar to the introduction of the AJCC/UICC staging manuals in 1997[4], the latest clinical guidelines still suggest that patients should have at least 12 rLNs to reliably define lymph node status, and patients should be re-examined if there are fewer than 12 rLNs[2,28]). However, this should be updated with the significant

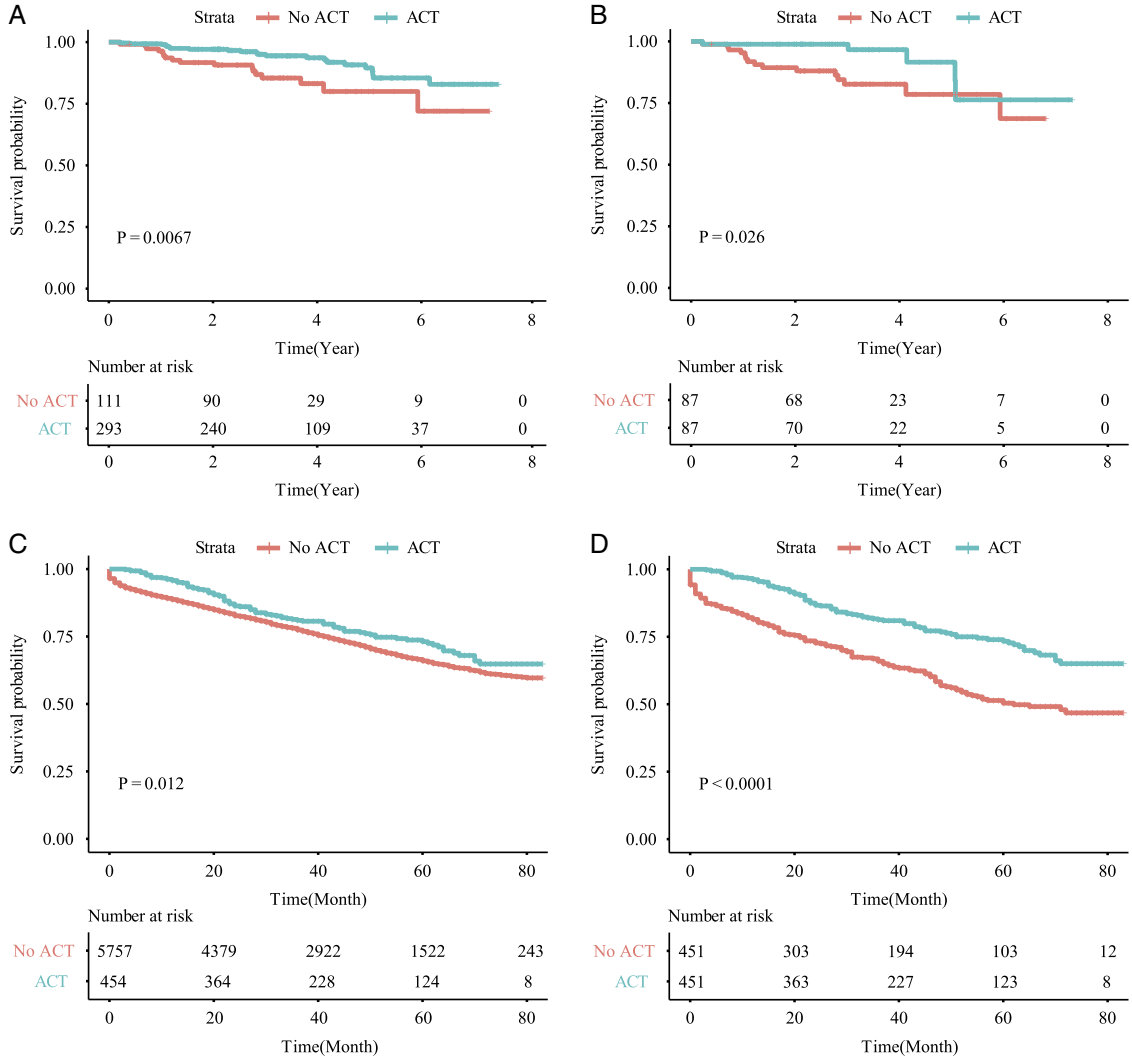

Figure 6. Prognostic differences between ACT and non-ACT groups in the N0 limited population of XJCRC and SEER cohorts. (A-B) K–M curves of ACT and non-ACT before (A) and after PSM (B) in the XJCRC cohort. (C–D) K–M curves of ACT and non-ACT before (C) and after PSM (D) in the SEER cohort. ACT, adjuvant chemotherapy; SEER, Surveillance, Epidemiology, and End Results; K–M, Kaplan–Meier; PSM, propensity score matching.

Table 5

Multivariate cox analysis of ACT in the N0 limited.

Group	Adjustment	HR (95% CI)	P
ACT for XJCRC cohort[a]	Nonadjusted	0.43 (0.22–0.80)	0.01
	T stage-adjusted	0.50 (0.26–0.94)	0.03
	neo-ACT-adjusted	0.36 (0.19–0.70)	0.00
	Tumor site-adjusted	0.43 (0.22–0.82)	0.01
	Fully-adjusted[b]	0.39 (0.19–0.77)	0.00
ACT for SEER Cohort[a]	Nonadjusted	0.78 (0.64–0.95)	0.01
	T stage-adjusted	0.43 (0.35–0.53)	0.00
	Grade adjusted	0.72 (0.59–0.87)	0.00
	Tumor site-adjusted	0.77 (0.64–0.95)	0.01
	Fully-adjusted[c]	0.43 (0.35–0.52)	0.00

[a]Non-ACT as reference.
[b]Fully adjusted by T stage + max tumor_size + CEA + CA199 + AFP + MSI + tumor site + CD34 + S100 + D240 + neoACT.
[c]Fully adjusted by T stage + Grade + race + tumor site.
ACT, adjuvant chemotherapy; HR, hazard ratio; MSI, microsatellite instability; neo-ACT, neoadjuvant chemotherapy.

increase in rLNs in the CRC. Our cohort included a large number of N0 patients ($N = 1103$), and the median rLNs varied from 13.5 to 17 in patients sampled from 2013 to 2019. We suggested that the minimum number of rLNs should be 16 for N0 patients and similar results were observed in the SEER cohort included in this study.

In N0 or N1a patients, those with greater than or equal to 16 rLNs (adequate group) had better OS than those with 1–15 rLNs (limited group). After adjusting for potential confounders, the limited group still had a worse prognosis. Interestingly, the probability of MSI-H was higher in the N0 adequate population than in the N0 limited population, which could explain the better prognosis in N0 adequate patients, consistent with recent findings[29–31]. The ratio of positive CD34 (microvessel density marker, MVD) and D240 (lymphatic vessel marker) was significantly lower in the N0 adequate patients than in their N0 limited counterparts. MVD is a possible predictor of LNM in patients with CRC[31]. Another study showed that tube-like structures co-expressing D240 and CD34 were often observed at the junctions between preinvasive and invasive CRC[32]. Lymphatic vessels (LV and D240 positive) have been found even in the mucosa of in situ CRC, indicating the presence of a low tumor burden in regional LNs[33]. We also observed that N0 limited patients had similar survival rates to N1a adequate patients in both the XJCRC and SEER cohorts, which were still significant after adjusting for basic factors.

ACT and neo-ACT are crucial to CRC prognosis, and neo-ACT can affect the number of harvested LNs[34,35]. In the XJCRC cohort, 12.5% of the N0 group and 16.3% of the N1a group received neo-ACT. Consistent with previous findings[17], the median number of rLNs in this study was significantly different from those who received neo-ACT (median: 15; average: 16.4) than in those who did not receive neo-ACT. In the RCS subgroup, neo-ACT had an optimal cutoff ($N = 15$), with significant prognostic differences between patients with less than 15 and with greater than 15 neo-ACT. The patients who underwent neo-ACT had at least 16 rLNs.

Tumor site or location is an irreplaceable predictor of rLNs and clinical prognosis[14,36]. In the XJCRC cohort, patients with right-sided tumors had more rLNs than patients with left-sided tumors (median: 19 vs. 16.5). Further, the optimal cutoff of rLNs for right-sided CRC was 19, consistent with the previous literature[12]. However, right-sided CRC patients always had a worse prognosis than left-sided CRC patients, consistent with a previous report[14]. Therefore, we adjusted for this confounder in the two cohorts, and the results suggested that 16 rLNs is the optimal cutoff for N0 and N1a and that the N0 or N1a limited population had a worse prognosis than their adequate counterparts. These prognostic differences showed that N0 patients could be upstaged to N1a stage disease if they did not have adequate rLNs to identify the LN status, consistent with a previous study that used 12 rLNs as the cutoff[37,38].

Although postoperative chemotherapy is not routinely administered to N0 patients, ACT is more beneficial than no ACT in N0 patients with less than 12 rLNs[39,40]. Hence, we examined whether N0 patients with <16 rLNs would benefit from ACT. Our findings indicated that patients who received ACT exhibited more favorable prognostic outcomes than those who did not. N0 patients with high-risk factors such as MSI low/microsatellite stable, perineural invasion, T4 stage, and vascular invasion need to undergo ACT. Therefore, we adjusted for confounders and found consistent results that N0 limited patients benefitted from ACT. Collectively, these findings support that ACT could prolong the survival of the N0 limited population, thus warranting further investigations.

In a recent study of patients with stage II right-sided colon cancer, compared with the group with greater than or equal to 19 rLNs, the group with fewer rLNs had a significantly worse cancer-specific survival rate and OS[12]. Another study recommended at least 18 rLNs in $T_{2\text{-}4}N_0M_0$ CRC patients. These studies focused on specific CRC patients. In contrast, the current study included patients with stage II ($T_{1\text{-}4}N_0M_0$) CRC and applied the PSM method and multivariate Cox models to balance the confounding factors.

Novel indexes have been designed to evaluate the prognosis of CRC with limited rLNs; these indexes include the lymph node ratio (LNR; i.e. ratio of metastases to rLNs)[41–43] and log odds of positive lymph nodes (LODDS; i.e. the log ratio between the number of positive and negative LNs)[20,44]. The LODDS is more accurate than the LNR for assessing CRC survival[44,45]. Additionally, the LNR is not applicable to N0 patients, whereas the LODDS can stratify the survival of N0 patients. However, both LN indices indicate that patients with more rLNs are precisely diagnosed with their N stage and have satisfactory prognoses. Therefore, the lowest number of rLNs for CRC, especially stage II, should be updated. An older study conducted 10 years ago reported that overall LN yields increased between 2 and 3% annually[36]. Further, based on the minimum of 12 rLNs in 2010, the optimal rLNs could be increased to ~16.

This study had some limitations. First, this was an observational study of a retrospective cohort, although it was one of the largest study cohorts of CRC without LNM. Second, although we minimized the measured confounders as efficiently as possible, there could still be other factors affecting LNM, such as tumor budding[46], tumor burden[47], KRAS mutations[48], and BRAF mutations[49]. Third, the SEER database did not include more detailed information about tumors, such as MSI, CD34, S100, and D240, which could affect the rLNs and CRC prognosis. In addition, the subgroup analysis also had limitations because of the small proportion of the neo-ACT subgroup (12.5%) and right-sided CRC subgroup (22.2%) in N0 patients. The optimal rLNs for patients who undergo neo-ACT and the right-sided CRC population should be investigated. Despite these limitations, this study elucidated the necessity of updating the least number of rLNs for node-negative CRC, which

could guide clinical surgeons and pathologists in resecting sufficient LNs and accurately identifying the LNM status.

Conclusion

At least 16 rLNs are needed to accurately determine LN status. N0 patients with an insufficient number of rLNs may potentially have similar prognoses to N1a patients. Fewer than 16 rLNs could supersede the current benchmark of 12 as an independent risk factor for stages I–II. This suggests that N0 limited patients might need a more assertive therapeutic chemotherapy.

Ethical approval

The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of the Air Force Medical University (No. KY20112170-C-1).

Patient consent

Not applicable.

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Author contribution

L.S. and J.P.L.: designed the study; Y.H.Q., J.Z., and T.H.H.: performed most of the results and completed the manuscript together; X.L.J., K.W., R.J.C., and Y.T.D.: helped with following-up and data analysis. All authors read and approved the final manuscript.

Conflicts of interest disclosures

None declared.

Research registration unique identifying number (UIN)

The study was approved by the Research Registry platform (https://www.researchregistry.com/browse-the-registry, Registration UIN: researchregistry9398) and the ChiCTR platform (https://www.chictr.org.cn/searchprojEN.html, registration number: ChiCTR2300070629).

Guarantor

Li Sun and Jipeng Li.

Data availability statement

The data used in this study are not publicly available. Access can be provided upon reasonable request with the consent of the corresponding author.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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