Impact of positive-to-total lymph node ratio on prognosis in stage 3 colorectal cancer.
A multicenter study

Ann. Ital. Chir., 2023 94, 4: 375-383
pii: S0003469X23039210

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BACKGROUND: *Colorectal cancer remains a health problem despite advances in diagnostic and treatment methods. This study aimed to determine the impact of positive-to-total lymph node ratio on survival in colorectal cancer.*
METHODS: *Patients with stage 3 colorectal cancer were included. Patients age; sex; operation type (emergency or elective); tumor size, grade, and location; TNM stage; vascular and perineural invasions; numbers of lymph nodes examined and negative lymph nodes, positive-to-total lymph node ratio, and administration of postoperative chemotherapy were examined.*
RESULTS: *Median follow-up period was 34.7 months. Most patients were in stage 3b (67.9%), and the median number of dissected lymph nodes was 15. The number of metastatic lymph nodes, positive lymph node ratio, and negative-to-positive lymph node ratio were 3, 16.7, 11, and 5, respectively. The overall survival rate was 48.6%. Mean life expectancy was 51.5 months. Multivariate Cox regression analysis revealed positive-to-total lymph node ratio >23.3%, age, and absence of postoperative chemotherapy as risk factors for overall survival (p<0.05). Positive-to-total lymph node ratio >23.3% was associated with poor overall survival and 3.726-fold poorer survival.*
DISCUSSION: *Positive-to-total lymph node ratio >23.3% is a risk factor affecting overall survival in stage 3 colorectal cancer. Increased positive-to-total lymph node ratio (>23.3%) is associated with poor overall survival.*

KEY WORDS: Colorectal Cancer, Overall Survival, Positive Lymph Node Ratio, Stage 3 Cancer

Introduction

Colorectal cancer (CRC) has a rate of 9.4% among all cancer cases and is the second leading type of cancer in women, while it accounts for 10.6% of all cancer cases and is ranked third among all cancers in men [1].

In the prognosis of CRC, patients' comorbidities, biological behavior and stage of the tumor, quality of the surgical intervention, and neo adjuvant and adjuvant oncological therapies play important roles [2]. En bloc surgical resection of the tumor and sufficient lymph node (LN) dissection at the local stage of the disease is an effective treatment method [3]. The eight edition of the American Joint Committee on Cancer Staging Manual recommends dissection of at least 12 LNs for the correct staging of CRC [2]. A study conducted on patients divided into subgroups of insufficient (<12 LNs) and sufficient (>12 LNs) LN dissections at stage 1-2-3 reported significant positive outcomes on disease-free survival F) at stage 1 and DFS and overall survival (OS) at stages 2 and 3 ($p > 0.001$) [4].
The concept of high-risk stage 2 was reported with bowel obstruction or perforation, invasion depth at T4, pos-

Pervenuto in Redazione Novembre 2022. Accettato per la pubblicazione Gennaio 2023
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itive vascular invasion, number of retrieved LNs < 12, and poorly differentiated adenocarcinoma [4], and such patients were recommenced to receive adjuvant chemotherapy (CT). The number of dissected LNs affects the clinical outcomes of patients with stage II and III disease; higher numbers of negative LNs are advantageous for survival [5,6]. The concept of negative LN count has recently attracted attention as a bowel, gastric, esophageal, and cervical indicator of prognosis in various types of cancer [7-10]. The present study aimed to determine the impact of the positive-to-total LN ratio (+LNR) on long-term prognosis in stage 3 CRC.

Materials and Methods

Patients and Ethics

A total of 109 patients who underwent surgery for CRC at our surgical clinics between 2010 and 2018 were included in the study. The patients' data were examined retrospectively.

The patients were evaluated for age, sex, operation type (emergency or elective), tumor size, tumor location, TNM stage, vascular invasion, perineural invasion, tumor grade, number of LNs examined, numbers of negative and positive LNs, positive LN-to-total LN ratio, and administration of postoperative CT. The ethics committees of Kanuni Sultan Süleyman Training and Research Hospital and Sakarya Training and Research Hospital approved the study (approval number 80929729-000-5628). Informed consent was not obtained because of the retrospective study design.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: patients with curative resection (R0), pathological tumor stage 3, diagnosed with adenocarcinoma, not receiving neoadjuvant CT, and age >18 years.

The exclusion criteria were as follows: Patients with secondary malignancy, history of polypectomy prior to surgery, mortal course during the past 30 days, and irregular follow-up +LNR.

The total number of LNs dissected from the resected specimen and the number of positive or negative LNs were assessed +LNR was determined for each patient OS. The primary endpoint was OS, which was defined as the period from the primary surgery until death due to any reason or the specified end date of the follow-up.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, USA). Continuous variables were analyzed for normal distribution using the Kolmogorov-Smirnov test. Categorical data were expressed as numbers (n) and percentage, and quantitative data were expressed as mean ± standard deviation and median (25^{th}-75th) percentiles. The optimal threshold for positive LN ratio (LNR) to predict prognosis (i.e., OS) was evaluated using receiver operating characteristic analysis as it provided the maximum sum of sensitivity and specificity for the significant test. Sensitivity, specificity, positive and negative predictive values, and accuracy levels for LNR were also determined.

Differences in LNR between two independent groups were compared using the Mann-Whitney U test, and the Kruskal-Wallis test was used for comparisons among more than two independent groups. When the p-values from the Kruskal-Wallis tests were statistically significant, the Dunn-Bonferroni test was performed to determine which group differed from which other groups. Categorical data were analyzed using the Fisher-Freeman-Halton test or the continuity correction 2 test, where appropriate. Spearman's rank-order coefficient of correlation was calculated to determine the degrees of association between tumor size and LNR. Kaplan-Meier survival analysis using the log-rank test was performed to determine whether LNR threshold and T and N stages had statistically significant effects on prognosis.

Crude survival ratios; cumulative survival rates for 1, 3, and 5 years; mean life expectancy; and 95% confidence intervals (CIs) were determined. Whether the potential factors had statistically significant effects on prognosis was investigated using univariate Cox proportional hazards regression models. Multiple Cox proportional hazards regression models were obtained to determine the best independent predictors with the highest effect on prognosis after adjustment for clinically important factors. In addition to biological factors such as age and sex, any variable whose univariate test had a p-value of <0.10 was accepted as a candidate for the multivariable model. Hazard ratios (HR), 95% CIs, and Wald statistics for each independent variable were also calculated. Unless otherwise stated, a p-value of <0.05 was considered statistically significant. However, for all possible multiple comparisons, the Bonferroni correction was applied for checking for Type I errors.

Results

Assessment of clinical and histopathological data. The mean age of the patients was 61.4 ± 14.0 years, and 64 (58.7%) patients were male and 45 (41.3%) were female. Of the patients, 78.9% underwent elective surgery. Based on frequency, the location-specific ranking of the tumors was as follows: tumors of the right colon (27.5%), sigmoid colon tumors (23.9%), and rectal tumors (22.0%). The median follow-up period was 34.7 (19.0-49.5)

TABLE I - *Demographic and clinical characteristics of the cases.*

	n=109
Age (years)	61.4 ± 14.0
Sex	
Male	64 (58.7%)
Female	45 (41.3%)
Operation Type	
Emergency	23 (21.1%)
Elective	86 (78.9%)
Tumor location	
Right colon	30 (27.5%)
Transverse colon	5 (4.6%)
Left colon	12 (11.0%)
Sigmoid colon	26 (23.9%)
Rectosigmoid colon	12 (11.0%)
Rectum	24 (22.0%)
Tumor size (cm)	5.0 (3.5%–6.0%)
Grade	
I	30 (32.6%)
II	49 (53.3%)
III	13 (14.1%)
Stage	
3a	4 (3.7%)
3b	74 (67.9%)
3c	31 (28.4%)
N stage	
N1	66 (60.6%)
N2	43 (39.4%)
Lymphovascular invasion	90 (82.6%)
Perineural invasion	56 (51.4%)
Lymph node count	15 (11-22)
Number of metastatic lymph nodes	3 (1–5)
Positive lymph node ratio (%)	16.7 (9.1–38.3)
Number of negative lymph nodes	11 (8-17)
Negative-to-positive lymph node ratio	5 (2-10)
Administration of postoperative chemotherapy	68 (62.4%)
Follow-up period (months)	34.7 (19.0-49.5)
Mortality	56 (51.4%)

TABLE II - *Positive lymph node ratios of the cases by clinical characteristics.*

	n	**Positive lymph node ratio (%)**	**p-value**
Age groups			0.771†
≤60 years	55	15.4 (8.8–38.5)	
>60 years	54	18.2 (9.7–36.9)	
Tumor location			0.155‡
Right colon	30	14.3 (8.1–26.9)	
Transverse colon	5	15.0 (5.3–26.0)	
Left colon	12	19.1 (11.7–40.8)	
Sigmoid colon	26	25.0 (14.3–58.3)	
Rectosigmoid	12	14.1 (6.8–30.3)	
Rectum	24	14.8 (9.3–37.4)	
Grade			0.047‡
I	30	15.5 (8.3–28.4)	
II	49	14.3 (8.6–23.7)a	
III	13	28.6 (16.6–59.1)a	
Tumor size			0.158†
<5 cm	54	15.4 (8.3–36.1)	
≥5 cm	55	20.0 (10.0–38.5)	
Lymphovascular invasion			0.749†
No	19	15.4 (9.1–38.1)	
Yes	90	17.4 (9.1–38.5)	
Perineural invasion			0.937†
No	53	15.4 (9.5–37.2)	
Yes	56	18.2 (8.7–40.9)	
Status			0.002†
Alive	53	13.8 (8.3–20.7)	
Exitus	56	24.4 (12.9–54.1)	

Data are shown as median (25th–75th) percentiles, † Mann–Whitney U test, ‡ Kruskal–Wallistest, a: Grade II vs. III (p = 0.043).

months. The majority (67.9%) of the patients were in stage 3b. The median tumor size was 5.0 (3.5-6.0) cm. Histopathologically, the tumors were mostly grade 2 (53.3%) and grade 3 (32.6%). The median number of dissected LNs was 15 [11-22]. The number of metastatic LNs, positive LN ratio, number of negative LNs, and negative-to-positive LN ratio (NPR) were 3 [1-5], 16.7 (9.1-38.3), 11 [8-17], and 5 [2-10], respectively. Moreover, 60.6% of the patients were N1 and 39.4% were N2. The rates of perineural invasion and lymphovascular invasion were 56 (51.4%) and 90 (82.6%), respectively (Table I). LN ratios, ROC analyses, and correlation of survival.

In total, 64 patients (62.4%) received CT, and the mortality rate was 51.4% (n = 56) When the LN ratio was compared based on the clinical characteristics of the cases, the cases did not significantly differ in age, tumor location, tumor size, perineural invasion, and lymphovascular invasion (p=0.771, p=0.155, p=0.158, p=0.749, and p=0.937, respectively). However, there was a statistically significant change in the positive LN ratio in terms of the tumor histopathological grade (p=0.047), which was because the positive LN ratio was higher in grade 2 than in grade 3 (p = 0.043). There was no statistically significant difference between grades 1 and 2 and between grades 1 and 3 (p > 0.999 and p = 0.117, respectively). The positive LN ratios were significantly higher in the exitus group than in the survival group (p = 0.002). The area under the ROC curve of the positive LN ratio for distinguishing the survival group and the exitus group was also statistically significant (area under the curve = 0.672; 95% CI: 0.571-0.774; p = 0.002); (Fig. 1). Based on the ROC analysis result, the optimal cutoff point of the positive LN ratio for distinguishing the survival group and the exitus group was 23.3%. The positive LN ratio had a sensitivity of 53.6%, specificity of 81.1%, positive predictive value of

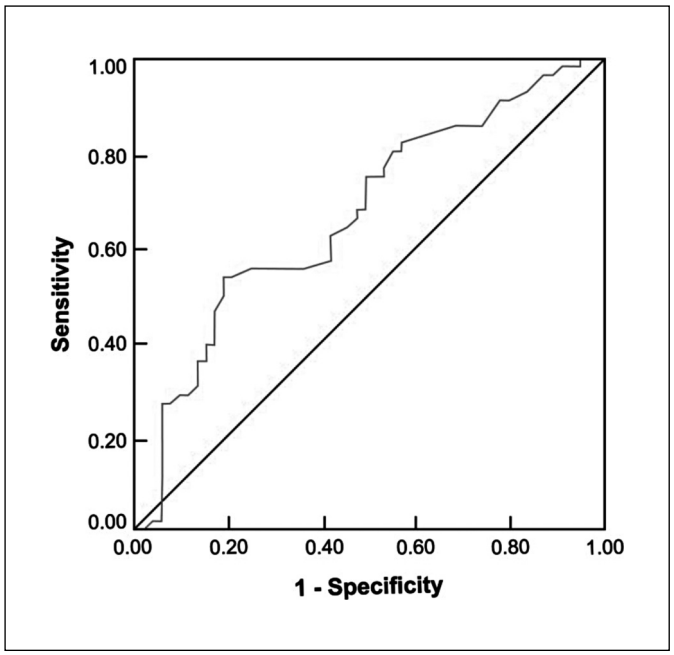

Fig. 1: Receiver operating characteristic curve of the positive lymph node ratio in predicting mortality

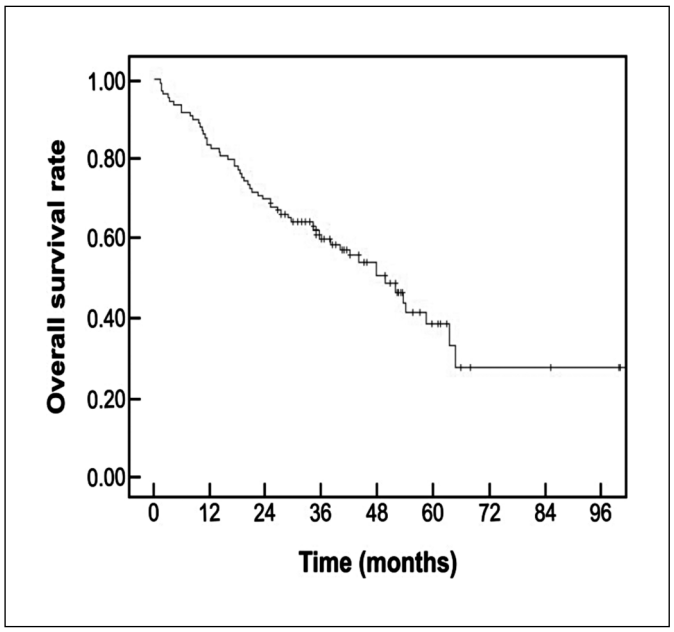

Fig. 2: Kaplan-Meier curve indicating the overall survival levels of all cases.

75.0%, negative predictive value of 62.3%, and diagnostic accuracy of 66.9% at this optimal cutoff point (Table II).

There was a statistically significant difference in the distribution of the positive LN rate according to the stage, and the possibility of having a positive LN rate of ≥23.3% increased cumulatively from stage 3a to stage 3c ($p < 0.001$). Similarly, the likelihood of a positive LN ratio of ≥23.3% was considerably increased in

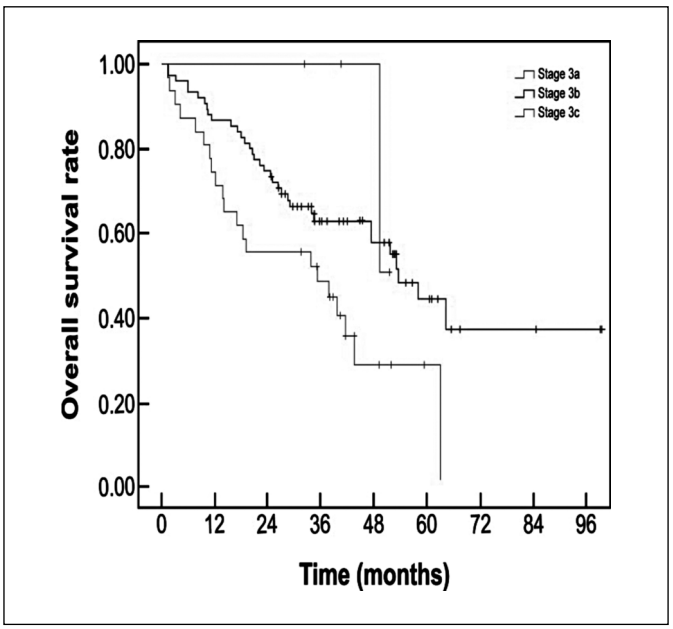

Fig. 3 – Kaplan-Meier curves indicating the overall survival results by stage. The approximate survival rate of the cases in stage 3a was 75.0%, their 1-year and 3-year survival rates were 100%, and their life expectancy was 50.6 (49.1-52.1) months. The approximate survival rate of the cases in stage 3b was 54.1%, and their 1-, 3-, and 5-year survival rates were 86.5%, 62.4%, and 43.6%, respectively. The mean life expectancy of the cases was 57.7 (47.6-67.8) months. The approximate survival rate of the cases in stage 3c was 32.3%, and their 1-, 3-, and 5-year survival rates were 74.2%, 47.7%, and 27.8%, respectively. The mean life expectancy of the cases was 33.0 (24.4-1.5) months.

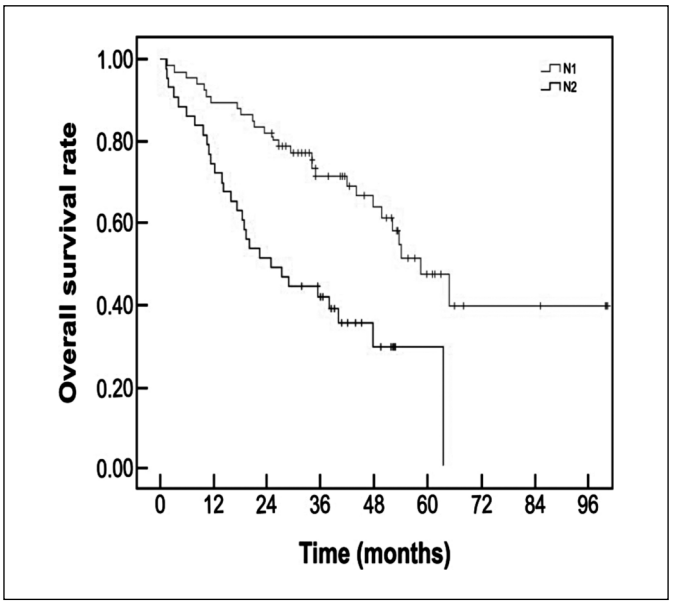

Fig. 4: Kaplan-Meier curves indicating the overall survival rates by N1 and N2 subgroups. The approximate survival rate of the cases in the N1 group was 59.1%, and their 1-, 3-, and 5- survival rates were 89.4%, 71.3%, and 47.1%, respectively. The mean life expectancy of the cases was 62.2 (51.8–72.5) months. The approximate survival rate of the cases in the N2 group was 32.6%, and their 1-, 3-, and 5-survival rates were 74.4%, 41.6%, and 29.3%, respectively. The mean life expectancy of the cases was 32.2 (25.0–39.4) months.

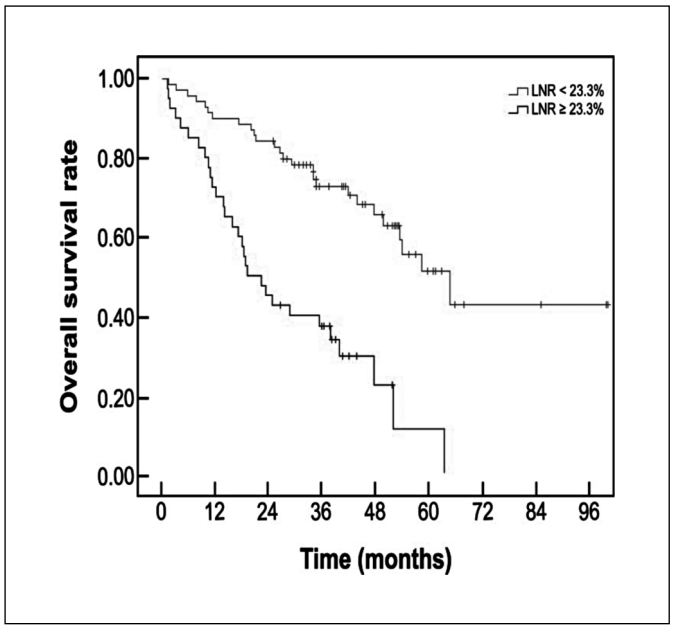

Fig. 5: Kaplan-Meier curves indicating the overall survival results in terms of lymph node ratio (LNR). The approximate survival rate of the cases with LNR <23.3% was 63.3%, and their 1-, 3-, and 5-year survival rates were 89.9%, 72.6%, and 51.1%, respectively. The mean life expectancy of the cases was 64.4 (53.9-74.9) months. The approximate survival rate of the cases with LNR 23.3% was 25.0%, and their 1-, 3-, and 5-year survival rates were 72.5%, 37.2%, and 11.1%, respectively. The mean life expectancy of the cases was 28.1 (21.4-34.8) months.

the N2 group compared with that in the N1 group ($p < 0.001$) (Table III).

The 1-, 3-, and 5-year survival rates of all the cases were 83.5%, 59.6%, and 38.3%, respectively (Fig. 2).

The mean life expectancy was 51.5 years (95% CI: 43.1-59.9). There was a statistically significant difference among the stages in terms of OS ($p = 0.0.021$); stage 3 had poorer prognosis than stage 3b ($p = 0.011$). There was no statistically significant difference between stage 3a and stage 3b and between stage 3a and stage 3c ($p = 0.485$ and $p = 0.122$, respectively) (Fig. 3).

Compared with the N1 group, the N2 group had poor prognosis in terms of survival ($p< 0.001$; Fig. 4), which was statistically significant. Compared with the group of LNR <23.3%, the group of LNR ≥23.3% had poor prognosis in terms of survival ($p < 0.001$), which was statistically significant. Among the cases with a positive LN ratio of 23.3%, the groups based on stage and the N1/N2 groups did not significantly differ in OS ($p < 0.05$) (Fig. 5).

Among the stage 3b cases, compared with the group of LNR <23.3%, the group of LNR ≥23.3% had significantly poor prognosis in terms of survival ($p < 0.001$).

Among the stage 3c cases, compared with the group of LNR <23.3%, the group of LNR ≥23.3% had poor prognosis in terms of survival, but this difference was not statistically significant ($p < 0.061$).

Because all stage 3a cases had LNR <23.3%, a comparison of LNR was not feasible in the relevant stage group.

In the N1 group, compared with the group of LNR <23.3%, the group of LNR ≥23.3% had poor prognosis in terms of survival, but this difference was not statistically significant according to Bonferroni correction ($p < 0.037$). In the N2 group, compared with the group of LNR <23.3%, the group of LNR ≥23.3% had poor prognosis in terms of survival, but this difference was not statistically significant according to Bonferroni correction ($p = 0.043$) (Table IV).

Increasing age was associated with a statistically significant increase in the mortality rate (risk ratio [RR] = 1.032; 95% CI: 1.011-1.054; $p = 0.003$). Each gradual increase between two consecutive stages (from 3a to 3b or from 3b to 3c) increased the mortality rate 2.038-fold (95% CI: 1.217-3.415; $p = 0.007$). The mortality rate was significantly increased 2.753-fold (95% CI: 1.595-4.751) in the N2 group compared with that in the N1 group ($p < 0.001$). The mortality rate was also

TABLE III - *Results of OS by T and N stage and positive lymph node ratio: results of Kaplan–Meier survival analysis.*

			Survival rates						
	Number of cases	**Number of incidents**	**Approximate**	**1-year**	**3-year**	**5-year**	**Life expectancy***	**Log-Rank**	**p-value**
Stage								7.748	0.021
3a	4	1	75.0%	100.0%	100.0%	N/A	50.6 (49.1–52.1)		
3b	74	34	54.1%	86.5%	62.4%	43.6%	57.7 (47.6–67.8)a		
3c	31	21	32.3%	74.2%	47.7%	27.8%	33.0 (24.4–41.5)a		
N stage								14.296	<0.001
N1	66	27	59.1%	89.4%	71.3%	47.1%	62.2 (51.8–72.5)		
N2	43	29	32.6%	74.4%	41.6%	29.3%	32.2 (25.0–39.4)		
LNR								24.555	<0.001
<23.3%	69	26	62.3%	89.9%	72.6%	51.1%	64.4 (53.9–74.9)		
≥23.3%	40	30	25.0%	72.5%	37.2%	11.1%	28.1 (21.4–34.8)		
General	109	56	48.6%	83.5%	59.6%	38.3%	51.5 (43.1–59.9)	-	-

* Data are expressed as mean (95% confidence interval), N/A: Not available, a: Stage 3b vs. 3c ($p = 0.011$).

TABLE IV - *Comparison of OS between the subgroups of LNRs, T stages, and N stages*

	Number of cases	Survival rates 1-year	3-year	5-year	Life expectancy*	Log-rank	p-value
LNR<23.3%						0.392	0.822†
Stage 3a	4	100.0%	100.0%	N/A	50.6 (49.1–52.1)		
Stage 3b	56	89.3%	70.1%	53.5%	65.0 (53.6–76.5)		
Stage 3c	9	88.9%	76.2%	N/A	45.5 (34.2–56.7)		
LNR≥23.3%						0.169	0.681†
Stage 3b	18	77.8%	38.1%	N/A	29.2 (20.7–38.0)		
Stage 3c	22	68.2%	36.4%	25.5%	28.2 (18.2–38.3)		
LNR<23.3%						0.037	0.847†
N1	60	90.0%	73.5%	50.8%	64.5 (53.7–75.4)		
N2	9	88.9%	66.7%	N/A	41.0 (30.1–52.0)		
LNR≥23.3%						0.380	0.538†
N1	6	83.3%	50.0%	0.0%	34.2 (17.4–51.0)		
N2	34	70.6%	35.3%	18.2%	27.8 (20.2–35.4)		
Stage 3b						12.397	<0.001‡
LNR<23.3%	56	89.3%	70.1%	53.5%	65.0 (53.6–76.5)		
LNR≥23.3%	18	77.8%	38.1%	N/A	29.2 (20.7–38.0)		
Stage 3c						3.514	0.061‡
LNR<23.3%	9	88.9%	76.2%	N/A	45.5 (34.2–56.7)		
LNR≥23.3%	22	68.2%	36.4%	25.5%	28.2 (18.2–38.3)		
N1						4.370	0.037†
LNR<23.3%	60	90.0%	73.5%	50.8%	64.5 (53.7–75.4)		
LNR≥23.3%	6	83.3%	50.0%	0.0%	34.2 (17.4–51.0)		
N2						4.087	0.043†
LNR<23.3%	9	88.9%	66.7%	N/A	41.0 (30.1–52.0)		
LNR≥23.3%	34	70.6%	35.3%	18.2%	27.8 (20.2–35.4)		

* Data are expressed as mean (95% confidence interval); N/A: Not available; † According to Bonferroni correction; p < 0.025 was considered statistically significant; ‡ p < 0.0167 was set as the statistical significance level in terms of the Bonferroni correction.

TABLE V - *Univariate and multivariate Cox proportional-hazard regression models related to factors thought to affect OS*

	Univariate			Multivariate		
	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.032	1.011–1.054	0.003	1.037	1.013–1.060	0.002
Female sex	1.193	0.696–2.048	0.521	1.284	0.731–2.255	0.384
Emergency	1.652	0.897–3.042	0.107	-	-	-
Transverse colon	2.774	0.868–8.867	0.085	-	-	-
Left colon	1.987	0.720–5.487	0.185	-	-	-
Sigmoid colon	1.672	0.729–3.834	0.225	-	-	-
Rectosigmoid	1.662	0.603–4.581	0.326	-	-	-
Rectum	2.050	0.938–4.482	0.072	-	-	-
Tumor size	1.114	0.991–1.253	0.072	1.119	0.998–1.255	0.055
Grade	1.403	0.891–2.210	0.144	-	-	-
Stage	2.038	1.217–3.415	0.007	1.378	0.716–2.649	0.337
N2	2.753	1.595–4.751	<0.001	1.499	0.562–4.000	0.419
LVI	1.549	0.700–3.426	0.280	-	-	-
PNI	1.321	0.771–2.261	0.311	-	-	-
LNR ≥23.3%	3.700	2.133–6.419	<0.001	3.726	1.542–9.003	0.003
Absence of postoperative chemotherapyadministration	2.022	1.184–3.453	0.010	2.613	1.402–4.867	0.002

HR: Hazard ratio; CI: Confidence interval

significantly increased 3.700-fold in those with a positive LN ratio of ≥23.3% compared with that in those with a positive LN ratio of <23.3% (95% CI: 2.133-6.419; p < 0.001). The absence of postoperative CT administration also resulted in a statistically significant increase in mortality rate (RR = 2.022; 95% CI: 1.184-3.453; p = 0.010; (Table III). However, sex, operation type, tumor location, tumor size, tumor grade, perineural invasion, lymphovascular invasion, and LN count did not have significant effects on OS (p > 0.05). On the other hand, the factors that affected OS most were advanced age, absence of postoperative CT administration, and a positive LN ratio of ≥23.3%.

When adjusted for other factors, the mortality rate continued to increase significantly as age increased (RR = 1.037; 95% CI: 1.013-1.060; p = 0.002). When the effects of other factors were kept constant, the absence of postoperative CT administration also continued to significantly increase the mortality rate (RR = 2.613; 95% CI: 1.402-4.867; p = 0.002).

Regardless of other factors, the mortality rate was significantly increased 3.726-fold (95% CI: 2.542-9.003) in those with a positive LN ratio of ≥23.3% compared with that in those with a positive LN ratio of <23.3% (p = 0.003; (Table V).

Discussion

The effective treatment method for local CRC is a combination of curative resection of the tumor and sufficient LN dissection [2]. The combination of systemic CT and surgical treatment can also be effective in cases of locally advanced tumors [2]. However, relapse can still occur despite curative (R0) resection in early-stage tumors, especially in tumors that are not LN metastases of T1-2-3 cancers. These relapses may occur due to the unrevealed aggressive biological characteristics of the tumors as well as because of insufficient surgical application, micro-metastases in LNs, and inadequate treatment (adjuvant therapy) for the tumor graded at a lower grade than the actual grade.

The surgical specimen should be resected from the embryological margins without damaging the mesenteric surface [3]. The experience level of the surgeon, high ligation of the arteries, and lateral pelvic LN dissection are some of the factors that affect the number of LNs in the resected piece [11,12].

The pathologist that examines the resected specimen is the second factor that might affect the staging. Pathologists should re-examine specimens that do not reach the 12 LN count, especially in stage 2 patients with no LN positivity [2].

Unexplored aspects in tumor biology, tumor location, tumor grade, perineural invasion, lymphovascular invasion, the mucinous component of the tumor are the tumor-related factors that affect prognosis [2].

Postoperative (adjuvant) CT administered after curative resection of CRC is aimed at eliminating micro-metastases and therefore minimizing the likelihood of relapse and maximizing the cure rate. The benefits of adjuvant CT reduces the risk of relapse by 30% and the mortality rate by 22%-32% in stage 3 (node-positive) cases [13] (up-to-date).

Although there is no consensus regarding the number of LNs required to be dissected for a complete oncological surgery in CRC, the National Comprehensive Cancer Network (NCCN) recommends dissection of 12 LNs [2]. According to the staging system of the NCCN, N1 is defined as 1-3 LNs and N2 as > 4 LNs [2]. The number of negative LNs dissected was excluded. Previous studies reported that higher numbers of negative LNs dissected were associated with better prognosis [14,15].

Previous studies that examined the positive-to-total LN ratio or NPR have included the negative LN count in tumor staging [14,15].

These studies revealed the effect of negative LN count particularly on the prognosis of stage 3 tumors. Among the factors that affected + LNR in the present study, age, lymphovascular invasion, perineural invasion, tumor size, and tumor location were found to not affect LNR (p > 0.05). We also found out that +LNR increased as the grade increased (p = 0.047). There was a significant difference in the +LNR ratio between the survival and exitus groups.

Some studies have reported that +LNR is a differentiating factor for the aggressive characteristic of tumors because it is associated with a higher percentage of lymphovascular invasion and malignant tumor differentiation [16-18]. Large tumor size and presence of T3-T4 tumors have been shown to cause inflammation at the mesentery and thus result in higher numbers of dissected LNs [4,19]. No correlation between +LNR and age, sex, tumor location, and T stage have been reported [16,17,20]. We also found that the stage and N stage had a significant effect on +LNR (p < 0.001). Stage, N stage, and LNR ≥23.3% and <23.3% had significant effects on OS (p < 0.001). In a study that analyzed 20,702 cases of stage 3 cancer, the disease-specific mortality rate significantly decreased as the negative LN count decreased in stage IIIB and IIIC patients; the 5-year cumulative cancer mortality rate was 27% in stage IIIB patients with a negative LN count of ≥13 and 45% in those with a negative LN count of ≤3 (p < 0.0001).

Among patients with stage IIIC cancer, the 5-year mortality rate was 42% in those with a negative LN count of ≥13 and 65% in those with a negative LN count of ≤3 (p < 0.0001).

There was no relationship between negative LN count and disease-specific survival in stage IIIA patients. After determining the positive LN count, a high number of negative LNs was found to be independently related to the specific DFS of the disease [7]. In the present study, patients with a +LNR of <23.3% or ≥23.3% were exam-

ined separately as stage 3a, 3b, and 3c and as N1 and N2, and no difference in OS were found.
In a study that investigated the effect of NPR on survival in 2,256 patients with stage 3 CRC, high NPR values were shown to have a protective effect on survival [14]. The same study also reported that NPR was more successful at predicting prognosis than +LNR [14].
In the present study, in the comparison of stages 3b and 3c and groups N1 and N2 in terms of LNR ratio, there was a significant difference in only stage 3b, in which OS differed significantly between the LNR< 23.3% and LNR ≥23.3% subgroups ($p < 0.001$). Among the stage 3c cases, compared with the group of LNR <23.3%, the group of LNR ≥23.3% had poor prognosis in terms of survival, but this difference was not statistically significant ($p < 0.061$).
As all stage 3a cases had LNR <23.3%, a comparison of LNR was not feasible in the relevant stage group.
There is no consensus regarding the reliable cutoff value of +LNR [15]. Moreover, as in our study, other studies did not indicate the comorbidities, which are important prognostic indicators in patients with cancer. An extensive cohort study that can stratify and assess different LNRs and identify minor differences in prognostic outcomes or an individual meta- analysis of patient data is required in CRC prognosis [15].
According to the univariate analysis in the present study, female sex, emergency surgery, tumor location, tumor size, tumor grade, and lymphovascular and perineural invasion did not have a prognostic effect on OS ($p > 0.05$). Age, +LNR >23.3%, stage, N2, and absence of CT administration were negative prognostic factors of OS ($p < 0.05$). According to the results of Cox regression analysis of OS performed as part of the univariate analysis of the prognostic factors, age, absence of postoperative CT administration, and LNR >23.3% were found to be significant ($p < 0.05$). Furthermore, +LNR >23.3% was associated with 3.726- fold poorer survival.
In a retrospective study of three Northern Centre for Cancer Care Group studies, +LNR was proven to be an independent factor of both local relapse and OS [21]. In this analysis of 673 patients with stage II/III rectal cancer, the patients were categorized according to their LNR values: LNR <0.25 (n = 355), ≤0.5 (n = 153), ≤0.75 (n = 66), and >0.75 (n = 68).
The incidence of local relapse increased as LNR increased: 10%, 14%, 34%, and 34%, respectively. Contrary to the findings of local relapse, the OS rates were 70%, 55%, 39%, and 37%, respectively [21].
The present study has some limitations. The study was a retrospective study with a small sample size, which we estimate may affect the numerical data of the +LNR ratio. The number of dissected LNs may vary because the surgical resections were performed during a period that also covered the surgeon's training period.
In conclusion, A +LNR value of >23.3% was found to be a risk factor affecting OS. Increased +LNR (>23.3%) is associated with poor OS. The effect of negative LN on prognosis in colorectal cancer should be evaluated with multicentric randomized controlled studies.

Acknowledgments

Not applicable.
Data availability statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Riassunto

Il cancro del colon-retto rimane un problema di salute nonostante i progressi nei metodi diagnostici e terapeutici. Questo studio mirava a determinare l'impatto del rapporto linfonodi positivi sul totale sulla sopravvivenza nel cancro del colon-retto.
Sono stati inclusi nello studio pazienti con cancro del colon-retto al III stadio, analizzando età dei pazienti, sesso, tempistica dell'intervento (di emergenza o di elezione), dimensioni, grado e posizione del tumore, stadio TNM, invasioni vascolari e perineurali. Infine sono stati esaminati il numero di linfonodi isolati, quelli negativi ed il rapporto tra linfonodi positivi e il totale, e la somministrazione o meno della chemioterapia postoperatoria.
Risultati: Il periodo mediano di follow-up è stato di 34,7 mesi. La maggior parte dei pazienti era allo stadio 3b (67,9%) e il numero mediano di linfonodi sezionati era 15. Il numero di linfonodi metastatici, il rapporto linfonodale positivo e il rapporto linfonodale negativo-positivo erano 3, 16,7, 11, e 5, rispettivamente. Il tasso di sopravvivenza globale è stato del 48,6%. L'aspettativa di vita media era di 51,5 mesi. L'analisi di regressione multivariata di Cox ha rivelato un rapporto linfonodi positivi/totali >23,3%, l'età e l'assenza di chemioterapia postoperatoria come fattori di rischio per la sopravvivenza globale ($p <0,05$). Il rapporto linfonodi positivi/totali >23,3% è stato associato a una sopravvivenza globale scarsa e a una sopravvivenza inferiore di 3,726 volte.
Discussione: il rapporto linfonodi positivi/totali >23,3% è un fattore di rischio che influenza la sopravvivenza globale nel cancro del colon-retto in stadio 3. L'aumento del rapporto linfonodi positivi/totali (> 23,3%) è associato a una scarsa sopravvivenza globale.

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