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Original Article

The Prognostic Value of Lymph Node Ratio after Neoadjuvant Chemotherapy in Patients with Locally Advanced Gastric Adenocarcinoma

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ABSTRACT

Purpose: This study aimed to investigate the prognostic value of lymph node ratio (LNR) in patients with locally advanced gastric cancer who received neoadjuvant chemotherapy.
Materials and Methods: We retrospectively enrolled gastric cancer patients treated with neoadjuvant chemotherapy and curative surgery at the First Affiliated Hospital of Zhejiang University from 2004 to 2015 as the study cohort. Patients with the same inclusion criteria treated in 2016–2017 were enrolled as the validation cohort. Kaplan-Meier curves were assessed using the log-rank test to analyze the differences in overall survival (OS). Multivariate survival analysis was performed using the Cox proportional hazards model. The areas under the receiver operating characteristic curve of ypN and LNR categories for predicting the actual 3-year OS were compared.
Results: A total of 265 patients were included in the proposal cohort. The median number of retrieved lymph nodes (rLNs) was 32. The number of positive lymph nodes (pLNs) increased as rLN increased (P=0.037), but the LNR remained relatively constant (P=0.462). The LNR was categorized into 4 groups according to the prognosis: ypNr0, node-negative with rLN>25; ypNr1, node-negative with rLN≤25 or 0<LNR≤0.1; ypNr2, 0.1<LNR≤0.3; and ypNr3, LNR>0.3. In the validation cohort of 43 enrolled patients, there was a clear distinction in OS that significantly (P<0.001) varied depending on the LNR values and LNR was the only independent prognostic factor in multivariate analysis (P<0.001).
Conclusions: LNR was an independent prognostic factor for survival of patients with gastric cancer after preoperative chemotherapy and might be an alternative predictor for ypN stage.

Keywords: Stomach neoplasms; Lymph node ratio; Neoadjuvant chemotherapy

INTRODUCTION

Gastric cancer is the fifth most common malignancy and the third leading cause of cancer-related deaths worldwide [1]. Most cases of gastric cancer are diagnosed at an advanced stage. Perioperative chemotherapy is an optional treatment for advanced gastric cancer. Despite multimodal treatment, the prognosis for gastric cancer remains poor, with recurrence or death within 3 years reported in half of the patients [2,3]. Very few studies have investigated the predictive factors for survival of patients with gastric carcinoma after preoperative

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Author Contributions

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

chemotherapy. It is important to determine these factors in order to tailor postoperative treatment strategies that would help prevent relapse.

The most commonly used predictive system was the tumor-node-metastasis (TNM) classification proposed by the Union for International Cancer Control and American Joint Committee on Cancer (AJCC) [4]. Lymph node (LN) metastasis after preoperative chemotherapy (ypN stage) is the most important prognostic factor in patients with gastric carcinoma [5]. However, the determination of ypN stage was based on the number of positiveLNs, by which stage migration phenomenon occurred in approximately 10%–25% of cases, especially in patients with insufficient LN dissection or harvesting (<15) [6]. The lymph node ratio (LNR), namely the ratio between positive lymph nodes (pLNs) and retrieved lymph nodes (rLNs), is an effective and simple index to assess the stage migration phenomenon. Several investigators have reported that LNR was an independent prognostic factor for gastric cancer patients with upfront surgery. Moreover, LNR showed a better prognostic value than the N stage [7,8]. However, few studies have investigated the prognostic value of LNR in patients with gastric cancer after preoperative chemotherapy [9].

In the present study, we evaluated the prognostic significance of LNR in patients with gastric carcinoma after preoperative chemotherapy.

MATERIALS AND METHODS

Patients

We conducted a retrospective collection of gastric cancer patients who received preoperative chemotherapy at the First Affiliated Hospital of Zhejiang University from July 2004 to May 2015 as a proposal cohort. Patients who received neoadjuvant chemotherapy in our center between January 2016 and December 2017 were enrolled as the validation cohort. The inclusion criteria included: (1) histologically confirmed gastric adenocarcinoma by endoscopic biopsy before treatment initiation; (2) the primary lesion invading the serosa or involvement of adjacent structures with or without LN metastasis ($cT_{4a/4b}N_{any}$), which was mainly evaluated by computed tomography; (3) R0 resection with D2 lymphadenectomy following chemotherapy; and (4) complete medical record and 3-year follow-up data. The exclusion criteria were as follows: (1) distant metastasis or unresectable disease before the treatment or confirmed during the surgery; (2) history of another malignancy, except cured basal cell carcinoma of the skin or cured carcinoma in situ of the uterine cervix, and (3) prior major stomach surgery.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University (No. 2020IIT-11). Patient data was anonymized. All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the recommendations laid out in the Helsinki Declaration (1964 and later versions).

Preoperative chemotherapy

The preoperative chemotherapy regimen was determined by an oncologist. The regimens of preoperative chemotherapy included S-1 combined with oxaliplatin (SOX), capecitabine combined with oxaliplatin (XELOX), and fluorouracil, leucovorin, and oxaliplatin (FOLFOX). SOX consisted of oxaliplatin 130 mg/m^2 as a 2-hour intravenous infusion on day 1, and S-1

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was administered orally twice daily for 2 weeks followed by a 7-day rest period. The dose of S-1 was 80 mg/day for body surface area (BSA) <1.25 m^2, 100 mg/day for BSA ≥1.25 and <1.5 m^2, and 120 mg/day for BSA ≥1.5 m^2. XELOX consisted of oxaliplatin 130 mg/m^2 as a 2-hour intravenous infusion on day 1, oral capecitabine (of 1,000 mg/m^2 twice daily on days 1–14). FOLFOX consisted of oxaliplatin 130 mg/m^2 as a 2-hour intravenous infusion on day 1, leucovorin 400 mg/m^2, and a bolus of 5-fluorouracil (5-FU) 400 mg/m^2 on day 1, followed by a 46-hour infusion of 5-FU at 2,400 mg/m^2. All regimens were repeated every 3 weeks.

Surgery

Two to 4 cycles of preoperative chemotherapy were scheduled before the surgery. Patients underwent curative resection 2 weeks after completion of the last cycle of preoperative chemotherapy. Surgery was also indicated when stable disease or disease progression was acquired, or emergency conditions such as perforation or massive hemorrhage occurred. Distal, total gastrectomy or combined resection was performed depending on the location and extent of the primary tumor. D2 lymphadenectomy was conducted by experienced surgeons according to criteria established by the Japanese Gastric Cancer Association [10]. All patients underwent surgery by experienced surgeons who perform more than 100 radical gastrectomies per year. The reconstruction type was determined based on the surgeon's decision. Postoperative chemotherapy was continued within 4–6 weeks after the surgery. The cycles and regimens were decided by the oncologist according to the response and adverse events. The yield pathological TNM (ypTNM) staging was evaluated according to the 8th edition of the AJCC Staging Handbook [4].

Follow-up

After completion of the treatment, patients were followed up every 3–6 months in the first 2 years, 6–12 months from the third to the fifth year, and then annually thereafter. Follow-up included complete blood counts, chemistry profiles, tumor markers, endoscopy, and radiological imaging examinations.

Statistical analysis

Overall survival (OS) was calculated from the start of treatment to the date of death from any cause or the day of the last follow-up. Progression-free survival was calculated from the start of treatment to the occurrence of the first event (local progression or recurrence, distant recurrence, or death from any cause). Patients who were alive with no evidence of progression or recurrence were censored. LNR was defined as the ratio between metastatic and dissected LNs.

The enumeration method was used to determine the cut-off value of the LNR. All patients were stratified into 10 LNR subgroups (0–0.9) defined with an interval of 0.1. Kaplan-Meier curves of neighborhood subgroups and different combinations were compared using the log-rank test.

Categorical variables were analyzed using the χ^2 test and Fisher's exact probability test. Continuous data are expressed as mean±standard deviation or median values. The student's t-test or the Mann-Whitney U-test was used to compare continuous variables. The relationship between pLNs, LNR, and rLN number was evaluated using the Wilcoxon test and Spearman correlation analysis. The areas under the receiver operating characteristic curve (AUC) of ypN and LNR categories for predicting the actual 3-year OS were compared. Kaplan-Meier curves were compared using the log-rank test for OS. Hazard ratios and 95% confidential intervals were calculated using Cox proportional hazards regression

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models. Multivariate survival analysis was performed using the likelihood ratio test of the Cox proportional hazards model. All statistical analyses were performed using IBM SPSS Statistics (version 25.0; IBM Corp., Armonk, NY, USA), and a 2-tailed P<0.05, was considered to indicate statistical significance.

RESULTS

Clinical and pathological characteristics in proposal cohort

In the proposed cohort, we identified 265 patients according to the inclusion and exclusion criteria. The clinical and pathological characteristics of the patients are summarized in **Table 1**. The median age was 62 years (range, 34–80 years). A total of 193 patients (72.8%) were men and 72 patients (27.2%) were women. The median number of neoadjuvant chemotherapy regimens was 3 (range, 1–7). Regarding the pathological findings, 21 patients (7.9%) were found to have complete tumor regression in the primary lesion (ypT0), of which 8 patients still had LN metastasis (ypT0N+) and the other 13 patients had a pathological complete response to preoperative chemotherapy. There were 19 ypT1 (7.2%), 35 ypT2 patients (13.2%), 3 ypT3 patients (1.1%), and 187 ypT4 patients (70.6%) patients, respectively. Furthermore, there were 32 patients (13.1%) with ypstage I, 59 (24.2%) with ypstage II, and 153 (62.7%) with ypstage III. A total of 217 patients (81.9%) continued postoperative chemotherapy, while 39 patients (14.7%) did not. The postoperative treatment status of the remaining 9 (3.4%) patients was unknown. The proportion of postoperative chemotherapy was not significantly different between the subgroups (P=0.078).

Nodal status and a cut-off value of LNR

The average number of rLNs was 34.2±13.5, with a median number of 32 (range, 9–86). The number of rLNs was >15 in 254 patients (95.8%). A total of 181 patients (68.3%) had pLNs after preoperative chemotherapy. This included 58 patients at ypN1 (21.9%), 59 patients were ypN2 (22.3%), and 64 patients were ypN3 (24.2%) respectively. The mean number of pLNs was 4.6±6.5, with a median number of 2 (range, 0–43). There was a significant correlation between the number of pLNs and rLNs according to the Spearman correlation test (r=0.128, P=0.037). However, LNR was not associated with the number of LNs removed (r=−0.045, P=0.462).

To determine the appropriate cut-off value of LNR at which the prognosis was most similar in the resulting subgroups and most differed among subgroups (**Table 2**). The analysis was conducted as follows: (1) All patients were stratified into 10 subgroups (0–0.9) based on an interval of 0.1. The 3-year-and 5-year OS rates are summarized in **Table 2**. Kaplan-Meier curves of neighborhood subgroups were compared using the log-rank test, and P-values for each neighborhood subgroup are summarized in **Table 2**. According to the P-value between the neighborhood subgroups, the first cut-off point of 0.1 was made. (2) The OS was lower in group 5 (LNR: 0.31–0.4) than in group 4 (LNR: 0.21–0.3), but the difference was not statistically significant (P=0.107). The enumeration method was used as the second cut-off value (**Supplementary Table 1**). According to the P-value between different combinations of the cut-off points, 0.3 was chosen as the second cut-off value to best discriminate the prognosis. (3) The prognostic effect of rLN in patients with ypN0 cancer was investigated. ypN0 patients were divided into 5 subgroups: patients with rLN of 0–15 were merged into the first group as at least 16 or greater LNs were recommended according to National Comprehensive Cancer Network guidelines, and the remaining patients were categorized

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Table 1. Clinical and pathological characteristics of the proposal cohort

Characteristics	ypNr0 (n=61)	ypNr1 (n=92)	ypNr2 (n=70)	ypNr3 (n=42)	Total (n=265)	P*
Age						0.804
Median (ranges)	63 (34–80)	61 (37–78)	61 (37–77)	63 (38–76)	62 (34–80)	
Gender						0.832
Man	47 (77)	67 (72.8)	49 (70)	30 (71.4)	193 (72.8)	
Woman	14 (23)	25 (27.2)	21 (30)	12 (28.6)	72 (27.2)	
ECOG status						0.290
0	26 (42.6)	36 (39.1)	19 (27.1)	21 (50)	102 (38.5)	
1	27 (44.3)	45 (48.9)	43 (32.6)	17 (40.5)	132 (49.8)	
2	8 (13.1)	11 (12)	8 (11.4)	4 (9.5)	31 (11.7)	
Primary tumor location						0.067
Upper	11 (18)	24 (26.1)	12 (17.1)	4 (9.5)	51 (19.2)	
Middle	10 (16.4)	17 (18.5)	5 (7.1)	11 (26.2)	43 (16.2)	
Lower	36 (59)	43 (46.7)	47 (67.1)	21 (50)	147 (55.5)	
MRI	4 (6.6)	8 (8.7)	6 (8.6)	6 (8.6)	24 (9.1)	
Regimen						0.002
FOLFOX	28 (45.9)	37 (40.2)	42 (60)	22 (52.4)	129 (48.7)	
XELOX	3 (4.9)	17 (18.5)	12 (17.1)	11 (26.2)	43 (16.2)	
SOX	30 (49.2)	38 (41.3)	16 (22.9)	9 (21.4)	93 (35.1)	
Preoperative cycles						0.137
Median (ranges)	2 (2–7)	3 (1–7)	3 (1–7)	3 (1–6)	3 (1–7)	
Gastrectomy						0.060
DG	39 (63.9)	41 (44.6)	42 (60)	20 (53.6)	142 (53.6)	
TG	22 (36.1)	51 (55.4)	28 (40)	22 (52.4)	123 (46.4)	
Combined resection						0.504
Yes	4 (6.6)	12 (13)	10 (14.3)	6 (14.3)	32 (12.1)	
No	57 (93.4)	80 (87)	60 (85.7)	36 (85.7)	233 (87.9)	
Differentiation†						0.001
Well	13 (21.3)	16 (17.4)	6 (8.6)	4 (9.5)	39 (14.7)	
Poorly	35 (57.4)	68 (73.9)	60 (85.7)	38 (90.5)	201 (75.8)	
Gx	13 (21.3)	8 (8.7)	4 (5.7)	0	25 (9.4)	
Retrieved lymph nodes						<0.001
Median (ranges)	36 (27–76)	29 (10–86)	29.5 (9–67)	31.5 (11–68)	32 (9–86)	
ypT stage						<0.001
ypT0	10 (16.4)	7 (7.6)	4 (5.7)	0	21 (7.9)	
ypT1	14 (23)	5 (5.4)	0	0	19 (7.2)	
ypT2/3	10 (16.4)	17 (18.5)	6 (8.6)	5 (11.9)	38 (14.3)	
ypT4	27 (44.3)	63 (68.5)	60 (85.7)	37 (88.1)	187 (70.6)	
ypTNM stage‡						<0.001
ypstage I	24 (47.1)	8 (9.4)	0	0	32 (13.1)	
ypstage II	27 (52.9)	27 (52.9)	5 (7.6)	0	59 (24.2)	
ypstage III	0	50 (58.8)	61 (92.4)	42 (100)	153 (62.7)	
Postoperative chemotherapy						0.078
Yes	56 (91.8)	77 (83.7)	49 (70)	35 (83.3)	217 (81.9)	
No	4 (6.6)	12 (13)	17 (24.3)	6 (14.3)	39 (14.7)	
Unknown	1 (1.6)	3 (3.3)	4 (5.7)	1 (2.4)	9 (3.4)	

Values are presented as number (%).
ECOG = Eastern Cooperative Oncology Group; MRI = multiple regions involved; FOLFOX = fluorouracil, leucovorin, and oxaliplatin; XELOX = capecitabine combined with oxaliplatin; SOX = S-1 combined with oxaliplatin; DG = distal gastrectomy;TG = total gastrectomy; ypTNM = yield pathological tumor-node-metastasis.
*P<0.05 was considered statistically significant; †Well differentiation included high-and moderately differentiated patients, and poorly differentiated patients included poor and undifferentiated patients. ‡The 21 patients' ypTNM stage cannot be classified according to the 8th edition of the TNM International Union Against Cancer classification: ypT0N0M0 in 13 patients and ypT0N+M0 in 8 patients.

with an interval of 10. The Kaplan-Meier curve of each subgroup is shown in **Supplementary Fig. 1**. The difference in OS by rLN was marginally significant in the entire model (P=0.055). The OS of the neighborhood subgroup was compared using the log-rank test, and the P-values are summarized in **Supplementary Table 2**. According to the P-value, patients with ypN0 who had more than 25 rLNs were assigned to one group (ypNr0). This is because the

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Table 2. The 3-year and 5-year OS rate of each subgroup of LNR

Subgroup No.	LNR	No. of patients	3-year OS (%)	5-year OS (%)	P-value*
1	0	84	86.9	84.2	
2	0.01–0.10	69	78.3	62.1	0.003
3	0.11–0.20	47	44.7	31.1	<0.001
4	0.21–0.30	23	52.2	33.2	0.832
5	0.31–0.40	19	26.3	6.6	0.107
6	0.41–0.50	11	27.3	13.6	0.684
7	0.51–0.60	5	6	2	0.122
8	0.61–0.70	3	0	0	0.004
9	0.71–0.80	1	0	0	0.182
10	0.81–0.90	3	0	0	0.515

LNR = lymph node ratio; OS = overall survival.
*P-value of each neighborhood subgroup using the log-rank test.

prognoses of these patients were significantly better than those with 25 or fewer retrieved nodes (P=0.004). Meanwhile, there were no statistical differences between node-negative patients with 25 or fewer rLNs and ypNr1 patients (3-year OS 73.9% vs. 78.3%, P=0.653). The LNR were finally categorized into ypNr0: node-negative with rLN>25; ypNr1: node-negative with rLN≤25 and 0<LNR≤0.1; ypNr2: 0.1<LNR≤0.3, ypNr3: LNR>0.3.

Table 1 shows the clinical and pathological characteristics of the different ypNr categories of the proposed cohort. Preoperative regimen, differentiation, rLNs, ypT stage, and ypTNM stage were found to be statistically significant among ypNr stages. Compared with the SOX regimen, FOLFOX and XELOX were associated with higher ypNr stages (P=0.001 and 0.007, respectively). Poorly differentiated tumors were associated with higher ypNr stages compared with well-differentiated tumors and Gx with P-values of 0.016 and 0.003, respectively. More LNs were harvested in the ypNr0 group than in the ypNr1 (P=0.016) and ypNr2 (P=0.003). The difference between the reset intergroup was not significant. This could be because all patients in the ypNr0 group retrieved more than 25 LNs according to the category method. Higher ypNr stages were observed along with higher ypT categories (P<0.001). Meanwhile, higher ypNr stages were associated with higher ypTNM staging (P<0.001). The differences between subgroups were also significant; the P-values between ypstage I and ypstage II, ypstage I and ypstage III, and ypstage II and III were 0.049, <0.001, and <0.001, respectively.

Follow-up and OS

All patients were regularly followed up until May 2018, and 127 patients (47.9%) died according to the data collected at the last follow-up. The median follow-up time was 42 months (range, 7–148 months). The 3-year-and 5-year OS rates of all patients were 64.9% and 52%, respectively. The 3-year OS rates of ypN0, ypN1, ypN2, and ypN3 were 86.9%, 74.1%, 62.7%, and 29.7%, respectively (**Fig. 1A**).

The OS estimation was well discriminated by the LNR according to the cut-off. The 3-year OS rates of ypNr0, ypNr1, ypNr2, and ypNr3 were 91.8%, 72.6%, 47.1% and 28.6%, respectively. The difference in OS by LNR was statistically significant, as in the entire model (**Fig. 1B**). For adjacent LNR categories, the difference was also significant between ypNr0 and ypNr1 patients (P<0.001), ypNr1 and ypNr2 patients (P<0.001), and between ypNr2 and ypNr3 patients (P<0.001).

The results of the univariate analysis of prognostic factors for OS are summarized in **Table 3**. Univariate analysis revealed that the prognostic factors included the preoperative

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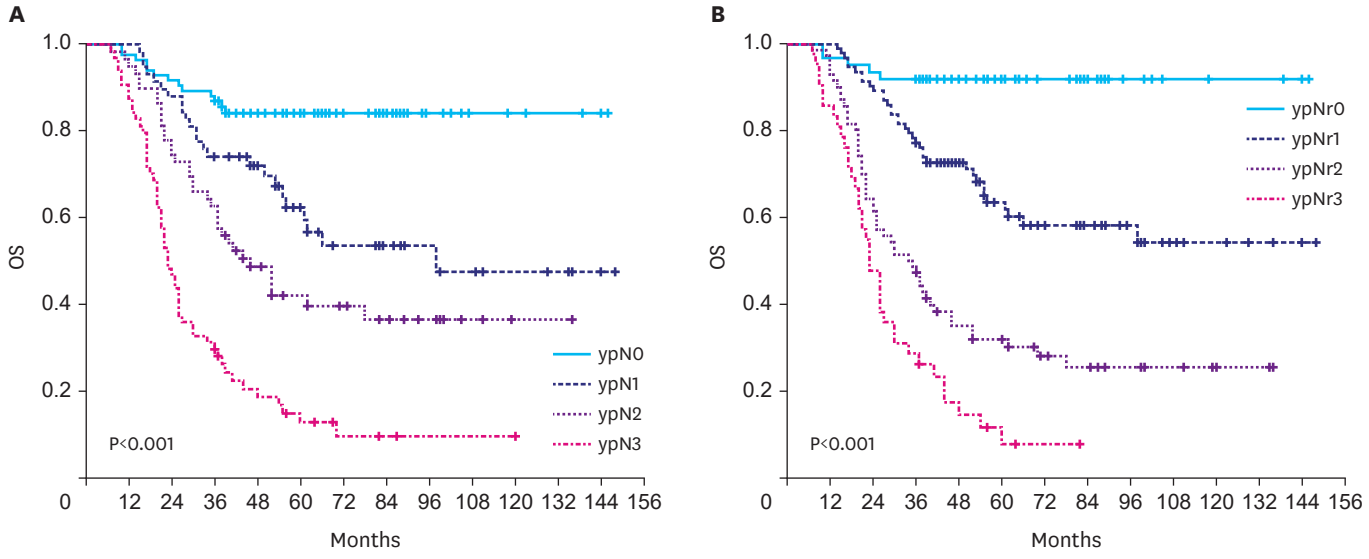

Fig. 1. (A) Kaplan-Meier estimates for OS according to ypN stage. (B) Kaplan-Meier estimates for OS according to ypNr stage.
OS = overall survival.

Table 3. Univariable and multivariable analysis of overall survival

Variables	Univariable		Multivariable	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Regimen		0.006		0.152
FOLFOX	Ref.		Ref.	
XELOX	0.972 (0.616–1.535)	0.904	0.644 (0.413–1.005)	0.052
SOX	0.507 (0.329–0.779)	0.002	0.853 (0.531–1.372)	0.513
Preoperative cycles		0.068		0.534
≥4	Ref.		Ref.	
3–4	0.569 (0.337–0.962)	0.035	0.844 (0.483–1.472)	0.550
1–2	0.540 (0.313–0.931)	0.027	0.731 (0.413–1.294)	0.282
Concomitant resection				
No	Ref.		Ref.	
Yes	1.616 (1.002–2.606)	0.049	0.720 (0.443–1.334)	0.184
ypT		<0.001		0.005
ypT4	Ref.		Ref.	
ypT0	0.161 (0.051–0.508)	0.002	0.278 (0.087–0.883)	0.030
ypT1	0 (0–1.79×10^{174})	<0.001	0 (0–1.49×10^{207})	<0.001
ypT2/3	0.279 (0.141–0.550)	<0.001	0.357 (0.180–0.708)	0.003
ypNr stage		<0.001		<0.001
ypNr0	Ref.		Ref.	
ypNr1	4.794 (1.878–12.238)	0.001	3.507 (1.371–8.967)	0.009
ypNr2	12.641 (5.037–31.720)	<0.001	7.535 (2.988–19.004)	<0.001
ypNr3	22.643 (8.860–57.865)	<0.001	13.735 (5.344–35.304)	<0.001
Histological grade		<0.001		0.433
Well-differentiated	Ref.		Ref.	
Poor-differentiated	2.007 (1.129–3.566)	0.018	1.553 (0.650–3.709)	0.322
Gx	0.320 (0.091–1.125)	0.076	0.566 (0.104–3.093)	0.511

HR = hazard ratio; CI = confidence interval; SOX = S-1 combined with oxaliplatin; FOLFOX = fluorouracil, leucovorin, and oxaliplatin; XELOX = capecitabine combined with oxaliplatin.

chemotherapy regimen (P=0.006), concomitant resection (P=0.049), ypT category (P<0.001), ypN category (P<0.001), ypTNM stage (P<0.001), ypNr category (P<0.001), and histological grade (P<0.001). Multivariable analysis with Cox backward regression for OS revealed the independent prognostic factors to be ypT (P=0.005) and the ypNr category (P<0.001).

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Table 4. 3-year-and 5-year overall survival according to the 8th edition American Joint Committee on Cancer N category and ypNr category

ypN stages	ypNr stage			
	ypNr0 (n=61) (91.8%/91.8%)	ypNr1 (n=92) (77.2%/63.4%)	ypNr2 (n=70) (47.1%/31.8%)	ypNr3 (n=42) (28.6%/7.8%)
ypN0 (n=84) (86.9%/84.2%)*	n=61 (91.8%/91.8%)	n=23 (73.9%/65.2%)	-	-
ypN1 (n=58) (74.1%/62.4%)	-	n=51 (76.5%/65.1%)	n=7 (57.1%/42.9%)	-
ypN2 (n=59) (62.7%/42.1%)	-	n=17 (76.5%/60.5%)	n=40 (55%/36.3%)	n=2 (50%/0%)
ypN3 (n=64) (29.7%/12.8%)	-	n=1 (100%/0%)†	n=23 (30.4%/20.3%)	n=40 (27.5%/8.3%)

*The 3-year overall survival/5-year overall survival; †Only 1 patient with ypN3 (7/86) was categorized as ypNr1, with an overall survival time of 55 months.

Table 4 shows the 3-year and 5-year OS of the ypN stage and ypNr category. We then compared the prognoses of ypN and ypNr categories using the log-rank test. First, each ypN category was stratified into subgroups according to the stage of ypNr (**Fig. 2**). ypN0 patients were stratified into 2 groups according to the previous definition. The OS was significantly different between the 2 groups (3-year OS 91.8% vs. 73.9%, P=0.004). In the ypN1 group, no ypNr3 patients were observed. ypNr1 showed better OS than ypNr2 patients, although the difference was not significant (3-year OS 76.1% vs. 57.1%, P=0.288). In the ypN2 group, there were only 2 patients with ypNr3, and both patients showed poor prognoses. Although the prognosis was not significantly different among ypNr subgroups in the whole model (P=0.078), ypNr1 patients still showed better OS than ypNr2 (3-year OS 76.1% vs. 55%, P=0.039). In the ypN3 group, only 1 patient with ypNr1 died 55 months after treatment initiation. OS was not significantly different among the ypNr subgroups (P=0.642). Conversely, when each ypNr category was stratified into ypN subgroups. The prognosis was not different among the different ypN stages (**Fig. 3**).

ROC curve according to actual 3-year survival

The AUC of ypNr and ypN stages was calculated to evaluate the predictive accuracy of 3-year OS. As shown in **Fig. 4**, the AUCs of the ypNr and ypN categories were 0.773 and 0.757, respectively.

Validation cohort

A total of 143 patients with complete follow-up data were retrospectively analyzed in the validation cohort. Baseline characteristics are summarized in **Supplementary Table 3**. The average number of rLNs was 36.3±16.0, with a median number of 34 (range, 10–100). The number of rLNs was >15 in 140 patients (97.9%).

All patients were followed up until November 2020, and 47 patients (32.9%) died at the last follow-up. The median follow-up time was 42 months (range, 3–62 months). The 3-year OS rate of all patients was 72%. The 3-year OS rates of ypNr0, ypNr1, ypNr2 and ypNr3 were 94.6%, 75.4%, 62.2% and 16.7% respectively. In the univariate analysis, preoperative chemotherapy regimen (P=0.003), ypN (P<0.001), and ypNr (P<0.001) (**Supplementary Fig. 2**). We used the chemotherapy regimen, ypT, and ypNr in the multivariable analysis with backward regression. ypNr was the only independent prognostic factor (P<0.001) (**Supplementary Table 4**).

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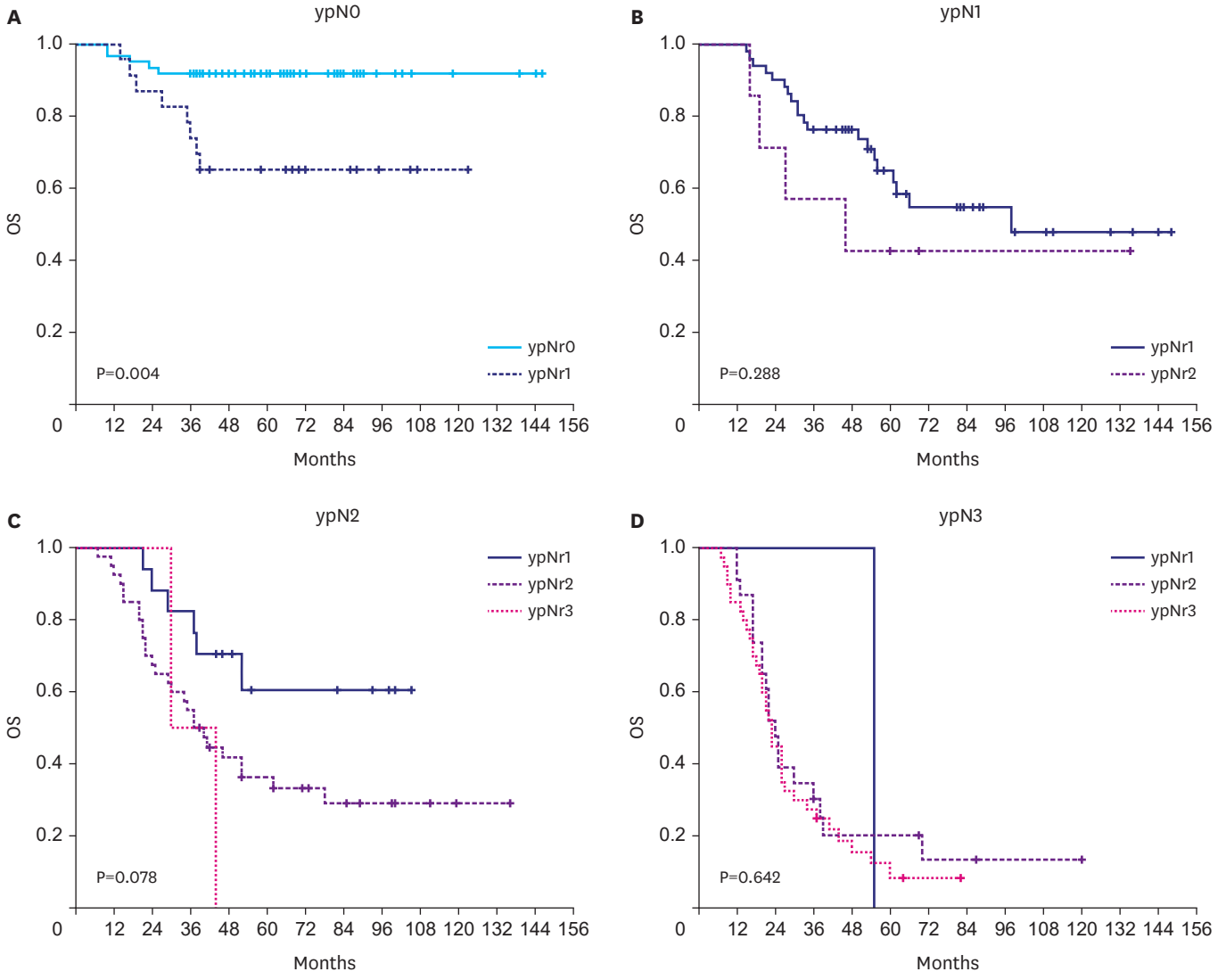

Fig. 2. Kaplan-Meier estimates of ypNr category stratified by ypN stage. (A) ypNr category in ypN0 stage, P=0.004, no ypNr2 nor ypNr3 in this group. (B) ypNr category in ypN1 stage, P=0.288, no ypNr3 in this group. (C) ypNr category in ypN2 stage, P=0.078, 2 ypNr3 patients in this group. (D) ypNr category in ypN3 stage, P=0.642, 1 ypNr1 patient in this group.
OS = overall survival.

DISCUSSION

In this retrospective study, the number of pLNs increased as rLN increased, but the LNR was relatively constant. LNR was an independent prognostic factor for OS in multivariate analysis and was not inferior to ypN stage in predicting the actual 3-year OS.

The TNM classification is an important predictive system for gastric cancer, and the 8th edition of the TNM staging system was released recently, in which a novel ypstage was created to discriminate the prognosis of patients after preoperative therapy [4]. As the ypN stage was determined by the number of pLNs, the stage migration phenomenon remains a possible problem in patients undergoing preoperative chemotherapy. Our results showed that the pLN count after chemotherapy increased, accompanied by an increase in the rLN count, which was consistent with previous studies [7,11]. However, most studies

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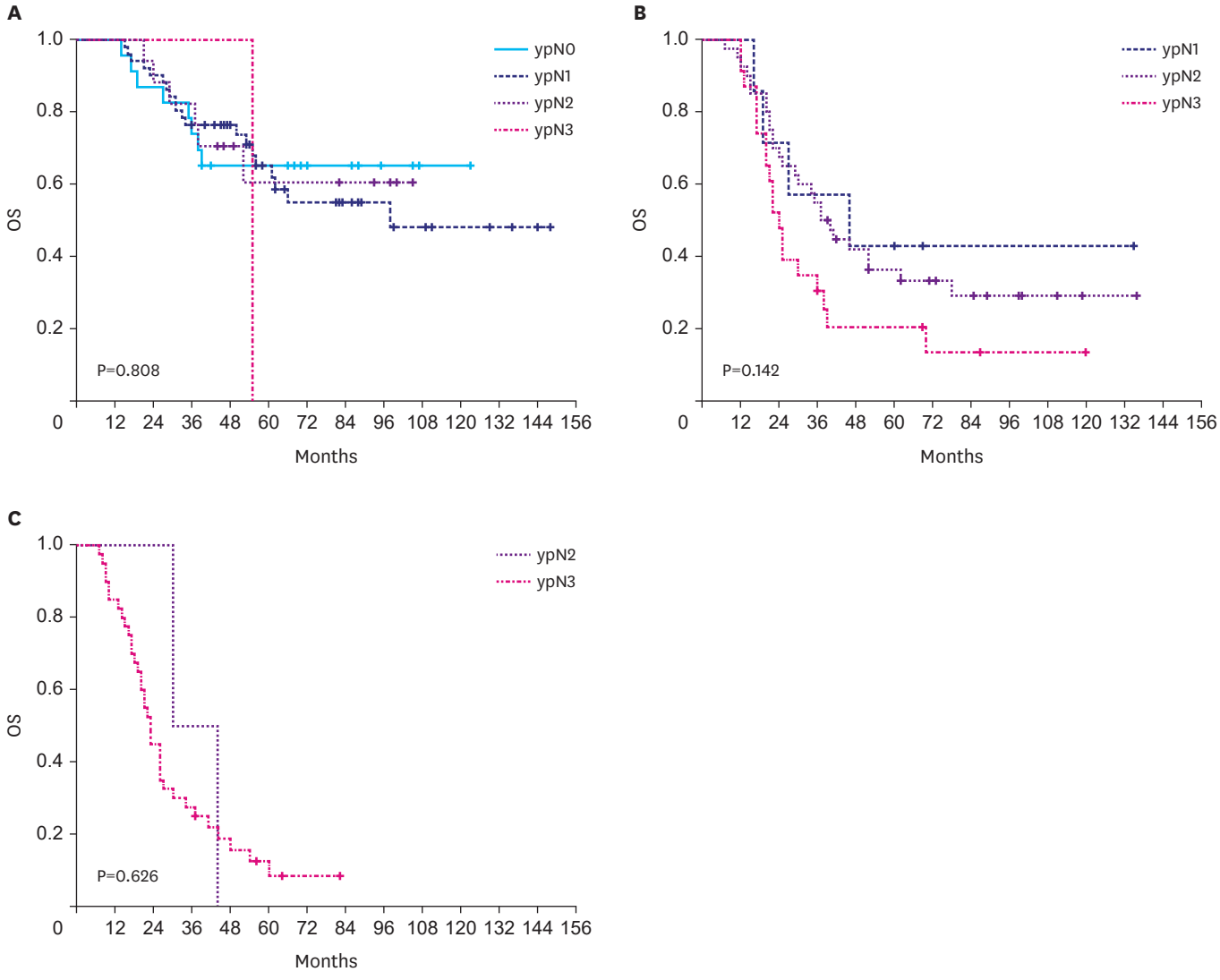

Fig. 3. Kaplan-Meier estimates of ypN stage stratified by ypNr category. (A) ypN stage in ypNr1 category, P=0808. (B) ypN stage in ypNr2, P=0.142. (C) ypN stage in ypNr3 stage, P=0.626, no ypN1 patients in this group.
OS = overall survival.

investigating the prognostic value of LNR excluded patients receiving preoperative therapy because neoadjuvant therapy might influence the total and pLN counts [12,13]. Recently, Li et al. [14] reported that neoadjuvant chemotherapy did not reduce the total LN count with D2 lymphadenectomy in patients with esophagogastric adenocarcinoma. In the current study, all patients underwent standard D2 lymphadenectomy. Additionally, the majority of patients had an rLN number of more than 15, and the median number of harvested LNs was 32. These results were comparable to those of previous studies in which patients underwent upfront surgery [14-16]. Thus, the total LN count might not be influenced by neoadjuvant chemotherapy. However, it is determined by the extent of lymphadenectomy and the effort of LN harvesting.

Although many studies have demonstrated that LNR was an independent prognostic factor for gastric cancer patients, the cut-off value varied between different studies [8,12,13,17,18]. There was still no optimal cut-off value for LNR in predicting prognosis. In addition, the

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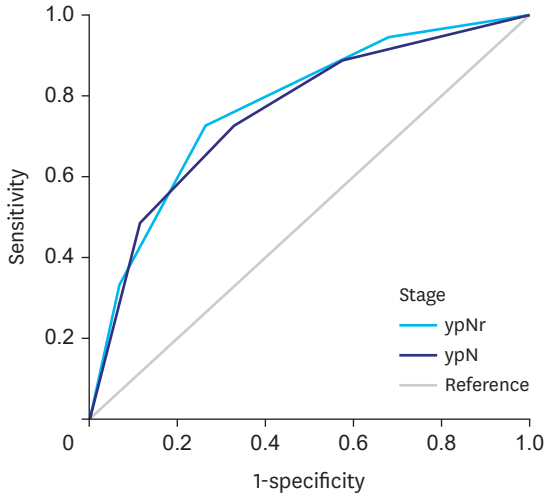

Fig. 4. ROC curves of ypN and ypNr category for prediction of actual 3-year OS of patients with preoperative chemotherapy.
OS = overall survival; ROC = receiver operating characteristic.

cut-off value in previous studies was not appropriate for our study, as they excluded patients receiving preoperative chemotherapy. In the present study, we first divided patients into 10 groups, with neighboring groups having similar OS merged. Then, the first cut-off value of 0.1 was made. Compared with group 4 (LNR: 0.21–0.3), the OS was lower in group 5 (LNR: 0.31–0.4). However, the difference was not statistically significant between the 2 groups. This might be due to the small number of cases that were included in each subgroup, thus statistical significance was not reached. Different combinations were compared, and 0.3 was the best cut-off value to discriminate the prognosis. Furthermore, the prognosis of ypNr2 was also significantly lower than that of ypNr3 in the validation cohort (P<0.001). The prognosis of patients with LNR>0.5, was extremely poor compared with other groups. However, we merged these patients into ypNr3 (LNR>0.3) because of their small number. With a larger sample size, ypNr3 might be divided into ypNr3a and ypNr3b. Nr0 was initially observed in patients with no regional LN metastasis. However, we observed that the prognosis of node-negative patients with 25 or less was worse than that of patients with more than 25 retrieved nodes, while the prognosis was similar to that of patients with Nr1. Many patients were found to have N0 and insufficiently examined LNs were not truly node-negative but rather under staged. Some studies have also demonstrated poor prognosis in node-negative patients with insufficient LN counts [19,20]. Hence, we merged these patients into the ypNr1. In the validation cohort, ypNr categories according to the proposed cut-off values could discriminate the prognosis well. Additionally, it was the only independent prognostic factor.

LNR might be an alternative, even a superior predictor for gastric cancer patients with insufficient LN harvest (<15) [21,22]. An increasing number of studies have demonstrated that the predictive value is better than the N stage classification even in patients with D2 lymphadenectomy and a sufficient number of LN dissections [7,23,24]. However, the predictive value of LNR in patients receiving neoadjuvant chemotherapy has not been reported. Recently, it was reported that LNR was associated with tumor diameter, Lauren classification, and tumor regression grade (TRG) [9]. Our results showed that LNR remained an independent prognostic factor for gastric cancer patients after neoadjuvant chemotherapy in both the proposal and validation cohorts. Each ypN stage was stratified into different ypNr subgroups, after which the patients with different subgroups showed heterogeneous 3-year/5-

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year OS in the ypN0–ypN2 stage. Conversely, in the same ypNr stage, patients with different ypN classifications showed homogenous-/3-year/5-year OS. However, in patients with the ypN3 stage, the predictive value of ypNr classification was attenuated. It might be because all patients received D2 lymphadenectomy with sufficient LNs harvest. Therefore, ypN3 represented an extremely poor prognosis which was partly reflected by the poor response to neoadjuvant chemotherapy. Meanwhile, in the ypN0–2 stage, one additional metastatic LN could cause stage migration. Hence, ypNr classification might have more predictive value in these patients. In this study, the 3-year OS of patients with ypN3 and ypNr1 was 100%, but the 5-year OS was 0%. This might be due to the limited sample size, in which only one patient with ypN3 was categorized as ypNr1. Moreover, it is a rare condition in that more than 70 rLNs were required in ypN3 patients in order to obtain the ypNr1 category. Hence, efforts to harvest enough LNs are recommended to determine the precise prognosis of patients with this condition.

The present study had limitations: (1) The retrospective cohort nature of the study, which might have resulted in patient selection bias. (2) The data of clinical response and TRG were not included in this study, and the relationship between response and ypNr, ypN could not be demonstrated. However, our results showed that were more ypT0/T1 patients in the ypNr0 and ypNr1 subgroups. This indirectly indicates that the LNR might be related to pathological response. Although this study showed that patients with higher LNR in the same ypN stage had an unfavorable prognosis, the sample size was not large enough in each subgroup to draw more solid conclusions. Many studies have reported that LNR is an independent prognostic factor. However, it has not been adopted in the TNM stage classification. This might be due to computational complexity, and no uniform cut-off was developed. Nevertheless, LNR is a simple tool used to minimize the stage migration phenomenon caused by an increase in rLN number. These results should be confirmed by a large sample-sized external prospective cohort.

In conclusion, LNR was an independent prognostic factor for patients with gastric adenocarcinoma after preoperative chemotherapy. It might be a simple alternative predictor for the ypN category of the 8th edition TNM staging system.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Combination of different LNR cut-off points with P-values using enumeration method

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Supplementary Table 2

The OS of each subgroup of ypN0 according to rLN number

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Supplementary Table 3

Baseline characteristics of validation cohort

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Supplementary Table 4

Univariable and multivariable analysis of overall survival in validation cohort

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Supplementary Fig. 1

Kaplan-Meier estimates for OS according to rLN number in ypN0 patients (P=0.055).

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Supplementary Fig. 2

(A) Kaplan-Meier estimates for OS according to ypN stage in validation cohort (P<0.001). (B) Kaplan-Meier estimates for OS according to ypNr stage in validation cohort (P<0.001).

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