Combination of the ratio between metastatic and harvested lymph nodes and negative lymph node count as a prognostic indicator in advanced gastric cancer: a retrospective cohort study

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Background: The aim of our study was to examine the impact of the combination of the ratio between metastatic and harvested lymph nodes (RML) and negative lymph node (NLN) count on overall survival (OS) in patients with advanced gastric cancer (GC).

Methods: The clinicopathological data of 2,952 advanced GC patients who received curative resection between 1994 and 2015 were collected. They were divided into four groups according to the RML: 0, 0–0.1, 0.1–0.4, and >0.4. We distinguished survival differences through Kaplan-Meier analysis among the subgroups to investigate the impacts of the RML on OS in advanced GC patients. OS was examined according to clinicopathological variables. Spearman's correlation coefficient was used to assess the relationships between the RML and metastatic lymph node (MLN) count and NLN count.

Results: A total of 1,182 patients were enrolled into the study. The median follow-up time was 39 months (interquartile range 20 to 68 months). The 5-year OS rate of all 1,182 GC patients was 54.4%. Kaplan-Meier survival analysis showed that the median OS declined significantly with increasing RML (5-year survival rate 81.2% *vs.* 69.1% *vs.* 42.8% *vs.* 13.1%, P<0.001). As the NLN count increased, the survival rate of GC patients increased (5-year survival rate 12.8% *vs.* 25.2% *vs.* 60.2%, P<0.05). The RML, not NLN count, was identified as an independent factor for OS (P<0.001) through multivariate analysis. Spearman correlation analysis suggested that the RML was positively correlated with the number of MLNs (ρ=0.973, P<0.001) and inversely associated with the NLN count (ρ=−0.513, P<0.001).

Conclusions: The RML is an independent prognostic predictor of OS in advanced GC patients, and the NLN count may serve as a supplementary strategy for the present tumor-node-metastasis (TNM) classification to further improve the prognostic prediction efficiency. The combination of the RML and NLN count should be an important predictor for current clinical applications.

Keywords: Gastric cancer (GC); prognosis; negative lymph node (NLN); metastatic lymph node (MLN)

Introduction

Gastric cancer (GC) is the fourth most common cancer in men and the fifth most common in women worldwide (1). It is also the second most common cause of death from cancer (2). Lymph node metastasis is the main metastatic mode of GC and an important cause of postoperative recurrence and death. The most important predictive factor of GC patient prognosis is lymph node status. More than half of GC patients are initially diagnosed with lymph node metastasis (3). Accurate postoperative staging and prognostic evaluation depend on a precise evaluation of lymph node status. Postoperative pathological lymph node (pN) staging should consider the number of metastatic lymph nodes (MLNs) rather than their anatomical site according to the American Joint Committee on Cancer (AJCC) (4). However, whether the current pN staging is the appropriate strategy for the assessment of overall survival (OS) is still controversial. Many researchers have reported that an insufficient number of harvested lymph nodes (HLNs) can induce stage migration (5). The current TNM staging strategy is dependent only on the number of MLNs; hence, it has an insufficient effect when the HLN count is not enough. Thus, we aimed to identify a sensible prognostic marker that does not rely on the range of lymphadenectomy (6). Previous studies have reported that the ratio between metastasis and harvested lymph nodes (RML) is a strong prognostic factor for advanced GC after radical gastrectomy (7-9). Considering that the HLNs of GC are comprised of metastases and negative lymph nodes (NLNs), both are removed from the specimen simultaneously. In theory, the RML should be related to the NLN count, and the NLN count can also serve as a prognostic indicator. However, to the best of our knowledge, no scholars have reported the potential relationship between the RML and NLN count.

Taking into account the above issues, we aimed to elucidate the potential influence of both the RML and NLN count on the prognosis of advanced GC and to determine whether the NLN count could increase the predictive effect of the RML on survival.

We present the following article in accordance with the STROBE reporting checklist (available at https://dx.doi.org/10.21037/jgo-21-212).

Methods

Patients who underwent curative resection for advanced GC from January 1994 to December 2015 were identified from the GC database of the First Affiliated Hospital of Sun Yat-sen University (FAHSYSU) in Guangzhou, China. The following patients were excluded: personal history of malignancy, remnant stomach cancer or recurrent carcinoma, preoperative chemotherapy, stage IV and distant metastasis (e.g., lung, liver, brain, or bone marrow metastasis) and early GC, non-curative resection, <15 HLNs and inadequate follow-up data.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval was obtained from the Medical Ethics Committee of the Seventh Affiliated Hospital of Sun Yat-sen University (No.: KY-2020-024-01). All patient records and information were anonymized and de-identified prior to analysis. Individual consent for this retrospective analysis was waived.

Clinical data collection and processing

The following variables were extracted directly from our GC database: age, sex, primary tumor site, tumor size, degree of tumor differentiation, preoperative serum carcinoembryonic antigen (CEA) level, Borrmann's classification, type of lymphadenectomy, number of HLNs, number of positive lymph nodes, and follow-up status. Moreover, postoperative pathological T stage (pT), N stage (pN), and final AJCC TNM stage were recalculated according to the eighth American Joint Committee on Cancer TNM staging system. Patients were divided into four subgroups according to the RML: 0, 0–0.1, 0.1–0.4, and >0.4, as reported by previous authors (10).

Follow-up and study end points

All participants were followed every three months in the first two years after hospital discharge, every six months in the subsequent three years, then every year or until death. Follow-up evaluations including: physical examination, serum tumor biomarkers, gastroscopy, and contrast-enhanced thorax and abdomen computed tomography (CT). The last follow-up date was December 2019.

The primary end point of this study was OS. OS was defined as the time elapsed between initiation of treatment and death from any cause. OS rates and 95% confidence intervals (CI) were calculated using the Kaplan-Meier estimator. The log-rank test was used to distinguish differences between the survival curves of different patients' groups.

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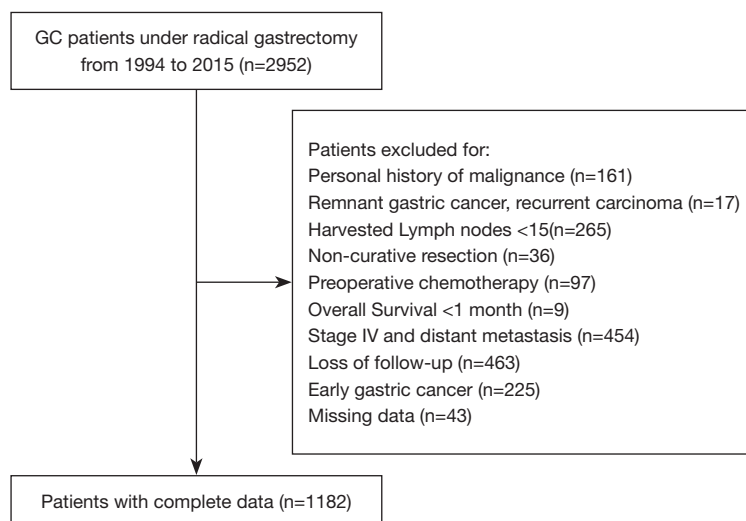
Figure 1 Flowchart describing patient enrollment and exclusion.

Statistical analysis

Categorical data were reported as absolute numbers (n) with proportion (%). Groups were compared using chi-square test and Fisher's exact test. Survival rates were analyzed using the Kaplan-Meier survival curves and log-rank test. Univariate analyses were performed with the unadjusted Cox proportional hazards model to calculate the hazard ratio (HR). To identify independent prognostic factors, all significant variables on univariate Cox regression analysis (P≤0.05) were subjected to multivariate Cox regression analysis. Data were analyzed using SPSS (Windows version 22.0; Chicago, IL, USA).

The following variables were analyzed: (I) sex (male or female); (II) age (<65 or ≥65 years); (III) CEA level (≤5 or >5 ng/mL); (IV) tumor size (<5 or ≥5 cm); (V) primary tumor site (lower third, middle third, upper third, or whole stomach); (VI) depth of primary tumor invasion (AJCC) (T2, T3, or T4); (VII) the number of positive lymph nodes (AJCC) (N0, N1, N2, or N3); (VIII) TNM stage (n0, n1, n2, or n3, AJCC, 8th ed., 2017); (IX) differentiation(well, moderate or poor); (X) Borrmann classification of primary tumor (I,II,III,IV); (11) RML (0, 0.1–10%, 10.1–40%, and >40%); (XI) number of NLNs (0 to 9, 10 to 14, and ≥15).

Results

Patient characteristics

In all, 1,182 patients were enrolled in our study, the participant flowchart are shown in *Figure 1*. The overall median follow-

up time was 39 months (interquartile range, 20 to 68 months). *Table 1* illustrates the demographic and clinical features of these patients. The 5-year OS rate of the study cohort was 54.4%. Of these 1182 patients, 798 were men. The mean age was 57.65 years (21–86 years). A total of 860 patients (72.8%) with MLNs were diagnosed through pathologic examination and assessment. The Borrmann classification was distributed among I (3.6%), II (19.9%), III (66.0%), and IV (9.2%). The primary tumor site was allocated among the upper third stomach (31.5%), middle third stomach (24.8%), lower third stomach (38.7%), and whole stomach (5.1%). The median size of the primary tumor was 4.5 cm (range, 0–23 cm). The total number of HLNs removed from patients in our study was 41,125 (median 32, range, 15–102), and 33,365 lymph nodes were negative. Baseline characteristics are summarized in *Tables 1* and 2.

Prognostic influence of the RML and NLN count in advanced GC after radical gastrectomy

In the present study, Kaplan-Meier survival analysis among different RML groups showed that the RML was associated with a poor prognosis (5-year survival rate 13.1% *vs.* 42.8% *vs.* 69.1% *vs.* 81.2%, P<0.001, *Figure 2A*). Sixty-eight patients with less than nine NLNs who presented with an RML >40% were distinguished. The median OS duration of these patients was fifteen months, and only eight patients were alive at the end of follow-up. Moreover, 96 patients had an NLN count of 10–14. Among these 96 patients, one had an RML of 0.1–10%, with a median OS duration of

Table 1 Baseline characteristics of RML among patients

Characteristics	n	RML				χ^2	P
		0	0–0.1	0.1–0.4	>0.4		
Age	1,182	322	270	398	192	11.74	0.008
<65	823	243	190	254	136		
≥65	359	79	80	144	56		
Gender	1,182	322	270	398	192	9.86	0.020
Male	798	224	188	275	111		
Female	384	98	82	123	81		
CEA[†]	1,181	321	270	398	192	24.15	<0.001
≤5	981	291	229	313	148		
>5	200	30	41	85	44		
Tumor size[‡]	1,147	314	261	388	184	31.31	<0.001
<5	582	192	141	181	68		
≥5	565	122	120	207	116		
Primary site	1,182	322	270	398	192	47.02	<0.001
Upper	372	96	104	131	41		
Middle	293	92	53	92	56		
Lower	457	122	109	155	71		
Whole	60	12	4	20	24		
pT stage	1,182	322	270	398	192	90.97	<0.001
T2	154	87	31	28	8		
T3	467	124	108	165	70		
T4	561	111	131	205	114		
pN stage	1,182	322	270	398	192	2169.00	<0.001
N0	322	322	0	0	0		
N1	209	0	197	12	0		
N2	255	0	72	182	1		
N3	396	0	1	204	191		
pTNM stage	1,182	322	270	398	192	790.50	<0.001
I	85	85	0	0	0		
II	384	226	120	38	0		
III	713	11	150	360	192		
Differentiation	1,182	322	270	398	192	60.55	<0.001
Well/moderate	257	109	68	67	13		
Poor	925	213	202	331	179		

Table 1 (*continued*)

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Table 1 (*continued*)

Characteristics	n	RML				χ^2	P
		0	0–0.1	0.1–0.4	>0.4		
Borrmann[§]	1,166	313	266	395	192	32.95	<0.001
I/II/III	1,057	291	255	356	155		
IV	109	22	11	39	37		
NLN	1,182	322	270	398	192	566.85	<0.001
0–9	68	0	0	0	68		
10–14	97	0	1	41	55		
≥15	1,017	322	269	357	69		

[†], CEA information missing for 1 patient (0.085%); [‡], tumor size information missing for 34 patients (2.8%); [§], Borrmann information missing for 16 patients (1.35%). CEA, carcinoembryonic antigen; NLN, negative lymph node; RML, ratio between metastatic and harvested lymph nodes.

Table 2 Baseline characteristics of negative lymph node counts among patients

Characteristics	n	NLN counts			Statistics	P
		0–9	10–14	≥15		
Age	1,182	68	97	1,017	0.68	0.712
<65	823	48	64	711		
≥65	359	20	33	306		
Gender	1,182	68	97	1,017	7.63	0.022
Male	798	40	56	702		
Female	384	28	41	315		
CEA[†]	1,181	68	97	1,016	15.65	<0.001
≤5	981	45	78	858		
>5	200	23	19	158		
Tumor size[‡]	1,147	62	96	989	8.07	0.018
<5	582	21	46	515		
≥5	565	41	50	474		
Primary site	1,182	68	97	1,017	21.79	0.001
Upper	372	14	32	326		
Middle	293	27	18	248		
Lower	457	19	38	400		
Whole	60	8	9	43		
pT stage	1,182	68	97	1,017	2.75	0.253
T2	154	3	7	144		
T3	467	29	44	394		
T4	561	36	46	479		

Table 2 (*continued*)

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Characteristics	n	NLN counts			Statistics	P
		0–9	10–14	≥15		
pN stage	1,182	68	97	1,017	180.58	<0.001
N0	322	0	0	322		
N1	209	0	8	201		
N2	255	0	27	228		
N3	396	68	62	266		
pTNM stage	1,182	68	97	1,017	90.85[e]	<0.001
I	85	0	0	85		
II	384	0	10	374		
III	713	68	87	558		
Differentiation	1,182	68	97	1,017	15.22	<0.001
Well/moderate	257	3	16	238		
Poor	925	65	81	779		
Borrmann[§]	1,166	68	96	1,002	12.60	0.002
I/II/III	1,057	54	84	919		
IV	109	14	12	83		
RML	1,182	68	97	1,017	316.94	<0.001
0	322	0	0	322		
0.001–0.1	270	0	1	269		
0.1–0.4	398	0	41	357		
>0.4	192	68	55	69		

[†], CEA information missing for 1 patient (0.085%); [‡], tumor size information missing for 35 patients (2.8%); [§], Borrmann information missing for 16 patients (1.35%). CEA, carcinoembryonic antigen; NLN, negative lymph node; RML, ratio between metastatic and harvested lymph nodes.

92 months, 41 had an RML of 10.1–40%, with a median OS duration of 47 months, and 54 had an RML >40%, with a median OS duration of 19 months. There was a clear trend that the larger the RML was, the shorter the median OS was. Last, 1,017 patients were determined to have ≥15 NLNs. Among these patients, 322 had an RML of 9, with a median OS duration of 142 months, 269 had an RML of 0.1–10%, with a median OS duration of 123 months, 357 had an RML of 10.1–40%, with a median OS duration of 44 months, and 69 had an RML >40%, with a median OS duration of 18 months. There was a significant correlation between the RML and OS, and the median OS decreased significantly as the RML increased (P<0.001) (5-year survival rate 81.2% *vs.* 69.1% *vs.* 42.8% *vs.* 13.1%).

After stratification by the AJCC/TNM and pN stage (*Figure 2B-2H*), we found significant survival differences between patients with stage II disease and an RML of 0 and those with an RML of 10.1–40% (5-year survival rate 78.3% *vs.* 54.7%, P=0.001), an RML of 0.1–10% and an RML of 10.1–40% (5-year survival rate 72.8% *vs.* 54.7%, P=0.019) (*Figure 2C*) and among patients with stage III disease and an RML of 0.1–10%, an RML of 10.1–40%, and an RML>40% (5-year survival rate 64.8% *vs.* 41.5 *vs.* 13.1%, P<0.001). The median OS showed a decreasing trend with increasing RML (5-year survival rate 36.1% *vs.* 13.2%, P<0.001) in stage pN3 (*Figure 2H*). Of note, in the analysis of patients with pN1 and pN2 disease, a high RML was not associated with inferior survival (P>0.05, *Figure 2F,2G*).
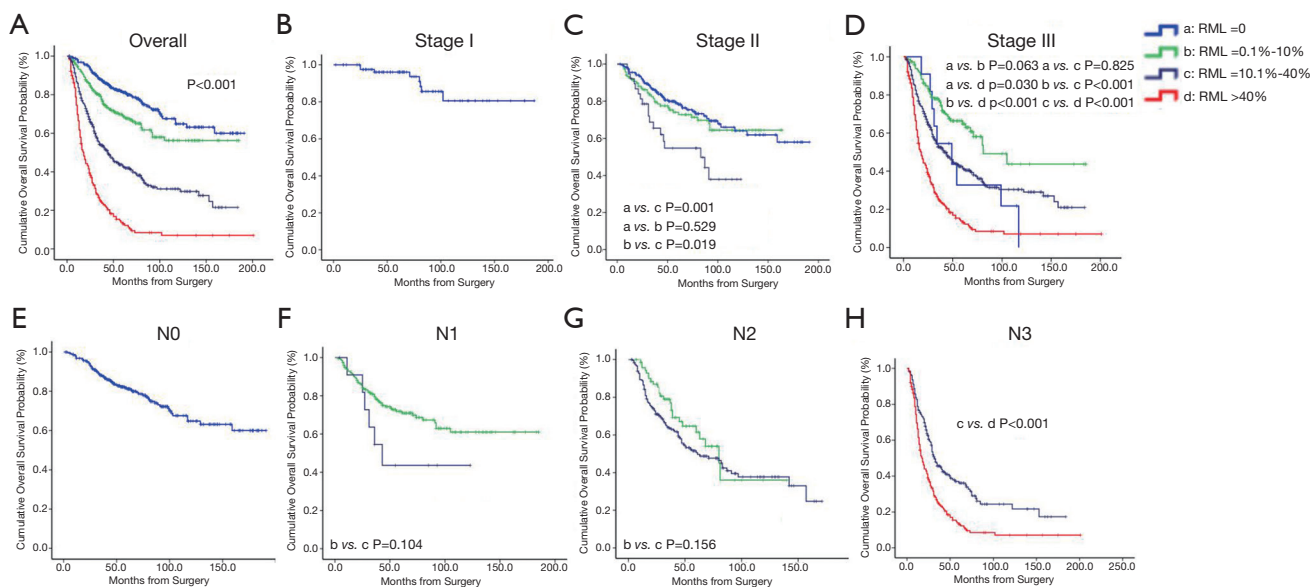
Figure 2 Survival curves of patients according to subgroups: (A) overall; (B) stage I; (C) stage II; (D) stage III; (E) stage pN0; (F) stage pN1; (G) stage pN2; (H) stage pN3. In addition, we observed the opposite trend in overall survival after evaluating differences among different NLN groups. The survival analysis revealed that the NLN count was correlated with increased survival rates of gastric cancer patients (5-year survival rate 12.8% *vs.* 25.2% *vs.* 60.2%, *Figure 3A*, P<0.05). After stratification by the AJCC/TNM and pN stage (*Figure 3B-3H*), we found a significant survival difference between patients with an NLN count =10–14 and those with an NLN count ≥15 (5-year survival rate 21.0% *vs.* 44.0%, *Figure 3D*, P<0.001). Remarkably, in our analysis of stage II, pN1 and pN2 gastric cancer patients, the current cutoff of the NLN count was not related with improved survival (P>0.05, *Figure 3C,3F,3G*). NLN, negative lymph node; AJCC, American Joint Committee on Cancer; TNM, tumor-node-metastasis.

Univariate and multivariate survival analyses of OS

In the univariate analysis, we found the following 12 clinicopathologic variables to be significantly related to OS: age, sex, CEA level, tumor size, degree of tumor differentiation, primary tumor site, depth of tumor invasion (pT), N stage (pN), AJCC pathological classification (pTNM classification), Borrmann's classification, NLN count, and RML (*Table 3*). pN stage was excluded from the multivariate analysis due to multicollinearity, and the remaining 11 variables were included in the multivariate Cox proportional hazards model (entry model) to adjust for the influence of covariates.

Five factors that were independently related with OS were revealed through the multivariate analysis: age at surgery (HR =1.403, P<0.001), primary tumor site (P=0.019), pT stage (P=0.024), Borrmann's classification (HR =1.584, P=0.001), and RML (P<0.001) (*Table 3*). Patients with an RML=0 had the highest 5-year survival rate (81.2%), followed by patients with an RML of 0.1–10% (69.1%), 10.1–40% (42.8%), and >40% (13.1%) (*Figure 2A*).

Regarding pT stage, the 5-year OS rate was highest in patients with stage T2 disease, followed by those with stage T3 and T4 disease. The 5-year OS rate was higher in patients with Borrmann I/II/III than in patients with Borrmann IV, in patients with a tumor size ≤5 cm than in patients with a tumor size >5 cm, and in patients <65 years old than in patients ≥65 years old.

To explore the correlations between the RML and number of MLNs and NLN count, we drew two scatter plots with the RML as a dependent variable. We found that the RML was highly associated with the number of MLNs (ρ=0.973, P<0.001, *Figure 5A*) and decreased as the NLN count increased (ρ=−0.513, P<0.001, *Figure 5B*).

Discussion

In our study, we found that the RML was an independent indicator of prognosis in advanced GC patients undergoing gastrectomy with no less than 15 HLNs. This result suggests that a high RML has prognostic value independent of both traditional clinicopathological factors and the
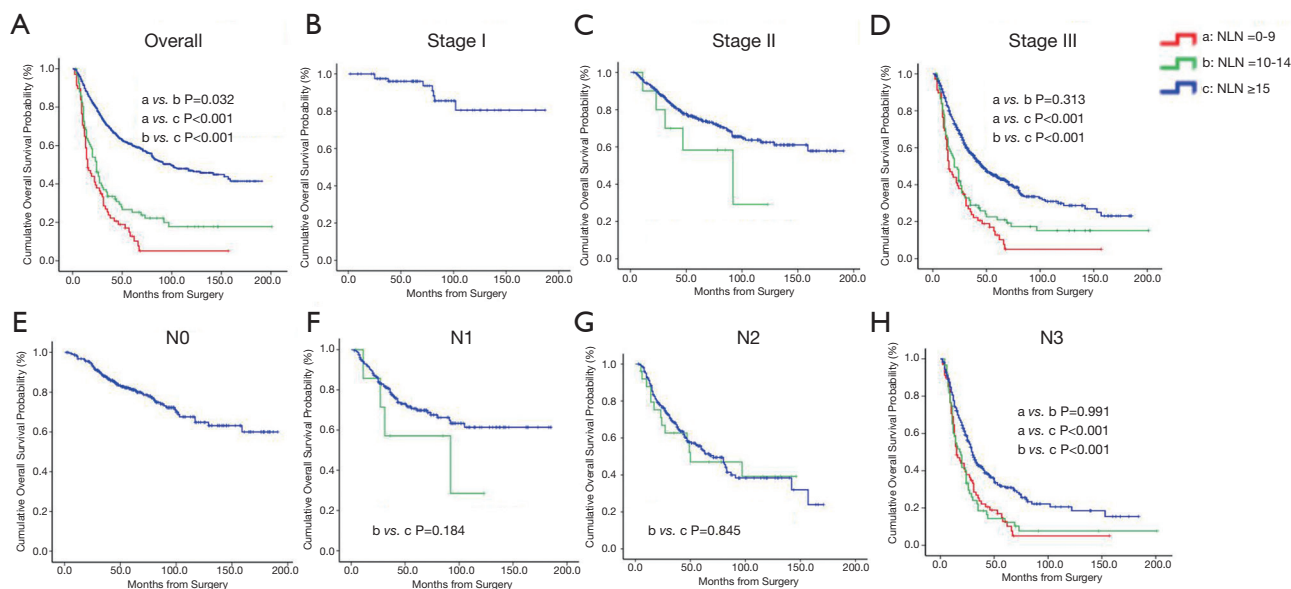
Figure 3 Survival curves of patients according to subgroups: (A) overall; (B) stage I; (C) stage II; (D) stage III; (E) stage pN0; (F) stage pN1; (G) stage pN2; (H) stage pN3. Ultimately, our study provides good evidence that the RML can enhance the prognostic prediction when combined with the NLN count (*Figure 4A-4C*). In the NLN =10–14 group, the RML was associated with inferior survival (5-year survival rate 41.1% *vs.* 11.9%, P<0.001, *Figure 4B*). The same result was true among the NLN ≥15 group (5-year survival rate 81.2% *vs.* 69.0% *vs.* 43.0% *vs.* 13.8%, *Figure 4C*, P<0.001). RML, the ratio between metastatic and harvested lymph node; NLN, negative lymph node.
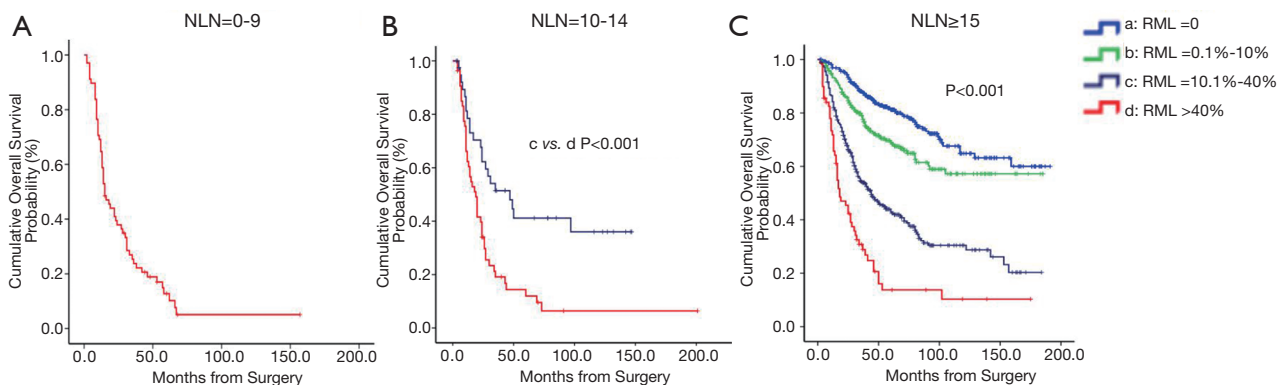
Figure 4 Survival curves of patients according to subgroups: (A) NLN: 0–9; (B) NLN: 10–14; (C) NLN ≥15. NLN, negative lymph node.

extent of lymphadenectomy. We estimated the best cutoff value of the RML to correlate with patient survival. We divided patients into four groups on the basis of the RML and found that a high RML indicated a poor prognosis. The 5-year survival rate was the lowest in the RML >40% group, higher in the RML 10.1–40% and 0.1–10% groups, and highest in the RML=0 group. Theoretically, a high RML may indicate a poor prognosis (11). Our findings are generally consistent with those from previous studies

from both Western and Japanese cohorts despite different cutoff values (9,12,13). Yu *et al.* (14), in 886 patients who were treated with curative resection, indicated that the RML cutoffs were 0, 1–25%, and >25%, with significant differences among the three groups. In a study by Kodera *et al.* (15), 656 advanced GC patients who underwent radical gastrectomy with D2 or higher lymphadenectomy were analyzed, and they chose four cutoffs for the RML: 0%, 1–19%, 20–60%, and >60%. Although there is no

**Table 3** Univariate and multivariate analysis for overall survival

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age				
<65	Referent		Referent	
≥65	1.399 (1.173–1.668)	<0.001	1.403 (1.164–1.692)	<0.001
Gender				
Male	Referent		Referent	
Female	1.204 (1.010–1.435)	0.039	1.189 (0.988–1.430)	0.067
CEA				
≤5	Referent		Referent	
>5	1.421 (1.154–1.750)	0.001	1.110 (0.892–1.382)	0.349
Tumor size				
<5	Referent		Referent	
≥5	1.749 (1.469–2.083)	<0.001	1.162 (0.966–1.397)	0.112
Primary site		<0.001		0.019
Upper	0.414 (0.298–0.575)	<0.001	0.933 (0.636–1.367)	0.720
Middle	0.320 (0.227–0.451)	<0.001	0.655 (0.445–0.963)	0.031
Lower	0.344 (0.248–0.477)	<0.001	0.811 (0.556–1.181)	0.274
Whole	Referent		Referent	
pT stage		<0.001		0.024
T2	Referent		Referent	
T3	3.229 (2.181–4.780)	<0.001	1.822 (1.123–2.958)	0.015
T4	4.158 (2.818–6.136)	<0.001	2.016 (1.217–3.339)	0.007
pTNM stage		<0.001		0.338
I	Referent		Referent	
II	3.312 (1.165–6.794)	0.001	1.358 (0.564–3.269)	0.495
III	10.819 (5.372–21.793)	<0.001	1.770 (0.646–4.846)	0.267
Differentiation				
Well/moderate	Referent		Referent	
Poor	1.623 (1.300–2.026)	<0.001	1.196 (0.944–1.516)	0.138
Borrmann				
I/II/III	Referent		Referent	
IV	2.497 (1.963–3.175)	<0.001	1.584 (1.193–2.103)	0.001

Table 3 (*continued*)

Table 3 (*continued*)

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
RML				<0.001
0	Referent		Referent	
0.001–0.1	1.614 (1.178–2.211)	0.003	1.268 (0.850–1.892)	0.244
0.1–0.4	3.704 (2.839–4.833)	<0.001	2.288 (1.471–3.561)	<0.001
>0.4	8.656 (6.529–11.475)	<0.001	4.587 (2.780–7.569)	<0.001
NLN		<0.001		0.747
0–9	Referent		Referent	
10–14	0.666 (0.471–0.941)	0.021	1.012 (0.697–1.471)	0.948
≥15	0.252 (0.191–0.331)	<0.001	0.910 (0.633–1.307)	0.610

CEA, carcinoembryonic antigen; NLN, negative lymph node; RML, ratio between metastatic and harvested lymph nodes; HR, hazard ratio; CI, confidence interval.
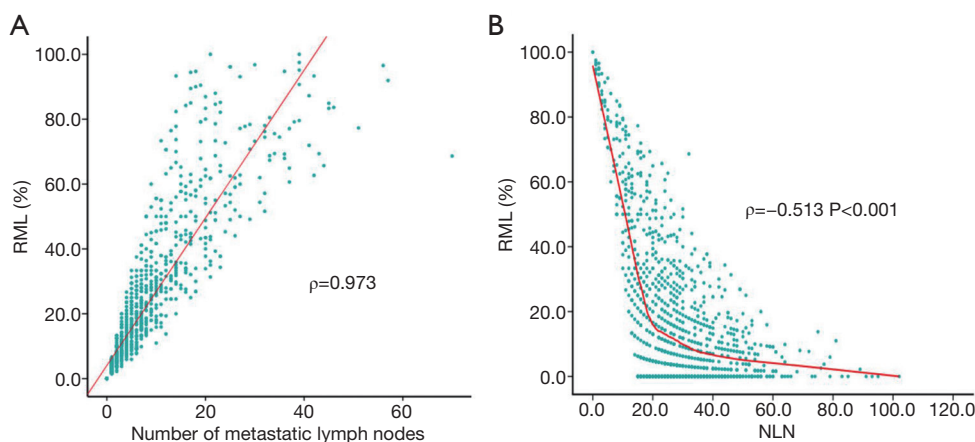
Figure 5 Correlation between RML and number of metastatic lymph nodes (A) and NLN count (B). RML, the ratio between metastatic and harvested lymph node; NLN, negative lymph node.

agreement on the reasonable cutoff values of the RML, all researchers emphasize its clinical significance in terms of simplicity, convenience, reproducibility, and prognostic importance (16).

Moreover, when stratified by the AJCC/TNM stage, we found that the higher the RML was, the worse the prognosis among patients with TNM stage II, III and pN3 disease. Moreover, when we considered patients in stages pN0, pN1 and pN2, no statistically significant differences in survival were observed. The findings of our study are generally in accordance with the results of previous studies (9). One possible reason for this is that the RML is distributed mostly near zero. Therefore, the RML is not an effective tool to distinguish GC of the pN0, pN1 and pN2 stages with less than 6 MLNs.

We divided patients into three groups according to the NLN count and discovered that a higher NLN was related to better survival through Kaplan-Meier survival analysis. Previous studies have reported that so-called NLNs might harbor micrometastasis from the primary tumor (17). In addition, lymph node micrometastasis cannot be diagnosed through a general pathological examination, revealing that the NLN count can be regarded as an important marker and might even be necessary in improving the prognostic

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ability of lymph node status (18). Previous researchers have reported the possibility of recurrence in advanced GC patients without lymph node metastasis even after extended lymphadenectomy (19). Saito *et al.* (20) suggested that lymph node micrometastasis was significantly related to relapse after radical gastrectomy. Here, we inferred that a higher NLN count means fewer residual tumor cells. However, we found that the NLN count could not serve as an independent factor in advanced GC, as estimated by the multivariate analysis. These results are discrepant with those of Deng (10) and Zhang (21). The possible reasons could be the diverse study population and the inclusion and exclusion criteria. We excluded patients with early GC, those who received preoperative chemotherapy and those with stage IV disease, distant metastasis, and <15 HLNs, which is more in line with the actual situation.

The mechanism underlying the correlation between the RML or NLN count and survival has not been determined. There are several possibilities. First, the RML and NLN count may be signs of adequate surgical, pathological, and medical care. Advanced surgeon skills and expertise may be related to more extensive lymph node dissection or a more intensive search for lymph nodes. These factors may contribute to improved outcomes and may reflect institutional endeavors in the therapy of GC patients. Thus, the RML and NLN count may be related to good medical care and may not directly influence the outcome. However, many studies have identified a relationship between the RML or NLN count and outcome, suggesting that when other variables are kept constant, they are still critical (22). Another reason is that an adequate number of lymph nodes rely on surgeons, who vary in attempt and skills as they search for lymph nodes. Many doctors tend to search only for nodes that are visible and palpable (23). Third, according to previous anatomic studies, the number of lymph nodes differs between patients and is affected by disease status (24). The tumor itself may cause lymph nodes to increase in size; germinal centers may also cause the development of lymph nodes, increasing the actual number. If true, the RML and NLN count would indicate potential tumor-host interactions and may have an independent influence on survival (25).

In our creative study, we found a significant relationship between the RML and prognosis in advanced GC patients after gastrectomy with systemic lymphadenectomy, and the RML was identified as an independent predictive indicator of outcome. Stratification analyses by TNM stage and pN stage indicated that both the RML and NLN count

could provide better prognostic information for patients with stage III and pN3 GC than those with other stages. Moreover, evaluation of the RML may obviate possible confounding factors associated with the number of lymph nodes excised and the number of gastric lymph nodes, which differ in each individual (26). Unlike the pN stage classification, the prognostic efficiency was significantly influenced by the number of removed lymph nodes. In summary, our findings emphasize the ability of the RML to distinguish prognostically homogeneous subsets of patients.

However, there were also some limitations to our study. First, the retrospective character of our cohort study is its major limitation. Second, surgical procedures, surgical instruments, surgical skills, examinations of lymph nodes and adjuvant chemotherapies changed during the evolution of the cohort, which may have influenced the findings; such evolution was inherent in long-term follow-up studies. Third, the cohort was comprised of the Chinese population, which may have led to racial bias. However, almost 42% of GC patients are from China. Therefore, we believe that our present results have representative significance.

In summary, our data suggest that the RML may serve as an independent prognostic indicator in advanced GC patients after radical gastrectomy with standard lymphadenectomy, especially those with stage III and pN3 disease. Moreover, the RML was highly correlated with the number of MLNs and inversely associated with the NLN count. According to the results presented herein, we suggest utilization of the combination of the RML and NLN count in the prognostic assessment of advanced GC, especially for stage III and pN3 GC patients.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval was obtained from the Medical Ethics Committee of the Seventh Affiliated Hospital of Sun Yat-sen University (No.: KY-2020-024-01). All patient records and information were anonymized and de-identified prior to analysis. Individual consent for this retrospective analysis was waived.

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