Impact of the extent of negative lymph nodes in gastric adenocarcinoma undergoing primary surgical resection: An institutional report

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Abstract

Background: Sub-total/total gastrectomy with lymph node dissection (LND) remains an effective therapeutic strategy for resectable gastric adenocarcinomas (GACs). Despite the prognostic significance of positive lymph nodes (PLNs) defined in N-status, few have appraised the impacts of negative lymph nodes (NLNs) and the percentage of NLN (=number of NLNs/number of total lymph nodes [TLNs], %), as well as the extent of TLNs to be dissected in GACs.

Methods: We retrospectively analyzed 62 GAC patients (mean age of 67.1 years; 41 men) undergoing primary sub-total/total gastrectomy from a single institute. Candidate variables, including the number of NLNs (≤9 and >9) and the percentage of NLN (≤37.5, 37.5-80.6 and >80.6, %), were evaluated to determine their prognostic impacts and hazard ratios (HRs).

Results: Under the multivariate Cox proportional-hazards regression model, tumor length exceeding 4 cm ($p = 0.017$; HR = 2.828), perineural invasion ($p = 0.037$; HR = 3.182), and lower percentage of NLN ($p = 0.016$ and $p = 0.060$; HRs = 1.000, 0.327, and 0.333 for subgroups ≤37.5, 37.5-80.6, and >80.6, respectively) were three independent predictors with elevated HRs for poor prognosis. GAC patients with the percentage of NLN > 80.6 were highly related to those with NLNs > 9 ($p < 0.001$), and GAC patients with NLNs > 9 were highly related to those with TLNs > 15 ($p < 0.001$). For all 62 GAC or 42 N(+) GAC patients, those who underwent LND with TLNs>15 tended to have more PLNs ($p = 0.018$, $p = 0.003$) and more NLNs ($p < 0.001$, $p = 0.029$) than did those with TLNs ≤ 15. Among the 42 GAC patients with TLNs > 15, a lower percentage of NLN ($p = 0.026$ and $p = 0.015$; HRs = 1.000, 0.272, and 0.180 for subgroups ≤37.5, 37.5-80.6, and >80.6, respectively) remained an independent predictor of poor prognosis.

Conclusion: The percentage of NLN could predict the prognosis of GAC patients properly. However, an accurate percentage of NLN needs a minimal requirement of TLNs > 15 to detect an adequate number of PLNs and sufficient number of NLNs.

Keywords: Adenocarcinoma; Lymph node excision; Lymph nodes; Prognosis

1. INTRODUCTION

Gastric adenocarcinoma (GAC) is one of the 10 leading causes of cancer-related deaths worldwide.[1,2] Surgical resection with an extensive lymph node dissection (LND) remained an optimal treatment modality for operable GAC.[3] Concerning the extent of dissected lymph nodes (LNs), quantified as the number of total LNs (TLNs), some would be diagnosed as nodal positive status N(+), quantified as the number of positive LNs (PLNs), and some would be diagnosed as nodal negative status N(−), quantified as the number of negative LNs (NLNs), after pathological examination (number of TLNs = number of PLNs + number of NLNs). In clinical practice, classifications of the dissected LNs, from the viewpoints of N(+), N(−), or their combinations, played an important role in predicting GAC prognosis.[4,5]

Generally, tumor (T) status, node (N) status, metastasis (M) status, and cancer stage defined in the American Joint Committee on Cancer (AJCC) manual remain the gold standard for making survival predictions and tailoring therapeutic strategies for GAC patients.[6] Nowadays, the N-status defined in the AJCC eighth edition emphasizes the number of PLNs (subgrouped 0, 1-2, 3-6, and ≥7 as N0, N1, N2, and N3, respectively) to predict survivals.[7] On the contrary, the role of NLN in predicting GAC prognosis has emerged in recent years.[8] Some demonstrated more NLNs might improve survival rates, especially the N(+) GAC patients; and some demonstrated a

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higher NLN ratio was related to a better survival.[5,9,10] As a result, reappraising the impacts of LN classifications seems of great clinical relevance.

In this retrospective analysis, we reappraise the possible prognostic variables, including the impacts of NLN and its classifications, for operable GAC patients undergoing primary surgery. The minimal requirement of TLNs was also evaluated.

2. METHODS

2.1. Collection of GAC patients

From January 2012 to December 2019, a total of 62 GAC patients who underwent upfront surgical resection in Taipei Hospital, Ministry of Health and Welfare, New Taipei City, Taiwan, were recruited in this retrospective study. None of them received preoperative neoadjuvant chemotherapy, radiotherapy, or both. Postoperative adjuvant chemotherapy, radiotherapy, or both were advocated if clinically indicated. The Institutional Review Board of Taipei Hospital approved this study (IRB number, TH-IRB-0018-0022) and all patients were completely followed until December 2019.

Routine chest radiography, computed tomographic (CT) scanning of the whole abdomen, upper gastro-intestinal pan-endoscopy, a complete blood count with cell differentials of leukocytes in the peripheral blood, prothrombin time/activated partial thromboplastin time, blood biochemistries and EKG ± cardiac ultrasound were routine examinations to access each patient's general and oncological conditions. Whole body bone scan or CT scanning of the brain was performed if clinically indicated.

The surgical modalities included laparoscopy or explore laparotomy for subtotal/total gastrectomy with Billroth-I, Billroth-II, or Roux-en-Y gastrojejuno/esophagojejuno anastomosis. D2 LN dissection was carried out along the left gastric artery, common hepatic artery, celiac artery, splenic artery, and hepatoduodenal ligament. The proximal and distal cut ends of the resected stomach were checked by an experienced pathologist during operation to confirm freedom from tumor invasion. The pathological T-/N-/M-status and cancer stage were set according to the AJCC eighth edition.[6]

2.2. Prognostic variables

Possible variables, including demographic data and tumor characteristics, were recorded in detail for analysis. Concerning the LN classifications, we compared the differences among (1) current N-status defined in the AJCC eighth edition, (2) the number of NLNs, and (3) the percentage of NLN (defined as number of NLNs/number of TLNs, %).

2.3. Statistical analysis

The continuous variables between two or more groups were compared using a *t*-test/Mann-Whitney *U* test or analysis of variance (ANOVA)/Kruskal-Wallis *H* test when appropriate. Categorical variables between/among groups were compared using a chi-square (χ^2) test, Fisher exact test, or chi-square (χ^2) test for trend (linear-by-linear association) when appropriate. Relationships between two continuous variables were evaluated by linear regression and presented with the Pearson correlation coefficient (Pcc) and R^2. The overall survivals were calculated from the date of surgery to the date of death or last follow-up in December 2019. Survival curves were plotted by the Kaplan-Meier method. The log-rank test was used to compare survival probabilities among different levels within each categorical variable, and the univariate Cox proportional-hazards regression method was used to examine their relative hazard ratios (HRs). Variables associated with survival probability at a significance level of 0.1 or less (≤0.1) in the log-rank test were included in the multivariate Cox proportional-hazards regression model. To subgroup the 62 GAC patients, the optimal cutoff points, including the number of NLNs or percentage of NLN to distinguish the current survival status and to predict the survival prognosis or the number of TLNs to distinguish NLNs ≤ or >9, were plotted by receiver operating characteristic (ROC) curves to figure out the highest/relatively highest Youden indexes (Youden index = sensitivity + specificity − 1).[11] Significant difference was defined as a $p < 0.05$.

Table 1

Demographic data of the 62 gastric adenorcinoma patients

Variable	Number (%)/mean ± SD
Gender	
Female	21 (33.9)
Male	41 (66.1)
Age, y	67.1 ± 13.6
Type of gastrectomy	
Subtotal	47 (75.8)
Total	15 (24.2)
Postoperative adjuvant therapy[a]	
Yes	24 (38.7)
No	38 (61.3)
Pathological findings	
Maximal tumor diameter, cm	4.6 ± 2.6
Cell differentiation	
Well	8 (12.9)
Moderate	22 (35.5)
Poor	32 (51.6)
T-status[b]	
T1	13 (21.0)
T2	6 (9.7)
T3	29 (46.8)
T4	14 (22.6)
N-status[a]	
N0	20 (32.3)
N1	3 (4.8)
N2	12 (19.4)
N3	27 (43.5)
M-status[a]	
M0	49 (79.0)
M1	13 (21.0)
Lymphovascular invasion	
No	22 (36.7)
Yes	38 (63.3)
Perineural invasion	
No	22 (37.3)
Yes	37 (62.7)
Number of TLNs	22.1 ± 11.5
Number of PLNs	8.4 ± 9.8
Number of NLNs	13.7 ± 10.1
Percentage of NLN	64.9 ± 34.2
Follow-up period, mo	23.1 ± 22.7
Survival, mo	45.1 ± 5.8

NLNs = negative lymph nodes; Percentage of NLN = number of NLNs/number of TLNs of each gastric adenocarcinoma (GAC) patient; PLNs = positive lymph nodes; SD = standard deviation; TLNs = total lymph nodes.

[a]Among the 62 GAC patients, 24 (38.7%) underwent postoperative adjuvant therapy, including 22 adjuvant chemotherapy, 1 adjuvant radiotherapy, and 1 concurrent adjuvant chemotherapy and radiotherapy.

[b]American Joint Committee on Cancer, eighth edition.

Table 2

HRs and prognostic impacts of the number of NLNs, percentage of NLN, and number of PLNs of the 62 gastric adenocarcinoma patients

	HRs		Cox proportional-hazards regression model	Survivals, mo		Survival difference
	Mean	95% CI	*p* value (univariate)	Mean	95% CI	*p* value (Log-rank)
Continuous model						
Number of NLDs (n = 62)	0.952	0.912-0.994	0.027			
Percentage of NLN (n = 62)	0.976	0.965-0.988	<0.001			
Number of PDLs (n = 62)	1.070	1.033-1.108	<0.001			
Categorical model						
Number of NLNs[a]						0.001
≤9 (n = 26)	1.000	1.000		18.6	10.5-26.7	
>9 (n = 36)	0.314	0.149-0.664	0.002	58.9	44.2-73.5	
Percentage of NLN[b]						<0.001
≤37.5 (n = 15)	1.000	1.000		9.6	6.1-13.2	
37.5-80.6 (n = 20)	0.315	0.133-0.747	0.009	26.7	18.7-34.8	
>80.6 (n = 27)	0.119	0.044-0.321	<0.001	66.5	58.8-82.2	
Number of PLNs[c]						0.009
0 (N0) (n = 20)	1.000	1.000		60.4	46.3-74.6	
<3 (1, 2) (N1) (n = 3)	1.624	0.190-13.909	0.658	62.6	12.6-112.7	
3-7 (3, 4, 5, 6) (N2) (n = 12)	3.694	1.205-11.327	0.022	33.2	13.2-53.3	
≥7 (7 or more) (N3) (n = 27)	4.965	1.782-13.829	0.002	19.1	12.2-26.0	

CI = confidence interval; HRs = hazard ratios; NLNs = negative lymph nodes; Percentage of NLN = number of NLNs/number of total lymph nodes of each gastric adenocarcinoma (GAC) patient; PLNs = positive lymph nodes.

[a]We tested various cutoff points for number of NLNs on receiver operating characteristic (ROC) curves (area under the curve [AUC] = 0.661, 95% confidence interval [CI] = 0.520-0.801, $p = 0.030$) to distinguish survival status among 62 GAC patients, and 9 (sensitivity = 0.516, specificity = 0.839) had the highest Youden index of 0.355. Then we divided the 62 GAC patients into two groups, including those with NLNs ≤ 9 (n = 26) and those with NLNs > 9 (n = 36).

[b]We tested various cutoff points for percentage of NLN on ROC curves (AUC = 0.728, 95% CI = 0.603-0.853, $p = 0.002$) to distinguish survival status among 62 GAC patients, and 37.5 (sensitivity = 0.419, specificity = 0.935) and 80.6 (sensitivity = 0.742, specificity = 0.613) had the relative highest Youden indexes of 0.354 and 0.355, respectively. Then we divided the 62 GAC patients into three groups, including those with percentage of NLN ≤ 37.5 (n = 15), those with percentage of NLN 37.5 to 80.6 (n = 20), and those with percentage of NLN > 80.6 (n = 27).

[c]According to the N-status defined in American Joint Committee on Cancer eighth edition.

3. RESULTS

3.1. Demographic data

From January 2012 to December 2019, a total of 62 GAC patients (mean age of 67.1 years, male/female: 41/21) undergoing upfront surgical resection (subtotal/total gastrectomy: 47/15) were analyzed in this retrospective study (Table 1). Among the 62 GAC patients, 24 (38.7%) underwent postoperative adjuvant therapy, including 22 adjuvant chemotherapy, 1 adjuvant radiotherapy, and 1 concurrent adjuvant chemotherapy and radiotherapy. As to the pathological diagnosis, their mean maximal tumor diameter was 4.6 cm and there were 13 (21.0%)/6 (9.7%)/29 (46.8%)/14 (22.6%) in T1/T2/T3/T4 status 20 (32.3%)/3 (4.8%)/12 (19.4%)/27 (43.5%) in N0/N1/N2/N3 status, and 49 (79.0%)/13 (21.0%) in M0/M1 status, respectively. Concerning cancer cell differentiation, there were 8 (12.9%), 22 (35.5%), and 32 (51.6%) GACs, harboring well, moderate, and poor differentiations, respectively. Thirty-eight (63.3%) GAC patients found to have lymphovascular invasion and 37 (62.7%) with perineural invasion. Concerning the distributions of dissected LNs, their mean numbers of TLNs, PLNs, and NLNs were 22.1, 8.4, and 13.7, respectively. Their mean percentage of NLN was 64.9%. Overall, their mean survival and follow-up period were 45.1 and 23.1 months, respectively.

3.2. HRs and prognostic impacts of the number of NLNs, percentage of NLN, and number of PLNs of the 62 GAC patients

Concerning the prognostic impacts, the HRs of the number of NLNs, percentage of NLN, and number of PLNs of the 62 GAC patients were analyzed through the continuous and categorical models, respectively (Table 2). Under the continuous model, we found one more NLN had a lower HR of 0.952 (95% confidence interval [CI] = 0.912-0.994, $p = 0.027$), one more percentage of NLN had a lower HR of 0.976 (95% CI = 0.965-0.988, $p < 0.001$), but one more PLN had a higher HR of 1.070 (95% CI = 1.033-1.108, $p<0.001$), respectively.

We tested various cutoff points for the number of NLNs on ROC curves (area under the curve [AUC] = 0.661, 95% CI = 0.520-0.801, $p = 0.030$) to distinguish the survival status among 62 GAC patients, and 9 (sensitivity = 0.516, specificity = 0.839) had the highest Youden index of 0.355. Then we divided the 62 GAC patients into 2 groups, including those with NLNs ≤ 9 (n = 26) and those with NLNs > 9 (n = 36). Under the categorical model, we found GAC patients with NLNs > 9 had a lower HR (HR = 0.314, 95% CI = 0.149-0.664 vs HR = 1.000, $p = 0.002$, univariate Cox regression) and a longer survival (58.9 months, 95% CI = 44.2-73.5 vs 18.6 months, 95% CI = 10.5-26.7, $p = 0.001$, Log-rank) than did those with NLNs ≤ 9 (Table 2).

Further, we tested various cutoff points for the percentage of NLN on ROC curves (AUC = 0.728, 95% CI = 0.603-0.853, $p = 0.002$) to distinguish the survival status among 62 GAC patients, and 37.5 (sensitivity = 0.419, specificity = 0.935) and 80.6 (sensitivity = 0.742, specificity = 0.613) had the relatively highest Youden indexes of 0.354 and 0.355, respectively. Then we divided the 62 GAC patients into three groups, including those with a percentage of NLN ≤ 37.5 (n = 15), those with a percentage of NLN 37.5 to 80.6 (n = 20), and those with a percentage of NLN > 80.6 (n = 27). Under the categorical model, we found GAC patients with a percentage of NLN > 80.6 had the lowest HR (HR = 0.119, 95% CI = 0.044-0.321 vs HR = 0.315, 95% CI = 0.133-0.747 vs HR = 1.000, $p < 0.001$ and $p = 0.009$, univariate Cox regression) and had the longest

Table 3

Prognostic variables and their HRs of the 62 gastric adenocarcinoma patients

	Survival differences			Cox proportional-hazards regression model			
	Survivals		Log-rank	Univariate		Multivariate	
Variable (case number)	Mean	95% CI	*p* value	HRs (95% CI)	*p* value	HRs (95% CI)	*p* value
Gender			0.684				
Male (n = 41)	47.1	33.3-60.8		0.857 (0.407-1.804)	0.685		
Female (n = 21)	29.7	18.7-40.8		1.000			
Age, y			0.383				
≤65 (n = 29)	50.4	34.8-65.9		1.000			
>65 (n = 33)	40.4	26.0-54.8		1.381 (0.666-2.862)	0.385		
Type of gastrectomy			0.938				
Subtotal (n = 47)	42.7	30.8-54.6		1.000			
Total (n = 15)	42.3	18.4-66.3		0.967 (0.415-2.255)	0.938		
Postoperative adjuvant therapy			0.464				
No (n = 38)	46.9	32.5-61.4		1.000			
Yes (n = 24)	41.0	24.5-57.6		1.308 (0.636-2.693)	0.465		
Pathological findings							
Maximal tumor diameter, cm			<0.001				
≤4 (n = 31)	60.5	46.3-74.6		1.000		1.000	
>4 (n = 31)	25.9	12.4-39.3		4.168 (1.890-9.191)	<0.001	2.828 (1.206-6.632)	0.017
Cell differentiation			0.054				
Well (n = 8)	59.6	41.1-78.2		1.000			
Moderate/poor (n = 54)	39.7	27.4-52.0		3.694 (0.875-15.584)	0.075		
T-status[a]			0.008				
T1 (n = 13)	62.5	46.5-78.5		1.000			
T2 (n = 6)	43.3	21.4-65.1		1.800 (0.310-10.778)	0.520		
T3 (n = 29)	22.8	12.3-33.1		5.984 (1.750-20.461)	0.004		
T4 (n = 14)	45.7	21.6-69.7		3.588 (0.920-13.999)	0.066		
N-status[a]			0.009				
N0 (n = 20)	60.4	46.3-74.6		1.000			
N1 (n = 3)	62.6	12.6-112.7		1.624 (0.190-13.909)	0.658		
N2 (n = 12)	33.2	13.2-53.3		3.694 (1.205-11.327)	0.022		
N3 (n = 27)	19.1	12.2-26.0		4.965 (1.782-13.829)	0.002		
M-status[a]							
M0 (n = 49)	52.4	39.6-65.1	<0.001	1.000			
M1 (n = 13)	9.4	5.1-13.6		4.295 (1.872-9.857)	0.001		
Lymphovascular invasion			0.004				
No (n = 22)	68.1	50.8-85.4		1.000			
Yes (n = 38)	31.0	18.7-43.3		3.506 (1.416-8.681)	0.007		
Perineural invasion			0.001				
No (n = 22)	68.4	51.3-85.5		1.000		1.000	
Yes (n = 37)	25.7	14.0-37.4		4.121 (1.639-10.366)	0.003	3.182 (1.070-9.462)	0.037
Number of NLNs			0.001				
≤9 (n = 26)	18.6	10.5-26.7		1.000			
>9 (n = 36)	58.9	44.2-73.5		0.314 (0.149-0.664)	0.002		
Percentage of NLN (%)			<0.001				
≤37.5 (n = 15)	9.6	6.1-13.2		1.000		1.000	
37.5-80.6 (n = 20)	26.7	18.7-34.8		0.315 (0.133-0.747)	0.009	0.327 (0.131-0.814)	0.016
>80.6 (n = 27)	66.5	58.8-82.2		0.119 (0.044-0.321)	<0.001	0.333 (0.106-1.048)	0.060

CI = confidence interval; HRs = hazard ratios; Percentage of NLN = number of NLNs/number of TLNs of each gastric adenocarcinoma patient.
[a]American Joint Committee on Cancer, eighth edition.

survival (66.5 months, 95% CI = 58.8-82.2 vs 26.7 months, 95% CI = 18.7-34.8 vs 9.6 months, 95% CI = 6.1-13.2, $p < 0.001$, Log-rank) than those with a percentage of NLN 37.5 to 80.6 and those with a percentage of NLN ≤ 37.5, in a stepwise pattern (Table 2).

According to the N-status defined in the AJCC eighth edition, we found N3 (number of PLNs ≥ 7, n = 27) GAC patients had the highest HR (HR = 4.965, 95% CI = 1.782-13.829 vs HR = 3.694, 95% CI = 1.205-11.327 vs HR = 1.624, 95% CI = 0.190-13.909 vs HR = 1.000, $p = 0.002$, $p = 0.022$ and $p = 0.658$, univariate Cox regression) and the shortest survival (19.1 months, 95% CI = 12.2-26.0 vs 33.2 months, 95% CI = 13.2-53.3 vs 62.6 months, 95% CI = 12.6-112.7 vs 60.4 months, 95% CI = 46.3-74.6, $p = 0.009$, Log-rank) compared to N2 (number of PLNs, 3, 4, 5, or 6, n = 12), N1 (number of PLNs, 1 or 2, n = 3) and N0 (number of PLN, 0, as reference, n = 20) GAC patients, in a stepwise pattern (Table 2).

3.3. Prognostic variables and their HRs for all GAC patients

As shown in Table 3, we demonstrated maximal tumor diameter (≤4 vs >4 cm, $p < 0.001$), cancer cell differentiation (well

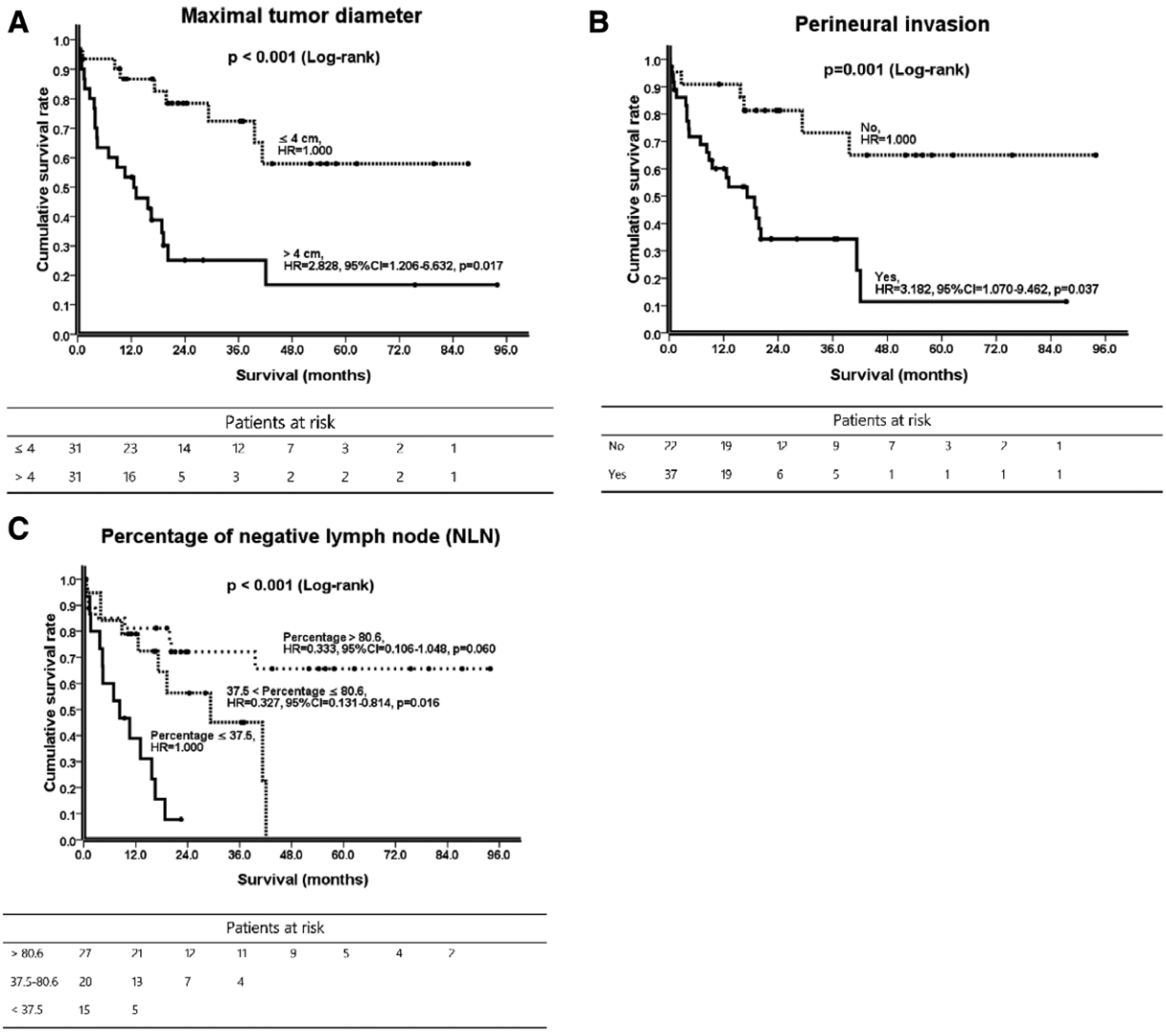

Fig. 1 Kaplan-Meier survival curves, *p*-values (log-rank test), hazard ratios (HRs) (including 95% confidence interval [CI], Cox proportional-hazards regression, multivariate) and patients at risk of the three independent factors in gastric adenocarcinoma (GAC) patients, the maximal tumor diameter (A), the perineural invasion (B), and the percentage of NLN (C), are illustrated. NLNs = negative lymph nodes; Percentage of NLN = number of NLNs/number of TLNs of each GAC patient.

vs moderate/poor, p = 0.054), pathological T-status (T1 vs T2 vs T3 vs T4, p = 0.008), pathological N-status (N0 vs N1 vs N2 vs N3, p = 0.009), pathological M-status (M0 vs M1, p < 0.001), lymphovascular invasion (no vs yes, p = 0.004), perineural invasion (no vs yes, p = 0.001), number of NLNs (≤9 vs >9, p = 0.001), and percentage of NLN (≤37.5 vs 37.5-80.6 vs >80.6, p < 0.001) as possible prognostic variables differentiating survivals.

The univariate Cox proportional-hazards regression model revealed patients with a maximal tumor diameter >4.0 cm (>4.0, HR = 4.168, 95% CI = 1.890-9.191, p < 0.001; ≤4 cm, HR = 1.000, Reference), a moderate/poor differentiation of GAC (moderate/poor, HR = 3.694, 95% CI = 0.875-15.584, p = 0.075; well, HR = 1.000, Reference), an advanced T status (T4, HR = 3.588, 95% CI = 0.920-13.999, p = 0.066; T3, HR = 5.984, 95% CI = 1.750-20.461, p = 0.004, T2, HR = 1.800, 95% CI = 0.310-10.778, p = 0.520; T1, HR = 1.000, Reference), an advanced N status (N3, HR = 4.965, 95% CI = 1.782-13.829, p = 0.002; N2, HR = 3.694, 95% CI = 1.205-11.327, p = 0.022; N1, HR = 1.624, 95% CI = 0.190-13.909, p = 0.685; N0, HR = 1.000, Reference), an M1 status (M1, HR = 4.295, 95% CI = 1.872-9.857, p = 0.001; M0, HR = 1.000, Reference), a lymphovascular invasion (yes, HR = 3.506, 95% CI = 1.416-8.681, p = 0.007; no, HR = 1.000, Reference), and a perineural invasion (yes, HR = 4.121, 95% CI = 1.639-10.366, p = 0.003; no, HR = 1.000) tended to have poorer prognosis and higher HRs.

Nevertheless, patients with a greater number of NLNs (>9, HR = 0.314, 95% CI = 0.149-0.664, p = 0.002; ≤9, HR = 1.000, Reference) and a higher percentage of NLN (>80.6, HR = 0.119, 95% CI = 0.044-0.321, p < 0.001; 37.5-80.6, HR = 0.315, 95% CI = 0.133-0.747, p = 0.009; ≤37.5, HR = 1.000, Reference) tended to have better prognosis and lower HRs.

Under Cox proportional-hazards regression model with multivariate analysis, we demonstrated maximal tumor diameter >4 cm (>4, HR = 2.828, 95% CI = 1.206-6.632, p = 0.017; ≤4, HR = 1.000, Reference, Fig. 1A), perineural invasion (yes, HR = 3.182, 95% CI = 1.070-9.462, p = 0.037; no, HR = 1.000, Reference, Fig. 1B), and lower percentage of NLN (≤37.5, HR = 1.000, Reference; 37.5-80.6, HR = 0.327, 95% CI = 0.131-0.814, p = 0.016; >80.6, HR = 0.333, 95% CI = 0.106-1.048, p = 0.060, Fig. 1C) were independent poor prognostic variables with elevated HRs.

Table 4

Distributions of the percentage of NLN according to the pathological T, N, and M status of the 62 gastric adenorcinoma patients

	Percentage of NLN					
	Continuous model		Categorical model			
Variable (case number, %)	Mean ± SD	*p* value	≤37.5 (n = 15)	37.5-80.6 (n = 20)	>80.6 (n = 27)	*p* value
T-status[a]		<0.001[b]				<0.001[c]
T1 (n = 13, 100.0)	96.5 ± 12.6		0 (0.0)	1 (7.7)	12 (92.3)	
T2 (n = 6, 100.0)	89.2 ± 23.4		0 (0.0)	1 (16.7)	5 (83.3)	
T3 (n = 29, 100.0)	56.7 ± 31.3		9 (31.0)	13 (44.8)	7 (24.1)	
T4 (n = 14, 100.0)	42.4 ± 32.2		6 (42.9)	5 (35.7)	3 (21.4)	
N-status[a]		<0.001[b]				<0.001[c]
N0 (n = 20, 100.0)	100.0 ± 0.0		0 (0.0)	0 (0.0)	20 (100.0)	
N1 (n = 3, 100.0)	90.9 ± 4.5		0 (0.0)	0 (0.0)	3 (100.0)	
N2 (n = 12, 100.0)	70.1 ± 20.5		1 (8.3)	7 (58.3)	4 (33.3)	
N3 (n = 27,100.0)	33.8 ± 22.6		24 (51.9)	13 (48.1)	0 (0.0)	
M-status[a]		<0.001[d]				<0.001[e]
M0 (n = 49,100.0)	73.9 ± 31.2		7 (14.3)	15 (30.6)	27 (55.1)	
M1 (n = 13,100.0)	31.0 ± 21.2		8 (61.5)	5 (38.5)	0 (0.0)	

NLNs = negative lymph nodes; Percentage of NLN = number of NLNs/number of TLNs of each gastric adenocarcinoma patient; SD = standard deviation.
[a]American Joint Committee on Cancer, eighth edition.
[b]Analysis of variance.
[c]Chi-square (χ^2) test for trend (linear-by-linear association).
[d]*t*-test.
[e]Chi-square (χ^2).

3.4. Distributions of the percentage of NLN according to the pathological T, N, and M status of the 62 GAC patients

As shown in Table 4, the mean percentages of NLN were 96.5, 89.2, 56.7, and 42.4 for T1, T2, T3, and T4 ($p < 0.001$), 100.0, 90.9, 70.1, and 33.8 for N0, N1, N2, and N3 ($p < 0.001$), and 73.9 and 31.0 for M0 and M1 ($p < 0.001$), respectively, and they were distributed in an ever decreasing pattern. Concerning those GAC patients with a percentage of NLN >80.6, their proportions decreased from 92.3%, 83.3%, 24.1% to 21.4% for T1, T2, T3, and T4 ($p < 0.001$), from 100.0%, 100.0%, 33.3% to 0.0% for N0, N1, N2, and N3 ($p < 0.001$), and from 55.1% to 0.0% for M0 and M1 ($p < 0.001$), respectively. The percentage of NLN could reflect the severity of T, N, and M status, simultaneously.

3.5. Associations among the percentage of NLN, number of NLNs, and number of TLNs

Through a continuous model with linear regression, we found a greater number of NLNs were related to a higher percentage of NLN (Pcc = 0.617, $R^2 = 0.381$, $p < 0.001$) and a greater number of NLNs were related to a higher number of TLNs (Pcc = 0.595, $R^2 = 0.354$, $p < 0.001$), respectively (Table 5, upper part).

Now that a greater number of TNLs was related to a higher number of NLNs, we tested various cutoff points for the number of TLNs on ROC curves (AUC = 0.757, 95% CI = 0.630-0.884, $p = 0.001$) to distinguish NLNs ≤ 9 or NLNs >9 among 62 GAC patients, and TLNs of 15 (sensitivity = 0.577, specificity = 0.917) had the highest Youden index of 0.494. Then we divided the 62 GAC patients into two groups, including those with number of TLNs ≤ 15 (n = 20) and those with number of TLNs > 15 (n = 42).

Through a categorical model with cross-table analysis, we found GAC patients with a number of NLNs > 9 was highly related to those with a percentage of NLN > 80.6 ($p < 0.001$) or a number of TLNs > 15 ($p < 0.001$), respectively (Table 5, lower part).

3.6. Distributions of the numbers of PLNs and NLNs according to the numbers of TLNs ≤ or >15 of GAC patients

For the 62 GAC patients, those with TLNs > 15 (n = 42) had

Table 5

Relationship between percentage of NLN and number of NLNs, and between number of NLNs and number of TLNs based on the continuous or categorical models of the 62 gastric adenocarcinoma patients

	Linear association		
Continuous model	Pearson correlation coefficient	R^2	*p* value
Percentage of NLN (n = 62) Number of NLNs (n = 62)	0.617	0.381	<0.001
Number of NLNs (n = 62) Number of TLNs (n = 62)	0.595	0.354	<0.001
	Cross-table		
Categorical model	Number of NLNs ≤ 9 (n = 26)	Number of NLNs > 9 (n = 36)	*p* value
Percentage of NLN (case number, %)			<0.001[b]
≤37.5 (n = 15, 100.0)	14 (93.3)	1 (6.7)	
37.5-80.6 (n = 20, 100.0)	7 (35.0)	13 (65.0)	
>80.6 (n = 27, 100.0)	5 (18.5)	22 (81.5)	
Number of TLNs (case number, %)[a]			<0.001[c]
≤15[a] (n = 20, 100.0)	15 (75.0)	5 (25.0)	
>15[a] (n = 42, 100.0)	11 (26.2)	31 (73.8)	

NLNs = negative lymph nodes; Percentage of NLN = number of NLNs/number of TLNs of each gastric adenocarcinoma (GAC) patient; TLNs = total lymph nodes.
[a]We tested various cutoff points for number of TLNs on receiver operating characteristic curves (area under the curve = 0.757, 95% confidence interval = 0.630-0.884, $p = 0.001$) to distinguish NLNs ≤ 9 or NLNs > 9 among 62 GAC patients, and TLNs of 15 (sensitivity = 0.577, specificity = 0.917) had the highest Youden index of 0.494. Then we divided the 62 GAC patients into two groups, including those with Number of TLNs ≤ 15 (n = 20) and those with number of TLNs > 15 (n = 42).
[b]Chi-square (χ^2) test for trend (linear-by-linear association).
[c]Chi-square (χ^2).

Table 6

Distributions of the numbers of PLNs and NLNs of the 62 gastric adenocarcinoma (GAC) patients and the 42 N(+) GAC patients according to the numbers of TLNs ≤ 15 or >15

	Number of TLNs		
Overall (n = 62)	**≤15 (n = 20)**	**>15 (n = 42)**	***p* value[a]**
Number of PLNs (mean ± SD)	3.4 ± 3.7	10.8 ± 10.9	0.018
Number of NLNs (mean ± SD)	7.5 ± 4.0	16.7 ± 10.7	<0.001
	Number of TLNs		
N(+) (n = 42)	**≤15 (n = 12)**	**>15 (n = 30)**	***p* value[a]**
Number of PLNs (mean ± SD)	5.7 ± 3.1	15.1 ± 10.0	0.003
Number of NLNs (mean ± SD)	6.6 ± 3.9	13.5 ± 10.2	0.029

NLNs = negative lymph nodes; PLNs = positive lymph nodes; SD = standard deviation; TLNs = total lymph nodes.
[a]*t*-test.

more PLNs (10.8 ± 10.9 vs 3.4 ± 3.7, $p = 0.018$) and more NLNs (16.7 ± 10.7 vs 7.5 ± 4.0, $p < 0.001$) than did those with TLNs ≤15 (n = 20) (Table 6).

Further, for the 42 N(+) GAC patients, those who underwent LND with TLNs > 15 (n = 30) had more PLNs (15.1 ± 10.0 vs 5.7 ± 3.1, $p = 0.003$) and more NLNs (13.5 ± 10.2 vs 6.6 ± 3.9, $p = 0.029$) than those who underwent LND with TLNs ≤ 15 (n = 12) (Table 6).

3.7. Prognostic impacts and their HRs of number of NLNs and percentage of NLN for 42 GAC patients with the number of TLNs > 15

As shown in Table 7, we demonstrated the possible prognostic variables for GAC patients with the number of TLNs > 15. Besides the reported variables, we found the number of NLNs (≤9 vs >9, $p < 0.001$) and percentage of NLN (≤37.5 vs 37.5-80.6 vs >80.6, $p < 0.001$) were prognostic variables for GAC patients undergoing LND with number of TLNs > 15. The univariate Cox proportional-hazards regression model revealed patients with a greater number of NLNs (>9, HR = 0.167, HR = 0.062-0.454, $p < 0.001$; ≤9, HR = 1.000, Reference) and a higher percentage of NLN (>80.6, HR = 0.083, 95% CI = 0.023-0.299, $p < 0.001$; 37.5-80.6, HR = 0.206, 95% CI = 0.067-0.630, $p = 0.006$; ≤37.5, HR = 1.000, Reference) tended to have better prognosis and lower HRs for GAC patients with a number of TLNs > 15.

Under the Cox proportional-hazards regression model with multivariate analysis, likewise, we identified a lower percentage of NLN (≤37.5, HR = 1.000, Reference; 37.5-80.6, HR = 0.272, 95% CI = 0.086-0.857, $p = 0.026$; >80.6, HR = 0.180, 95% CI = 0.045-0.716, $p = 0.015$) as an independent poor prognostic variable with an elevated HR for GAC patients with a number of TLNs > 15.

4. DISCUSSION

Nowadays, the T-/N-/M-status and cancer stage defined in AJCC have worldwide consensus in predicting the prognosis of GAC patients.[12] Besides, we demonstrated the maximal tumor diameter > 4 cm, perineural invasion and lower percentage of NLN (subgrouped ≤37.5, 37.5-80.6, >80.6, %) were three independent and poor prognostic variables in this retrospective study (Table 3, Figure). Their clinical significances and implications deserve to be further addressed.

Similar to other cancers of the digestive tract, esophageal cancer,[13,14] colorectal cancer,[15] and gastric cancer demonstrated that, in the current study, longer tumor lengths or larger tumor sizes were related to shorter survivals. Although we defined a cutoff value of 4 cm to differentiate survivals and to estimate HRs, different lengths have been reported by other research groups.[16] Despite these mute alterations, they emphasized the poor prognostic effects of longer tumor length. Of note, among the 62 GAC patients, we found the proportions of maximal tumor diameter of >4 cm increased from T1, T2, T3 to T4 (7.7%, 0.0%, 65.5%, 78.6%, $p < 0.001$), from N0, N1, N2 to N3 (20.0%, 33,3%, 50.0%, 74.1%, $p < 0.001$), and from M0 to M1 (38.8%, 92.3%, $p = 0.001$) stepwise (data not shown). In other words, maximal tumor diameter of >4 cm might be an effective surrogate to reflect the severity of the T, N, and M status of GAC, simultaneously. This might explain why the maximal tumor diameter of >4 cm was identified as an independent prognostic factor in this study cohort.

Perineural invasion could be found in various malignant tumors, including esophageal cancer,[17] colorectal cancer,[18,19] or gastric cancer.[20] It is a sign of tumor metastasis and invasion and portends the poor prognosis of cancer patients.[21] Compatible with the reported literature, we demonstrated perineural invasion as an independent poor prognostic factor for GAC patients. Although the detailed pathogenesis of the perineural invasion remains unclear, exploring its mechanism has great significance for blocking tumor progression and improving patient survival.[21]

Different classifications about N-status have been set, and they emphasized the impacts of detected "PLNs,"[22] including the Japanese classification focusing on the involvements of nodal stations[23] or the AJCC on the number of involved LNs,[24] to reflect the severity of the cancer and the prognosis. Nowadays, AJCC classification is used most widely, because of its simplicity, reliability, and stratification and can provide a more accurate estimation of prognosis.[25] However, few studies have discussed the role of "NLNs" in GACs. In this retrospective analysis, we kept an eye on both PLNs and NLNs, synchronously. In Table 2, we have shown the HRs and prognostic impacts of GAC patients based on the number of PNLs (subgrouped, 0, <3, 3-7, and ≥7; N0, N1, N2, and N3 defined in AJCC eighth edition), number of NLNs (≤9 and >9), and percentage of NLN (≤37.5, 37.5-80.6, >80.6, %), and they all showed significant predictive powers. Thereafter, we incorporated them into other reported prognostic variables as shown in Table 3, and the percentage of NLN (≤37.5, 37.5-80.6, >80.6) became an independent one. These findings suggested fewer PLNs and more NLNs, which is reflected as a higher percentage of NLN (TNLs = PLNs + NLNs; percentage of NLN = number of NLNs/number of TLNs) may more accurately predict a better GAC prognosis.

Actually, such a situation is usually encountered in the clinical practice, for example, GAC patients harboring the nodal conditions as case A: 1/4/5/80% (represented for PDLNs/NLNs/TDLNs/percentage of NLN) vs case B: 1/19/20/95% vs case C: 4/1/5/20%. From the viewpoint of PLNs defined in AJCC, cases A and C belonged to the N1 and N2 status, respectively. Undoubtedly, case C may have a worse outcome than case A due to the advanced N status.[26] From the viewpoint of NLNs, although both case A and case B belong to the N1 status, case B harbored more NLNs. As a result, some speculated case B may have better survival, as demonstrated by several research groups.[5,9] Similarly, our analysis revealed those GAC patients who owned NLNs > 9 tended to have longer survivals than those ≤9 (Tables 2 and 3). The cutoff value of 9 NLNs has ever been reported by other research group.[9] Such a situation indicated the pathological N1 status of case B is more accurate than case A, because case A might be understaged due to fewer NLNs.[27] In other words, a greater number of NLNs may improve the accuracy of the N(+) status based number of PLNs. To balance the disconcordance between the poor prognostic effect of PLNs and the better prognostic effect of NLNs, the ratio between

Table 7

Prognostic variables and their HRs of the 42 gastric adenocarcinoma patients with number of total lymph nodes >15

	Survival differences			Cox proportional-hazards regression model			
	Survivals		Log-rank	Univariate		Multivariate	
Variable (case number)	Mean	95% CI	*p* value	HRs (95% CI)	*p* value	HRs (95% CI)	*p* value
Gender			0.662				
Male (n = 29)	42.5	27.8-57.3		0.816 (0.328-2.031)	0.662		
Female (n = 13)	23.1	13.1-33.1		1.000			
Age, y			0.215				
≤65 (n = 21)	52.7	34.1-71.3		1.000			
>65 (n = 21)	26.3	16.7-35.9		1.728 (0.721-4.144)	0.220		
Type of gastrectomy			0.844				
Subtotal (n = 30)	41.2	26.5-55.9		1.000			
Total (n = 12)	27.6	14.6-40.6		0.909 (0.350-2.356)	0.844		
Postoperative adjuvant therapy			0.896				
No (n = 22)	30.2	20.4-40.0		1.000			
Yes (n = 20)	44.3	25.8-62.7		0.945 (0.405-2.206)	0.896		
Pathological findings							
Maximal tumor diameter, cm			<0.001				
≤4 (n = 19)	66.7	49.7-83.7		1.000		1.000	
>4 (n = 23)	19.4	8.8-30.0		6.505 (2.166-19.535)	0.001	4.075 (1.216-13.622)	0.023
Cell differentiation			0.178				
Well (n = 4)	57.1	27.6-86.6		1.000			
Moderate/poor (n = 38)	37.2	24.0-50.3		3.648 (0.487-27.328)	0.208		
T-status[a]			0.002				
T1 (n = 8)	51.8	44.9-58.6		1.000			
T2 (n = 3)	[a]			[a]			
T3 (n = 20)	18.9	8.3-29.5		13.044 (1.716-99.136)	0.013		
T4 (n = 11)	46.0	20.2-71.8		5.946 (0.687-51.436)	0.105		
N-status[b]			0.026				
N0 (n = 12)	49.5	37.0-62.0		1.000			
N1 (n = 1)	[a]			[a]			
N2 (n = 7)	42.7	13.9-71.5		2.973 (0.660-13.384)	0.156		
N3 (n = 22)	18.2	10.7-25.6		5.482 (1.534-19.592)	0.009		
M-status[b]			0.001				
M0 (n = 32)	49.0	34.8-63.2		1.000			
M1 (n = 10)	8.3	3.4-13.2		4.760 (1.796-12.611)	0.002		
Lymphovascular invasion			0.103				
No (n = 13)	44.4	30.3-58.6		1.000			
Yes (n = 27)	35.2	20.2-50.1		2.452 (0.807-7.282)	0.114		
Perineural invasion			0.009				
No (n = 13)	59.9	44.6-75.1		1.000			
Yes (n = 27)	27.6	13.9-41.4		4.646 (1.329-16.237)	0.016		
Number of NLNs			<0.001				
≤9 (n = 11)	8.9	5.1-12.6		1.000			
>9 (n = 31)	51.8	37.1-66.6		0.167 (0.062-0.454)	<0.001		
Percentage of NLN (%)			<0.001				
≤37.5 (n = 12)	8.3	4.6-11.9		1.000		1.000	
37.5-80.6 (n = 13)	30.2	20.0-40.4		0.206 (0.067-0.630)	0.006	0.272 (0.086-0.857)	0.026
>80.6 (n = 17)	62.5	44.4-80.6		0.083 (0.023-0.299)	<0.001	0.180 (0.045-0.716)	0.015

CI = confidence interval; HRs = hazard ratios; NLNs = negative lymph nodes; Percentage of NLN = number of NLNs/number of TLNs of each GAC patient.

[a]All were alive at the time of follow-up.

[b]American Joint Committee on Cancer, eighth edition.

NLNs and PLNs has been advocated to adjust. Some authors reported a higher NLNs/PLNs ratio or lower PLNs/NLNs ratio was an important factor reflecting survival differences among GAC patients[10] as well as breast cancer[28] or colorectal cancers.[29] Similar but mute modification, our results showed a higher percentage of NLN (subgrouped ≤37.5, 37.5-80.6, >80.6, %) was related to a better prognosis and regarded as an independent one. Of note, as shown in Table 4, a lower percentage of NLN may be an effective surrogate reflecting the severity of the T-/N-/M-status, simultaneously.

Thereafter, how to figure out a proper number of NLNs becomes an important issue. As shown in Table 5 (upper part), we found more NLNs would contribute to a higher percentage of NLN (Pcc = 0.617, $p < 0.001$), and more NLNs needed more TLNs to achieve (Pcc = 0.595, $p < 0.001$). In other words, an adequate LND with sufficient TNLs might be an important step in achieving a proper percentage of NLN. Through a step-by-step categorical analysis (Table 5, lower part), we demonstrated GAC patients who underwent TLNs > 15 had a higher chance of achieving NLNs > 9 ($p < 0.001$), and those

who had NLNs > 9 tended to harbor a percentage of LNL > 80.5% ($p < 0.001$). Taken together, we recommend a minimal requirement of TLNs > 15, that is, 16 TLN, for GAC patients undergoing LND.

Whether the TLN of 15/16 is sufficient remains to be validated. Interestingly, our preliminary data showed if GAC patients undergoing LND with TLNs > 15, and more PLNs would be found, as compared with those undergoing LND with TLNs ≤ 15, regardless of whether they were GAC patients ($p = 0.018$) or N(+) GAC patients ($p = 0.003$) (Table 6). This indicated a minimal requirement of TLN of 15/16 is necessary for N staging. We further demonstrated if GAC patients undergoing LND with TLNs > 15, and more NLNs would be found, as compared with those undergoing LND with TLNs ≤ 15, regardless of whether they were GAC patients ($p < 0.001$) or N(+) GAC patients ($p = 0.029$) (Table 6). This indicated TLN > 15 would help in obtaining accurate N staging and avoiding downstaging bias. Compatible with the reported literature, a minimal requirement of 15/16 TLN has been reported by several scientific groups[30,31] and recommended in the AJCC seventh edition.[6] All these indicated TLN > 15 would guarantee accurate N staging without downstage bias.

Further, to test the consistency of the number of NLNs and the percentage of NLN in predicting the prognosis in GAC patients, we focused on those GAC patients undergoing TLN > 15, suggesting an adequate LND. Significantly, NLNs > 9 and the percentage of NLN > 80.6 related to better prognosis with lower HRs, and the percentage of NLN > 80.6 remained an independent factor (Table 7).

Several reasons have been advocated to explain the favorable outcomes of more NLNs or higher percentage of NLN in resectable GAC patients. The first is avoiding the possibility of downstage, understage, or stage-migration bias as demonstrated in above paragraphs and in the literature.[9,32] According to the equation TNLs = PLNs + NLNs, it indicates more NLNs require an extensive LND to achieve. An extensive LND could offer sufficient LNs to be examined and establish accurate N staging. Further, some authors claimed an extensive LND could achieve better local-regional control, eliminate undetectable lesions, and perhaps prolong survival.[33,34] Second is the hint of curative surgical technique and higher hospital quality. Since an extensive LND needs a skilled surgeon to carry it out and a well-trained pathologist for the examination, it suggests a higher quality of clinical service.[35,36] The third is the plausible interactions between the tumor and host immunity.[32] Host LNs played an important role in the surveillance of cancer cells and defending against cancer invasion. Cancer stimulation may trigger host immunity to develop new LNs or cause the LNs to increase in size, increasing the number of NLNs or facilitating detection by surgeons or pathologists.[37,38]

Due to the hospital volume limitation, we retrieved only 62 consecutive GAC patients who underwent primary gastrectomy for evaluation. However, our preliminary results did offer and represent several scientific findings for surgeons and oncologists.

In conclusion, the percentage of NLN is an optimal variable predicting the prognosis of GAC patients. However, an accurate percentage of NLN requires a minimal requirement of TLNs, exceeding 15 to detect an adequate number of PLNs and a sufficient number of NLNs.

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