A Reappraisal of Lymph Node Dissection for Gastric Adenocarcinoma during Upfront Gastrectomy–An Institutional Report

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AIM: The role of lymph node dissection (LND) in gastric adenocarcinoma (GAC) remained some controversy. This study reappraised the clinical implications of LND for GAC in terms of the numbers of total lymph node (TLN), positive lymph node (PLN) and negative lymph node (NLN).

METHODS: A total of 106 GAC patients receiving an upfront gastrectomy (laparoscopic/laparotomy surgery, 19/87) with LND (D1/D1+/D2 dissection, 5/53/48) between Jan 2017 and Dec 2021 in Cathay General Hospital, Taipei were analyzed. The surgical-pathological T-/N-/M-status and cancer stage were determined according to the American Joint Committee on Cancer (AJCC) 8th edition. The numbers of TLN, PLN and NLN were recorded for analysis (TLN = PLN + NLN). The associations between prognosis and related variables, including pathological findings, the clinical implications of TLN/PLN/NLN and extent for LND, were all deeply studied.

RESULTS: Advanced T-status ($p < 0.001$), N-status ($p = 0.025$), M-status ($p = 0.001$) and cancer stage ($p = 0.001$) had a negative effect on survival. The severity of N-status was associated with the progression of T-status ($p < 0.001$), M-status ($p = 0.015$) and cancer stage ($p < 0.001$). For all 106 GAC patients ($p = 0.002/0.017$) and the 25 T1 GAC patients ($p = 0.052/0.015$), those undergoing TLN >17 (≥ 18) had a more PLN and a higher rate of N(+) than those ≤ 17. For 65 N(+) GAC patients, an extension of TLN to ≥ 23 allowed the detection of a median value for PLN of 7, the N3-status. NLN >9 (≥ 10) was related to a better prognosis ($p = 0.066$) and lower HR ($p = 0.073$) for N(+) GAC patients. TLN with threshold value ≥ 20 owned the best power to distinguish NLN >9 (≥ 10) from ≤ 9 among N(+) GAC patients ($p < 0.001$). A D2 dissection could achieve these thresholds, including TLN ≥ 18 ($p = 0.001$) for GAC patients, TLN ≥ 23 ($p = 0.028$) for N(+) GAC patients, and NLN ≥ 10 ($p = 0.012$) as well as TLN ≥ 20 ($p = 0.011$) for N(+) GAC patients, more effectively than a D1/D1+ dissection.

CONCLUSIONS: A value of TLN ≥ 18 is necessary for *de-novo* GAC patients during gastrectomy to detect possible N(+) status, and ≥ 23 is recommended for N(+) GAC patients to identify the possible N3-status. A value of NLN ≥ 10 could reach a better survival for N(+) GAC patients and it requires a value of TLN ≥ 20 to achieve. A D2 dissection is recommended for GAC patients during gastrectomy. LND establishes adequate N-status staging and increases survival for GAC patients.

Keywords: GAC; gastrectomy; LND; TLN; PLN; NLN

Introduction

The incidence of gastric adenocarcinoma (GAC) had decreased in recent decades but it remained one of the most common malignancies, with high mortality rates, worldwide [1]. The Taiwanese national cancer registry database reflected a similar decrease in incidence, but GAC persisted one of the top 10 leading causes of cancer related deaths in

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Taiwan [2]. Better therapeutic strategies seemed necessary for GAC patients.

Gastrectomy with lymph node dissection (LND) is the preferred treatment of choice for operable GAC and it might be combined with pre/post-operative chemo/radio/immunotherapy, if required clinically [3,4]. For types of abdominal surgery, gastrectomy is usually performed through a laparotomy or laparoscopic surgery (incision). Depending on the invasiveness and the location of the GAC in the stomach, a proximal, a subtotal or a total gastrectomy is performed. Regarding the extent of the LND, D1, D1+ or D2 dissections are commonly executed [3,4]. The resected specimen are examined by a pathologist to confirm the T-/N-/M-status and cancer stage, as defined in the American Joint Committee on Cancer (AJCC) manual 8th edition, which is used to predict the survival and to tailor optimally personalized neo/adjuvant therapies for GAC patients [5,6].

N-status played a pivotal variable to establish the cancer stage for GAC [7]. Accurately determining the surgical-pathological N-status requires an extensive of LND, an identification and counting of the dissected lymph nodes (LNs) and an examination of the dissected LNs. These procedures depend on skilled surgeons and experienced pathologists to execute [8]. All dissected LNs constitute the number (value) of total lymph node (TLN). Among these dissected LNs, some are determined to be positive for malignant cells and are quantified as the number (value) of positive lymph node (PLN), and some are determined to be negative for malignant cells and are quantified as the number (value) of negative lymph node (NLN). The equation is TLN = PLN + NLN. Currently, AJCC 8th edition defined the number (value) of PLN, including subgroups of 0, 1–2, 3–6, and $\geq$7 as N0-, N1-, N2- and N3-status to correlate the severity and prognosis of GAC patients. Nevertheless, the number (value) of NLN was not discussed [5,6].

A high PLN quantity, either in terms of the absolute number or the percentage/ratio, correlated to a poor prognosis for GAC patients had been reported in the literature [7,9]. On the contrary, other studies showed that a high NLN quantity, either in terms of the absolute number or the percentage/ratio, was associated with a better prognosis for GAC patients [10,11]. In the clinical practice, an optimal and adequate quantity of PLN and NLN requires a sufficient number of TLN to achieve. Of note, the number of TLN would be affected by the extensiveness of D1, D1+ or D2 LND [12]. The reciprocal relationships between the amounts of TLN, PLN and NLN for GAC are worthy of study.

This retrospective study determined the prognostic variables, T-/N-/M-status and cancer stage, and the relationships between TLN, PLN and NLN for operable GAC patients. The requirements and extents of TLN to achieve an accurate N-status, and the role of NLN in N(+) GAC were studied. The extent of D1/D1+/D2 dissection to achieve an adequate amount for TLN was also evaluated.

Materials and Methods

Information of GAC Patients

Candidate patients who were more than 18 years old, who had pathologically proven GAC and who initially underwent an elective gastrectomy were included. Patients who were with sump gastric cancer, who underwent preoperative neoadjuvant therapies, who underwent palliative gastrectomy, who underwent synchronous resection of intra-abdominal organs due to other malignancies (e.g., colorectal cancer or ovary cancer) or who had coexisted active malignancy other than GAC were excluded from the study. Data base of the Division of General Surgery, Department of Surgery, Cathay General Hospital, Taipei with de-identification, from Jan 2017 to Dec 2021, produced a total of 108 GAC patients who underwent upfront gastrectomy for GAC in terms of the inclusion and exclusion criteria. Two GAC patients (2/108, 1.9%) who died less than one month after surgery-surgical mortalities were also excluded. A total of 106 operable GAC patients without definite distant organ metastasis who underwent an upfront gastrectomy plus LND as the primary treatment modality were studied. Postoperative adjuvant therapies were undertaken if clinically required. In accordance with the Declaration of Helsinki, the Institute Review Board (IRB) of Cathay General Hospital approved this study (IRB No: CGH-P112022) and the waiver of the participants' consent. All the patients were followed until 31 January 2023.

Preoperative Workup

The preoperative workups, including upper gastrointestinal endoscopic examination with/or without upper gastrointestinal series, endoscopic ultrasound (EUS), chest roentgenogram with /or without low dosage chest computed tomography (CT) scan, abdominal CT scan and whole abdominal sonography and tumor makers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9), were undertaken to determine each patient's oncological condition. Neck ultrasound, whole body bone scan or CT scanning of the brain was performed if clinically indicated. The complete blood count with cell differentials, prothrombin time/activated partial thromboplastin time, blood biochemistries and electrocardiogram (EKG) with/or without cardiac ultrasound were used to determine each patient's general status. Patients would undergo surgical resection after completing the preoperative workup and providing written consent.

Surgical Modalities and Surgical-Pathological Status

Gastrectomy

Surgery involved laparoscopic (n = 19) or laparotomy (n = 87) abdominal surgery for proximal (n = 6)/subtotal (n = 84)/total (n = 16) gastrectomy with gastro-esophageal, Billroth-I gastroduodenal or Billroth-II/Roux-en-Y gastro-jejunal/esophagojejunal anastomosis.

Lymph Node Dissection

Regional LNs are classified as right and left pericardial LN (cardioesophageal, Station 1 and 2), lesser curvature LN along the left and right gastric arteries (Station 3a and 3b), greater curvature LN along the short gastric, left gastroepiploic and right gastroepiploic arteries (Station 4sa, 4sb and 4d), suprapyroric LN (Station 5), infrapyloric LN (Station 6), root of the left gastric artery LN (Station 7), the common hepatic artery LN (Station 8a), the celiac artery LN (Station 9), splenic hilum LN (Station 10), splenic artery LN, proximal and distal segment (Station 11p and 11d) and hepatoduodenal ligament LN along the proper hepatic artery, bile duct and portal vein (Station 12a, 12b and 12p) [13]. According to these LN Stations and the Japanese gastric cancer treatment guidelines 2021 (6th edition) [14], D1 (n = 5), D1+ (n = 53) and D2 (n = 48) LND were performed during gastrectomy.

For proximal gastrectomy, D1 dissection included Stations 1, 2, 3, 4 and 7, D1+ included those in D1 and Stations 8a, 9 and 11p, and D2 included those in D1+ and Station 11d LNs. For the subtotal gastrectomy, D1 dissection included Stations 1, 3, 4, 5, 6 and 7, D1+ included those in D1 and Stations 8a and 9, and D2 included those in D1+ and Stations 11p and 12a LNs. For total gastrectomy, D1 dissection included Stations 1, 2, 3, 4, 5, 6 and 7, D1+ dissection included those in D1 and Stations 8a, 9 and 11p, and D2 included those in D1+ and Stations 11d and 12a LNs. The final surgical-pathological T-/N-/M-status and cancer stage were determined according to AJCC 8th edition.

Pathological Findings and LN Descriptions

In addition to the T-/N-/M-status and cancer stage, as defined by the AJCC 8th edition, the pathological condition in terms of lymphovascular or perineural invasion were determined. In terms of the quantity of dissected/or removed LNs, values for TLN, PLN and NLN were measured and counted. Concerning the GAC patients in the current study, those harbored PLN with value equals to 0, the N0 status, were classified as negative nodal status, the N(–); and those harbored PLN with value $\geq$1, the N1, N2 or N3 status, were classified as positive nodal status, the N(+), respectively.

Prognostic Variables

Possible prognostic variables, including sex, age, types of abdominal surgery, gastrectomy and LND, and the pathological findings, were analyzed. The effects of TLN/NLN on the accurate staging of N-status were also determined.

Statistical Analysis

The overall survivals were calculated from the date of surgery until death or the last follow-up on 31 January 2023. Survival curves were plotted by using the Kaplan-Meier method. A Log-rank test was used to compare the survival rate for the groups for each categorical variable. A univariate Cox proportional-hazards regression model (Cox's regression model) was used to calculate the relative hazard ratios (HRs) for survival for each variable. Variables with a p-value of less than ($\leq$) 0.1 under the Log-rank test were entered into a multi-variate Cox's regression model to determine the independent prognostic variables and to determine the HRs for survival. Categorical variables compared for two, three or more groups were analyzed by using a χ^2 test, corrected χ^2 test (Yates's χ^2 test) or a Fisher exact test (2 $\times$ 2 table), or a Fisher-Freeman-Halton exact test (multi-way contingency tables larger than 2 $\times$ 2, the R $\times$ C table, R and C $\geq$2 as well as either R or C $\geq$3, an extension for Fisher exact test), when appropriate. These categorical variables were represented as case number (n) and percentage (%). The normality of the continuous variable was determined by using a Kolmogorov-Smirnov test (for sample size >50). The distribution is considered to be not normal (non-normal) distribution if the p-value $<$ 0.05 [15]. Continuous variables compared for two groups were analyzed by using a t-test or a Mann-Whitney U test when appropriate. These continuous variables were represented as Mean $\pm$ standard deviation (SD) or Median (25th percentile (25P), 75th percentile (75P)) or Mean (95% confidence interval (CI)). The relationship between two continuous variables was defined in terms of the Pearson or Spearman's Rho correlation coefficient (*Pcc* or *Scc*) when appropriate. The optimal threshold values for TLN to disti nguish N(+) from N(–) patients among GAC patients or to distinguish patients with a value of NLN >9 ($\geq$10) from NLN $\leq$9 among N(+) GAC patients were determined using the area under the curve (AUC) and Youden index from a receiver operating characteristic (ROC) curve [16]. Statistical significance is defined if the p value is less than ($<$) 0.05. Statistical analysis was performed with SPSS software (SPSS Statistics for Windows, Version 17.0, SPSS Inc, Chicago, IL, USA).

Results

Demographic Data

As shown in Table 1, a total of 106 GAC patients (Male/female = 58/48) with a mean age of 69.3 years who underwent upfront gastrectomy with LND were retrospectively studied. In terms of the types of abdominal surgery, gastrectomy and LND, 87 (82.1%) underwent laparotomy surgery and 19 (17.9%) laparoscopic surgery, 6 (5.7%) underwent a proximal gastrectomy, 84 (79.2%) a subtotal gastrectomy and 16 (15.1%) a total gastrectomy, and 5 (4.7%) underwent D1 dissection, 53 (50.0%) D1+ dissection and 48 (45.3%) D2 dissection, respectively. In terms of the surgical-pathological T-/N-/M-status and cancer stage, 25 (23.6%)/15 (14.2%)/32 (30.2%)/34 (32.1%) showed T1/T2/T3/T4 status, 41 (38.7%)/18 (17.0%)/13 (12.3%)/34 (32.1%) showed N0/N1/N2/N3 status, 101 (95.3%)/5 (4.7%) showed M0/M1 status, and 32 (30.2%)/27 (25.5%)/42 (39.6%)/5 (4.7%) were identified as being in cancer stages I, II, III or IV, respectively. Eighty-

Table 1. Demographic data for the 106 GAC patients.

Variable	n (%)/Mean ± SD/Median (25P, 75P)/Mean (95% CI)
Sex	
Male/female	58 (54.7)/48 (45.3)
Age (years)	69.3 ± 12.8
≤65/>65	40 (37.7)/66 (62.3)
Type of abdominal surgery	
Laparotomy/laparoscopic	87 (82.1)/19 (17.9)
Type of gastrectomy	
Proximal/subtotal/total	6 (5.7)/84 (79.2)/16 (15.1)
Type of LND	
D1/D1+/D2	5 (4.7)/53 (50.0)/48 (45.3)
Pathological findings (AJCC 8th edition)	
T-status	
T1/T2/T3/T4	25 (23.6)/15 (14.2)/32 (30.2)/34 (32.1)
N-status	
N0/N1/N2/N3	41 (38.7)/18 (17.0)/13 (12.3)/34 (32.1)
M-status	
M0/M1	101 (95.3)/5 (4.7)
Cancer stage	
I/II/III/IV	32 (30.2)/27 (25.5)/42 (39.6)/5 (4.7)
Lymphovascular invasion	
No/Yes	25 (23.6)/81 (76.4)
Perineural invasion	
No/Yes	44 (41.5)/62 (58.5)
Dissected LN	
Number of TLN	19.0 (16.0, 25.0)
Number of PLN	2.0 (0.0, 9.0)
Number of NLN	16.0 (9.0, 22.3)
Duration of follow-up (months)	40.0 (18.2, 58.6)
Survival (months)	54.2 (48.8–59.5)
N(–) (n = 41, 38.7)	60.8 (54.0–67.5)
N(+) (n = 65, 61.3)	48.3 (41.0–55.5)
1-year/2-year/5-year survival rate (%)	89.6/72.7/62.3

25P, 25th percentile; 75P, 75th percentile; SD, standard deviation; CI, confidence interval; GAC, gastric adenocarcinoma; LN, lymph node; LND, lymph node dissection; TLN, total LN; PLN, positive LN; NLN, negative LN; AJCC, American Joint Committee on Cancer.

one (76.4%) exhibited a lymphovascular invasion and 62 (58.5%) exhibited a perineural invasion. The respective Median values for TLN, PLN and NLN were 19.0, 2.0 and 16.0. All patients were followed for a median period of 40.0 months. The mean respective survivals were 54.2, 60.8 and 48.3 months for all, N(–) and N(+) GAC patients. The 1-/2-/5-year survival rates were 89.6%, 72.7% and 62.3%, respectively.

Prognostic Variables that Affect Survival

Prognostic variables (variable, mean survival, Log-rank test) that might affect survivals are listed in Table 2 (Left side). The results showed that patients who were male (Male vs. female, 45.9 vs. 63.7 months, $p = 0.001$), older than 65 years (>65 vs. ≤65, 50.5 vs. 60.1 months, $p = 0.089$), with advanced T-status (T4 vs. T3 vs. T2 vs. T1, 40.5 vs. 54.3 vs. 60.9 vs. 66.3 months, $p < 0.001$), with advanced N-status (N3 vs. N2 vs. N1 vs. N0, 45.6 vs. 42.6 vs. 55.4. vs. 60.8 months, $p = 0.025$), with M1 status (M1 vs. M0, 20.7 vs. 55.7 months, $p = 0.001$), with late cancer stage (IV vs. III vs. II vs. I, 20.7 vs. 46.8 vs. 56.1 vs. 64.7 months, $p = 0.001$), exhibited lympovascular invasion (Yes vs. No, 50.1 vs. 66.3 months, $p = 0.020$) and exhibited perineural invasion (Yes vs. No, 47.8 vs. 61.1 months, $p = 0.015$) tended to have a shorter survival (Table 2, Left side). The type of abdominal surgery ($p = 0.627$), gastrectomy ($p = 0.552$) or LND ($p = 0.177$) did not affect survival.

Prognostic variables that had a p-value of less than (≤) 0.1 for the Log-rank test (Table 2, Left side) were incorporated into Cox's regression model for multi-variate analysis. The result showed that most variables had a mutual effect on each other and none of the variables was identified as being

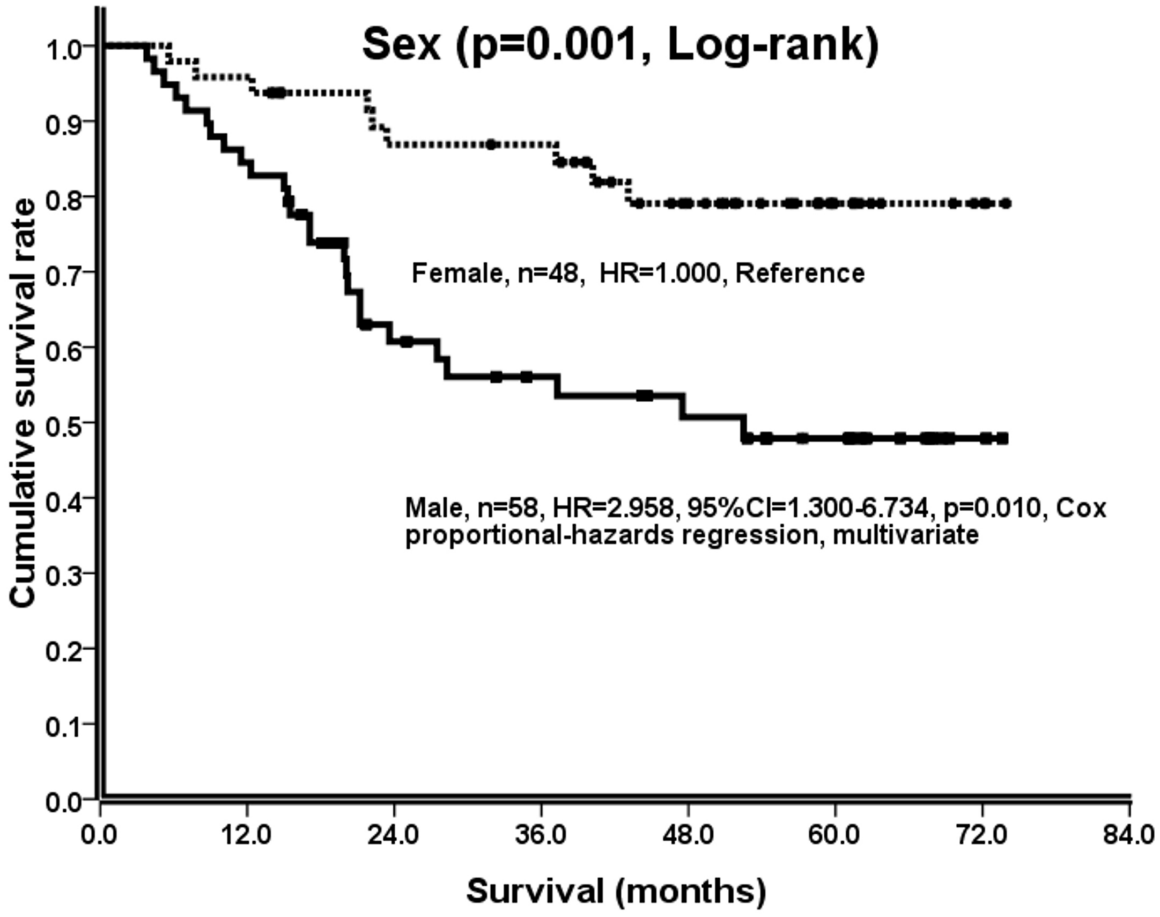

Fig. 1. Kaplan-Meier survival curve, *p*-value (Log-rank test), relative HR (including the 95% CI and Cox proportional-hazards regression model, multi-variate) for the independent factor of sex for GAC patients are shown. CI, confidence interval; GAC, gastric adenocarcinoma; HR, hazard ratio.

independent (All's $p > 0.05$, Table 2, Right side) except for sex ($p = 0.010$). Male patients had a higher HR value than female patients (HR = 2.958, 95% CI = 1.300–6.734, $p = 0.010$, Fig. 1).

The Effect of N-Status on Other Pathological Findings

As shown in Table 3, for T1, T2, T3 to T4 GAC patients, the percentage of N0 status decreased from 76.0%, 66.7%, to 18.8% and then 17.6%, but the percentage of N3 status increased from 4.0%, 13.3% to 31.3% and then 61.8% ($p < 0.001$). The severity of the T-status was highly correlated with an increase in N-status. M1 status ($p = 0.015$), the progression of the cancer stage ($p < 0.001$), lymphovascular invasion $p < 0.001$) or perineural invasion ($\chi^2 = 23.806$, $p < 0.001$) were all highly correlated with an increase in N-status (Table 3).

Cutoff Value for TLN and Results for Analyzed LN

For the 106 GAC patients, 65 N(+) GAC patients had a greater value of TLN than the other 41 N(–) GAC patients (Median, 21.0 vs. 18.0, $p = 0.063$: data not shown). The ROC curve (AUC = 0.608, 95% CI = 0.497–0.718, $p = 0.063$) figured out that a threshold value of 17.5 for TLN gave the highest Youden index of 0.226 and gave an optimal power to distinguish N(+) from N(–) GAC patients (Sensitivity = 0.738, 1-Specificity = 0.512). Therefore, the effects of increasing cutoff value for TLN, from 14 to 17 and then 20 (i.e., 17 $\pm$ 3), on the results of PLN, NLN and N(–)/N(+) status were evaluated.

Using the cutoff values of 14 ($Z = -2.143$, $p = 0.032$), 15 ($Z = -1.927$, $p = 0.054$), 16 ($Z = -2.704$, $p = 0.007$), 17 ($Z = -3.069$, $p = 0.002$), 18 ($Z = -2.426$, $p = 0.015$), GAC patients who underwent LND with a value of TLN exceeding ($>$) 14, 15, 16, 17, and 18 had a higher value for PLN than those who underwent LND with a value of TLN less than ($\leq$) 14, 15, 16, 17 and 18, respectively (Table 4, Left side). However, there was no difference for a cutoff value of 19 ($Z = -1.487$, $p = 0.137$) or 20 ($Z = -1.718$, $p = 0.086$) (Table 4, Left side).

For cutoff values of 14 ($Z = -4.884$, $p < 0.001$), 15 ($Z = -5.507$, $p < 0.001$), 16 ($Z = -5.195$, $p < 0.001$), 17 ($Z = -5.041$, $p < 0.001$), 18 ($Z = -5.489$, $p < 0.001$), 19 ($Z = -6.429$, $p < 0.001$) and 20 ($Z = -6.336$, $p < 0.001$), GAC patients who underwent LND with a value of TLN exceed-

Table 2. Prognostic variables that affect survival among the 106 GAC patients.

	Survival (months)	Survival difference	HRs	Cox's regression model
Variable (n, %)	Mean (95% CI)	*p*-value (Log-rank)	Mean (95% CI)	*p*-value (multi-variate)
Sex		0.001		0.010
Female (n = 48, 45.3)	63.7 (57.6–69.8)		1.000	
Male (n = 58, 54.7)	45.9 (38.0–53.7)		2.958 (1.300–6.734)	
Age		0.089		0.220
≤65 (n = 40, 37.7)	60.1 (52.1–68.0)		1.000	
>65 (n = 66, 62.3)	50.5 (43.6–57.4)		1.640 (0.744–3.617)	
Type abdominal surgery		0.627		
Laparotomy (n = 87, 82.1)	53.4 (47.4–59.3)			
Laparoscopic (n = 19, 17.9)	56.7 (44.9–68.5)			
Type of gastrectomy		0.552		
Proximal (n = 6, 5.7)	53.2 (30.3–76.2)			
Subtotal (n = 84, 79.2)	55.3 (49.4–61.2)			
Total (n = 16, 15.1)	41.9 (31.0–52.8)			
Type of LND		0.177		
D1 (n = 5, 4.7)	41.5 (25.6–57.4)			
D1+ (n = 53, 50.0)	50.3 (42.3–58.3)			
D2 (n = 48, 45.3)	57.7 (50.5–64.9)			
Pathological findings				
T-status		<0.001		0.530
T1 (n = 25, 23.6)	66.3 (59.8–72.8)		1.000	
T2 (n = 15, 14.2)	60.9 (50.1–71.6)		1.724 (0.317–9.380)	0.529
T3 (n = 32, 30.2)	54.3 (44.5–64.1)		3.137 (0.211–46.654)	0.406
T4 (n = 34, 32.1)	40.5 (30.4–50.6)		6.014 (0.335–107.874)	0.223
N-status		0.025		0.981
N0 (n = 41, 38.7)	60.8 (54.0–67.5)		1.000	
N1 (n = 18, 17.0)	55.4 (43.4–67.4)		1.206 (0.318–4.572)	0.783
N2 (n = 13, 12.3)	42.6 (28.9–56.3)		1.465 (0.152–14.099)	0.741
N3 (n = 34, 32.1)	45.6 (35.3–55.9)		1.325 (0.144–12.170)	0.804
M-status		0.001		0.844
M0 (n = 101, 95.3)	55.7 (50.4–61.0)		1.000	
M1 (n = 5, 4.7)	20.7 (2.5–38.8)		1.432 (0.040–51.711)	
Cancer stage		0.001		0.839
I (n = 32, 30.2)	64.7 (58.3–71.1)		1.000	
II (n = 27, 25.5)	56.1 (45.7–66.5)		0.502 (0.043–5.872)	0.583
III (n = 42, 39.6)	46.8 (38.0–55.7)		0.399 (0.015–10.874)	0.586
IV (n = 5, 4.7)	20.7 (2.5–38.8)		–	
Lymphovascular invasion		0.020		0.598
No (n = 25, 23.6)	66.3 (59.9–72.8)		1.000	
Yes (n = 81, 76.4)	50.1 (43.6–56.6)		1.486 (0.340–6.490)	
Perineural invasion		0.015		0.998
No (n = 44, 41.5)	61.1 (55.2–67.1)		1.000	
Yes (n = 62, 58.5)	47.8 (39.9–55.8)		1.003 (0.372–2.707)	

CI, confidence interval; Cox's regression model, Cox proportional-hazards regression model; HRs, hazard ratios; LND, lymph node dissection.

ing (>) 14, 15, 16, 17, 18, 19 and 20 had a greater value for NLN than those who underwent LND with a value of TLN less than (≤) 14, 15, 16, 17, 18, 19 and 20, respectively (Table 4, Middle side).

For cutoff values of 16 ($\chi^2 = 4.579$, $p = 0.032$) or 17 ($\chi^2 = 5.665$, $p = 0.017$), GAC patients who underwent LND with a value of TLN exceeding (>) 16 or (>) 17, N(+) GAC patients were more effectively distinguished from N(–) GAC patients than those who underwent LND with a value of TLN less than (≤) 16 and (≤) 17, respectively (Table 4, Right side).

Table 3. The effect of N-status on other pathological findings of 106 GAC patients.

Pathological findings (n, %*)	N-status (AJCC 8th edition, n, %**) N0 (n = 41, 38.7)	N1 (n = 18, 17.0)	N2 (n = 13, 12.3)	N3 (n = 34, 32.1)	Total (n = 106, 100.0)	χ^2	*p*-value
T-status (AJCC 8th edition)							<0.001[a]
T1 (n = 25, 23.6)	19 (76.0)	4 (16.0)	1 (4.0)	1 (4.0)	25 (100.0)		
T2 (n = 15, 14.2)	10 (66.7)	3 (20.0)	0 (0.0)	2 (13.3)	15 (100.0)		
T3 (n = 32, 30.2)	6 (18.8)	11 (34.4)	5 (15.6)	10 (31.3)	32 (100.0)		
T4 (n = 34, 32.1)	6 (17.6)	0 (0.0)	7 (20.6)	21 (61.8)	34 (100.0)		
M-status (AJCC 8th edition)							0.015[a]
M0 (n = 101, 95.3)	41 (40.6)	18 (17.8)	13 (12.9)	29 (28.7)	101 (100.0)		
M1 (n = 5, 4.7)	0 (0.0)	0 (0.0)	0 (0.0)	5 (100.0)	5 (100.0)		
Stage (AJCC 8th edition)							<0.001[a]
I (n = 32, 30.2)	29 (90.6)	3 (9.4)	0 (0.0)	0 (0.0)	32 (100.0)		
II (n = 27, 25.5)	11 (40.7)	15 (55.6)	1 (3.7)	0 (0.0)	27 (100.0)		
III (n = 42, 39.6)	1 (2.4)	0 (0.0)	12 (28.6)	29 (69.0)	42 (100.0)		
V (n = 5, 4.7)	0 (0.0)	0 (0.0)	0 (0.0)	5 (100.0)	5 (100.0)		
Lymphovascular invasion							<0.001[a]
No (n = 25, 23.6)	22 (88.0)	3 (12.0)	0 (0.0)	0 (0.0)	25 (100.0)		
Yes (n = 81, 76.4)	19 (23.5)	15 (18.5)	13 (16.0)	34 (42.0)	81 (100.0)		
Perineural invasion						23.806	<0.001[b]
No (n = 44, 41.5)	28 (63.6)	8 (18.2)	2 (4.5)	6 (13.6)	44 (100.0)		
Yes (n = 62, 58.5)	13 (21.0)	10 (16.1)	11 (17.7)	28 (45.2)	62 (100.0)		

[a] Fisher-Freeman-Halton exact test, [b] χ^2 test; *Percentage of subgroups of different pathological findings among 106 GAC patients, **Percentage of N0, N1, N2 and N3 status within subgroups of each pathological finding.

AJCC, American Joint Committee on Cancer.

A value of TLN >16 or 17, i.e., ≥17 or 18, gave the optimal power to distinguish the PLN amount, NLN amount and N(+)/N(–) status for GAC patients, simultaneously, and a threshold value of TLN ≥18 gave the best result (Table 4).

Cutoff Value for TLN and Results for Analyzed LN for 25 T1 GAC Patients

T1 GAC patients who underwent LND with a value of TLN exceeding (>) 17 had a greater PLN value than those who underwent LND with a value of TLN less than (≤) 17 (Z = –2.615, p = 0.052, Table 5, Left side) but there was no difference if the cutoff value was 14 (Z = –1.005, p = 0.497), 15 (Z = –1.361, p = 0.336), 16 (Z = –1.867, p = 0.175), 18 (Z = –1.481, p = 0.285), 19 (Z = –0.680, p = 0.637) or 20 (Z = –0.680, p = 0.637 (Table 5, Left side).

For T1 GAC patients who underwent LND with a value of TLN exceeding (>) 17, N(+) T1 GAC patients were more effectively distinguished (p = 0.015) than for T1 GAC patients who underwent LND with a value of TLN less than (≤) 17 (Table 5, Right side). However, there was no difference if the cutoff value was 14 (p = 0.554), 15 (p = 0.289), 16 (p = 0.129), 18 (χ^2 = 1.106, p = 0.293), 19 (χ^2 = 0.110, p = 0.740) or 20 (χ^2 = 0.110, p = 0.740) (Table 5, Right side). Therefore, a threshold value of TLN >17, i.e., ≥18, could distinguish the number of PLN and N(+)/N(–) status for T1 GAC patients with optimal detection power (Table 5).

With regards to the 25 T1 GAC patients, there were 19 with T1N0 and 6 with T1N(+). Among the 19 T1N0 GAC patients, 12 patients underwent a value of TLN ≤17 and 2 (2/12, 16.7%) died of disease; 7 patients underwent a value of TLN >17 (≥18) and none (0/7, 0.0%) died during the post-operative follow-up (p = 0.509, Fisher's exact test, data not shown). Although there was not significantly different in the small cohort, a value of TLN ≥18 tended to guarantee the survival benefit for true T1N0 GAC patients.

Distribution of PLN Values for 65 N(+) GAC Patients after Partitioning the TLN Value

The results in Table 6 showed that if the up-limit value for TLN increased, the integral number of Median value for PLN also increased. In terms of the integral number of the median, the PLN (ranged 6–7) for those who underwent LND with a value of TLN ≤19 or more (ranged ≤19–≤47) were significantly more than the PLN (ranged 1–4) for those who underwent LND with a value of TLN ≤18 or fewer (ranged ≤3–≤18). The integral median would reach a relative plateau value of 7 (i.e., the N3 status) if the value for TLN exceeded (≥) 23. A threshold value of TLN ≥23 is optimal for the diagnosis of possible N3 status for N(+) GAC patients.

Table 4. Cutoff value for TLN and results for analyzed LN among 106 GAC patients.

Sub-groups (n, %*)				Value of LN, Median (25P, 75P)			Sub-groups (n, %**)	N-status (n, %**)			
Cutoff value of TLN	PLN	*Z*	*p*-value[a]	NLN	*Z*	*p*-value[a]	Cutoff value of TLN	N(–)	N(+)	χ^2	*p*-value[b]
≤14 (n = 16, 15.1)	0.5 (0.0, 2.0)	–2.143	0.032	6.0 (5.0, 9.8)	–4.884	<0.001	≤14 (n = 16, 100.0)	8 (50.0)	8 (50.0)	1.018	0.313
>14 (n = 90, 84.9)	2.5 (0.0, 10.0)			17.0 (11.8, 24.3)			>14 (n = 90, 100.0)	33 (36.7)	57 (63.3)		
≤15 (n = 24, 22.6)	0.5 (0.0, 5.0)	–1.927	0.054	6.5 (5.0, 11.5)	–5.507	<0.001	≤15 (n = 24, 100.0)	12 (50.0)	12 (50.0)	1.676	0.195
>15 (n = 82, 77.4)	2.5 (0.0, 10.0)			17.5 (13.0, 25.0)			>15 (n = 82, 100.0)	29 (35.4)	53 (64.6)		
≤16 (n = 29, 27.4)	0.0 (0.0, 2.5)	–2.704	0.007	9.0 (5.0, 15.0)	–5.195	<0.001	≤16 (n = 29, 100.0)	16 (55.2)	13 (44.8)	4.579	0.032
>16 (n = 77, 72.6)	3.0 (0.0, 10.5)			19.0 (12.5, 25.0)			>16 (n = 77, 100.0)	25 (32.5)	52 (67.5)		
≤17 (n = 37, 34.9)	0.0 (0.0, 3.0)	–3.069	0.002	10.0 (5.5, 15.5)	–5.041	<0.001	≤17 (n = 37, 100.0)	20 (54.1)	17 (45.9)	5.665	0.017
>17 (n = 69, 65.1)	4.0 (0.0, 11.0)			20.0 (12.5, 26.0)			>17 (n = 69, 100.0)	21 (30.4)	48 (69.6)		
≤18 (n = 44, 41.5)	1.0 (0.0, 5.5)	–2.426	0.015	10.0 (6.0, 16.0)	–5.489	<0.001	≤18 (n = 44, 100.0)	21 (47.7)	23 (52.3)	2.597	0.107
>18 (n = 62, 58.5)	4.0 (0.0, 11.3)			20.0 (13.8, 26.3)			>18 (n = 62, 100.0)	20 (32.3)	42 (67.7)		
≤19 (n = 55, 51.9)	1.0 (0.0, 8.0)	–1.487	0.137	11.0 (6.0, 16.0)	–6.429	<0.001	≤19 (n = 55, 100.0)	24 (43.6)	31 (56.4)	1.184	0.276
>19 (n = 51, 48.1)	3.0 (0.0, 11.0)			23.0 (15.0, 28.0)			>19 (n = 51, 100.0)	17 (33.3)	34 (66.7)		
≤20 (n = 58, 54.7)	1.0 (0.0, 8.0)	–1.718	0.086	11.5 (6.0, 16.0)	–6.336	<0.001	≤20 (n = 58, 100.0)	26 (44.8)	32 (55.2)	2.041	0.153
>20 (n = 48, 45.3)	3.5 (0.0, 11.0)			23.0 (15.5, 28.0)			>20 (n = 48, 100.0)	15 (31.3)	33 (68.8)		

[a] Mann-Whitney *U* test, [b] χ^2 test, *Percentage of subgroups of different cutoff value of TLN among the 106 GAC patients, **Percentage of N(–) and N(+) status within subgroups of each cutoff value of TLN.

PLN, positive LN; NLN, negative LN; N(–), negative nodal status; N(+), positive nodal status.

Table 5. Cutoff value for TLN and results for analyzed LN for 25 T1 GAC patients.

Sub-groups (n, %*)	Value of LN, Median (25P, 75P)			Sub-groups (n, %**)	N-status (n, %)			
Cutoff value of TLN	PLN	*Z*	*p*-value[a]	Cutoff value of TLN	N(–)	N(+)	χ^2	*p*-value
≤14 (n = 3, 12.0)	0.0 (0.0, 0.0)	–1.005	0.497	≤14 (n = 3, 100.0)	3 (100.0)	0 (0.0)		0.554[b]
>14 (n = 22, 88.0)	0.0 (0.0, 1.0)			>14 (n = 22, 100.0)	16 (72.7)	6 (27.3)		
≤15 (5, 20.0)	0.0 (0.0, 0.0)	–1.361	0.336	≤15 (n =5, 100.0)	5 (100.0)	0 (0.0)		0.289[b]
>15 (20, 80.0)	0.0 (0.0, 1.0)			>15 (n =20, 100.0)	14 (70.0)	6 (30.0)		
≤16 (n = 8, 32.0)	0.0 (0.0, 0.0)	–1.867	0.175	≤16 (n = 8, 100.0)	8 (100.0)	0 (0.0)		0.129[b]
>16 (n = 17, 68.0)	0.0 (0.0, 1.5)			>16 (n = 17, 100.0)	11 (64.7)	6 (35.3)		
≤17 (n = 12, 48.0)	0.0 (0.0, 0.0)	–2.615	0.052	≤17 (n = 12, 100.0)	12 (100.0)	0 (0.0)		0.015[b]
>17 (n = 13, 52.0)	0.0 (0.0, 2.0)			>17 (n = 13, 100.0)	7 (53.8)	6 (46.2)		
≤18 (n = 15, 60.0)	0.0 (0.0, 0.0)	–1.481	0.285	≤18 (n = 15, 100.0)	13 (86.7)	2 (13.3)	1.106	0.293[c]
>18 (n = 10, 40.0)	0.0 (0.0, 2.0)			>18 (n = 10, 100.0)	6 (60.0)	4 (40.0)		
≤19 (n = 16, 64.0)	0.0 (0.0, 0.0)	–0.680	0.637	≤19 (n = 16, 100.0)	13 (81.3)	3 (18.8)	0.110	0.740[c]
>19 (n = 9, 36.0)	0.0 (0.0, 1.0)			>19 (n = 9, 100.0)	6 (66.7)	3 (33.3)		
≤20 (n = 16, 64.0)	0.0 (0.0, 0.0)	–0.680	0.637	≤20 (n = 16, 100.0)	13 (81.3)	3 (18.8)	0.110	0.740[c]
>20 (n = 9, 36.0)	0.0 (0.0, 1.0)			>20 (n = 9, 100.0)	6 (66.7)	3 (33.3)		

[a] Mann-Whitney *U* test, [b] Fisher's exact test, [c] corrected χ^2 test (Yates's χ^2 test), *Percentage of subgroups of different cutoff value of TLN among the 25 T1 GAC patients, **Percentage of N(–) and N(+) status within subgroups of each cutoff value of TLN.

The Effect of NLN on Survival HR and Its Relationship with TLN for GAC Patients

For the 106 GAC patients, a greater value for NLN was correlated with a lower value for survival HR (HR = 0.949, 95% CI = 0.910–0.990, $p = 0.016$, Table 7, Left side). This was also represented for the 65 N(+) GAC patients (HR = 0.960, 95% CI = 0.915–1.006, $p = 0.086$), but not for the 41 N(–) GAC patients ($p = 0.386$) (Table 7, Left side).

In terms of the quantitative relationship between NLN and TLN for all 106 GAC patients, a greater NLN value was associated with a greater TLN value (*Scc* = 0.716, $p < 0.001$, Table 7, Right side). The same positive correlation was observed for 65 N(+) GAC patients (*Scc* = 0.733, $p < 0.001$, Table 7, Right side). For the 41 N(–) GAC patients, the NLN value is equal to the TLN value because the PLN value is 0 (*Scc* = 1.000, $p < 0.001$, Table 7, Right side).

Differences in the HR Value, Survival and TLN for Different Cutoff Values for NLN for 65 N(+) GAC Patients

Concerning the distribution for the value of NLN among the 65 N(+) GAC patients, the values of the 25th percentile and the 75th percentile were 7 and 20, respectively. Using the cutoff values of 7 to 20, the relative HRs, survival differences and TLN values were shown in Table 8 (categorical model). Compared to N(+) GAC patients with a value for NLN ≤9, N(+) GAC patients with a value for NLN > 9 (i.e., ≥10) owned a lower HR (HR = 0.477, 95% CI = 0.213–1.070, $p = 0.073$, Table 8, Left side, Fig. 2), a longer survival (52.7, 95% CI = 44.3–61.0 vs. 35.2, 95% CI = 24.1–46.2 months, $p = 0.066$, Table 8, Middle side), and a greater value for TLN (Median, 23.0 vs. 17.0, $Z = -4.344$, $p < 0.001$, Table 8, Right side). The ROC curve (AUC = 0.827, 95% CI = 0.722–0.933, $p < 0.001$) showed that a cutoff value of 19.5 (i.e., 20 in round number) for TLN gave the highest Youden index of 0.540 and gave an optimal power to distinguish N(+) GAC patients with a value of NLN ≤9 from N(+) GAC patients with a value of NLN >9 (≥10) (Sensitivity = 0.826, 1-Specificity = 0.286). A value of TLN more than 20 (≥20) seemed a minimal requirement for N(+) GAC patients to harvest a value of NLN >9 (≥10).

Distribution of LN for 106 GAC and 65 N(+) GAC Patients for Different Types of LN Dissection

For all the 106 GAC patients (Table 9, upper part), the proportions for TLN >17 (i.e., ≥18) were greater stepwise for those who underwent D1 (40.0%) to D1+ (50.9%) and further D2 (83.3%) dissection ($\chi^2 = 13.084$, $p = 0.001$). For the 65 N(+) GAC patients (Table 9, lower part), the proportions for NLN >9 (i.e., ≥10) were greater stepwise for those who underwent D1 (0.0%) to D1+ (56.7%) and further D2 (78.1%) dissection ($p = 0.012$).

In terms of the proportions for TLN >19 (i.e., ≥20) or TLN > 22 (i.e., ≥23) among the 65 N(+) GAC patients, they were greater stepwise for those who underwent D1 (0.0%, 0.0%) to D1+ (40.0%, 26.7%) and further D2 (68.8%, 53.1%) dissection ($p = 0.011$; $p = 0.028$) (Table 9, Middle part).

D2 dissection reached the values of TLN ≥18 for GAC patients to determine N(+) status, TLN ≥23 for N(+) GAC patients to determine N3 status, and NLN ≥10 as well as TLN ≥20 for N(+) GAC patients to increase survival, more effectively than did D1/D1+ dissection, respectively (Table 9).

Table 6. Distribution of the PLN value for 65 N(+) GAC patients after partitioning the value for TLN from ≤3 to ≤47.

	Value of PLN	
TLN (Cumulative case number, %)	Median (25P, 75P)	Integral No
≤3 (n = 1, 1.5)*	- (-, -)	-
≤4 (n = 2, 3.1)*	- (-, -)	-
≤7 (n = 5, 7.7)	1.0 (1.0, 2.0)	1
≤11 (n = 6, 9.2)	1.5 (1.0, 3.0)	2
≤12 (n = 7, 10.8)	2.0 (1.0, 2.0)	2
≤14 (n = 8, 12.3)	2.0 (1.0, 5.0)	2
≤15 (n = 12, 18.5)	4.0 (1.3, 9.0)	4
≤16 (n = 13, 20.0)	3.0 (1.5, 9.0)	3
≤17 (n = 17, 26.2)	3.0 (1.5, 9.0)	3
≤18 (n = 23, 35.4)	4.0 (2.0 9.0)	4
≤19 (n = 31, 47.7)	7.0 (2.0, 10.0)	7
≤20 (n = 32, 49.2)	7.0 (2.0, 10.0)	7
≤21 (n = 36, 55.4)	6.0 (2.0, 10.0)	6
≤22 (n = 40, 61.5)	6.0 (2.0 10.0)	6
≤23 (n = 42, 64.6)	6.5 (2.0, 10.0)	7
≤24 (n = 45, 69.2)	7.0 (2.0, 10.5)	7
≤25 (n = 47, 72.3)	7.0 (2.0, 11.0)	7
≤28 (n = 50, 76.9)	6.5 (2.0, 11.0)	7
≤30 (n = 51, 78.5)	7.0 (2.0, 11.0)	7
≤31 (n = 54, 83.1)	6.5 (2.0, 11.3)	7
≤33 (n = 55, 84.6)	6.0 (2.0, 11.0)	6
≤34 (n = 56, 86.2)	6.5 (2.0, 11.8)	7
≤36 (n = 59, 90.8)	7.0 (2.0, 12.0)	7
≤40 (n = 60, 92.3)	6.5 (2.0, 11.8)	7
≤41 (n = 63, 96.9)	6.0 (2.0, 12.0)	6
≤43 (n = 64, 98.5)	6.5 (2.0, 11.8)	7
≤47 (n = 65, 100.0)	7.0 (2.0, 12.0)	7

*Concerning the TLN ≤3 and ≤4 groups, there were only 1 and 2 cases, respectively, as a result, there were missing Median, 25P, 75P and Integral No.

Table 7. The effect of the NLN value on survival HR and its relationship with the value for TLN for GAC patients.

Value of NLN	HR	Cox's regression model	Association to value of TLN	
Continuous model (n, %)	Mean (95% CI)	*p* (uni-variate)	*Scc*	*p*-value
Overall (n = 106, 100.0)	0.949 (0.910–0.990)	0.016	0.716	<0.001
N(–) (n = 41, 38.7)	0.962 (0.881–1.050)	0.386	1.000	<0.001
N(+) (n = 65, 61.3)	0.960 (0.915–1.006)	0.086	0.733	<0.001

Scc, Spearman's Rho correlation coefficient.

Discussion

AJCC T-/N-/M-status and cancer stage remained the well-accepted standards to predict the survival of GAC patients [5,6,17]. To determine an accurate N-status, an adequate LND to harvest sufficient value of TLN is necessary. Although we recommend a minimal requirement of a TLN value of 18, it was slightly different from the value of 16 suggested in the AJCC manual, 8th edition and other studies [6,10]. The minimal requirement about TLN was also reported for other cancers of the digestive tract, such as esophageal cancer [18] and colorectal cancer [19], and ever defined in the AJCC manual, 8th edition [6]. Nevertheless, the threshold value of TLN remained an issue for repeated debate in the literature. Different types of gastrectomy showed that the optimal respective threshold value for TLN was 16 for subtotal gastrectomy and was 21 for total gastrectomy [20]. Other studies showed that a value for TLN ≥30 was recommended for selective stage II GAC [21] or ≥24 for T2-4 GAC [22]. The Chinese Society of Clinical Oncology (CSCO) recommended a value of TLN at least ≥16 for proper staging for GAC patients undergoing radical gastrectomy and the value must be greater than 30 to ensure accurate cancer stage [23]. These heterogeneous

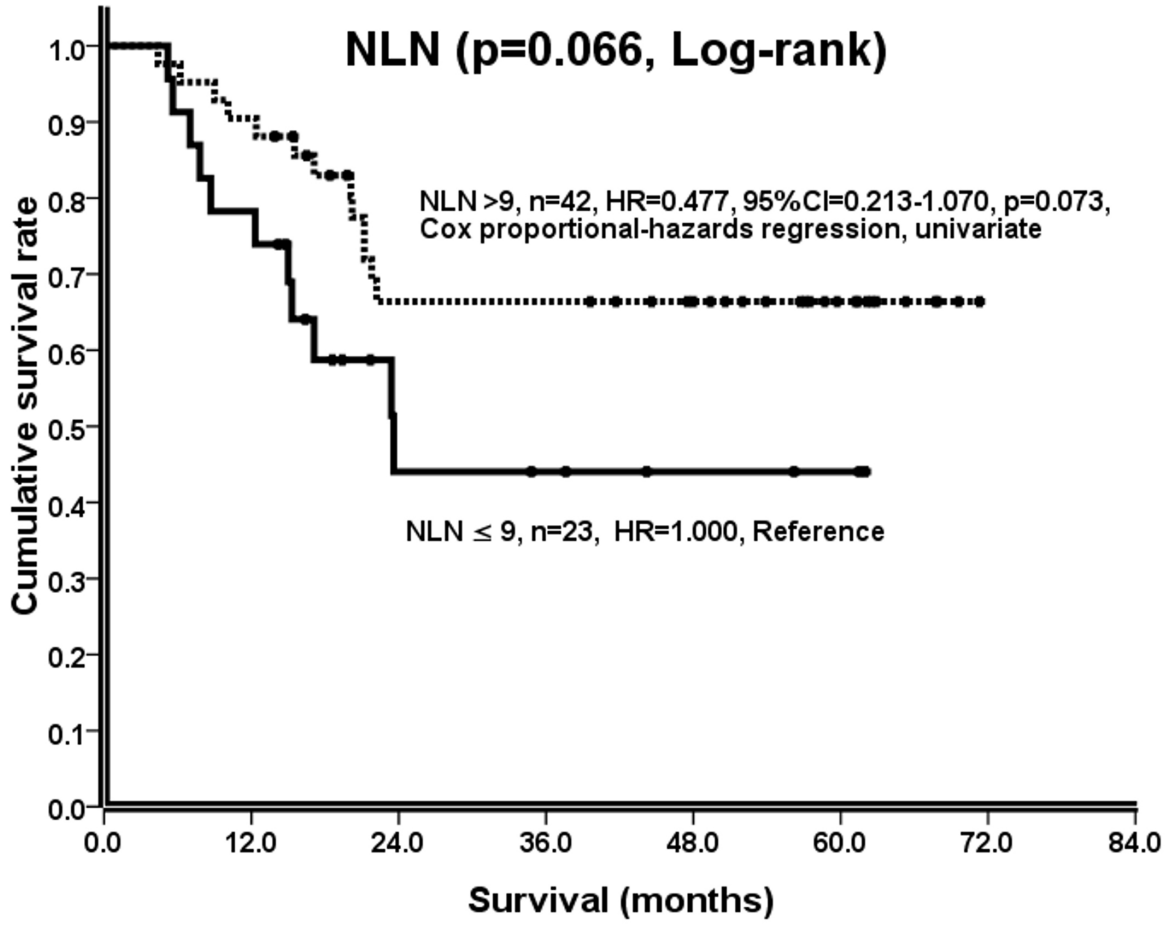

Fig. 2. Kaplan-Meier survival curve, *p*-value (Log-rank test), relative HR (including the 95% CI and the Cox proportional-hazards regression, uni-variate) for the prognostic variable NLN (>9 or ≤9) for N(+) GAC patient are shown. CI, confidence interval; GAC, gastric adenocarcinoma; HR, hazard ratio; LN, lymph node; NLN, negative LN.

reports accounted for the threshold of TLN ≥18 determined in this study was different to ≥16 in the AJCC 8th edition, because it consisted different types of gastrectomy, different cancer stages and a small sample size.

There is an increased likelihood of identifying an N(+) or even a positive N3 status, if a more extensive LND is executed. Nevertheless, previous studies showed that an extensive LND might be associated with a greater incidence of surgical complications and greater peri-operative mortality, which might hinder survival benefit [24,25]. But the results of the Italian Gastric Cancer Study Group demonstrated that the morbidity and mortality did not increase even if an extensive LND was carried out [26]. Similarly to the Italian report, our study showed that 2 (1.9%) patients died within 30 days of surgery, which was much less than the value for other studies [27,28]. Because there were no obvious harmful consequences in the clinical practice, a minimal required and reasonable LND during gastrectomy seemed necessary. Furthermore, we found that an increase of TLN to ≥23 could identify the suspicious N3 GAC patients, to whom a multi-modality therapy might be indicated to improve their survivals. In the literature, an increase in the TLN value to 30 or 45 were ever reported by other studies for advanced GAC [21,29]. These differences in the threshold values about TLN emphasized the following viewpoints, including adequate lymph node staging and tailoring suitable adjuvant therapy.

An adequate LND to improve survival of GAC patients remained another issue to discuss. Through an optimal LND with sufficient amounts of LNs, the 5-year survival rate of this cohort was 62.3%, with a mean survival period of 54.2 months, and it was not inferior to that for other studies [30]. Therefore, we appraised the survival benefits of TLN from the viewpoint of NLN. Recently, the role of NLN was paid attention to and reported studies emphasized the increased number of NLN to improve survivals for GAC patients [31,32]. Our data reproduced the favorable effect of NLN in survival for all GAC patients, but some diverse existed between N(–) and N(+) patients. For the N(+) GAC patients, this study showed a better survival for a value for NLN ≥10, and it was possible that more NLN could offer an accurate N-status to distinguish stage-specific survivals. If the nodal condition for patient A was described as 1/5/4 (numbers of PLN/TLN/NLN) and for patient B was 1/10/9,

Table 8. Differences between HRs, survivals and TLNs for different cutoff values of NLN among 65 N(+) GAC patients.

Categorical model	HR	Cox's regression model	Survival (months)	Survival difference	TLN		
Cutoff value of NLN Subgroups (n, %)	Mean (95% CI)	*p* (Univariate)	Mean (95% CI)	*p* (Log-rank)	Median (25P, 75P)	*Z*	*p*-value
≤7 (n = 18, 27.7)	1.000	0.103	33.9 (21.5–46.2)	0.096	15.0 (7.0, 19.0)	–4.892	<0.001
>7 (n = 47, 72.3)	0.501 (0.218–1.151)		51.7 (43.6–59.8)		23.0 (19.0, 31.0)		
≤8 (n = 20, 30.8)	1.000	0.279	37.5 (26.0–49.0)	0.274	15.0 (8.0, 19.0)	–4.842	<0.001
>8 (n = 45, 69.2)	0.633 (0.276–1.449)		50.8 (42.4–59.2)		23.0 (19.0, 32.0)		
≤9 (n = 23, 35.4)	1.000	0.073	35.2 (24.1–46.2)	0.066	17.0 (11.0, 19.0)[a]	–4.344	<0.001
>9 (n = 42, 64.6)	0.477 (0.213–1.070)		52.7 (44.3–61.0)		23.0 (19.0, 31.5)[a]		
≤10 (n = 27, 41.5)	1.000	0.118	38.8 (27.9–49.7)	0.111	17.0 (12.0, 19.0)	–4.695	<0.001
>10 (n = 38, 58.5)	0.526 (0.235–1.176)		52.6 (43.9–61.3)		24.0 (19.0, 33.8)		
≤11 (n = 30, 46.2)	1.000	0.152	40.1 (29.9–50.2)	0.145	18.0 (13.5 20.5)	–4.364	<0.001
>11 (n = 35, 53.8)	0.554 (0.247–1.242)		52.6 (43.5–61.6)		24.0 (19.0, 36.0)		
≤12 (n = 31, 47.7)	1.000	0.225	44.2 (33.2–55.3)	0.219	18.0 (14.0, 20.0)	–4.429	<0.001
>12 (n = 34, 52.3)	0.607 (0.271–1.359)		50.8 (41.8–59.9)		24.5 (20.5, 36.0)		
≤13 (n = 33, 50.8)	1.000	0.226	44.0 (33.4–54.6)	0.220	18.0 (14.5, 21.0)	–4.543	<0.001
>13 (n = 32, 49.2)	0.605 (0.268–1.365)		51.3 (42.0–60.6)		26.5 (21.0, 36.0)		
≤14 (n = 39, 60.0)	1.000	0.493	46.5 (36.8–56.2)	0.491	18.0 (15.0, 22.0)	–4.589	<0.001
>14 (n = 26, 40.0)	0.748 (0.327–1.714)		49.9 (39.6–60.3)		28.0 (21.0, 37.0)		
≤15 (n = 40, 61.5)	1.000	0.298	45.6 (35.9–55.2)	0.293	18.5 (15.0, 22.0)	–4.601	<0.001
>15 (n = 25, 38.5)	0.636 (0.271–1.492)		51.6 (41.3–61.8)		28.0 (21.0, 38.0)		
≤16 (n = 43, 66.2)	1.000	0.354	46.1 (37.0–55.3)	0.350	18.0 (15.0, 22.0)	–5.320	<0.001
>16 (n = 22, 33.8)	0.659 (0.272–1.593)		51.5 (40.4–62.5)		31.0 (22.0, 40.3)		
≤17 (n = 45, 69.2)	1.000	0.275	45.6 (36.5–54.6)	0.269	18.0 (15.0, 21.5)	–5.512	<0.001
>17 (n = 20, 30.8)	0.597 (0.236–1.509)		52.8 (41.6–64.1)		32.0 (25.0, 40.8)		
≤18 (n = 46, 70.8)	1.000	0.375	46.2 (37.4–55.1)	0.371	18.5 (15.0, 22.0)	–5.514	<0.001
>18 (n = 19, 29.2)	0.657 (0.260–1.662)		51.9 (40.2–63.6)		33.0 (28.0, 41.0)		
≤19 (n = 47, 72.3)	1.000	0.240	45.4 (36.6–54.2)	0.232	19.0 (15.0, 22.0)	–5.443	<0.001
>19 (n = 18, 27.7)	0.552 (0.206–1.485)		53.8 (42.0–65.6)		34.5 (28.0, 41.0)		
≤20 (n = 50, 76.9)	1.000	0.293	45.9 (37.5–54.3)	0.286	19.0 (15.8, 22.0)	–5.648	<0.001
>20 (n = 15, 23.1)	0.562 (0.191–1.648)		54.0 (40.9–67.1)		36.0 (31.0, 41.0)		

[a] The receiver operating characteristic (ROC) curve (area under the curve (AUC) = 0.827, 95% CI = 0.722–0.933, $p < 0.001$) showed that a cutoff value of 19.5 for TLN gave the highest Youden index of 0.540 and gave an optimal power to distinguish N(+) GAC patients with a value of NLN ≤9 from N(+) GAC patients with a value of NLN >9 (≥10) (Sensitivity = 0.826, 1-Specificity = 0.286).

Table 9. Distribution of LN for all 106 and for 65 N(+) GAC patients for different types of LN dissection.

LN and cutoff value (n, %*)	All GAC patients (n = 106)				
	Type of LND (n, %**)				
	D1 (n = 5, 100.0)	D1+ (n = 53. 100.0)	D2 (n = 48, 100.0)	χ^2	*p*-value
TLN (n = 106, 100.0)					
≤17 (n = 37, 34.9)	3 (60.0)	26 (49.1)	8 (16.7)	13.084	0.001[a]
>17 (n = 69, 65.1)	2 (40.0)	27 (50.9)	40 (83.3)		
LN and cutoff value (n, %*)	N(+) GAC patients (n = 65)				
	Type of LND (n, %**)				
	D1 (n = 3, 100.0)	D1+ (n = 30. 100.0)	D2 (n = 32, 100.0)		
TLN (n = 65, 100.0)					
≤19 (n = 31, 47.7)	3 (100.0)	18 (60.0)	10 (31.3)		0.011[b]
>19 (n = 34, 52.3)	0 (0.0)	12 (40.0)	22 (68.8)		
≤22 (n = 40, 61.5)	3 (100.0)	22 (73.3)	15 (46.9)		0.028[b]
>22 (n = 25, 38.5)	0 (0.0)	8 (26.7)	17 (53.1)		
NLN (n = 65, 100.0)					
≤9 (n = 23, 35.4)	3 (100.0)	13 (43.3)	7 (21.9)		0.012[b]
>9 (n = 42, 64.6)	0 (0.0)	17 (56.7)	25 (78.1)		

[a] χ^2 test, [b] Fisher-Freeman-Halton exact test, *Percentage of subgroups of cutoff value of TLN (≤17/>17) of the 106 GAC patients or cutoff value of TLN (≤19/>19, ≤22/>22) or NLN (≤9/>9) of the 65 N(+) GAC patients, **Percentage of subgroups of cutoff value of TLN (≤17/>17) of the D1, D1+ or D2 group of 106 GAC patients or cutoff value of TLN (≤19/>19, ≤22/>22) or NLN (≤9/>9) of the D1, D1+ or D2 group of the 65 N(+) GAC patients.

and they were both classified as N1 status. Patient B had a greater value for NLN and the N1-status for Patient B was much more accurate than that for Patient A. There was a smaller possibility to underestimate the cancer stage of Patient B. Because there were another 5 LNs needed to be dissected for Patient A to reach the same value for TLN as Patient B. As a result, there might be new results for the 5 new LNs: either all negative to maintain same N status or emerging positive nodes to cause progressed N status. This phenomenon were also reported for colorectal and gastric cancers [10,19]. Because the value of NLN was highly related to the value of TLN and we figured out a value for TLN ≥20 was required to achieve NLN ≥10 to guarantee better survival for N(+) GAC patients. A similar conclusion about NLN for N(+) GAC patients to influence survival was also reported in the literature [10]. To conjecture, adequate LND with sufficient amount of TLN could offer both accurate cancer staging and survival benefit for N(+) GAC patients. Nevertheless, for N(–) GAC patients, including the T1N0, this study showed no significant survival benefit from the viewpoint of NLN. It might be the small sample size or most patients had undergone sufficient LND exceeding the required value. For N(–) situation, the NLN equals to TLN. In the literatures, some studies showed that an extensive LND could improve local-regional control and may eliminate the occurrence of undetected tumor foci, as evidence for esophageal squamous cell carcinoma, colorectal cancer and gastric cancer [18,33,34]. The role of LND in N(–) GAC deserved further evaluation in the future.

There were many factors affecting the value for harvested TLN in the literature. In terms of hospital volume, high-volume centers could have more adequate lymphadenectomy [35]. In terms of the LNs inside surgical specimens that was collected in the operating room (OR) by surgeons or in the pathological department by pathologists, more TLN was collected in the OR [36]. Due to advances in image quality and the maturing of surgical techniques, several studies showed laparoscopic LND gave similar results to laparotomy and caused less postoperative comorbidities [37,38]. The use of 3-D imaging further improved quality of LND to a better surgical outcome [39]. A new technique using indocyanine green (ICG) guided laparoscopic LND collected significantly more TLN [40].

Besides the up mentioned factors to affect the harvested TLN, the extent of LND, from D1 to (D1+)/D2 and further to D3 according to the regional LN map, was the most discussed in the literatures [41,42]. For the United States Surveillance Epidemiology and End Results (SERR), Memorial Sloan-Kettering Cancer Center (MSKCC), National cancer center, Japan (NCC/Japan) and Seoul National University Hospital, Korea (SNUH/Korea) databases, a D2 dissection gave a greater value for TLN than a D1 dissection [41]. In line with the Japanese Gastric Cancer Treatment Guidelines 2021 (6th edition) [14], this study demonstrated a stepwise increase in TLN from the value for D1 to D1+ and then D2 dissections (Median, 15.0, 18.0, 24.0; data not shown). A D2 dissection owned the greatest power to achieve required thresholds, including TLN ≥18/20/23 and NLN ≥10, compared to those for D1/D1+ dissection.

A D2 dissection is indicated for all gastrectomy procedures to obtain a sufficient TLN. For a limited but increasing experiences in our institute, using laparoscopic surgery, the guiding of the ICG image, a high quality 4K image monitor and a well-trained surgeon, we could perform adequate D2 LND to gain more TLN without an increase in postoperative comorbidity.

Female gender was determined to be an independent prognostic variable to better survival for the study cohort, and it was a subject for study in the literature [43]. Several observations may explain this sex disparity. In terms of cancer locations, cardiac and proximal gastric cancers have a poorer prognostic effect and occur most commonly in Asian male patients [44]. Alpha Thalassemia/mental Retardation syndrome X-linked (ATRX) gene mutation or microsatellite instability-high (MSI-H) in female GAC are correlated with a better response to immune checkpoint inhibitors or neoadjuvant therapies [45,46]. Female estrogen is assumed to have some anti-cancer effect on several human cancers but there is no evidence that it affects gastric cancer [47]. This sex disparity in GAC prognosis requires more data to enable future study.

However, this study had several limitations. This was a retrospective study and it required a prospective protocol with propensity score matching for validation. Only a small number of GAC cases were analyzed and a multi-center or nationwide database was required. Some GAC patients received adjuvant therapies after gastrectomy and an analysis of disease-free survival might give more meaningful results than the overall survival, as demonstrated in the current study. Different surgeons and surgical equipment also affected results. These limitations should be considered by future studies.

Conclusions

The effects of LND on GAC patients differs, depending on the clinical implications. A value for TLN $\geq$18 is necessary to distinguish whether a *de-novo* GAC patient has N(–) or N(+) status and an increase to a value for TLN $\geq$23 is necessary for N(+) GAC patients to determine the possible N3 status. A value for NLN $\geq$10 improves survival rates for N(+) GAC patients and a value for TLN $\geq$20 is necessary. D2 dissection is recommended to achieve these requirements. LND has a dual role in the accurate determination of the N-status stage and improving survival for GAC patients.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

Author Contributions

CHL and HTL participated in the study design, analyzed the data, organized the materials and wrote the manuscript; STY analyzed the data and revised the statistic results; CYT participated in the study design and manuscript revision; HHL and CSL participated in the study design and revised the study design, analyzed the data, revised the manuscript. All authors read and approved the final manuscript. All authors contributed to important editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

In accordance with the Declaration of Helsinki, the Institute Review Board (IRB) of Cathay General Hospital approved this study (IRB No: CGH-P112022) and the waiver of the participants' consent. All the patients were followed until 31 January 2023.

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Conflict of Interest

The authors declare no conflict of interest.

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