Original Article

Validation of clinical significance of examined lymph node count for accurate prognostic evaluation of gastric cancer for the eighth edition of the American Joint Committee on Cancer (AJCC) TNM staging system

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Abstract

Objective: To validate the necessity of increasing the examined lymph node (ELN) count for enhancing the accuracy of prognostic evaluation of gastric cancer (GC) patients after curative gastrectomy in multiple medical centers of China.

Methods: The clinicopathological data of 7,620 patients who underwent the curative resection for GC between 2001 and 2011 were included to demonstrate whether the ELN count is indispensable for enhancing the accuracy of prognostic evaluation of GC patients after surgery. After a meticulous stratification by using the cut-point survival analysis, all included 7,620 patients were allocated into three groups as: less than 16 (<16), between 16 and 30 (16–30), and more than 30 (>30) ELNs. Survival differences among various subgroups of GC patients were analyzed to assess the impact of the ELN count on the stage migration in accordance with the overall survival (OS) of GC patients.

Results: Survival analyses revealed that the ELN count was positively correlated with the OS (P=0.001) and was an independent prognostic predictor (P<0.01) of 7,620 GC patients. Stratum analysis showed that the accuracy of prognostic evaluation could be enhanced when the ELN count was no less than 16 (≥16) for node-negative patients and >30 for node-positive patients. Stage migrations were mainly detected in the various subgroups of patients with specific pN stages as follows: pN0 with 16–30 ELNs ($pN0_{16–30}$) and pN0 with >30 ELNs ($pN0_{>30}$), pN0 with <16 ELNs ($pN0_{<16}$) and $pN1_{>30}$, $pN1_{<16}$ and $pN2_{16–30}$, $pN1_{16–30}$ and $pN2_{>30}$, $pN3a_{<16}$ and $pN3b_{16–30}$, and $pN3a_{<16}$ and $pN3b_{>30}$. These findings indicate that increasing the ELN count is a prerequisite to guarantee precisely prognostic evaluation of GC patients.

Conclusions: The ELN count should be proposed to be >30 for acquiring the accurate prognostic evaluation for GC patients, especially for node-positive patients.

Keywords: Stomach; neoplasm; lymph node; metastasis; prognosis

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Introduction

Intraoperatively dissected lymph node (DLN) count is an essential parameter to ensure the curative quality of gastrectomy for gastric cancer (GC) (1). This parameter corresponds to the number of lymph nodes dissected by surgeons intraoperatively within a complete tissue specimen. Actually, DLNs should be individually separated from a complete tissue sample for detailed pathological examination after surgery because this procedure requires patience and endurance. Consequently, the examined lymph node (ELN) count after curative surgery for GC is frequently lower than the DLN count, and this condition may directly impede the accurate evaluation of the curative degree of surgery for GC (2). In theory, three essential elements can affect the ELN count, which may induce the migration of pN stage after surgery: 1) extent of intra-operative lymphadenectomy or the degree of lymph node dissection; 2) number of lymph nodes postoperatively separated from a complete tissue specimen, and 3) pathological confirmation of ELNs. A consensus for the extent of lympadenectomy in standard GC curative surgery should be recommended for D2 lymphadenectomy but not for all early diseases (3). However, a consensus on the sufficient ELN count based on the DLN count for accurate evaluation of the pN stage has not been researched.

Several studies have revealed the significantly positive association of ELN count with the number of metastatic lymph nodes in GC, but the quantitative assessment of the effects of ELN count on GC has remained controversial (4,5). The minimum ELN count required for proper staging is not mandatory according to the 8th edition TNM classification for GC (6), although an ELN count of ≥16 has been proposed by the American Joint Commission for Cancer (AJCC) to guarantee the accurate prognosis of pN stage since 2009 (7). Recently, Sano *et al*. (8) reported an international gastric cancer association staging project including 25,411 patients mainly from Japan (41.85%) and Korea (42.98%) to propose that the optimal ELN count had better achieve to be 30 or more. We also noticed that the mean values of ELN count of GC patients in Japan (39.4) and Korea (33.0) were identified to be higher than those in other Asia countries (24.8) and West countries (29.5) in that manuscript (8). It must be sure that no pN classification system can supersede the performance of an adequate lymph node dissection of ≥16 nodes as endorsed by any oncology practice guidelines [such as National Comprehensive Cancer Network (NCCN) guidelines] (9).

Considering the important effects of the number of metastatic lymph nodes on the accurate evaluation of pTNM classification and prognosis of GC patients after surgery, we should define the cut-off values of the ELN count to prevent the migration of pN stage in clinical settings. The present study aimed to elucidate whether the sub-classifications of ELN count should be designed to enhance the accuracy of both postoperative staging and accurate prognostic evaluation for GC patients. Survival differences among various subgroups of GC patients were analyzed on the basis of various cut-off values of the ELN count from three high-volume medical centers in North and South China.

Materials and methods

Patients

Between January 2001 and December 2011, 2,864 GC patients underwent surgical resection in the Department of Gastric Cancer in the Tianjin Medical University Cancer Hospital (TJMUCH), 3,043 GC patients underwent surgical resection in the Department of Surgical Oncology in the First Affiliated Hospital of China Medical University (CMUFAH) and 2,977 GC patients underwent surgical resection in the Department of Gastric and Pancreatic Surgery in the Sun Yat-sen University Cancer Center (SYSUCC), respectively. After approval from the institutional review boards of the TJMUCH, the CMUFAH and the SYSUCC, data from the cancer registries of three hospitals was obtained. Informed consent was obtained from all patients for being included in the study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

Eligibility criteria included: 1) histologically proven primary adenocarcinoma of the stomach; 2) no history of gastrectomy or other malignancy; 3) absence of non-curative surgical factors including distant metastasis, positive peritoneal cytology, or peritoneal dissemination; 4)

no Siewert-I or II esophagogastric junction (EGJ) tumor; 5) pathologically negative resection margins (R0 resection); 6) remaining alive during the initial hospital stay and during the first postoperative month; and 7) no administration with neoadjuvant radiochemotherapy. After applying these criteria, we found that 71 patients had the history of gastrectomy, 46 patients had suffered from other malignant diseases, 117 patients presented with distant metastasis in the operation, 106 patients presented with the peritoneal dissemination, 503 patients were diagnosed as the Siewert-I EGJ tumor after surgery, 105 patients were identified as the R1 resection cases, 38 patients were identified as the R2 resection cases, 74 patients died during the first postoperative month, and 204 patients were administered with neoadjuvant treatments. Ultimately, 7,620 GC patients were included in the study (*Supplementary Figure S1*).

Surgical management

Primary tumors were resected *en bloc* by means of lymphadenectomy according to the guidelines of the Japanese Gastric Cancer Association (10). The surgical procedures were based mainly on the Japanese Gastric Cancer Treatment Guidelines (11). Patients with clinical T1 and N0 tumors underwent D1 or D1+ lymphadenectomy, and patients with clinical T2 or more advanced tumors and/or those with N1 or more advanced tumors underwent D2 or D2+ lymphadenectomy. Each medical center has a surgical expert who can perform the standard gastrectomy (including D1, D1+, D2, or D2+ lymph node dissection) in accordance with Japanese Gastric Cancer Treatment Guidelines.

Follow-up

After undergoing curative surgery, all 7,620 patients were followed up every 3 or 6 months for 2 years, then every 6 months for next 3 years, and annually thereafter until death. The median follow-up time for the entire cohort was 87 (range, 2–186) months. Follow-up of all patients included in this study was completed in October 2015.

Stage migration

Stage migrations were mainly defined as: 1) no statistical significance of the survival differences to be detected in several subgroups of patients with different ELNs in the specific pN stages, or 2) the significant survival differences to be detected in several subgroups of patients with the same ELNs in the different pN stages.

Statistical analysis

The cut-point survival analysis was adopted to determine the most appropriate cut-off values for the ELN count (12). Clinicopathological characteristics significantly related to patients' survival were evaluated by the Kaplan-Meier method and Cox proportional hazards analysis. Chi-square test, likelihood ratio test and multinomial logistic regression were used for the correlation analysis of ELN count and the various clinicopathological characteristics. The Bayesian information criterion (BIC) value within a multinomial logistic regression model was calculated for each category to measure its discriminatory ability. A smaller BIC value indicated a better model for predicting outcome (13). Stratum analysis was adopted to evaluate the influence of the clinicopathological characteristics on the efficiency of prognostic prediction of the ELN count for patients and to demonstrate the migration of pN stage of patients in accordance with the varieties of the ELN count. Significance was defined as a P value <0.05. All statistical analyses were performed using IBM SPSS Statistics (Version 19.0; IBM Corp., New York, USA).

Results

General results

The 5-year survival rate (YSR) of 7,620 GC patients was 54.6%, with a median overall survival (OS) of 81.0 months (*Supplementary Figure S2A*). Of the 7,620 GC patients, 2,793 (36.7%) were pN0 stage (node-negative) with 80.1% of 5-YSR, 1,364 (17.9%) were pN1 stage with 58.1% of 5-YSR, 1,487 (19.5%) were pN2 stage with 44.6% of 5-YSR, 1,337 (17.5%) were pN3a with 27.5% of 5-YSR, and 639 (8.4%) were pN3b stage with 9.9% of 5-YSR (*Supplementary Figure S2B*). The data from 7,620 GC patients were analyzed, and the clinicopathological characteristics of patients are shown in *Table 1*. Of the 7,620 GC patients, 2,318 (including 96.3% advanced cases) with 42.6% of 5-YSR underwent curative surgery in the TJMUCH, 2,868 (including 81.4% advanced cases) with 57.6% of 5-YSR were subjected to curative surgery in the CMUFAH, and 2,434 (including 86.6% advanced cases) with 62.9% of 5-YSR had their curative surgery in the SYSUCC. A total of 3,244 (42.6%) patients died at the end of the follow-up. The curve correlation between the number of metastatic lymph nodes and the ELN count is illustrated in *Supplementary Figure S2C*. In addition, the correlation between pN stage and ELN count was identified to be statistical significance in all 7,620 GC

Table 1 Clinicopathological characteristics and survival analyses of GC patients (N=7,620)

Characteristics	n	5-YSR (%)	χ^2	Univariate P value	HR (95% CI)	Multivariate P value
Gender			2.443	0.118	2.227 (2.081–2.376)	0.136
Male	5,378	54.0				
Female	2,242	56.0				
Age at surgery (year)			82.616	<0.001	1.369 (1.276–1.469)	<0.001
<60	3,986	59.4				
≥60	3,634	49.3				
Tumor location			320.657	<0.001	0.950 (0.914–0.988)	0.011
Upper third	2,023	49.1			Reference	Reference
Middle third	1,474	52.2			0.761 (0.636–0.911)	0.003
Lower third	3,487	62.9			0.830 (0.730–0.944)	0.004
>2/3 stomach	636	32.4			0.857 (0.744–0.988)	0.034
Tumor size (cm)*			513.623	<0.001	1.345 (1.245–1.452)	<0.001
≤4.0	3,626	68.4				
>4.0	3,994	42.2				
Lauren classification			90.692	<0.001	1.143 (1.061–1.230)	<0.001
Intestinal	3,215	61.2				
Diffuse	4,405	49.8				
Type of gastrectomy			439.340	<0.001	0.790 (0.746–0.836)	<0.001
TG	1,741	36.4			Reference	Reference
DG	4,230	63.3			1.288 (1.093–1.518)	0.003
PG	1,649	50.7			0.813 (0.665–0.993)	0.042
pT stage			938.880	<0.001	1.355 (1.303–1.410)	<0.001
T1	945	95.7			Reference	Reference
T2	1,409	67.8			0.122 (0.089–0.167)	<0.001
T3	1,248	53.6			0.527 (0.454–0.612)	<0.001
T4a	3,528	42.4			0.603 (0.524–0.695)	<0.001
T4b	490	26.9			0.726 (0.644–0.818)	<0.001
pN stage			1,829.794	<0.001	1.313 (1.227–1.405)	<0.001
N0	2,793	80.1			Reference	Reference
N1	1,364	58.1			0.177 (0.059–0.532)	0.002
N2	1,487	44.6			0.479 (0.388–0.591)	<0.001
N3a	1,338	27.5			0.524 (0.446–0.615)	<0.001
N3b	638	15.4			0.747 (0.658–0.847)	<0.001
Ratio between metastatic lymph nodes and ELNs (%)			2,036.660	<0.001	1.302 (1.206–1.406)	<0.001
0	2,797	80.0			Reference	Reference
0.1–10.0	1,101	66.0			0.915 (0.306–2.732)	0.874
10.1–40.0	2,037	43.3			0.531 (0.438–0.675)	<0.001
>40.0	1,685	19.5			0.751 (0.674–0.837)	<0.001
ELN count*			13.604	0.001	0.772 (0.726–0.822)	<0.001
<16	2,292	51.7			Reference	Reference
16–30	3,482	55.3			1.630 (1.436–1.851)	<0.001

Table 1 (*continued*)

Table 1 (*continued*)

Characteristics	n	5-YSR (%)	χ^2	Univariate P value	HR (95% CI)	Multivariate P value
>30	1,846	57.2			1.244 (1.127–1.372)	<0.001
Adjuvant chemotherapy						
Yes	4,487	53.5	0.815	0.367	0.541 (0.503–0.572)	0.283
No	3,133	51.6				

GC, gastric cancer; TG, total gastrectomy; DG, distal gastrectomy; PG, proximal gastrectomy; ELN, examined lymph node; 5-YSR, 5-year survival rate; HR, hazard ratio; 95% CI, 95% confidence interval; *, median of tumor diameter: 4.5 (0.1–35.0) cm; **, median of ELN count: 21.0 (1–118); The eighth edition of TNM classification for GC was adopted for postoperatively pathological stages of all included patients.

patients (P<0.001, *Supplementary Table S1*). According to the 7th edition TNM classification for GC, the minimal prerequisite for the prognostic evaluation of patients should be 16. However, the most appropriate cut-off value of the ELN count was 31 (>30), which were calculated in accordance with the method reported by Okajima (14) (*Supplementary Table S2* and *Figure 1A, B*).

Survival analysis

Univariate analysis revealed that the following nine clinicopathological characteristics were significantly associated with OS after the 7,620 GC patients undergoing curative surgery: age at surgery, tumor location, tumor size, Lauren classification, type of gastrectomy, depth of tumor invasion (pT stage), pN stage, ratio between metastatic lymph nodes and ELNs, and ELN count (*Table 1*). These characteristics were then included in a multivariate Cox proportional hazard model (forward stepwise procedure) to adjust for the effects of covariates. Multivariate analysis indicated that the ELN count [hazard ratio (HR), 0.772, P<0.001], age at surgery, tumor location, tumor size, Lauren classification, type of gastrectomy, pT stage, pN stage, and ratio between metastatic lymph nodes and ELNs (*Table 1*) were independent predictors of the OS of all GC patients postoperatively.

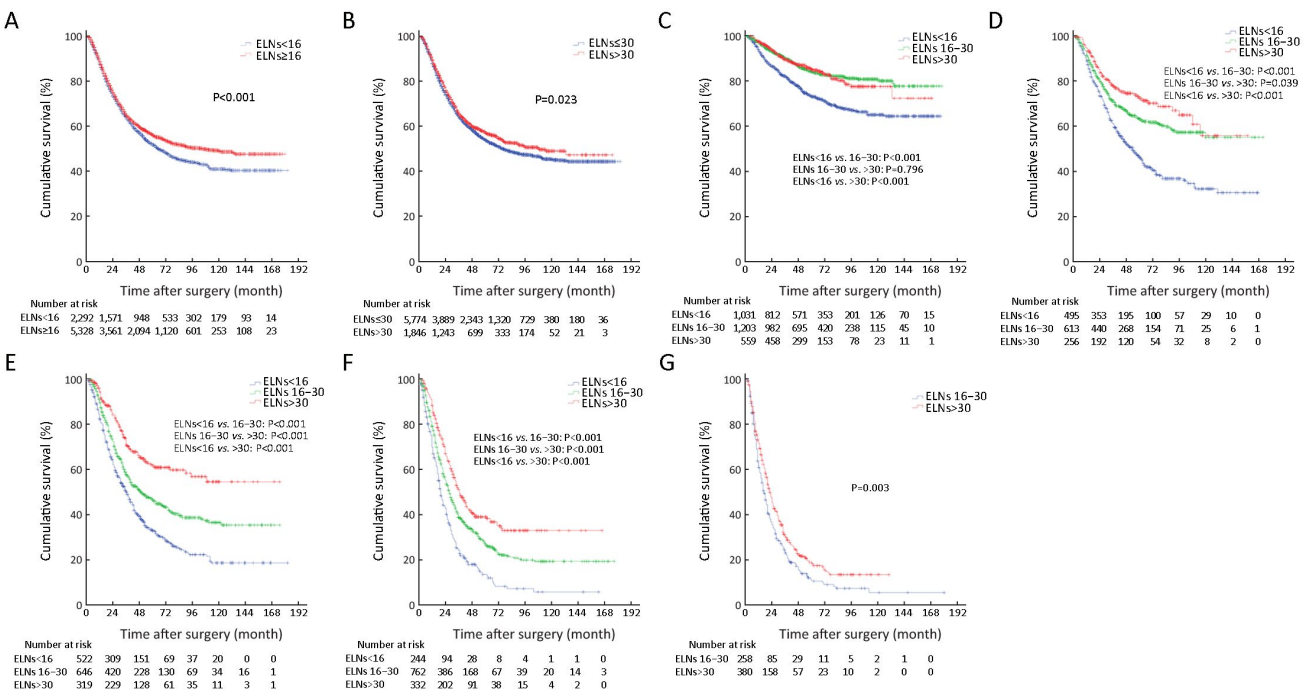

Figure 1 Survival curve of patients according to cut-off value and pN stage. (A) Survival curve of patients according to the cut-off value of 16 examined lymph nodes (ELNs) (P<0.001); (B) Survival curve of patients according to the cut-off value of 31 ELNs (P=0.023); (C) Survival curve of patients with pN0 stage according to the ELN count; (D) Survival curve of patients with pN1 stage according to the ELN count; (E) Survival curve of patients with pN2 stage according to the ELN count; (F) Survival curve of patients with pN3a stage according to the ELN count; (G) Survival curve of patients with pN3b stage according to the ELN count (P=0.003).

Relationship between clinicopathological characteristics and ELN count of GC patients

With the Chi-square test analysis, we demonstrated that gender, age at surgery, tumor location, tumor size, Lauren classification, type of gastrectomy, pT stage and pN stage were significantly related to the ELNs of all 7,620 included GC patients. Furthermore, the multinominal logistic regression model showed that both gender and tumor size presented the smallest BIC values indicating the most intensive relationship to the ELNs of GC patients (*Table 2*).

Table 2 Relationship between clinicopathological characteristics and ELN count of GC patients (N=7,620)

Characteristics	Cases for ELN count [n (%)]			χ^2	P	Likelihood ratio test P value	BIC value
	<16	16–30	>30				
Gender				22.949	<0.001	0.041	6,180
Male	1,699 (22.3)	2,432 (31.9)	1,247 (16.4)				
Female	593 (7.8)	1,050 (13.8)	599 (7.9)				
Age at surgery (year)				90.274	<0.001	<0.001	6,223
<60	1,020 (13.4)	1,883 (24.7)	1,083 (14.2)				
≥60	1,272 (16.7)	1,599 (21.0)	763 (10.0)				
Tumor location				398.163	<0.001	<0.001	6,223
Upper third	899 (11.8)	857 (11.2)	267 (3.5)				
Middle third	283 (3.7)	688 (9.0)	503 (6.6)				
Lower third	900 (11.8)	1,656 (21.7)	931 (12.2)				
>2/3 stomach	210 (2.8)	281 (3.7)	145 (1.9)				
Tumor size (cm)				19.963	<0.001	0.029	6,181
≤4.0	1,126 (14.8)	1,705 (22.4)	795 (10.4)				
>4.0	1,166 (15.3)	1,777 (23.3)	1,051 (13.8)				
Lauren classification				16.818	<0.001	0.392	
Intestinal	1,034 (13.6)	1,465 (19.2)	716 (9.4)				
Diffuse	1,258 (16.5)	2,017 (26.5)	1,130 (14.8)				
Type of gastrectomy				500.060	<0.001	<0.001	6,291
TG	360 (4.7)	774 (10.2)	607 (8.0)				
DG	1,129 (14.8)	2,018 (26.5)	1,083 (14.2)				
PG	803 (10.5)	690 (9.1)	156 (2.0)				
pT stage				66.037	<0.001	<0.001	6,195
T1	276 (3.6)	455 (6.0)	214 (2.8)				
T2	383 (5.0)	654 (8.6)	372 (4.9)				
T3	313 (4.1)	560 (7.3)	375 (4.9)				
T4a	1,189 (15.6)	1,576 (20.7)	763 (10.0)				
T4b	131 (1.7)	237 (3.1)	122 (1.6)				
pN stage				745.581	<0.001	<0.001	6,902
N0	1,031 (13.5)	1,203 (15.8)	559 (7.3)				
N1	495 (6.5)	613 (8.0)	256 (3.4)				
N2	522 (6.9)	646 (8.5)	319 (4.2)				
N3a	244 (3.2)	762 (10.0)	332 (4.4)				
N3b	0 (0)	258 (3.4)	380 (5.0)				

ELN, examined lymph node; GC, gastric cancer; TG, total gastrectomy; DG, distal gastrectomy; PG, proximal gastrectomy; BIC, Bayesian information criterion. The eighth edition of TNM classification for GC was adopted for postoperatively pathological stages of all included patients.

Effects of other clinicopathological characteristics on ELN count for predicting prognosis of GC patients

Stratum analysis through Kaplan-Meier analysis was conducted to investigate the potential effects of other clinicopathological characteristics on the ELN count prognostic prediction for all GC patients. In *Table 3*, ELN≥16 was considered as the minimal and optimal prerequisite for the accurate prognostic evaluation of the following subgroups of patients: pN0 stage, age at surgery ≥60 years, tumor sizes of ≤4.0 cm, diffuse Lauren classification, and 0% ratio between metastatic lymph nodes and ELNs.

We also found that the 5-YSR of node-negative patients with ELNs≥16 was significantly higher than those with ELNs<16 (P<0.001), while the 5-YSR of node-negative patients with ELNs 16–30 was not significantly different from that of the node-negative patients with ELNs>30 (P=0.796; *Figure 1C*). On the other hand, the 5-YSR of node-positive patients (pN1–3a) with ELNs 16–30 was significantly higher than that of the patients with ELNs<16 (P<0.001), and the 5-YSR of node-positive patients (pN1–3a) with ELNs>30 was also significantly higher than that of the patients with ELNs 16–30 (P<0.05; *Figures 1D–F*). In addition, the 5-YSR of pN3b patients with ELNs 16–30 was significantly different from that of pN3b patients with ELNs>30 (10.8% *vs.* 18.7%, P=0.003; *Figure 1G*).

In *Table 3*, ELNs>30 was also identified as a potentially optimal prerequisite for the prognostic evaluation of patients with tumor sizes of >4.0 cm (*Figure 2A*), and as the minimal and optimal prerequisite for the prognostic evaluation of patients who underwent total gastrectomy, and pT4b stage patients (*Figure 2B, C*).

In order to make the above results more convincing, we have adopted the univariate COX proportional hazards analysis to evaluate the impact of the ELN count on GC patients' prognosis (*Table 4*). Ultimately, we found that the ELNs>30 had the significant impact on discriminating the prognosis of subgroups of patients in clinicopathological characteristics, such as tumor size (P=0.031), type of gastrectomy (P=0.015), and pN stage (P<0.001).

Stage migration analysis of ELN count

Univariate survival analysis revealed that the survival of patients with ELNs 16–30 was not significantly different from that of patients with ELNs>30 in the all 7,620 included patient cohort (P=0.262) (*Figure 2D*), although ELN count was identified as an independent predictor of patients' prognostic evaluation in this study. In *Tables 1* and *2*, several stage migrations were observed in the pN stage of all GC patients. Stage migrations were mainly detected in several subgroups of patients with specific pN stages: 1) pN0 with ELNs 16–30 ($pN0_{16-30}$) and pN0 with ELNs>30 ($pN0_{>30}$) (P=0.796); 2) pN0 with ELNs<16 ($pN0_{<16}$) and $pN1_{>30}$ (P=0.444); 3) $pN1_{<16}$ and $pN2_{16-30}$ (P=0.857); 4) $pN1_{16-30}$ and $pN2_{>30}$ (P=0.815); 5) $pN3a_{<16}$ and $pN3b_{16-30}$ (P=0.302); and 6) $pN3a_{<16}$ and $pN3b_{>30}$ (P=0.060) (*Figures 3A–F*).

In addition, stage migration of pN stage was also analyzed in different ELN count in all 7,620 GC patients by using the univariate survival analysis. We demonstrated that the stage migration of pN stage was only found in subgroup of patients with ELNs>30 as between pN1 and pN2 stage patients (*Figures 4A–C*, *Supplementary Table S3*).

Discussion

Nodal involvement, which is one of the strongest predictors of GC prognosis, is mainly the potential root of disease relapse among patients after surgery (15-19). Our previous study showed that patients with ELNs≥16 had a significantly median OS than those with ELNs<16 after curative surgery because of the underestimation of the N stage of patients with ELNs<16 (20). We also demonstrated that the insufficient ELN count may be a potential risk factor of the postoperative recurrence of GC patients even in node-negative patients (17). Consistent with the 6th edition of Union for International Cancer Control (UICC)/AJCC TNM classification for GC, the median survival of patients with perigastric lymph node metastasis, serosal involvement, and ratio of positive lymph nodes less than 25% or patients without adjuvant chemotherapy in the ELN>15 group was comparatively longer than that of patients with homologous clinicopathologic variables in the ELN<15 group (1). We further demonstrated that ELNs<16 are more significantly associated with high rates of local regional recurrence and peritoneal dissemination in node-negative GC patients than ELNs≥16 (21).

At present, the clinicopathological data of 7,620 GC patients from three medical centers in South and North China represented the basic disease information and general therapeutic level of GC, especially advanced disease, in China. Similar to other researchers, we found that the 5-YSR of GC patients with ELNs≥16 was significantly higher than that of patients with ELNs<16

Table 3 Effects of clinicopathological characteristics on ELN count for predicting prognosis of GC patients

Characteristics	ELN count	5-YSR (%)	ELN<16		ELN 16–30	
			χ^2	P	χ^2	P
Age at surgery (year)						
<60	<16	58.5	–	–	–	–
	16–30	60.0	0.611	0.434	–	–
	>30	59.1	0.045	0.832	0.175	0.676
≥60	<16	46.2	–	–	–	–
	16–30	49.7	4.178	0.041	–	–
	>30	54.5	10.214	0.001	3.007	0.083
	≥16	51.2	8.504	0.004	–	–
Tumor location						
Upper third	<16	45.7	–	–	–	–
	16–30	51.8	6.765	0.009	–	–
	>30	51.5	2.869	0.090	0.044	0.834
Middle third	<16	53.7	–	–	–	–
	16–30	49.7	0.504	0.478	–	–
	>30	54.8	0.152	0.679	1.575	0.209
Lower third	<16	61.9	–	–	–	–
	16–30	63.0	0.225	0.635	–	–
	>30	63.8	0.527	0.468	0.184	0.668
>2/3 stomach	<16	31.5	–	–	–	–
	16–30	33.8	1.264	0.261	–	–
	>30	30.9	0.594	0.441	0.044	0.835
Tumor size (cm)						
≤4.0	<16	65.0	–	–	–	–
	16–30	69.6	4.734	0.030	–	–
	>30	70.9	6.090	0.014	0.747	0.388
	≥16	70.0	7.063	0.008	–	–
>4.0	<16	39.0	–	–	–	–
	16–30	41.7	4.526	0.033	–	–
	>30	46.9	13.728	<0.001	4.515	0.034
Lauren classification						
Intestinal	<16	59.2	–	–	–	–
	16–30	62.4	1.117	0.291	–	–
	>30	62.2	0.955	0.328	0.052	0.820
Diffuse	<16	45.6	–	–	–	–
	16–30	50.3	10.764	0.001	–	–
	>30	54.0	17.336	<0.001	2.370	0.124
	≥16	51.6	17.444	<0.001	–	–
Type of gastrectomy						
TG	<16	32.4	–	–	–	–
	16–30	35.2	0.818	0.366	–	–
	>30	40.5	7.883	0.005	5.413	0.020

Table 3 (*continued*)

Table 3 (*continued*)

Characteristics	ELN count	5-YSR (%)	ELN<16		ELN 16–30	
			χ^2	P	χ^2	P
DG	<16	61.6	–	–	–	–
	16–30	62.8	1.121	0.290	–	–
	>30	66.4	5.928	0.015	3.128	0.077
PG	<16	46.5	–	–	–	–
	16–30	54.8	13.042	<0.001	–	–
	>30	53.4	3.661	0.056	0.027	0.868
pT stage						
pT1	<16	95.0	–	–	–	–
	16–30	95.9	0.053	0.818	–	–
	>30	96.3	0.015	0.903	0.131	0.717
pT2	<16	64.3	–	–	–	–
	16–30	67.4	2.635	0.105	–	–
	>30	72.0	5.093	0.024	0.920	0.338
pT3	<16	54.5	–	–	–	–
	16–30	54.9	0.007	0.935	–	–
	>30	50.8	2.122	0.945	2.571	0.109
pT4a	<16	40.6	–	–	–	–
	16–30	42.9	1.853	0.173	–	–
	>30	44.3	3.696	0.055	1.305	0.253
pT4b	<16	20.1	–	–	–	–
	16–30	26.7	0.967	0.325	–	–
	>30	37.1	8.337	0.004	5.350	0.021
pN stage						
pN0	<16	73.0	–	–	–	–
	16–30	84.0	43.089	<0.001	–	–
	>30	85.1	22.618	<0.001	0.067	0.796
	≥16	84.3	51.499	<0.001	–	–
pN1	<16	46.1	–	–	–	–
	16–30	63.0	32.420	<0.001	–	–
	>30	72.2	36.869	<0.001	4.271	0.039
pN2	<16	32.9	–	–	–	–
	16–30	46.5	24.280	<0.001	–	–
	>30	61.5	69.240	<0.001	22.399	<0.001
pN3a	<16	13.6	–	–	–	–
	16–30	27.2	27.983	<0.001	–	–
	>30	38.9	62.465	<0.001	16.391	<0.001
pN3b	16–30	10.8	–	–	–	–
	>30	18.7	–	–	8.957	0.003
Ratio between metastatic lymph nodes and ELN (%)						
0	<16	73.0	–	–	–	–

Table 3 (*continued*)

Table 3 (*continued*)

Characteristics	ELN count	5-YSR (%)	ELN<16		ELN 16–30	
			χ^2	P	χ^2	P
	16–30	84.0	43.089	<0.001	–	–
	>30	85.1	22.618	<0.001	0.067	0.796
	≥16	84.3	51.499	<0.001	–	–
0.1–10.0	<16	58.5	–	–	–	–
	16–30	65.7	1.222	0.269	–	–
	>30	69.6	3.047	0.081	1.184	0.276
10.1–40.0	<16	41.3	–	–	–	–
	16–30	44.0	1.640	0.200	–	–
	>30	44.6	2.770	0.096	0.539	0.463
>40.0	<16	21.6	–	–	–	–
	16–30	18.6	2.104	0.147	–	–
	>30	18.3	2.068	0.150	0.031	0.860

ELN, examined lymph node; GC, gastric cancer; TG, total gastrectomy; DG, distal gastrectomy; PG, proximal gastrectomy; 5-YSR, 5-year survival rate. The eighth edition of TNM classification for GC was adopted for postoperatively pathological stages of all included patients.

(P<0.001) (*Figure 1A*). There is still no consensus to mandate a minimal requirement of ELN count for the accurate staging and prognostic evaluation of GC, although ELNs≥16 has been proposed in the latest edition GC pTNM classification.

In this study, patients were divided into two groups based on cut-off ELN numbers between 5 and 40. For each cut-off value of ELNs, the survival rates of patients between these two groups were compared. When the cut-off values of ELNs was between 7 and 31, the prognosis of patients who had less than the cut-off values of ELNs was significantly worse than that of patients who had the cut-off values or more ELNs (*Supplementary Table S2*), suggesting that a retrieval of more than 30 ELNs might be recommended as a sufficient ELN count for lymph node staging. Note that this is different from the Japanese researchers' report that a majority of GC patients in China were advanced stage cases at the time of initial diagnosis (14), which may incur stage migration of lymph node metastasis in Chinese GC patients with insufficient ELN count in theory. *Table 2* shows that pN stage had the significant relationship with ELN count of GC patients, indicating that increasing the ELN count could enhance the detection ratio of positive nodes partly. In *Supplementary Figure S2C*, the general trend of the correlation between the metastatic lymph nodes and the ELN count implied that the number of positive lymph nodes was partly based on the ELN count. In *Table 3*, pN stage was remarkably influenced by the ELN count during the evaluation of the survival of GC patients.

ELNs>30 could be considered to enhance the discrimination of the survival difference among some subgroups of node-positive patients (pN1–3), although the survival of node-negative patients (pN0) with ELNs 16–30 was not significantly different from that of patients with ELNs>30 (P=0.796, *Table 3* and *Figure 1C*). Therefore, we analyzed that the curve of ELNs>30 should be considered as the essential threshold to guarantee the accuracy of pN^+ stage for inhibiting the stage migration of positive nodal involvement, which might be contributed to precisely prognostic evaluation of GC patients. *Table 3* and *Figures 1D–G* also show that ELNs>30 significantly contributed to the improvement of the survival discrimination in all node-positive GC patients. *Figure 4* shows the survival rate differences in accordance with pN stage among the various subgroups of patients with different ELN count. It seems to be deduced that the 5-YSR of all GC patients with ELNs<16 can be increased by 12.1% for pN0 cases, 26.1% for pN1 cases, 28.6% for pN2 cases, and 25.3% for pN3a cases when ELN count increases to more than 30 for all patients with ELNs<16. Other GC patient subgroups, including those with tumor size >4.0 cm, those who underwent total gastrectomy, and those in pT4b stage also contributed to enhancing the survival discrimination by ELNs>30 (*Figure 2*).

Stage migrations in lymph node metastasis were also identified among several subgroups of GC patients in this

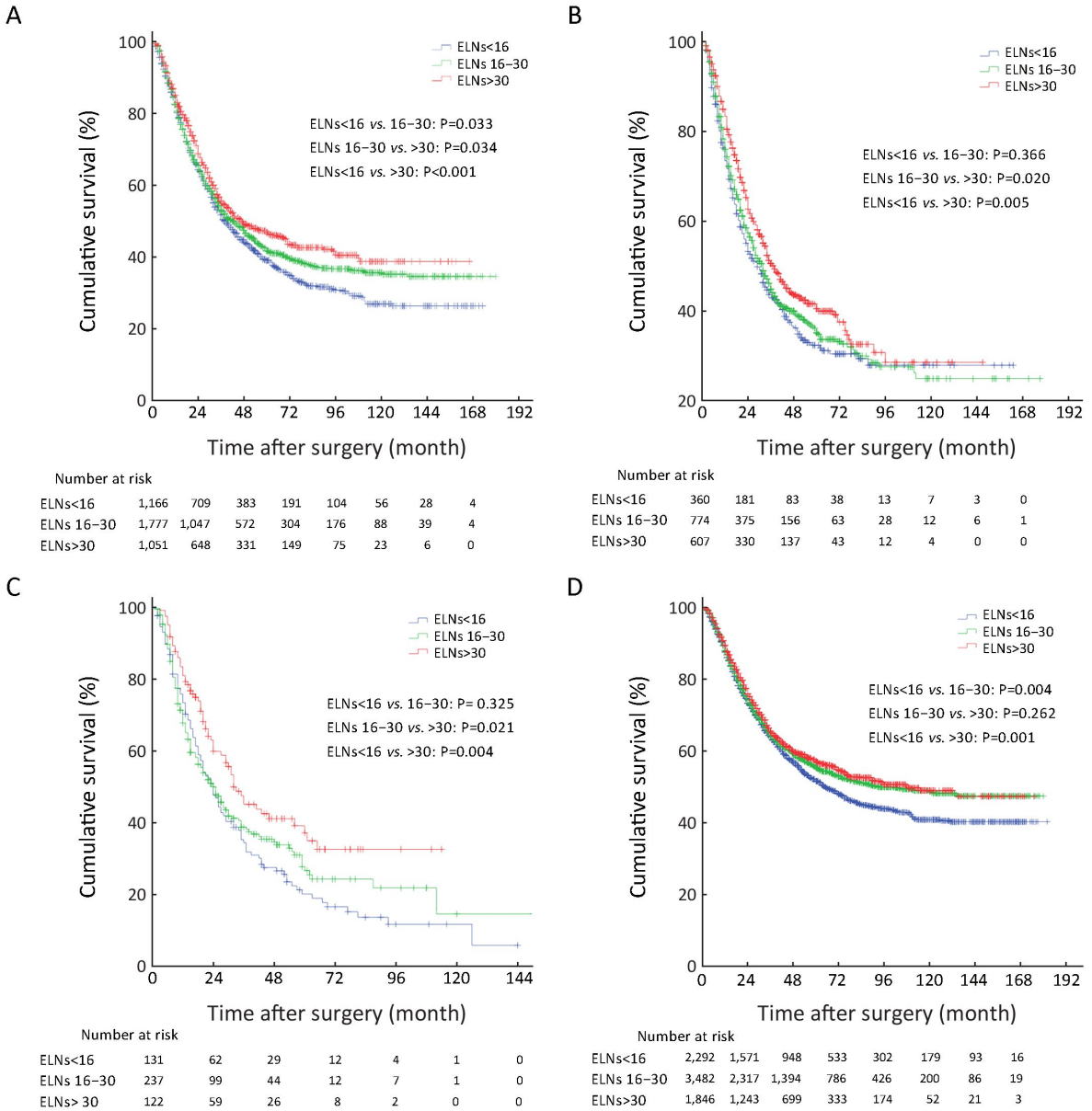

Figure 2 Superiorities of the examined lymph nodes (ELNs) >30 for prognostic evaluation in patients. (A) Superiorities of the ELNs>30 for prognostic evaluation in patients with tumor size of >4.0 cm; (B) Superiorities of the ELNs>30 for prognostic evaluation in patients underwent total gastrectomy; (C) Superiorities of the ELNs>30 for prognostic evaluation in pT4b stage patients; (D) Superiorities of the ELNs>30 for prognostic evaluation in all included 7,620 patients.

study. *Figure 3B* indicates that node-negative patients with ELNs<16 were potentially high-risk false node-negative cases in this patient cohort because of the smaller ELN count with a lower accuracy of pN staging. Similarly, $pN1_{<16}$ stage should be defined as ELNs>30 for the comparatively accurate prognostic discrimination of patients (*Figure 3C, D*). Patients with $pN1_{16-35}$ or $pN3a_{<16}$ demonstrated pN staging migration as the ELN count increased (*Figure 3E, F*). Therefore, an increase in ELN count could enhance the accuracy of prognostic evaluation by pN stage through the inhibition of stage migration for node-positive GC patients.

Of course, we have to admit that there are some limitations to this study. All patients are come from Chinese population in this study, which perhaps result in little bias of detection results comparing to the other race.

Table 4 ELN count predicting prognosis of GC patients (univariate Cox regression)

Characteristics	ELN<16		ELN 16–30		ELN>30	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age at surgery	Reference	Reference	1.130 (1.028–1.242)	0.012	1.037 (0.948–1.135)	0.432
Tumor location	Reference	Reference	1.118 (1.015–1.232)	0.024	1.033 (0.944–1.131)	0.480
Tumor size	Reference	Reference	1.243 (1.131–1.367)	<0.001	1.104 (1.009–1.267)	0.031
Lauren classification	Reference	Reference	1.210 (1.101–1.330)	<0.001	1.064 (0.973–1.164)	0.173
Type of gastrectomy	Reference	Reference	1.268 (1.150–1.398)	0.001	1.118 (1.022–1.225)	0.015
pT stage	Reference	Reference	1.139 (1.036–1.252)	0.007	1.052 (0.962–1.152)	0.269
pN stage	Reference	Reference	2.176 (1.960–2.415)	<0.001	1.354 (1.234–1.486)	<0.001
Ratio between metastatic and ELNs	Reference	Reference	1.151 (1.046–1.266)	0.004	1.031 (0.942–1.228)	0.510

ELN, examined lymph node; GC, gastric cancer; 5-YSR, 5-year survival rate; HR, hazard ratio; 95% CI, 95% confidence interval; The eighth edition of TNM classification for GC was adopted for postoperatively pathological stages of all included patients.

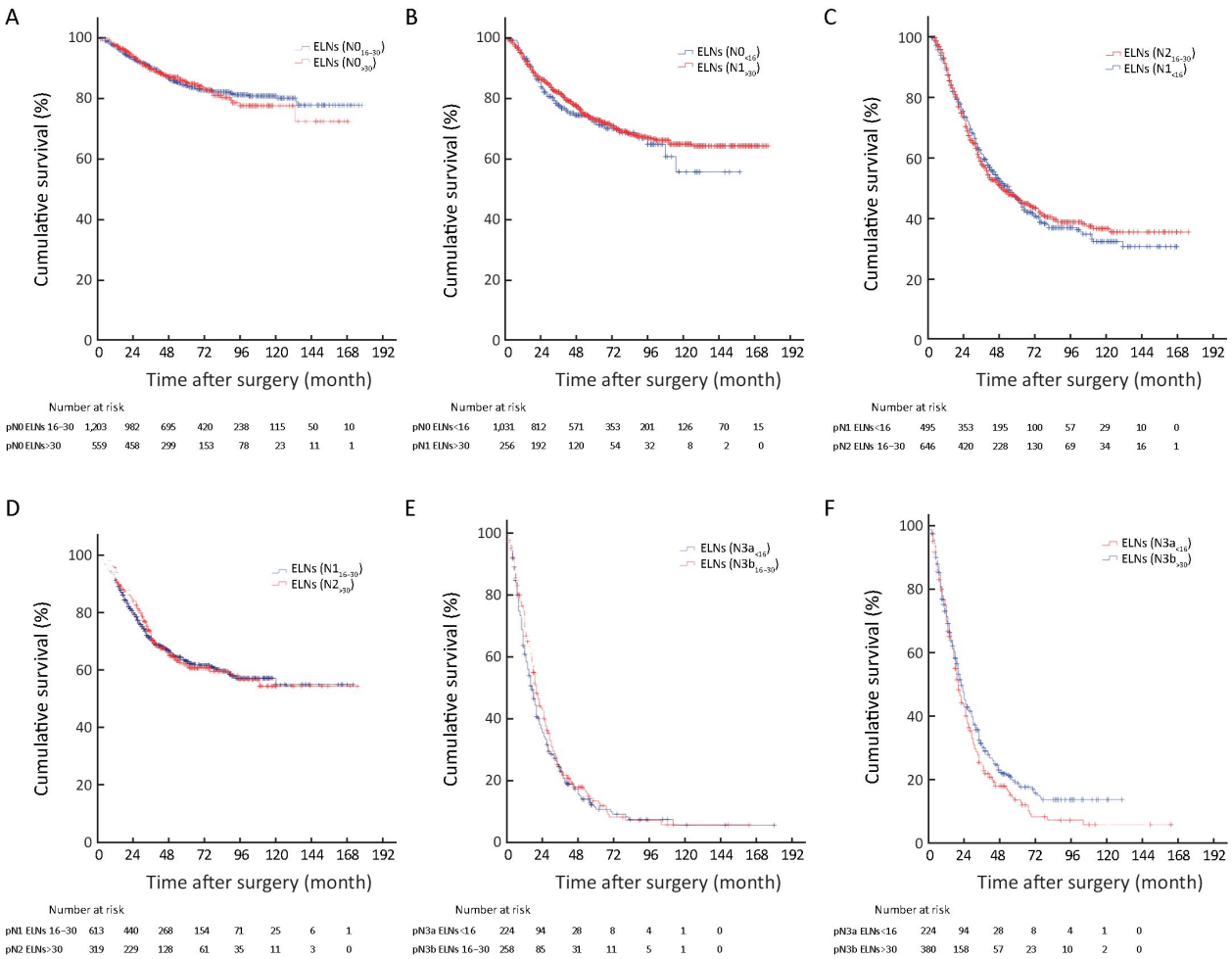

Figure 3 Stage migrations impact on the patients' survival in the various subgroups of patients between different pN stages. (A) Stage migrations impact on the patients' survival in the various subgroups of patients between $pN0_{16-30}$ and $pN0_{>30}$ (P=0.796); (B) Stage migrations impact on the patients' survival in the various subgroups of patients between $pN0_{<16}$ and $pN1_{>30}$ (P=0.444); (C) Stage migrations impact on the patients' survival in the various subgroups of patients between $pN1_{<16}$ and $pN2_{16-30}$ (P=0.857); (D) Stage migrations impact on the patients' survival in the various subgroups of patients between $pN1_{16-30}$ and $pN2_{>30}$ (P=0.815); (E) Stage migrations impact on the patients' survival in the various subgroups of patients between $pN3a_{<16}$ and $pN3b_{16-30}$ (P=0.302); (F) Stage migrations impact on the patients' survival in the various subgroups of patients between $pN3a_{<16}$ and $pN3b_{>30}$ (P=0.060).

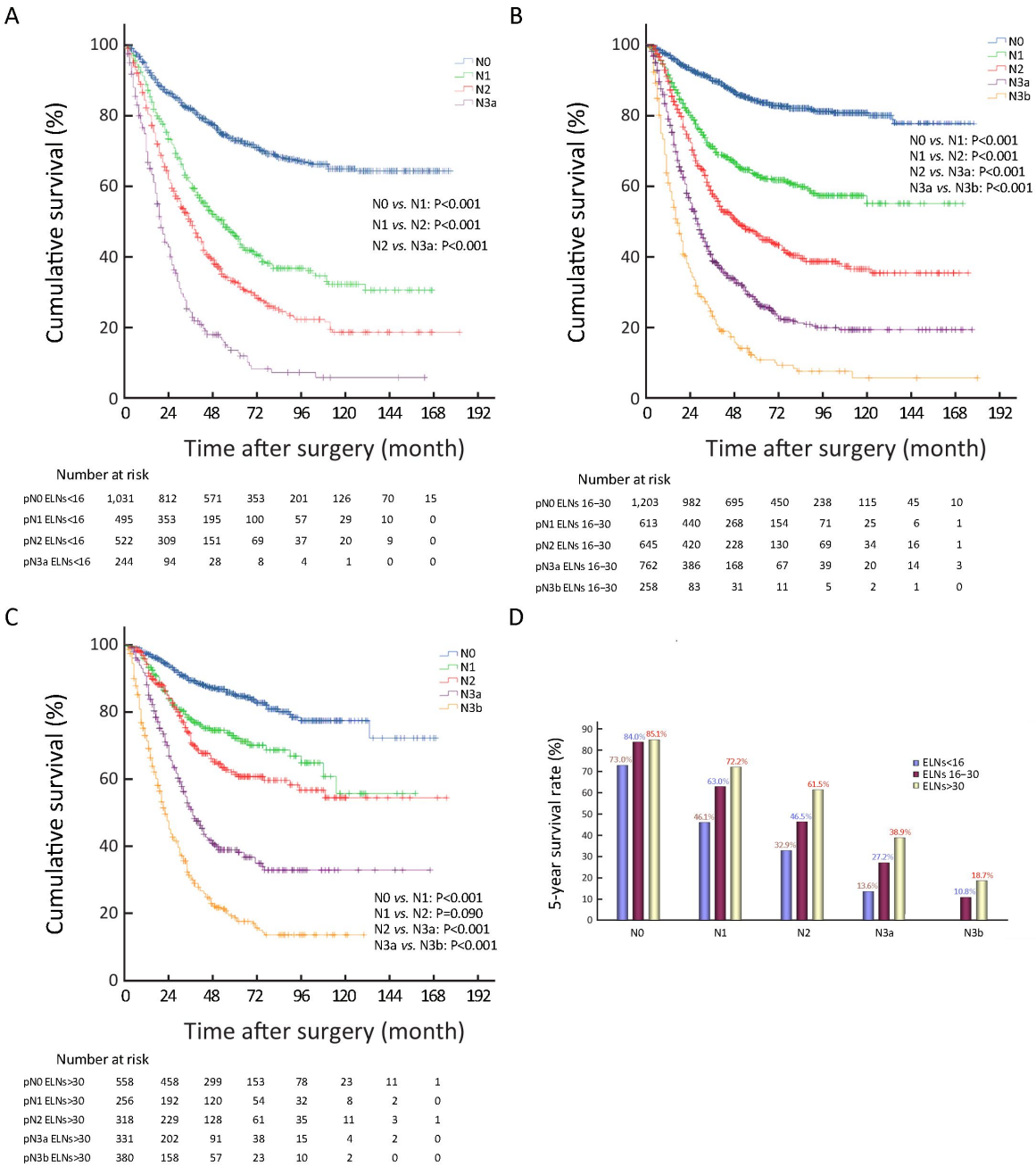

Figure 4 Stage migrations in different subgroups of patients with various examined lymph node (ELN) count survival curve of patients with different ELNs according to pN stage. (A) Stage migrations in different subgroups of patients with various ELN count survival curve of patients with ELNs<16 according to pN stage; (B) Stage migrations in different subgroups of patients with various ELN count survival curve of patients with ELNs 16–30 according to pN stage; (C) Stage migrations in different subgroups of patients with various ELN count survival curve of patients with ELNs>30 according to pN stage; (D) Stage migrations in the different subgroups of patients with various ELN count histograms of patients' 5-year survival rate according to pN stage in various ELN counts.

In viewing of about 42% of worldwide GC patients occurring in China, the large scale patient-based cases are capable of possessing certain representative significance.

Conclusions

Our analysis suggests that ELN count should be

determined to accurately assess the status of lymph node metastasis among GC patients. For node-positive GC patients, ELN>30 should be recommended to avoid stage migration after surgery.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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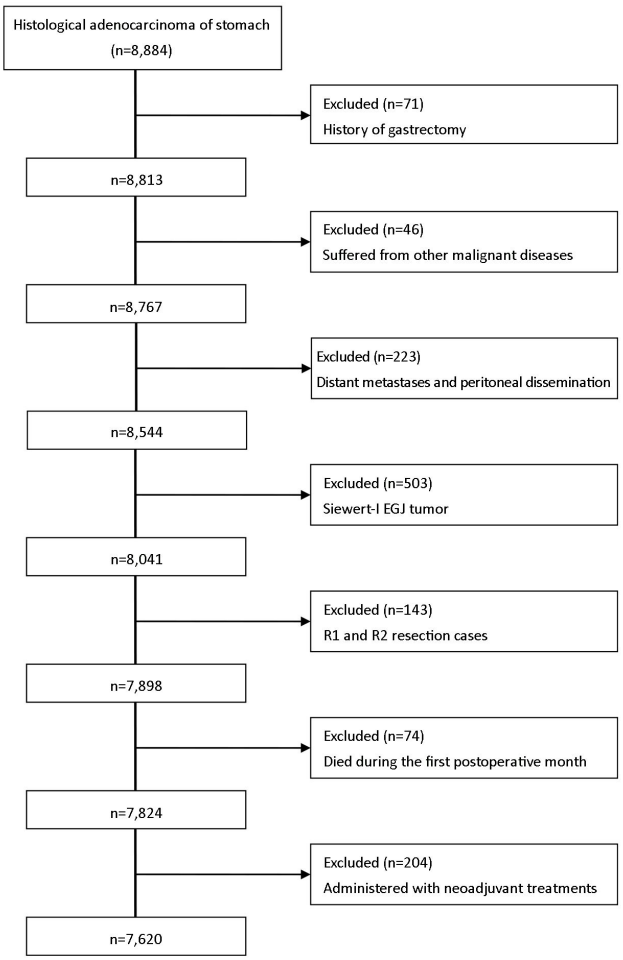

Figure S1 Scheme of included patients after curative gastrectomy.

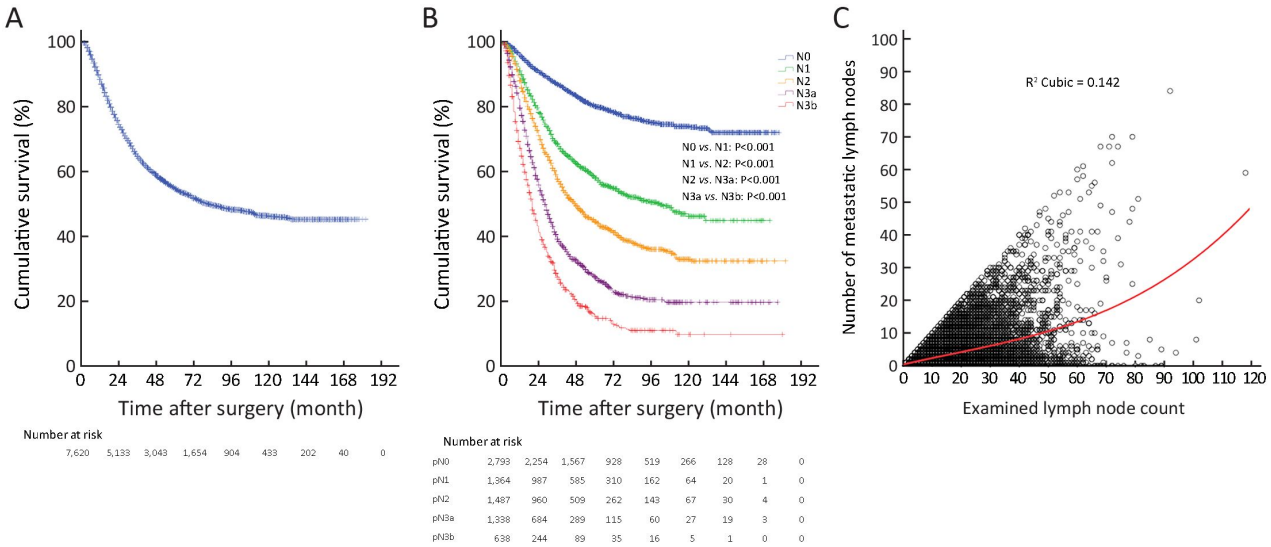

Figure S2 Survival curve and correlation between the number of metastatic lymph nodes and the ELN count. (A) Survival curve of patients included in this study; (B) Survival curve of patients according to the N stage; (C) Curve correlation between the number of metastatic lymph nodes and examined lymph node (ELN) count.

Table S1 Correlation between clinicopathological characteristics and pN stage of GC patients (N=7,620)

Characteristics	pN stage (n)					χ^2	P
	N0	N1	N2	N3a	N3b		
Gender						15.823	0.003
Male	1,985	986	1,065	932	410		
Female	808	378	422	406	228		
Age at surgery (year)						26.076	<0.001
<60	1,522	656	732	715	361		
≥60	1,271	708	755	623	277		
Tumor location						210.795	<0.001
Upper third	741	410	444	311	117		
Middle third	507	224	260	314	169		
Lower third	1,389	642	636	584	236		
>2/3 stomach	156	88	147	129	116		
Tumor size (cm)						636.873	<0.001
≤4.0	1,812	636	592	431	155		
>4.0	981	728	895	907	483		
Lauren classification						152.489	<0.001
Intestinal	1,363	620	610	438	184		
Diffuse	1,430	744	877	900	454		
Type of gastrectomy						363.556	<0.001
TG	435	250	355	407	294		
DG	1,739	767	768	692	264		
PG	619	347	364	239	80		
pT stage						1,334.542	<0.001
T1	792	84	51	18	0		
T2	637	267	259	177	69		
T3	365	256	286	225	116		
T4a	915	674	785	780	374		
T4b	84	83	106	138	79		
ELN count						745.581	<0.001
<16	1,031	495	522	244	0		
16–30	1,203	613	646	762	258		
>30	559	256	319	332	380		

GC, gastric cancer; TG, total gastrectomy; DG, distal gastrectomy; PG, proximal gastrectomy; ELN, examined lymph node. The eighth edition of TNM classification for GC was adopted for postoperatively pathological stages of all included patients.

Table S2 Prognostic effects in 7,620 GC patients depending on different cut-off ELN counts

Cut-off ELN count	Number of patients with ≤ cut-off ELN count	χ^2	P
5	337	0.284	0.594
6	499	2.742	0.098
7	632	4.364	0.037
8	799	6.166	0.013
9	990	7.439	0.006
10	1,166	7.202	0.007
11	1,361	9.352	0.002
12	1,575	11.621	0.001
13	1,797	12.401	<0.001
14	1,991	8.568	0.001
15	2,292	11.879	0.001
16	2,624	12.522	<0.001
17	2,892	7.803	0.005
18	3,176	6.436	0.011
19	3,421	12.681	<0.001
20	3,681	16.524	<0.001
21	3,978	19.309	<0.001
22	4,205	18.388	<0.001
23	4,420	17.481	<0.001
24	4,645	13.527	<0.001
25	4,852	9.702	0.002
26	5,041	11.965	0.001
27	5,255	11.050	0.001
28	5,456	11.112	0.001
29	5,623	7.944	0.005
30	5,774	5.949	0.015
31*	5,903	5.191	0.023
32	6,055	3.681	0.055
33	6,182	3.353	0.067
34	6,303	1.716	0.190
35	6,424	1.998	0.157
36	6,526	2.601	0.107
37	6,616	1.018	0.313
38	6,699	1.446	0.229
39	6,780	1.026	0.311
40	6,852	0.798	0.372
41	……	……	……

P values were calculated by the log-rank test for survival curves that were generated by the Kaplan-Meier method. *, the most appropriate cut-off value of the ELN count was 31 ($P<0.05$).

Table S3 Stage migration of pN stage in different ELN count in all 7,620 GC patients by using univariate survival analysis

ELN count	n	pN0		pN1		pN2		pN3a		pN3b	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
<16											
pN0	1,031										
pN1	495	121.562	<0.001								
pN2	522	273.329	<0.001	20.801	<0.001						
pN3a	244	473.813	<0.001	117.991	<0.001	49.616	<0.001	–			
pN3b	–	–		–		–		–		–	–
16–30											
pN0	1,203										
pN1	613	104.777	<0.001								
pN2	646	314.039	<0.001	32.409	<0.001						
pN3a	762	720.338	<0.001	166.551	<0.001	58.686	<0.001				
pN3b	258	930.922	<0.001	283.744	<0.001	163.020	<0.001	45.069	<0.001	–	–
>30											
pN0	559										
pN1	256	19.348	<0.001								
pN2	319	46.828	<0.001	2.879	0.090						
pN3a	332	201.424	<0.001	51.863	<0.001	36.004	<0.001				
pN3b	380	462.036	<0.001	159.319	<0.001	147.778	<0.001	42.406	<0.001	–	–

ELN, examined lymph nodes; GC, gastric cancer. The eighth edition of TNM classification for GC was adopted for postoperatively pathological stages of all included patients.