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Original Article

At least 16 lymph nodes are recommended to examine during pancreaticoduodenectomy in ampullary adenocarcinoma

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Abstract: The minimum number of lymph nodes to be examined during pancreaticoduodenectomy (PD) for patients with ampullary adenocarcinoma (AC) is still debatable due to limited clinical data. Therefore, here we explored the relationship between the number of examined lymph node (ELN) and the current N staging (American Joint Committee on Cancer staging system, AJCC, 8 edition) after PD for AC as well as determined the minimum number of examined lymph nodes (MNELN) to ensure the accurate detection of nodal involvement. Patients underwent PD for AC in the National Cancer Center cohort of China (NCC cohort of China) from 1998 to 2020 and in the Surveillance, Epidemiology, and End Results database (SEER database) from 2010 to 2018 were retrospectively reviewed, and a total of 452 eligible patients were included in this study. The MNELN was evaluated by binomial probability law and best survival separation methods. Furthermore, the cut-off value of MNELN was validated in the NCC cohort of China using Least Absolute Shrinkage and Selection Operator (LASSO) regression. Our analysis indicated that the median number of ELN was 14, and the number of ELN was positively correlated with N stage. The MNELN was 16, whereas the best survival separation of ELN was 38 in node-positive patients and 3 in node-negative patients. In the validation cohort, the number of 16 ELNs was identified as a predictive variable for lymph node metastasis with nonzero coefficients in the LASSO-logistic regression model. Together, we concluded that a greater number of ELN was associated with more accurate nodal status assessment in PD for AC patients. A minimum of 16 lymph nodes were required to during PD in AC patients.

Keywords: Examined lymph node, stage migration, ampullary adenocarcinoma, pancreaticoduodenectomy

Introduction

Ampullary adenocarcinoma (AC) is a rare malignant neoplasm accounting for 7% of pancreatic head and periampullary cancers [1, 2]. Pancreaticoduodenectomy (PD) is one of the standard treatment procedures for the resection of AC disease. However, the prognosis for resected AC is relatively poor with a previously reported 5-year survival rate of 45% [3, 4]. Several studies have demonstrated that AC patients with positive lymph nodes (LNs) have higher risk of recurrence and worse survival compared to LN-negative patients [5, 6].

The number of examined lymph nodes (ELNs) is significantly associated with nodal staging and long-term survival for pancreatic cancer patients who underwent PD [7]. Adequate number of ELNs is critical to ensure the accurate nodal assessment. Until now, the minimum number of ELNs (MNELNs) required during PD in AC patients is still undetermined due to limited clinical data. Currently, the National Comprehensive Cancer Network (NCCN) guidelines recommend MNELNs as 17 based on a retrospective cohort from National Cancer Database (NCDB, USA) [8]. Similarly, an retrospective study in Italy recommends that at least 12

LNs should be harvested during PD for AC patients [9]. However, the bulk of information is limited either by the influence of ethnicity or by the small sample size. Moreover, the methods for identifying the cut-off of MNELN are not sufficiently robust due to the stage migration.

Here, we explored the relationship between the number of ELN and the current N staging (American Joint Committee on Cancer staging system, AJCC, 8 edition) after PD for AC patients. In addition, we determined the MNELN sufficient to accurately detect the nodal involvement. Furthermore, the predictive value of MNELN for LN metastasis and long-term survival was validated in the NCC cohort of China.

Methods

Sources and study subjects

NCC cohort of China: The clinicopathological information of 324 patients who underwent surgery for ampullary carcinoma in the National Cancer Center of China, Cancer Hospital, from January 1998 to December 2020 were retrieved and evaluated for eligibility to this study. The patient inclusion criteria were: (1) Pathologically confirmed ampullary adenocarcinoma; (2) At least 1 LN was examined; (3) Patients who underwent curative PD. The patient exclusion criteria were: (1) Patients with positive surgical margins; (2) Patients with distant metastasis; (3) Patients died during the perioperative period; (4) Patients with histological types other than adenocarcinoma, such as squamous cell carcinoma and neuroendocrine carcinoma; (5) Patients with incomplete or unavailable clinical information; (6) Patients with multiple primary cancers. Due to the retrospective nature of this study, the informed consent was waived by the ethics committee. At the end, 246 patients were enrolled into the study.

SEER database: The clinical information of AC patients deposited in the Surveillance, Epidemiology, and End Results database (SEER database) from 2010 to 2018 were retrospectively reviewed. Institutional review board approval was not required as the SEER database is openly accessible. The sample inclusion criteria were: (1) Patients who were microscopically confirmed as ampullary adenocarcinoma; (2) Patients who underwent PD. The sample exclusion criteria were: (1) Patients with distant metastasis; (2) Patients with incomplete or unavailable clinical information, such as race, degree of tumor differentiation, tumor size, and follow-up information; (3) The number of ELN was 0 or not recorded; (4) The number of positive lymph nodes (PLN) was not recorded; (5) Patients with multiple primary cancers. Overall, 206 patients were eligible and were included in this study.

Participant follow-up

The postoperative follow-up was performed by telephone review, clinic visit, and death registration system. The median overall survival (OS) time for the patients of the NCC cohort of China and the SEER database was 42 months and 43 months, respectively.

Statistical analysis

Categorical variables were represented by frequency as well as percentage and were compared by Chi-square test or Fisher exact test. Continuous variables were expressed as medians with inter-quartile range (IQR) or as means with standard deviation (SD). Mann-Whitney U test or t test was used to compare the difference between groups and continuous variables. All statistical tests were two-sided at the significant level of 0.05.

The threshold number of ELNs required to detect at least one positive LN was calculated by binomial probability law, which was previously described [10]. Briefly, the formula used for calculation is $P=(1-p)^n$, where n is the number of ELNs, while P is the positive lymph node ratio (LNR) for node-positive patients. In this study, the threshold number of ELNs was defined as the required number of LNs to be dissected with at least 95% likely to detect one or more positive LNs (P=5%). According to the threshold, we grouped the number of ELNs and plotted the survival curve.

To determine the optimal cut-off number of ELNs for survival, we established the best survival separation model using the “Survival” and “Survminer” packages in R software (version 4.0.3). The corresponding *P*-value and hazard ratio (HR) were calculated with each number of ELNs as the cut-off value, and the smallest

P-value indicated the best survival separation cut-off number of ELNs.

Lastly, we determined the MNELN according to the threshold number of ELNs and the best survival separation cut-off number of ELNs. The least absolute shrinkage and selection operator (LASSO) regression analysis was conducted to identify the predictive value of MNELN for LN metastasis and long-term survival via the "glmnet" package in the NCC cohort of China.

In this study, the results were described as hazard ratio (HR), 95% confidence interval (CI), and *P*-value. A *P*-value less than 0.05 was considered statistically significant. All statistical analyses were performed using R software (version 4.0.3).

Results

Clinicopathologic characteristics of the study patients

The flowchart of the patient selection and the study design was presented in Figure S1. A total of 452 AC patients who underwent PD were included in this study. The baseline characteristics of patients in the SEER database and in the NCC cohort of China were summarized in **Table 1**. Overall, 215 AC patients (47.6%) had LNs metastasis (N1: 156 patients, 34.5%; N2: 59 patients, 13.1%). The median number of ELN was 14. The number of ELN was significantly different between the SEER database and the NCC cohort of China (15 vs 12; P=0.005) (**Figure 1**). For patients with positive LN, the median LNR was 0.150 (IQR: 0.077~0.273) as 80 patients (17.7%) had a LNR between 0 and 0.1, while 58 patients (12.8%) had a LNR between 0.1 and 0.2. The other 77 patients (17.0%) had a LNR greater than 0.2.

Number of ELNs and stage migration

According to the number of ELNs, AC patients were divided into four groups: 0<ELN≤10, 10< ELN≤20, 20<ELN≤30, and ELN>30. We found that as the number of ELN increased, the proportion of patients with N1 and N2 stage was also significantly increased. Specifically, the percentage of N1 and N2 stage patients was 24.0% and 4.0%, respectively, when the number of ELNs was between 1 and 10; however, it was 39.7% and 16.2%, respectively, when the number of ELNs was between 11 and 20. Furthermore, when the number of ELNs was between 21 and 30, it was 37.8% and 21.6%, respectively, and it was 45.8% and 16.7%, respectively, for ELN>30 (P<0.01). Moreover, increasing number of ELNs was associated with increasing proportion of LNR≤0.1 and decreasing proportion of LNR>0.2 (P<0.01) (**Figure 2**). Hence, our data indicated that more LN dissection is significant for the accurate assessment of lymph node status and for a lower probability of stage migration.

The MNELN for nodal status assessment

For the LN-positive AC patients who underwent PD, the overall LNR was 0.172. According to the binomial probability law, the threshold number of ELNs to be dissected with at least 95% likelihood to detect one or more positive LNs was 16. Therefore, we defined the MNELN for nodal status assessment was 16.

Number of ELNs and survival

The median OS for the entire patients included in this study was 36 months, with 3- and 5-year cumulate survival rate of 59.5% and 48.5%, respectively. There was no significant difference in survival between patients with less LN resection (<16) and more LN resection (≥16) in both the SEER database and the NCC cohort of China (**Figure 3**).

Furthermore, according to the best survival separation model, we calculated the optimal cut-off number of ELNs with the most prominent survival difference. We found that the difference in survival was most pronounced with a cut-off number of 3 LN dissected for LN-negative AC patients (*P*=0.002) and 38 LN dissected for the LN-positive AC patients (*P*=0.026) (**Figure 4**).

Validation of MNELN for predicting LNs metastasis and OS

From the analysis above, we determined the MNELN for AC patients with PD was 16. Since important clinical information was missing in the SEER database, we further validated the clinical value of MNELN in the NCC cohort of China. The ELN was divided into two groups according to the MNELN: ELN<16 and ELN≥

Table 1. Comparison in the baseline characteristics of ampullary adenocarcinoma patients from SEER database and National Cancer Center cohort of China

Variables	Total (n=452)	SEER Database (n=206)	NCC Cohort (China) (n=246)	*P*-value	Methods
Sex, n (%)				0.75	Chi-square test
Male	256 (56.637)	115 (55.825)	141 (57.317)		
Female	196 (43.363)	91 (44.175)	105 (42.683)		
Race, n (%)				nan	Chi-square test
White	159 (35.177)	159 (77.184)	0 (0.000)		
Black	21 (4.646)	21 (10.194)	0 (0.000)		
Other	272 (60.177)	26 (12.621)	246 (100.000)		
Age, median [IQR]	61.000 [53.000, 69.000]	65.000 [57.000, 75.000]	58.000 [50.000, 65.000]	<0.001	Mannwhitney-U
Differentiation, n (%)				0.005	Chi-square test
Poor	173 (38.274)	73 (35.437)	100 (40.650)		
Moderate	212 (46.903)	112 (54.369)	100 (40.650)		
Well	67 (14.823)	21 (10.194)	46 (18.699)		
Tumor size, mean (SD)	2.376 (1.112)	2.349 (1.217)	2.398 (1.018)	0.644	t-test
ELN, median [IQR]	14.000 [9.000, 19.000]	15.000 [11.000, 20.000]	12.000 [8.000, 19.000]	0.005	Mannwhitney-U
ELN group, n (%)				<0.001	Chi-square test
0<ELN≤10	137 (30.310)	38 (18.447)	99 (40.244)		
10<ELN≤20	209 (46.239)	115 (55.825)	94 (38.211)		
20<ELN≤30	81 (17.920)	39 (18.932)	42 (17.073)		
ELN>30	25 (5.531)	14 (6.796)	11 (4.472)		
PLN, median [IQR]	0.000 [0.000, 2.000]	1.000 [0.000, 3.000]	0.000 [0.000, 1.000]	<0.001	Mannwhitney-U
N Stage (AJCC, 8 edition), n (%)				<0.001	Chi-square test
N0	237 (52.434)	72 (34.951)	165 (67.073)		
N1	156 (34.513)	91 (44.175)	65 (26.423)		
N2	59 (13.053)	43 (20.874)	16 (6.504)		
LNR, median [IQR]	0.000 [0.000, 0.143]	0.079 [0.000, 0.211]	0.000 [0.000, 0.065]	<0.001	Mannwhitney-U
LNR group, n (%)				<0.001	Chi-square test
LNR=0	237 (52.434)	72 (34.951)	165 (67.073)		
0<LNR≤0.1	80 (17.699)	46 (22.330)	34 (13.821)		
0.1<LNR≤0.2	58 (12.832)	36 (17.476)	22 (8.943)		
LNR>0.2	77 (17.035)	52 (25.243)	25 (10.163)		
Survival months, mean (SD)	42.223 (33.142)	42.961 (27.310)	41.595 (37.385)	0.665	t-test

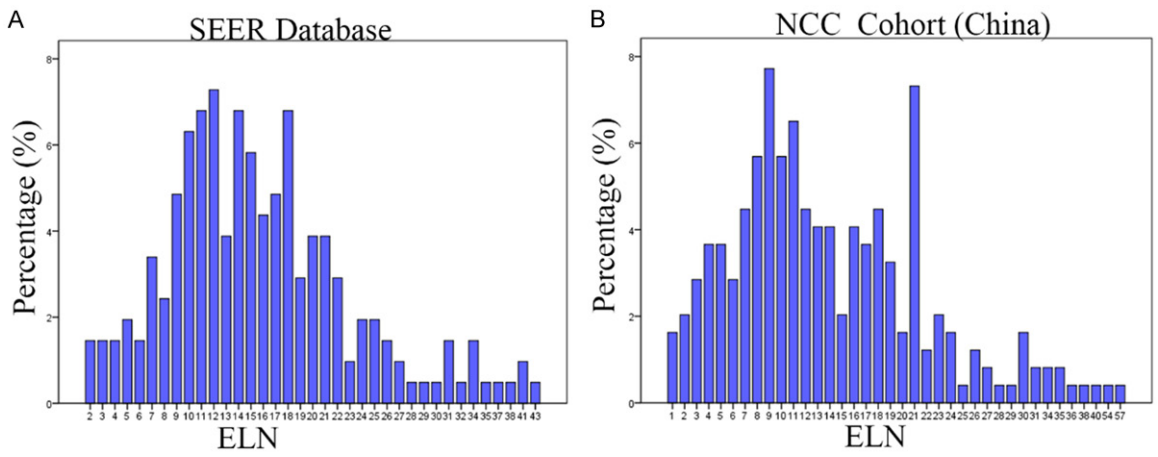

Figure 1. The distribution of the number of ELN in SEER database and the NCC cohort of China.

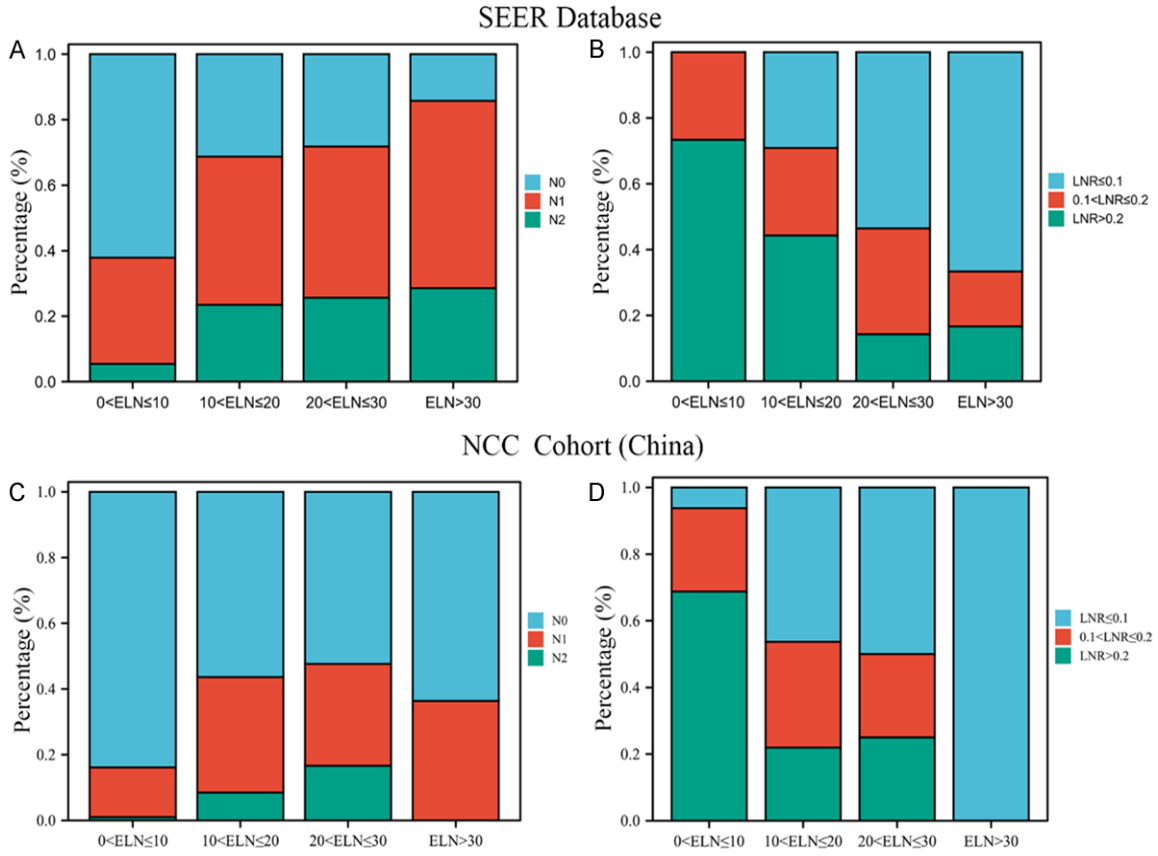

Figure 2. The distribution of nodal status and LNR with ELNs in the SEER database and the NCC cohort of China. The relationship between LNR and ELN was assessed only in patients with positive nodes (n=215).

16. The clinicopathologic characteristics of LN-positive and LN-negative AC patients who underwent PD were compared and illustrated in **Table 2**. The median number of ELN was 12, while the number of ELN was significantly lower in the LN-negative patients than in the LN-positive patients (10 vs 17; $P<0.001$).

In the NCC cohort of China, three potential predictors for LN metastasis: T stage, differentia-

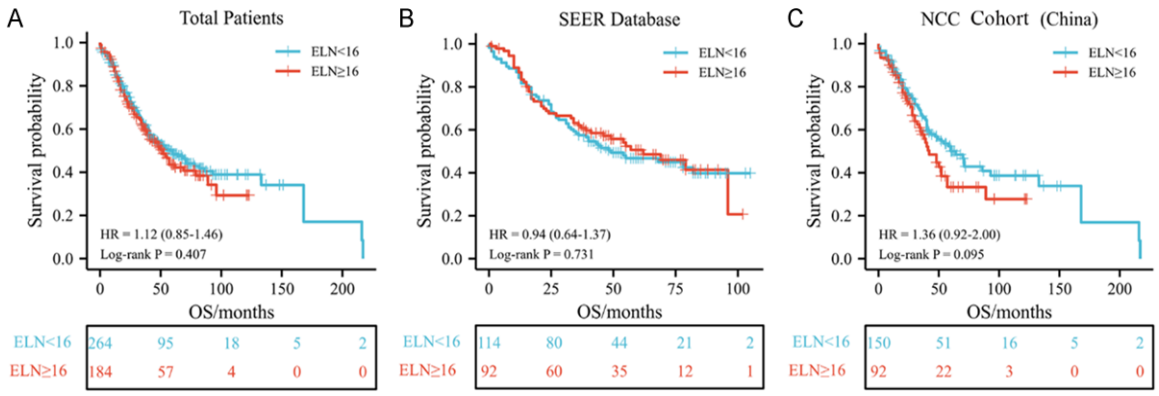

Figure 3. The overall survival curves according to the level of lymph node dissection performed.

tion, and ELN group, were identified with non-zero coefficients by the LASSO-logistic regression model (**Figure 5A** and **5B**; Table S1). However, in the LASSO-cox regression model, the N stage instead of the ELN group was identified as a prognostic factor for OS in the NCC cohort of China (**Figures 5C** and **5D**; Table S2).

Discussion

Currently, there is no definitive guideline regarding MNELN for the accurate lymph node staging of ampullary adenocarcinoma. Previous studies have reported MNELN of AC patients; however, the conclusions were mainly drawn from the perspective of survival difference, and thus the results are quite different [8, 9, 11-14]. In this study, the stage migration analysis demonstrated that an increasing number of ELNs was significantly correlated with a higher proportion of more advanced N stage in AC patients. By analyzing the stage migration and survival differences simultaneously, we determined 16 as the minimal number of lymph nodes to be dissected during PD in AC patients.

Lymph node assessment is crucial for postoperative adjuvant therapy and the prognosis of ampullary carcinoma. Examining sufficient number of ELNs can avoid the stage migration, thus ensuring the accurate lymph node assessment. Previous studies have indicated that when the number of ELNs is suboptimal, stage migration is prone to occur and may affect the long-term survival in various cancers, including distal bile duct cancer [15], pancreas ductal adenocarcinoma [16, 17], esophageal squamous cell carcinoma [18], and non-small-cell lung cancer [19]. Nevertheless, our study is the first to evaluate the association between the number of ELNs and stage migration. We demonstrated that the effects of the number of ELNs on nodal assessment could be reflected from multiple perspectives. First, the number of ELNs was significantly higher in node-positive AC patients than in node-negative patients, both in the SEER database and the NCC cohort of China. Second, the proportions of N1 and N2 stage were significantly increased with increasing number of ELNs. Third, for the node-positive AC patients, increasing number of ELNs was significantly associated with increasing proportion of lower LNR value and decreasing proportion of higher LNR value. Collectively, our data indicated the importance of assessing enough number of ELNs to accurately evaluate the nodal status of AC patients.

Furthermore, our study determined the MNELN as 16 during PD for AC patients based on the nodal evaluation and survival analysis. Although this result appears to be inconsistent with several published studies, in which different number of ELNs, ranging from 12-17, was suggested to be adequate [8, 9, 11-14], both a retrospective cohort study conducted in Italy [14] and a SEER based study [12] also recommended that at least 16 LNs should be removed during PD for AC patients to improve the long-term survival. Multiple reasons may account for the discrepancy. First, although the binomial probabilistic models have been confirmed to be robust to evaluate the MNELN for patients underwent PD [10], previous studies used survival difference to determine the MNELN through Cox regression models, which had lower sensi-

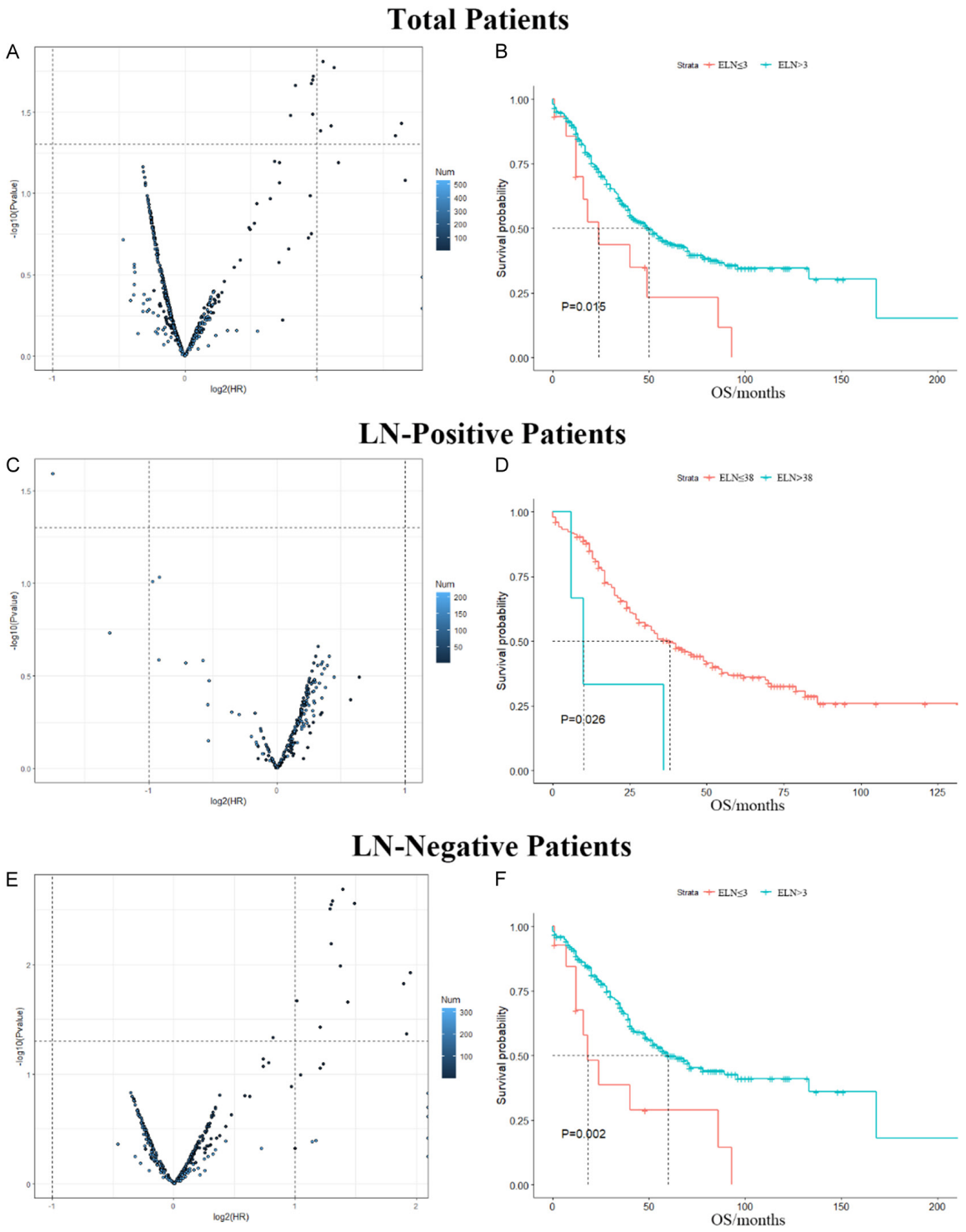

Figure 4. The optimal cut-off number of ELNs according to the best separation model. A. Volcano plot to determine the cut-off of ELNs for total patients; B. Survival curves according to the cut-off of ELNs for total patients; C. Volcano plot to determine the cut-off of ELNs for LN-positive patients; D. Survival curves according to the cut-off of ELNs for LN-positive patients; E. Volcano plot to determine the cut-off of ELNs for LN-negative patients; F. Survival curves according to the cut-off of ELNs for LN-negative patients.

tivity. Second, the technique for pathological examination of LNs did differ among different individuals and centers. A study conducted in Methodist Dallas Medical Center reported that

Table 2. Clinicopathological characteristics of lymph nodes negative patients and lymph nodes positive patients in National Cancer Center cohort of China

Variables	Total (n=246)	LN-Negative (n=165)	LN-Positive (n=81)	*P*-value	Methods
Sex, n (%)				0.225	Chi-square test
Male	141 (57.317)	99 (60.000)	42 (51.852)		
Female	105 (42.683)	66 (40.000)	39 (48.148)		
Age, median [IQR]	58.000 [50.000, 65.000]	57.000 [49.000, 64.000]	59.000 [53.000, 65.000]	0.105	Mannwhitney-U
Preoperative jaundice, n (%)				0.025	Chi-square test
No	68 (27.642)	53 (32.121)	15 (18.519)		
Yes	178 (72.358)	112 (67.879)	66 (81.481)		
Tumor size, median [IQR]	2.200 [1.500, 3.000]	2.000 [1.500, 3.000]	2.500 [1.800, 3.000]	0.034	Mannwhitney-U
Differentiation, n (%)				<0.001	Chi-square test
Poor	100 (40.650)	54 (32.727)	46 (56.790)		
Moderate	100 (40.650)	69 (41.818)	31 (38.272)		
Well	46 (18.699)	42 (25.455)	4 (4.938)		
Preoperative platelet count (10^9/L), median [IQR]	249.000 [205.000, 313.000]	253.000 [202.000, 324.000]	247.000 [213.000, 292.000]	0.593	Mannwhitney-U
Preoperative lymphocyte count (10^9/L), median [IQR]	1.520 [1.170, 2.000]	1.550 [1.210, 2.000]	1.450 [1.140, 1.930]	0.157	Mannwhitney-U
Preoperative neutrophil count (10^9/L), median [IQR]	4.060 [3.220, 5.760]	4.050 [3.220, 5.770]	4.090 [3.320, 5.710]	0.718	Mannwhitney-U
Preoperative CEA (ng/mL), median [IQR]	2.600 [1.820, 3.780]	2.590 [1.750, 3.670]	2.750 [1.890, 4.480]	0.356	Mannwhitney-U
Preoperative CA199 (U/mL), median [IQR]	68.360 [20.320, 198.000]	55.800 [17.160, 169.600]	78.680 [28.580, 292.700]	0.046	Mannwhitney-U
ELN, median [IQR]	12.000 [8.000, 19.000]	10.000 [7.000, 16.000]	17.000 [11.000, 21.000]	<0.001	Mannwhitney-U
Operation time, n (%)				0.673	Chi-square test
≤3 h	3 (1.220)	2 (1.212)	1 (1.235)		
>3 h and ≤6 h	173 (70.325)	119 (72.121)	54 (66.667)		
>6 h	70 (28.455)	44 (26.667)	26 (32.099)		
Blood transfusion, n (%)				0.558	Chi-square test
No	121 (49.187)	79 (47.879)	42 (51.852)		
Yes	125 (50.813)	86 (52.121)	39 (48.148)		
T stage (AJCC, 8 edition), n (%)				<0.001	Chi-square test
T1	32 (13.008)	31 (18.788)	1 (1.235)		
T2	80 (32.520)	62 (37.576)	18 (22.222)		
T3	126 (51.220)	71 (43.030)	55 (67.901)		
T4	8 (3.252)	1 (0.606)	7 (8.642)		
TNM stage (AJCC, 8 edition), n (%)				nan	Chi-square test
I	93 (37.805)	93 (56.364)	0 (0.000)		
II	103 (41.870)	70 (42.424)	33 (40.741)		
III	50 (20.325)	2 (1.212)	48 (59.259)		

		Proper lymph node dissection			
Vessel invasion, n (%)				<0.001	Chi-square test
No	177 (71.951)	139 (84.242)	38 (46.914)		
Yes	69 (28.049)	26 (15.758)	43 (53.086)		
Postoperative complications, n (%)				0.347	Chi-square test
No	141 (57.317)	98 (59.394)	43 (53.086)		
Yes	105 (42.683)	67 (40.606)	38 (46.914)		
Adjuvant treatment, n (%)				<0.001	Chi-square test
No	153 (62.195)	125 (75.758)	28 (34.568)		
Yes	68 (27.642)	33 (20.000)	35 (43.210)		
Unknown	25 (10.163)	7 (4.242)	18 (22.222)		
Recurrence, n (%)				0.008	Chi-square test
No	139 (56.504)	103 (62.424)	36 (44.444)		
Yes	107 (43.496)	62 (37.576)	45 (55.556)		

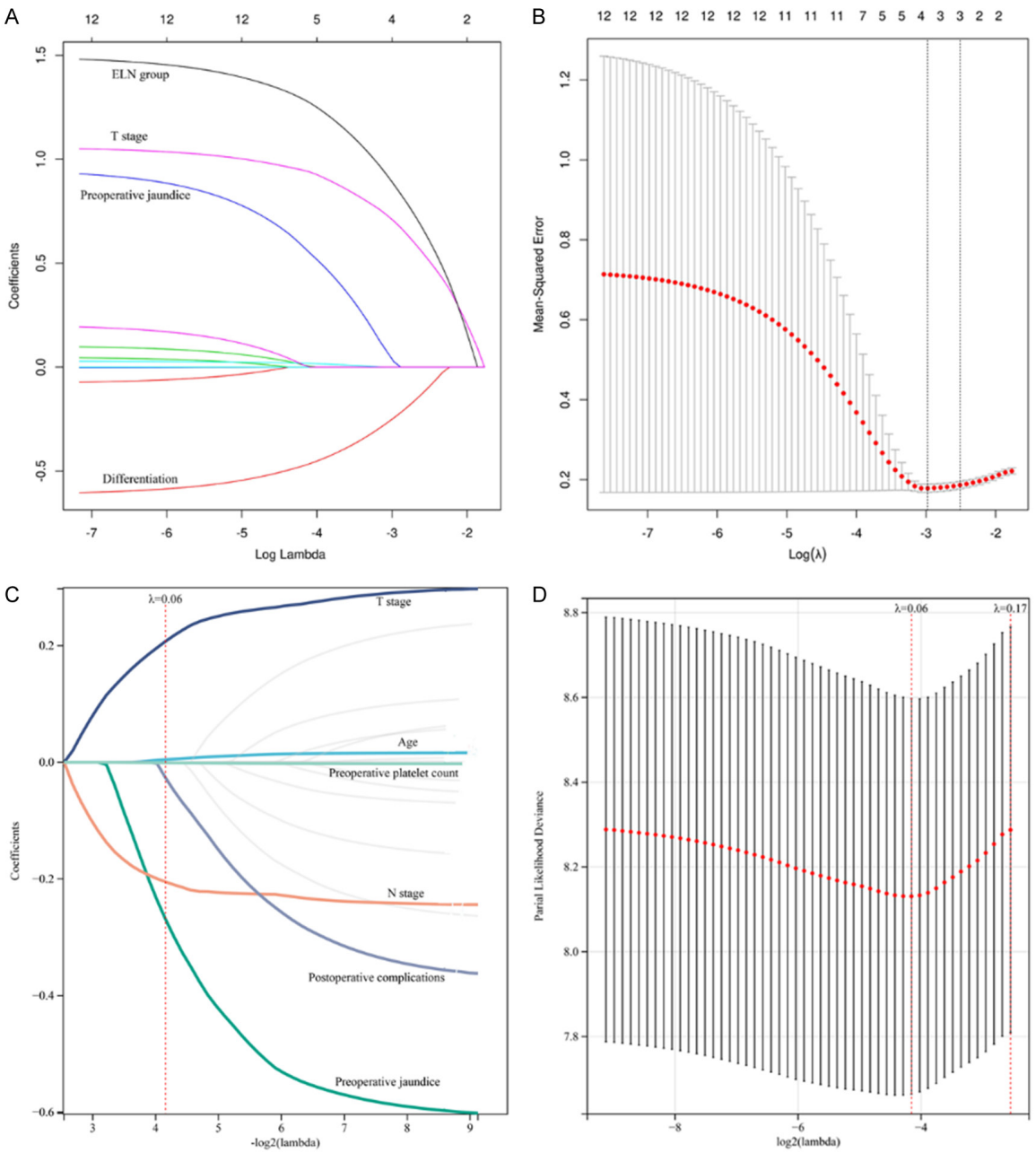

Figure 5. Plots for LASSO regression coefficients over different values of the penalty parameter. A. LASSO-logistic analysis to select the predictors of LN metastasis; B. Cross validation plot in LASSO-logistic model; C. LASSO-cox analysis to select the predictors of OS; D. Cross validation plot in LASSO-cox model.

the median numbers of LNs retrieved from the PD specimens by pathologists were obviously improved (from 11 to 22) after training [20]. Third, multiple factors may influence the prognosis of AC patients, which may lead to certain differences of the MNELN determined among different studies. For example, lymph node status is an important indicator of postoperative adjuvant therapy [2]; however, when the number of dissected lymph nodes is insufficient, the stage migration will occur and influence the selection of postoperative adjuvant therapy, thereby affecting the overall prognosis. Four, the biological differences among races [21] may influence the study outcomes of survival and MNELNs. In our study, we combined the SEER database with the NCC cohort of China to reduce this bias. Finally, the relatively small

sample size in some previous studies might potentially reduce the statistical power.

Our study was also the first to apply binomial probabilistic model to determine the MNELN for AC patients. To further validate our results clinically, we constructed the best survival separation model separately for node-negative and positive patients. To address potential confounding, we performed the LASSO regression model and further validated the accuracy and reliability our MNELN. Although the MNELN in our study was not an independent prognostic factor, it was an independent predictor of LN metastasis. Therefore, we speculated that the difference in survival due to the number of ELNs is mainly caused by the insufficient assessment of LN metastasis.

The major limitations of the current study were its retrospective nature and relatively small sample size. In addition, the SEER database also lacks several important clinical information, including the surgical margin status, detailed adjuvant treatment, and recurrence; hence, we were unable to investigate the key relationship between ELNs and tumor recurrence. Furthermore, two potential biases might cause the miscount of the ELNs, which should be addressed in future study. One comes from either the fusion of LNs which results in the undercounts of ELNs, or the incomplete LNs which result in increased counts of ELNs. The other bias lies in the proficiency of pathologists who are extremely important for ELN counts, as trained pathologists count significantly more ELNs than untrained pathologists [20].

In conclusion, a greater number of ELNs were significantly associated with nodal status assessment in PD for AC patients. We recommend that at least 16 lymph nodes should be examined during PD in AC patients in order to evaluate the postoperative N staging and long-term survival.

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Disclosure of conflict of interest

None.

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References

[1] Ecker BL, Vollmer CM Jr, Behrman SW, Allegrini V, Aversa J, Ball CG, Barrows CE, Berger AC, Cagigas MN, Christein JD, Dixon E, Fisher WE, Freedman-Weiss M, Guzman-Pruneda F, Hollis RH, House MG, Kent TS, Kowalsky SJ, Malleo G, Salem RR, Salvia R, Schmidt CR, Seykora TF, Zheng R, Zureikat AH and Dickson PV. Role of adjuvant multimodality therapy after curative-intent resection of ampullary carcinoma. JAMA Surg 2019; 154: 706-714.

[2] Moekotte AL, Malleo G, van Roessel S, Bonds M, Halimi A, Zarantonello L, Napoli N, Dreyer SB, Wellner UF, Bolm L, Mavroeidis VK, Robinson S, Khalil K, Ferraro D, Mortimer MC, Harris S, Al-Sarireh B, Fusai GK, Roberts KJ, Fontana M, White SA, Soonawalla Z, Jamieson NB, Boggi U, Alseidi A, Shablak A, Wilmink JW, Primrose JN, Salvia R, Bassi C, Besselink MG and Abu Hilal M. Gemcitabine-based adjuvant chemotherapy in subtypes of ampullary adenocarcinoma: international propensity score-matched cohort study. Br J Surg 2020; 107: 1171-1182.

[3] Yachida S, Wood LD, Suzuki M, Takai E, Totoki Y, Kato M, Luchini C, Arai Y, Nakamura H, Hama N, Elzawahry A, Hosoda F, Shirota T, Morimoto N, Hori K, Funazaki J, Tanaka H, Morizane C, Okusaka T, Nara S, Shimada K, Hiraoka N, Taniguchi H, Higuchi R, Oshima M, Okano K, Hirono S, Mizuma M, Arihiro K, Yamamoto M, Unno M, Yamaue H, Weiss MJ, Wolfgang CL, Furukawa T, Nakagama H, Vogelstein B, Kiyono T, Hruban RH and Shibata T. Genomic sequencing identifies ELF3 as a driver of ampullary carcinoma. Cancer Cell 2016; 29: 229-240.

[4] Zimmermann C, Wolk S, Aust DE, Meier F, Saeger HD, Ehehalt F, Weitz J, Welsch T and Distler M. The pathohistological subtype strongly predicts survival in patients with ampullary carcinoma. Sci Rep 2019; 9: 12676.

[5] Balci S, Basturk O, Saka B, Bagci P, Postlewait LM, Tajiri T, Jang KT, Ohike N, Kim GE, Krasinskas A, Choi H, Sarmiento JM, Kooby DA, El-Rayes BF, Knight JH, Goodman M, Akkas G, Reid MD, Maithel SK and Adsay V. Substaging nodal status in ampullary carcinomas has significant prognostic value: proposed revised

staging based on an analysis of 313 well-characterized cases. Ann Surg Oncol 2015; 22: 4392-4401.

[6] Kwon J, Kim K, Chie EK, Kim BH, Jang JY, Kim SW, Oh DY and Bang YJ. Prognostic relevance of lymph node status for patients with ampullary adenocarcinoma after radical resection followed by adjuvant treatment. Eur J Surg Oncol 2017; 43: 1690-1696.

[7] Tol JA, Gouma DJ, Bassi C, Dervenis C, Montorsi M, Adham M, Andrén-Sandberg A, Asbun HJ, Bockhorn M, Büchler MW, Conlon KC, Fernández-Cruz L, Fingerhut A, Friess H, Hartwig W, Izbicki JR, Lillemoe KD, Milicevic MN, Neoptolemos JP, Shrikhande SV, Vollmer CM, Yeo CJ, Charnley RM; International Study Group on Pancreatic Surgery. Definition of a standard lymphadenectomy in surgery for pancreatic ductal adenocarcinoma: a consensus statement by the international study group on pancreatic surgery (ISGPS). Surgery 2014; 156: 591-600.

[8] Nassour I, Christie A, Choti MA, Mansour JC, Minter RM, Polanco PM, Augustine MM, Porembka MR, Xie XJ and Wang SC. Determining the adequate examined lymph node count in resected ampullary adenocarcinoma-a national cohort study. J Gastrointest Surg 2018; 22: 792-801.

[9] Partelli S, Crippa S, Capelli P, Neri A, Bassi C, Zamboni G, Barugola G and Falconi M. Adequacy of lymph node retrieval for ampullary cancer and its association with improved staging and survival. World J Surg 2013; 37: 1397-1404.

[10] Vuarnesson H, Lupinacci RM, Semoun O, Svrcek M, Julié C, Balladur P, Penna C, Bachet JB, Resche-Rigon M and Paye F. Number of examined lymph nodes and nodal status assessment in pancreaticoduodenectomy for pancreatic adenocarcinoma. Eur J Surg Oncol 2013; 39: 1116-1121.

[11] Huang XT, Huang CS, Li JH, Xu QC and Yin XY. Use of nodal staging score in evaluating the accuracy of pathologic nodal status in node-negative ampullary carcinoma. J Gastrointest Surg 2021; 25: 1001-1009.

[12] Feng J, Wu R, Zhang G, Yang Z and Zhang L. Prognostic importance of numbers of retrieved lymph nodes and positive lymph nodes for ampulla of vater cancer (AVC) in 2347 patients from the surveillance, epidemiology, and end results (SEER) database. PLoS One 2021; 16: e0244987.

[13] Chen SC, Shyr YM, Chou SC and Wang SE. The role of lymph nodes in predicting the prognosis of ampullary carcinoma after curative resection. World J Surg Oncol 2015; 13: 224.

[14] Falconi M, Crippa S, Domínguez I, Barugola G, Capelli P, Marcucci S, Beghelli S, Scarpa A, Bassi C and Pederzoli P. Prognostic relevance of lymph node ratio and number of resected nodes after curative resection of ampulla of vater carcinoma. Ann Surg Oncol 2008; 15: 3178-3186.

[15] Kang JS, Higuchi R, He J, Yamamoto M, Wolfgang CL, Cameron JL, Han Y, Son D, Lee S, Choi YJ, Byun Y, Kim H, Kwon W, Kim SW, Park T and Jang JY. Proposal of the minimal number of retrieved regional lymph nodes for accurate staging of distal bile duct cancer and clinical validation of the three-tier lymph node staging system (AJCC 8th edition). J Hepatobiliary Pancreat Sci 2020; 27: 75-83.

[16] Pu N, Gao S, Beckman R, Ding D, Wright M, Chen Z, Zhu Y, Hu H, Yin L, Beckman M, Thompson E, Hruban RH, Cameron JL, Gage MM, Lafaro KJ, Burns WR, Wolfgang CL, He J, Yu J and Burkhart RA. Defining a minimum number of examined lymph nodes improves the prognostic value of lymphadenectomy in pancreas ductal adenocarcinoma. HPB (Oxford) 2021; 23: 575-586.

[17] Malleo G, Maggino L, Ferrone CR, Marchegiani G, Mino-Kenudson M, Capelli P, Rusev B, Lillemoe KD, Bassi C, Fernàndez-Del Castillo C and Salvia R. Number of examined lymph nodes and nodal status assessment in distal pancreatectomy for body/tail ductal adenocarcinoma. Ann Surg 2019; 270: 1138-1146.

[18] Zheng YZ, Li XQ, Wang JY, Yang H, Wen J, Zhai WY, Yuan LX, Fu SS, Liao HY and Fu JH. Impact of examined lymph node count for esophageal squamous cell carcinoma in patients who underwent right transthoracic esophagectomy. Ann Surg Oncol 2021; 28: 3025-3033.

[19] Liang W, He J, Shen Y, Shen J, He Q, Zhang J, Jiang G, Wang Q, Liu L, Gao S, Liu D, Wang Z, Zhu Z, Ng CS, Liu CC, Petersen RH, Rocco G, D'Amico T, Brunelli A, Chen H, Zhi X, Liu B, Yang Y, Chen W, Zhou Q and He J. Impact of examined lymph node count on precise staging and long-term survival of resected non-small-cell lung cancer: a population study of the US SEER database and a Chinese multi-institutional registry. J Clin Oncol 2017; 35: 1162-1170.

[20] Jeyarajah DR, Khithani A, Siripurapu V, Liu E, Thomas A and Saad AJ. Lymph node retrieval in pancreaticoduodenectomy specimens: does educating the pathologist matter? HPB (Oxford) 2014; 16: 263-266.

[21] Spratt DE, Chan T, Waldron L, Speers C, Feng FY, Ogunwobi OO and Osborne JR. Racial/Ethnic disparities in genomic sequencing. JAMA Oncol 2016; 2: 1070-1074.

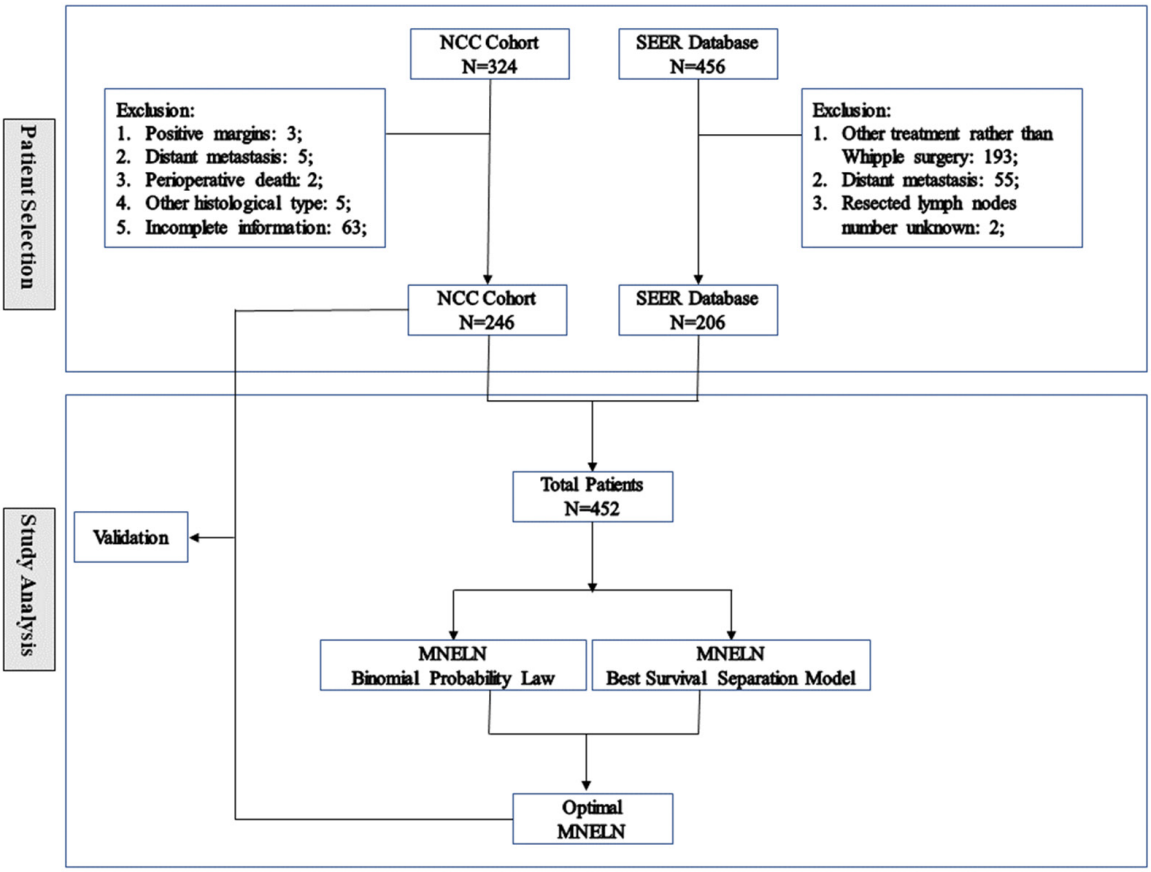

Figure S1. The flow-chart of the patient selection and study analysis.

Table S1. Coefficients of clinicopathologic values in the Lasso-logistic model

Variables	Coef.
T stage	-0.08
ELN group	0.135
Differentiation	-0.018

Table S2. Coefficients of clinicopathologic values in the Lasso-Cox model

Variables	Coef.
Age	0.005
Preoperative jaundice	-0.269
T stage	0.207
N stage	-0.205
Postoperative complications	-0.027
Preoperative platelet count	-0.001