BMJ Open

Prognostic value of different lymph node staging methods for node positive cardia gastric cancer

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-050378
Article Type:	Original research
Date Submitted by the Author:	22-Feb-2021
Complete List of Authors:	Wang, Xiao-Qing; Anhui Medical College Bao, Min; Anhui Medical College Zhang, Cheng; First Affiliated Hospital of Anhui Medical University
Keywords:	Gastrointestinal tumours < ONCOLOGY, Gastrointestinal tumours < GASTROENTEROLOGY, SURGERY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title page

Article title: Prognostic value of different lymph node staging methods for node positive cardia gastric cancer

Author information: Xiao-Qing Wang 1, Min Bao 1, Cheng Zhang 2

1 Anhui Medical College, Hefei, Anhui, China PR

2 Anhui Provincial Cancer Institute, the First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China PR

Corresponding to: Cheng Zhang, ahmuzc@sina.cn

Disclaimer: The views expressed in the submitted article are our own and not an official position of the institution or funder.

Word count: 2052 (excluding its abstract, acknowledgments, tables, figure legends, and references) abstract 274 .

Number of figures and tables: This draft included 4 figures and 2 tables.

Disclosure of relationships and activities: None.

Abstract

Objective: To investigate the prognostic efficacy of lymph node ratio (LNR) and log odds of positive lymph nodes (LODDS) in node positive cardia gastric adenocarcinoma (CGA).

Design: A SEER database review

Participants: A total of 1 038 patients with node positive CGA were enrolled from SEER database. Seventy percent of the entire patients were randomly assigned to training set (N = 723) and the rest was assigned to validating set (N = 315).

Interventions: The major endpoint was cancer specific survival (CSS). Optimal cut-off values were determined by X-tile software. The prognostic power was evaluated using Akaike Information Criterion (AIC) and Harrell concordance index (C-index). Cox stepwise regression analysis was performed to construct nomogram for prediction of 1-, 2-, and 5-year CSS.

Results: The training set and validating set are similar in terms of clinical and demographic features. The optimal cut-off values for LNR were 0.09 and 0.33, and for LODDS were -2.09 and -0.65. CSS was significantly different by N, LNR and LODDS categories. The C-index of N stage was lower than that of LNR or LODDS. The AIC of N stage was higher than that of LNR or LODDS. Independent predictors included age, race, tumor grade, T stage, M stage and LNR (or LODDS) and they were incorporated in nomograms for 1-, 2- and 5-year CSS prediction. Calibration plots showed satisfied results of internal and external validity of the nomogram.

Conclusions: LNR and LODDS staging methods have better prognostic efficacy than traditional N staging method in CGA patients with regional node metastasis. Besides, the two values are promising substitute for N staging in nomogram development when other independent prognostic factors are incorporated.

Key words: Cardia; Adenocarcinoma; Stomach Neoplasms; Lymph Node Ratio; Neoplasm Staging

Strengths and limitations of this study

- This study used national cancer registry data for cardia gastric adenocarcinoma research;
- Novel staging methods based on the number of positive lymph node was established for prognostic prediction;
- Nomograms based on the new staging methods were constructed and validated;
- This study needs to be confirmed by other populations.

Patient consent form: The SEER database review is granted exemption from obtaining patients' consents.

Word count: 2052 (excluding its abstract, acknowledgments, tables, figure legends, and references) abstract 274 .

Introduction

Gastric cancer (GC) generally includes 2 topographical categories: non-cardia GC that arises from more distant sites and cardia GC that arises in gastroesophageal junction (GEJ). In contrast to steady decline of non-cardia GC incidence, cardia GC occurs more frequently, particular in high-income countries (1, 2). This trend is associated with obesity, gastroesophageal reflux disease (GERD), and Barrett esophagus (2). In addition to different incidence trend, clinicalpathological feature and long-term survival vary between the two GC subtypes (3). For accurate prediction of survival, precise staging is required. The Tumor-Node-Metastasis (TNM) classification 7th edition by the American Joint Committee on Cancer (AJCC) recommends at least 15 lymph nodes (LN) collection for N staging (4, 5). However inadequate LN harvest frequently occurs due to many conditions, thus precise staging cannot be obtained sometimes. It has been demonstrated that LN ratio (LNR) could better estimate survival of GC patients after curative gastrectomy, regardless of the number of LN examined (6), and may be promising for aiding TNM staging system (7). Apart from that, log odds of positive LN (LODDS) outperformed N and LNR staging system when predicting survival of GC patients (8-10). Therefore the traditional N staging classification may be substituted with different methods, with even improved performance. Nevertheless little evidence evaluates the performance of the two LN staging systems aforementioned in cardia GC, since it has distinct clinical characteristics and epidemiology from overall GC.

Here we use nationwide cancer registry data to appraise the prognostic value of LNR and LODDS in patients with node positive cardia gastric adenocarcinoma (CGA), and, if possible, construct nomogram for survival prediction based on the new LN staging system.

Methods

Selection and Description of Participants

The inclusion criteria were as follows: 1) the International Classification of Disease for Oncology, Third Edition (ICD-O-3) for primary tumor site was C16.0 (cardia); 2) broad histological recode was 8140-8389: adenomas and adenocarcinomas; 3) diagnostic confirmation was positive histology; 4) surgery was performed; 5) diagnosed during 2010-2015; 6) the definite number of regional positive nodes was clear and not zero. We excluded cases with unknown race, T stage information, tumor size and grade. As shown in Figure 1, the final cohort enrolled 1 038 patients with node positive CGA, of whom 857 were male and 181 were female. Three hundred and thirty eight (32.56%) were over 70 years old. Eight hundred and ninety six (86.32%) were white, 64 were black and 78 were other races. Next 70% of the entire patients were randomly assigned to training set (N = 723) and the rest was assigned to validating set (N = 315).

Patient and Public Involvement

This study is a data review based on SEER program, so it was granted exemption from requiring informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the Anhui Medical College. We used SEER*Stat (version 8.3.8) to access to Incidence - SEER Research Data, 18 Registries, Nov 2019 Sub 2000-2017 (SEER 18 database) (11) for collection of node positive CGA patients (username: 21268-Nov2019). Patients were not involved in the recruitment to and conduct of the

study. The findings of the study will be disseminated to all study participants by online article.

Technical Information

The main outcome was cancer specific survival (CSS), which was referred to as death specifically due to CGA and the period between first diagnosis and death. In addition, we extracted the following variables for analysis: sex, race, age, AJCC 7th TNM stage information, tumor size, grade, number of regional nodes examined and number of regional nodes positive. LNR and LODDS were calculated as previously reported (12). Briefly, LNR was defined as the ratio of the number of positive nodes divided by the total number of examined nodes. LODDS was calculated using the formula: log(NPLN+0.50)/(NDLN−NPLN+0.50), in which 0.50 was added to both the numerator and denominator to avoid an infinite number.

The optimal thresholds for cutting LNR and LODDS into trichotomous variables were determined by X-tile software (version 3.6.1) (13), which were based on the maximal log-rank chi-square value that represented the greatest group difference of CSS probability.

Statistics

The distributions of baseline features between training set and validating set were described and compared by chi-square test. Survival curves, median survival and CSS rates were generated using the Kaplan-Meier method. Outcome difference between groups was analyzed by the log-rank test. Multivariable Cox regression model was used to establish prognostic model for CSS. The prognostic power was evaluated using Akaike Information Criterion (AIC) and Harrell concordance index (C-index). A predictive model with lower AIC indicated better model fit, while with higher C-index indicated better discriminative ability. A value of C-index of 0.5 indicates no predictive power, and an index of 1.0 indicates complete differentiation. Cox stepwise regression analysis was also performed to construct nomogram for prediction of 1-, 2-, and 5-year CSS. Validation of nomogram was performed by internal and external calibration plots (14). Bootstraps with 1 000 resample were used for validation activities. All statistical analyses were performed using R software (version 3.5.3). A two-tailed P value of less than 0.05 was considered statistically significant.

Results

Table 1 summarized the demographic and clinical feature of the participants. Six hundred and twenty eight patients (60.50%) were diagnosed with a tumor less than 5cm. Six hundred and forty patients (61.66%) were with grade III or IV. The numbers of patients with T1, T2, T3 and T4 respectively were 94, 125, 717 and 102. The numbers of patients with N1, N2 and N3 respectively were 488, 331 and 219. Seventy five patients (7.23%) were with distant metastasis at presentation. The median CSS was 27 months. The 1-, 2- and 5-year CSS rates were 76.8%, 53.0% and 29.2%, respectively. There was no statistical difference of baseline characteristics between training set and validating set. The detailed information of the two sets was also presented in Table 1.

Table 1. Baseline information of the included patients with node positive CGA, N(%).

Groups	Training set (N = 723)	Validating set (N = 315)	P-value

Sex			
Male	596 (82.43)	261 (82.86)	0.939
Female	127 (17.57)	54 (17.14)	
Age			
<70	490 (67.77)	210 (66.67)	0.781
≥70	233 (32.23)	105 (33.33)	
Race			
White	628 (86.86)	268 (85.08)	
Black	40 (5.53)	24 (7.62)	0.437
Others	55 (7.61)	23 (7.30)	
Tumor size			
<5cm	442 (61.13)	186 (59.05)	0.573
≥5cm	281 (38.87)	129 (40.95)	
Grade			
I-II	279 (38.59)	119 (37.78)	0.859
III-IV	444 (61.41)	196 (62.22)	
T stage			
T1	70 (9.68)	24 (7.62)	
T2	83 (11.48)	42 (13.33)	0.600
T3	501 (69.30)	216 (68.57)	
T4	69 (9.54)	33 (10.48)	
N stage			
N1	348 (48.13)	140 (44.44)	
N2	225 (31.12)	106 (33.65)	0.545
N3	150 (20.75)	69 (21.91)	
M stage			
M0	678 (93.78)	285 (90.48)	0.079
M1	45 (6.22)	30 (9.52)	
Median survival (months)	28 (25, 32)	25 (21, 32)	0.361
CSS rate (%)			
1-year	77.0 (74.0, 80.2)	76.3 (71.6, 81.2)	
2-year	53.7 (50.1, 57.5)	51.4 (46.0, 57.5)	
5-year	30.3 (26.7, 34.5)	26.4 (20.9, 33.4)	

Abbreviation: CGA, cardia gastric adenocarcinoma; CSS, cancer-specific survival

According to X-tile software results, the optimal cut-off values for LNR were 0.09 and 0.33, and for LODDS were -2.09 and -0.65. Thus patients were separated into low (R1), medium (R2) or high LNR (R3) group, or low (L1), medium (L2) or high LODDS (L3) group. Next we illustrated the survival curves of the patients according to N, LNR or LODDS staging system. As shown in Figure 2 training set section, CSS was significantly different by all the three staging systems (all the log-rank P values < 0.0001); however the 95% CIs of N2 and N3 survival curve initially separated and partly overlapped afterwards. The inferior discriminative ability of N system was further supported by AIC and C-index. As shown in Table 2, the C-index of N stage was lower than that of LNR or LODDS. Similarly, the AIC of N stage was higher than that of LNR or

LODDS. The prognostic value of adjusted model was better than crude mode generally. In addition, the value of LNR system seemed to be worse than LODDS system; however the difference was not noticeable, so we considered both of the systems into nomogram construction.

Table 2. Prognostic values of variables for patients with node positive CGA (N = 1 038).

Variables	Crude model			Adjusted model		
	HR (95% CI)	C-index	AIC	HR (95% CI)	C-index	AIC
Training set (N = 723)						
N stage		0.572	5412		0.633	5379
N1	1 (ref)			1 (ref)		
N2	1.44 (1.17, 1.79)			1.34 (1.07, 1.66)		
N3	1.98 (1.57, 2.51)			1.71 (1.34, 2.19)		
LNR*		0.607	5376		0.655	5343
R1	1 (ref)			1 (ref)		
R2	1.88 (1.44, 2.44)			1.83 (1.40, 2.39)		
R3	3.02 (2.30, 3.97)			2.74 (2.07, 3.63)		
LODDS*		0.609	5373		0.656	5339
L1	1 (ref)			1 (ref)		
L2	1.93 (1.48, 2.51)			1.86 (1.42, 2.44)		
L3	3.13 (2.38, 4.13)			2.87 (2.16, 3.81)		
Validating set (N = 315)						
N stage		0.603	1953		0.681	1926
N1	1 (ref)			1 (ref)		
N2	1.89 (1.36, 2.63)			1.88 (1.34, 2.64)		
N3	2.34 (1.62, 3.38)			2.18 (1.47, 3.24)		
LNR*		0.646	1927		0.702	1902
R1	1 (ref)			1 (ref)		
R2	2.20 (1.47, 3.30)			2.14 (1.41, 3.23)		
R3	4.16 (2.76, 6.28)			4.00 (2.59, 6.17)		
LODDS*		0.647	1927		0.703	1901
L1	1 (ref)			1 (ref)		
L2	2.07 (1.39, 3.09)			2.08 (1.38, 3.14)		
L3	4.22 (2.79, 6.39)			4.10 (2.65, 6.34)		

Abbreviations: CGA, cardia gastric adenocarcinoma; HR, hazard ratio; CI, confidence interval; AIC, Akaike information criterion; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes.

Adjusted model considered age, sex, race, tumor size, grade, T stage and M stage.

* cut-off values for LNR were 0.09 and 0.33, and for LODDS were -2.09 and -0.65.

Stepwise Cox regression analysis showed age, race, tumor grade, T stage, M stage and LNR (or LODDS) were independent predictors, so these factors were included in nomograms. For both LNR and LODDS, the total score was 40, and higher score suggested lower survival (Figure 3A and 4A). Next calibration plot was used to assess the internal and external validity of the nomogram (Figure 3B, 3C, 4B and 4C). Since the cross-spot line was generally close to the grey

reference line, we concluded the predicted CSS was well correlated with the actual situation.

Discussion

The present study analyzes national cancer registry databases and demonstrates that survival of patients with node-positive CGA is well predicted when the traditional N staging method is substituted with LNR or LODDS system. This finding both exists in training and validating sets. In training set, the survival curves separate clearly when patient grouping is implemented by LNR or LODDS method, which is not achieved by traditional N staging system. Adjusted model that simultaneously considers staging, clinical and demographic features outperforms crude model that only takes staging into account. Therefore multiple independent survival factors are incorporated in nomogram construction, which suggests older age at diagnosis, white, higher grade, greater tumor infiltration, higher proportion of positive LN, and metastasis as risk factors. The nomograms perform steadily in 1-, 2- and 5-year CSS prediction as the validation plots show.

Previous studies have demonstrated the superiority of LNR or LODDS for prognostic prediction in GC after surgical resection (8-10, 15-17). However the GC patients are not further separated and investigated according to primary tumor site, since there is much difference between cardia and non-cardia GC in terms of tumor features, etiological factors, and biological behaviors (3). In AJCC cancer staging 7th edition, tumors involving EGJ was categorized as esophagus cancer (5), which was however argued by the viewpoint that GC staging system has a better ability to predict survival of EGJ tumor (18, 19). In the latest 8th edition (20), a tumor that has its epicenter within 2 cm of EGJ and involves the EGJ (Siewert type I/II) is classified as esophageal cancer. Other situation, including a tumor with epicenter more than 2 cm from EGJ or a tumor located with 2 cm of EGJ but does not involve EGJ, is classified as stomach cancer. The superiority of the new system is confirmed by a retrospective observational study from two high-volume institutions in China, regardless of Siewert type (21). In terms of Siewert type II junctional adenocarcinoma, a marginal advantage of the esophagus cancer system is found in discriminating survival rates after 3 and 5 years, however the advantage of GC system lies in division of the N3 category into N3a and N3b, so the authors concludes neither the esophageal nor the stomach staging system is flawless in predicting survival in Siewert type II junctional cancer (22). Above all, CGA is probably a special entity that has a different biological property compared with genuine gastric and genuine esophageal cancer. To the best of our knowledge, the present study first reveals a superior performance of prognostic prediction based on LNR or LODDS in node positive CGA patients. Unfortunately we are unable to consider Siewert type due to unavailable information from SEER database; therefore we encourage further studies to pay special attention on tumor location.

LNR and LODDS have been proved to be the strongest indictors of survival in gastric adenocarcinoma when LN harvest is inadequate (16, 17). It is demonstrated that in general, more LN resected is associated with better survival, which may be the result of either improved N classification or a therapeutic effect of lymphadenectomy. For esophageal cancer, worldwide data shows that yielding 10 nodes for pT1, 20 for pT2, and 30 or more for pT3/T4 is recommended for maximum 5-year survival (23). For GC, greater LN harvest also shows improved survival (24). It is suggested that at least 16 nodes be assessed pathologically and evaluation of more than 30 nodes is desirable (25). Overall it is encouraged to harvest as many LN as possible, balancing the

extent of LN resection necessary for accurate N staging and maximum survival without unnecessarily increasing the morbidity of radical lymphadenectomy. Nevertheless, many conditions would lead to insufficient LN harvest. It is estimated that only one fifth GC patients have sufficient LN examined in Iran (26), while more than 15 LNs are examined in 64% of patients in the US (25). The LNR and LODDS staging methods do not require adequate number of LN assessment. In fact, the new N category method is stable when nodal assessment is insufficient during surgery not only for GC (8, 15-17) but also for colorectal cancer (27), esophageal cancer (28), oral squamous cell carcinoma (29), gallbladder cancer (30), etc.

One limitation of this study is that the recruited patients were diagnosed during 2010-2015 and staged based on TNM 7th edition that defined 3 N categories. In 8th edition, N category of GC includes N1, N2, N3a and N3b, which improves survival prediction in patients with junctional cancer (22). So whether LNR or LODDS based staging system outperforms TNM 8th edition needs to be further investigated. Another limitation is that our results are based on training set and confirmed by validating set; however the features of the two groups are similar. So this finding needs to be proved among populations with distinct features.

In conclusion, LNR and LODDS staging methods have better prognostic efficacy than traditional N staging method in CGA patients with regional node metastasis. Besides, the two values are promising substitute for N staging in nomogram development when other independent prognostic factors are incorporated.

a. **Contributorship statement:** WXQ work conception, data interpretation, critical review for important content, final approval of the manuscript and agreement to be accountable for all aspects of the work; BM administrative work, funding, critical review for important content, final approval of the manuscript and agreement to be accountable for all aspects of the work; ZC acquisition, analysis of data, drafting the work, final approval of the manuscript and agreement to be accountable for all aspects of the work.
b. **Competing interests:** None.
c. **Funding:** This work was supported by [Major Project of Natural Science of Anhui Provincial Department of Education] grant number [KJ2019ZD73].
d. **Data sharing statement:** Dataset available from the SEER website (https://seer.cancer.gov/data-software/).

Acknowledgement: We thank all patient advisers for their special contribution.

References:

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. [Journal Article]. 2018 2018-11-01;68(6):394-424.
2. Abdi E, Latifi-Navid S, Zahri S, Yazdanbod A, Pourfarzi F. Risk factors predisposing to cardia gastric adenocarcinoma: Insights and new perspectives. Cancer Med. [Journal Article; Research Support, Non-U.S. Gov't; Review]. 2019 2019-10-01;8(13):6114-26.
3. Amini N, Spolverato G, Kim Y, Squires MH, Poultsides GA, Fields R, et al. Clinicopathological features and prognosis of gastric cardia adenocarcinoma: a multi-institutional US study. J SURG ONCOL. [Comparative Study; Journal Article; Multicenter Study]. 2015 2015-03-01;111(3):285-92.

4. Sobin L, Gospodarowicz M, Wittekind C. TNM classification of malignant tumours. 7th edition.: Wiley-Blackwell; 2009.
5. Edge S, Byrd D, Compton C, Fritz A, Greene F, Trotti A. AJCC cancer staging manual. 7th edition.: Springer; 2009.
6. Hou Y, Wang X, Chen J. Prognostic significance of metastatic lymph node ratio: the lymph node ratio could be a prognostic indicator for patients with gastric cancer. WORLD J SURG ONCOL. [Journal Article]. 2018 2018-10-04;16(1):198.
7. Zhu J, Xue Z, Zhang S, Guo X, Zhai L, Shang S, et al. Integrated analysis of the prognostic role of the lymph node ratio in node-positive gastric cancer: A meta-analysis. INT J SURG. [Journal Article; Meta-Analysis; Review]. 2018 2018-09-01;57:76-83.
8. Sun Z, Xu Y, Li DM, Wang ZN, Zhu GL, Huang BJ, et al. Log odds of positive lymph nodes: a novel prognostic indicator superior to the number-based and the ratio-based N category for gastric cancer patients with R0 resection. CANCER-AM CANCER SOC. [Journal Article; Research Support, Non-U.S. Gov't]. 2010 2010-06-01;116(11):2571-80.
9. Zhao E, Zhou C, Chen S. Prognostic nomogram based on log odds of positive lymph nodes for gastric carcinoma patients after surgical resection. FUTURE ONCOL. [Journal Article]. 2019 2019-12-01;15(36):4207-22.
10. Gu P, Deng J, Sun Z, Wang Z, Wang W, Liang H, et al. Superiority of log odds of positive lymph nodes (LODDS) for prognostic prediction after gastric cancer surgery: a multi-institutional analysis of 7620 patients in China. SURG TODAY. [Journal Article]. 2021 2021-01-01;51(1):101-10.
11. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence - SEER Research Data, 18 Registries, Nov 2019 Sub (2000-2017) - Linked To County Attributes - Time Dependent (1990-2017) Income/Rurality, 1969-2018 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, released April 2020, based on the November 2019 submission..
12. Yu Y, Zhang P, Yao R, Wang J, Wang P, Xue X, et al. Prognostic value of log odds of positive lymph nodes in node-positive lung squamous cell carcinoma patients after surgery: a SEER population-based study. Transl Lung Cancer Res. [Journal Article]. 2020 2020-08-01;9(4):1285-301.
13. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. CLIN CANCER RES. [Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.]. 2004 2004-11-01;10(21):7252-9.
14. Iasonos A, Schrag D, Raj GV, Panageas KS. How to build and interpret a nomogram for cancer prognosis. J CLIN ONCOL. [Journal Article; Review]. 2008 2008-03-10;26(8):1364-70.
15. Jian-Hui C, Shi-Rong C, Hui W, Si-le C, Jian-Bo X, Er-Tao Z, et al. Prognostic value of three different lymph node staging systems in the survival of patients with gastric cancer following D2 lymphadenectomy. Tumour Biol. [Journal Article]. 2016 2016-08-01;37(8):11105-13.
16. Aurello P, Petrucciani N, Nigri GR, La Torre M, Magistri P, Tierno S, et al. Log odds of positive lymph nodes (LODDS): what are their role in the prognostic assessment of gastric adenocarcinoma? J GASTROINTEST SURG. [Comparative Study; Journal Article]. 2014 2014-07-01;18(7):1254-60.
17. Kilic MO, Gundogdu SB, Ozden S, Saylam B, Tez M. The prognostic value of different node staging systems in patients with </=15 lymph nodes following surgery for gastric adenocarcinoma. ACTA CHIR BELG. [Journal Article]. 2018 2018-02-01;118(1):1-6.
18. Suh YS, Han DS, Kong SH, Lee HJ, Kim YT, Kim WH, et al. Should adenocarcinoma of the

esophagogastric junction be classified as esophageal cancer? A comparative analysis according to the seventh AJCC TNM classification. ANN SURG. [Comparative Study; Journal Article; Research Support, Non-U.S. Gov't]. 2012 2012-05-01;255(5):908-15.

19. Huang Q, Shi J, Feng A, Fan X, Zhang L, Mashimo H, et al. Gastric cardiac carcinomas involving the esophagus are more adequately staged as gastric cancers by the 7th edition of the American Joint Commission on Cancer Staging System. Mod Pathol. [Journal Article; Research Support, Non-U.S. Gov't; Validation Study]. 2011 2011-01-01;24(1):138-46.

20. Amin M, Edge S, Greene F, Byrd D, Brookland R. AJCC cancer staging manual. 8th edition.: Springer; 2016.

21. Liu K, Feng F, Chen XZ, Zhou XY, Zhang JY, Chen XL, et al. Comparison between gastric and esophageal classification system among adenocarcinomas of esophagogastric junction according to AJCC 8th edition: a retrospective observational study from two high-volume institutions in China. GASTRIC CANCER. [Comparative Study; Journal Article; Observational Study]. 2019 2019-05-01;22(3):506-17.

22. Karstens KF, Ghadban T, Sawez S, Konczalla L, Woestemeier A, Bachmann K, et al. Comparison of the 8th UICC staging system for esophageal and gastric cancers in Siewert type II junctional adenocarcinomas. Eur J Surg Oncol. [Comparative Study; Journal Article]. 2020 2020-04-01;46(4 Pt A):638-43.

23. Rizk NP, Ishwaran H, Rice TW, Chen LQ, Schipper PH, Kesler KA, et al. Optimum lymphadenectomy for esophageal cancer. ANN SURG. [Journal Article; Research Support, Non-U.S. Gov't]. 2010 2010-01-01;251(1):46-50.

24. Macalindong SS, Kim KH, Nam BH, Ryu KW, Kubo N, Kim JY, et al. Effect of total number of harvested lymph nodes on survival outcomes after curative resection for gastric adenocarcinoma: findings from an eastern high-volume gastric cancer center. BMC CANCER. [Journal Article; Research Support, Non-U.S. Gov't]. 2018 2018-01-12;18(1):73.

25. Schoenleber SJ, Schnelldorfer T, Wood CM, Qin R, Sarr MG, Donohue JH. Factors influencing lymph node recovery from the operative specimen after gastrectomy for gastric adenocarcinoma. J GASTROINTEST SURG. [Journal Article]. 2009 2009-07-01;13(7):1233-7.

26. Khanjani N, Mirzaei S, Nasrolahi H, Hamedi SH, Mosalaei A, Omidvari S, et al. Insufficient lymph node assessment in gastric adenocarcinoma. J Egypt Natl Canc Inst. [Journal Article]. 2019 2019-10-22;31(1):2.

27. Pei JP, Zhang CD, Fan YC, Dai DQ. Comparison of Different Lymph Node Staging Systems in Patients With Resectable Colorectal Cancer. FRONT ONCOL. [Journal Article]. 2018 2018-01-20;8:671.

28. Cao J, Yuan P, Ma H, Ye P, Wang Y, Yuan X, et al. Log Odds of Positive Lymph Nodes Predicts Survival in Patients After Resection for Esophageal Cancer. ANN THORAC SURG. [Journal Article; Multicenter Study]. 2016 2016-08-01;102(2):424-32.

29. Jin W, Zhu Z, Wu Y, Ding X, Wu H, Song X, et al. Prognostic value of log odds of positive lymph nodes in patients with resectable oral squamous cell carcinoma. ORAL ONCOL. [Journal Article; Research Support, Non-U.S. Gov't]. 2020 2020-09-01;108:104709.

30. Li J, Sun Y, Zhao B, Tang C, Fan D, Jiang W, et al. Lymph Node Ratio-Based Staging System for Gallbladder Cancer With Fewer Than Six Lymph Nodes Examined. FRONT ONCOL. [Journal Article]. 2020 2020-01-20;10:542005.

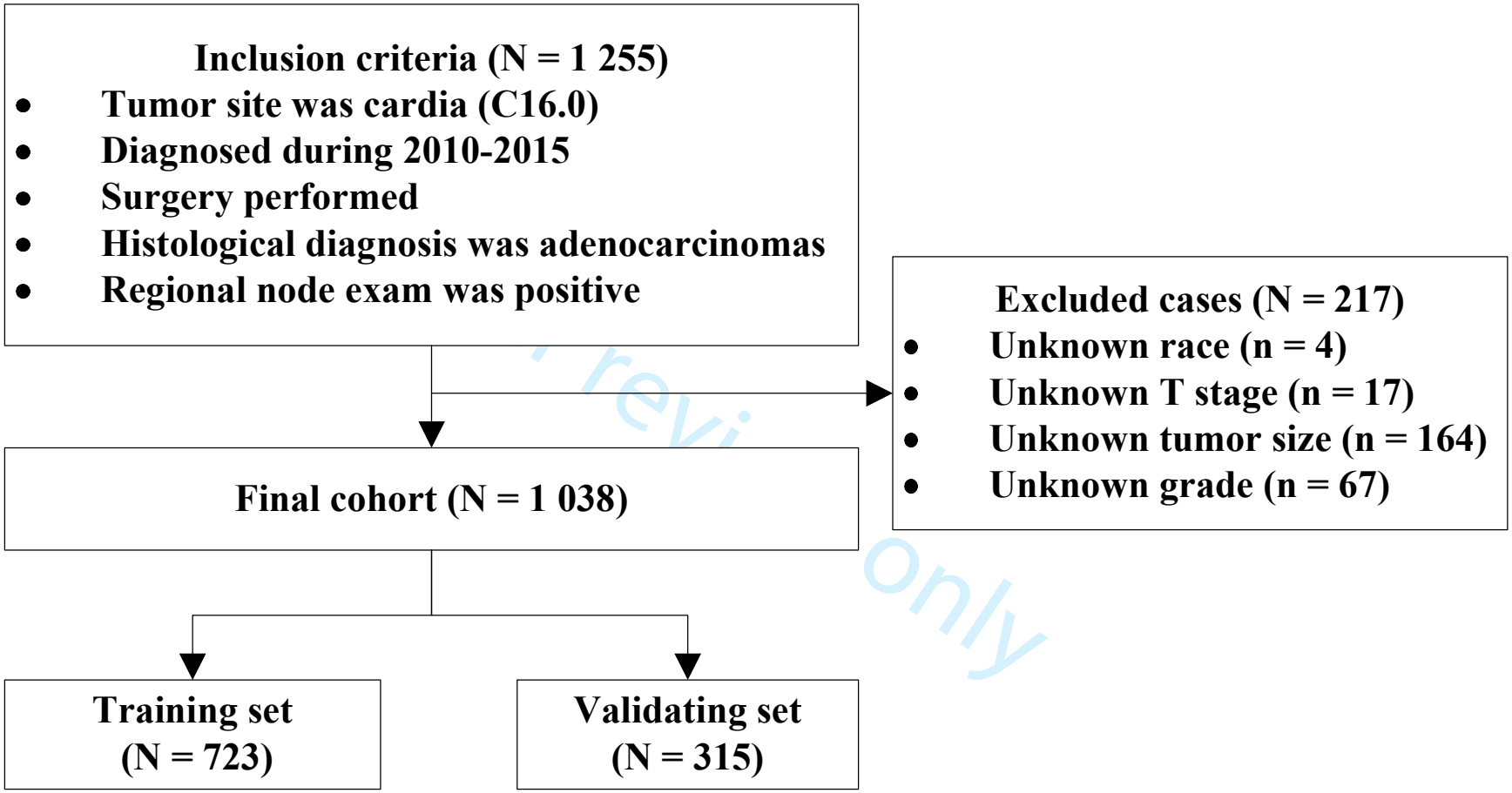
Illustrations

Figure 1. Flow diagram of patient selection and grouping.

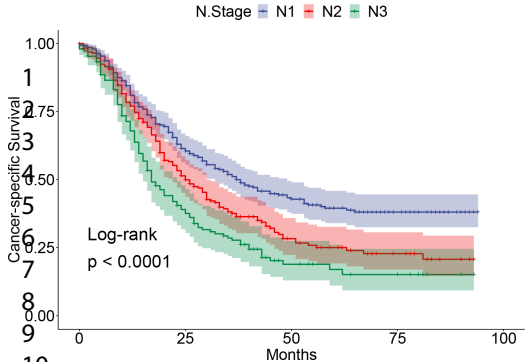
Figure 2. Survival curves of training and validating sets by different staging systems.

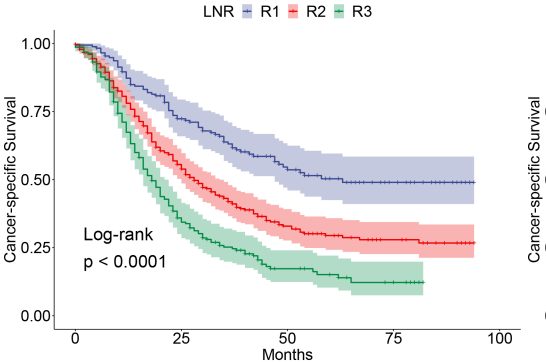
Figure 3. Construction and validation of nomogram based on Tumor-Lymph node ratio-Metastasis stating system. B) Internal validation; C) External validation.

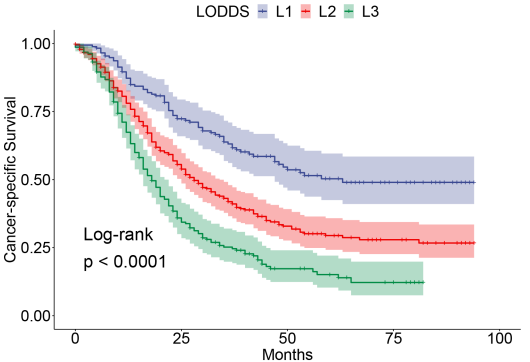
Figure 4. Construction and validation of nomogram based on Tumor-Log odds of positive lymph node-Metastasis stating system. B) Internal validation; C) External validation.



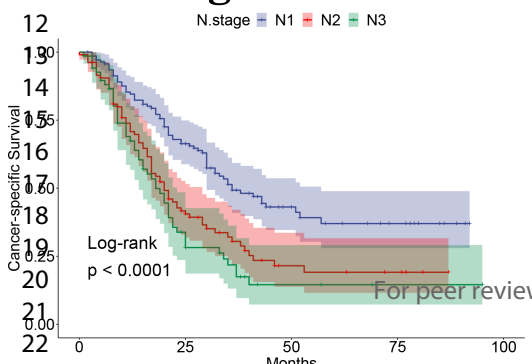
Training Set

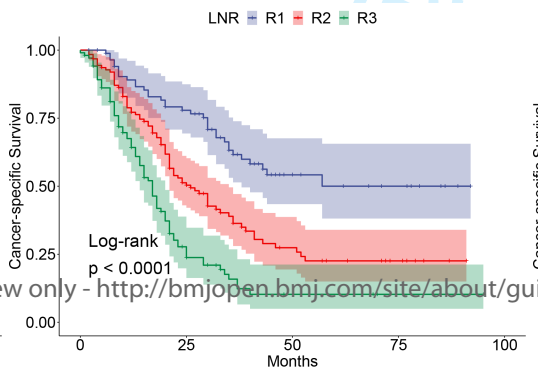


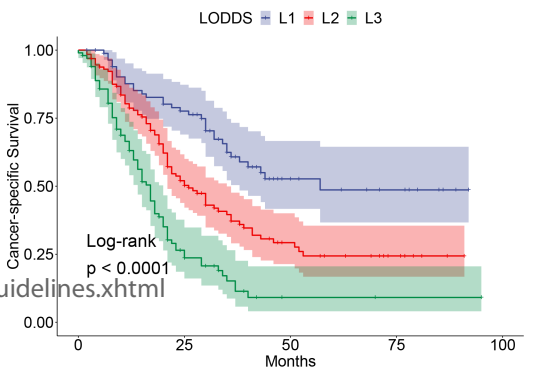




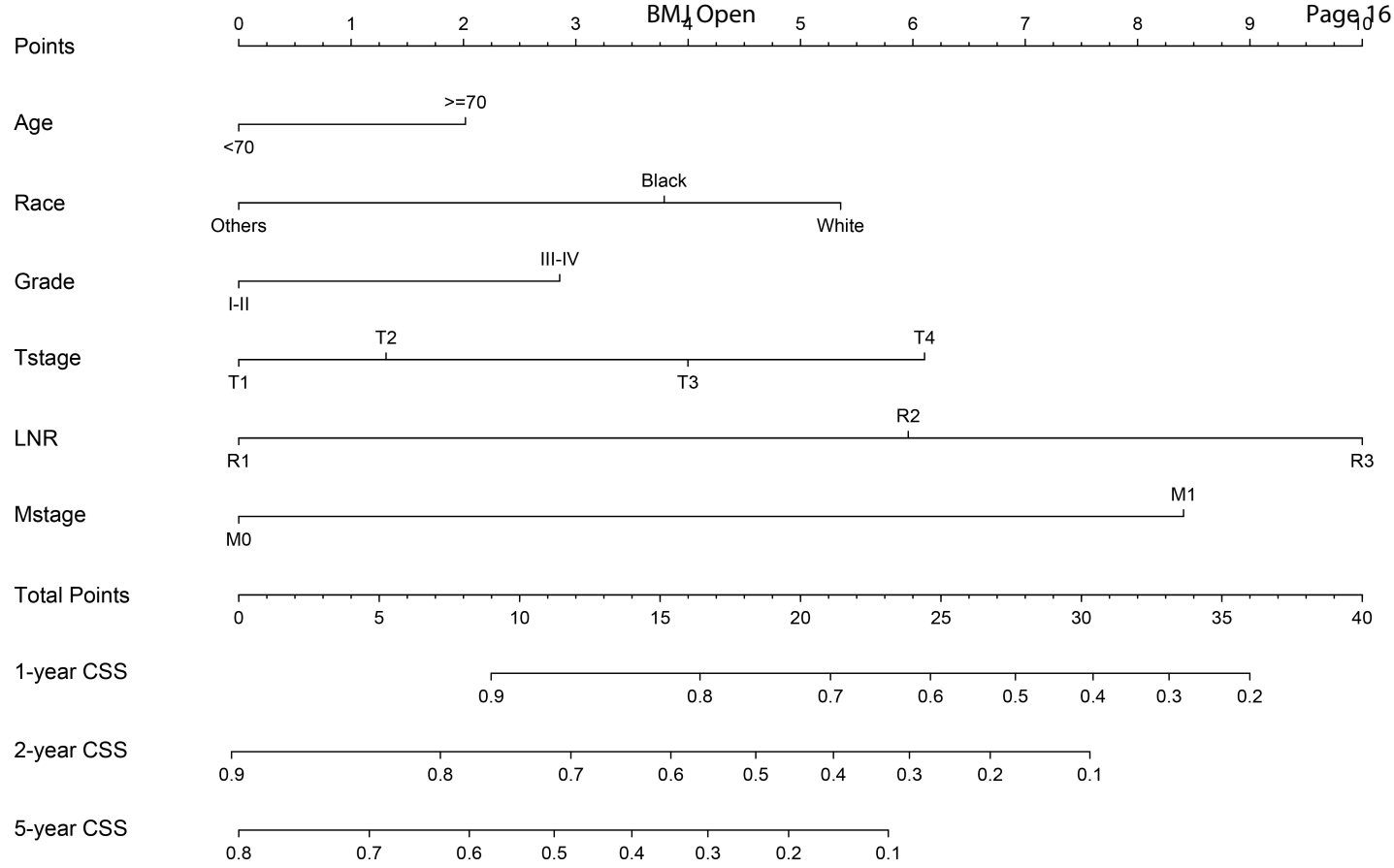
Validating Set



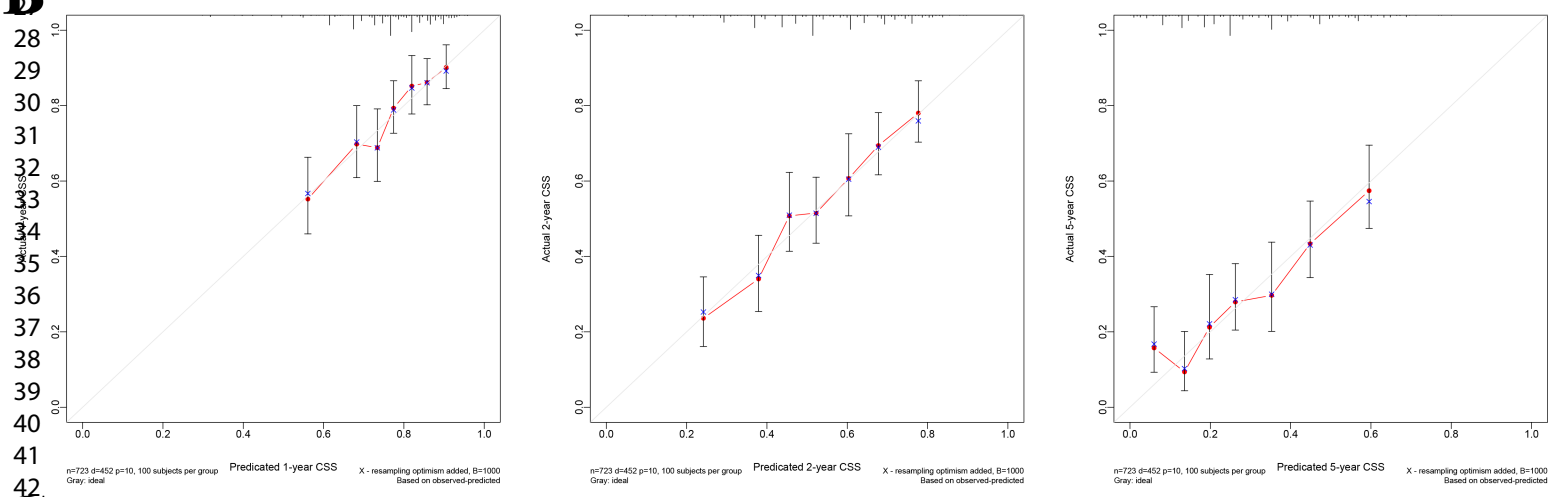




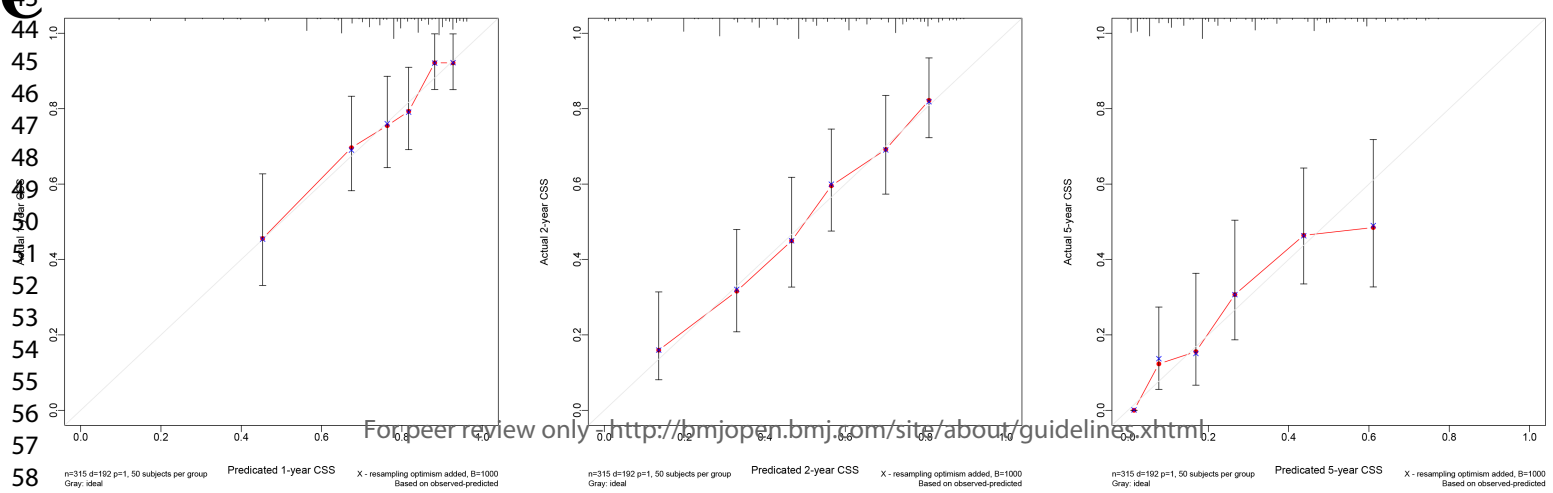
A



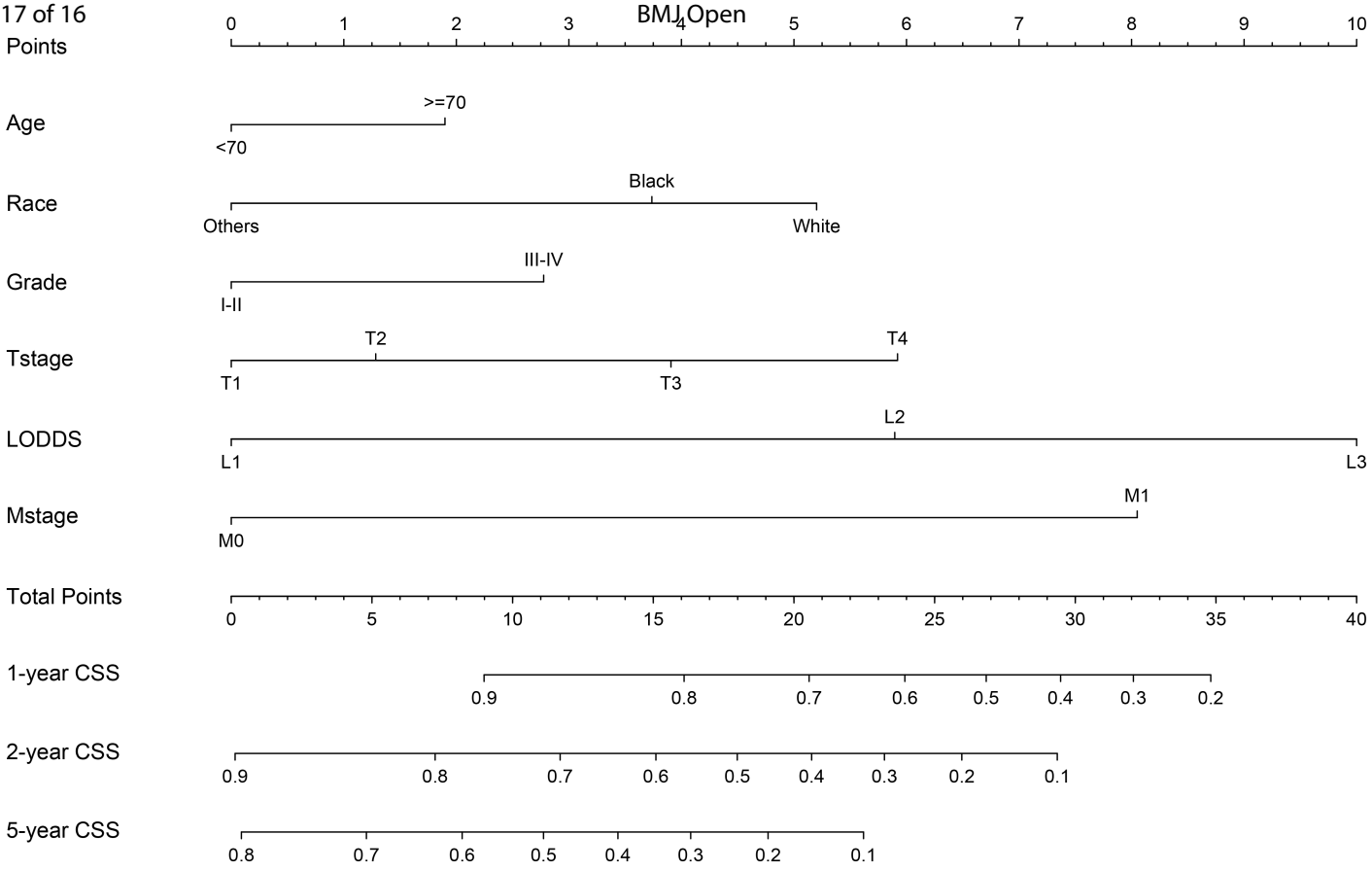
B



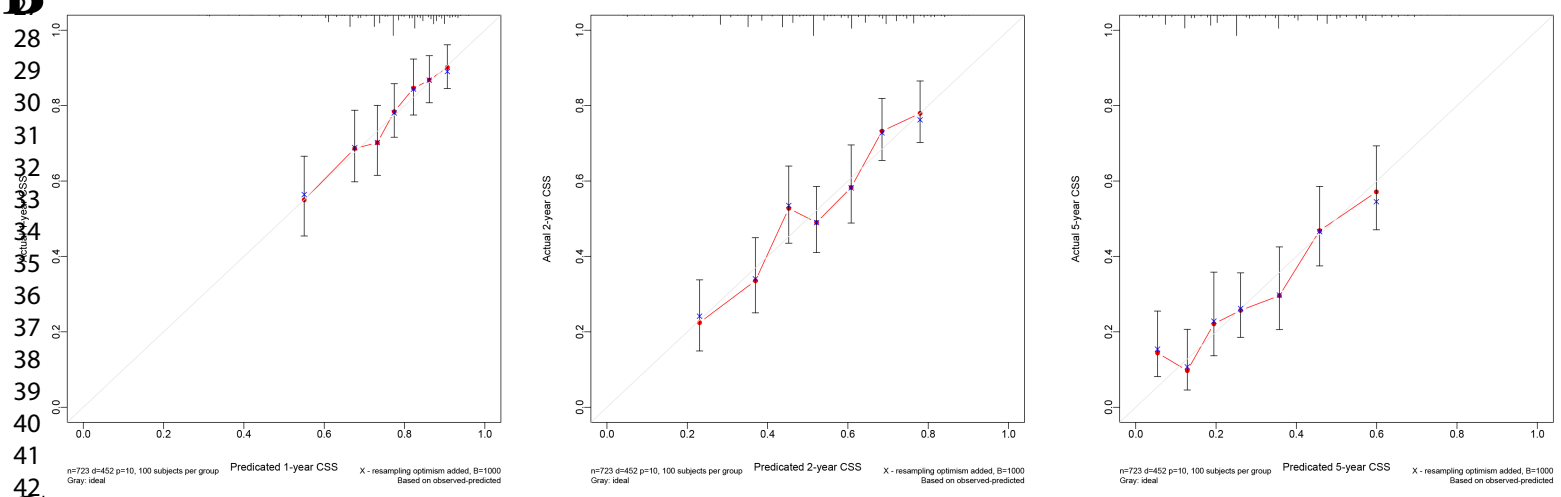
C



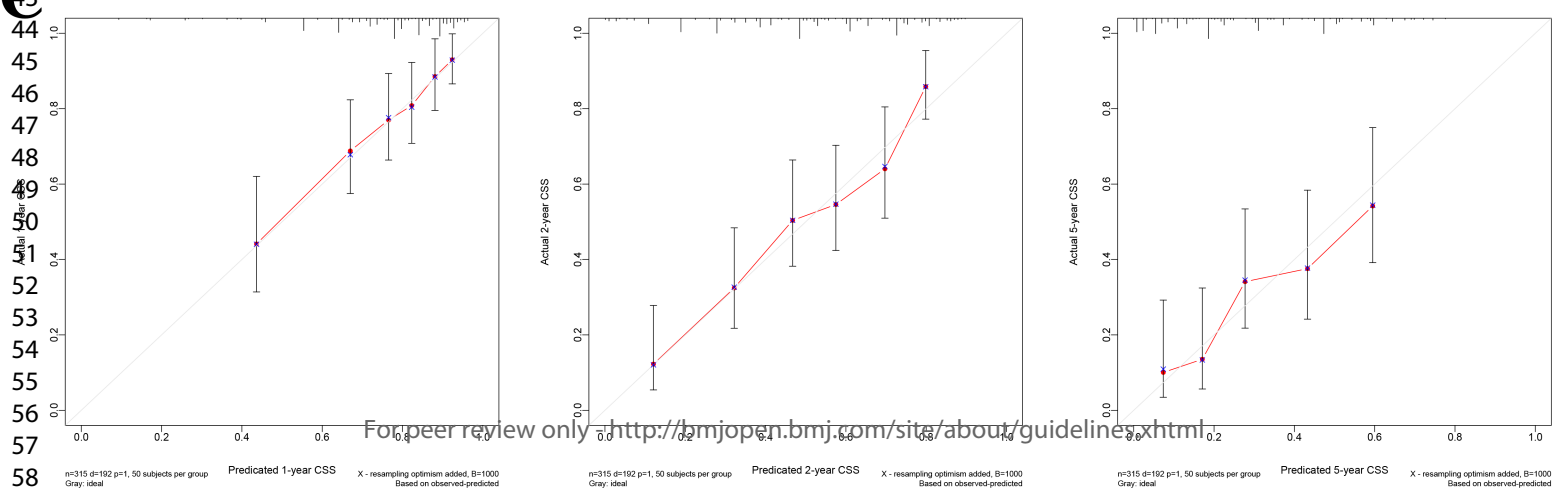
A



B



C



BMJ Open

Prognostic value of different lymph node staging methods for node positive cardia gastric cancer: a register-based retrospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-050378.R1
Article Type:	Original research
Date Submitted by the Author:	18-Jun-2021
Complete List of Authors:	Wang, Xiao-Qing; Anhui Medical College Bao, Min; Anhui Medical College Zhang, Cheng; First Affiliated Hospital of Anhui Medical University
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Gastroenterology and hepatology
Keywords:	Gastrointestinal tumours < ONCOLOGY, Gastrointestinal tumours < GASTROENTEROLOGY, SURGERY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title page

Article title: Prognostic value of different lymph node staging methods for node positive cardia gastric cancer: a register-based retrospective cohort study

Author information: Xiao-Qing Wang 1, Min Bao 1, Cheng Zhang 2

1 Anhui Medical College, Hefei, Anhui, China PR

2 Anhui Provincial Cancer Institute, the First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China PR

Corresponding to: Cheng Zhang, ahmuzc@sina.cn

Disclaimer: The views expressed in the submitted article are our own and not an official position of the institution or funder.

Word count: 3191 (excluding its abstract, acknowledgments, tables, figure legends, and references) abstract 281 .

Number of figures and tables: This draft included 3 figures and 2 tables.

Disclosure of relationships and activities: None.

Abstract

Objective: To investigate the prognostic efficacy of lymph node ratio (LNR) and log odds of positive lymph nodes (LODDS) in node positive cardia gastric adenocarcinoma (CGA).

Design: A register-based retrospective cohort study.

Participants: A total of 1 038 patients with node positive CGA were enrolled from SEER database, and randomly assigned (7:3) in training set (N = 723) or validating set (N = 315).

Interventions: The major endpoint was cancer specific survival (CSS). Optimal cut-off values were determined by X-tile software. The prognostic power was evaluated using Akaike Information Criterion (AIC) and Harrell concordance index (C-index). Cox stepwise regression analysis was performed to construct nomogram for prediction of 1-, 2-, and 5-year CSS. The prediction model was further evaluated by calibration curve, receiver operator characteristic (ROC) curve and decision curve analysis (DCA) plot.

Results: The training set and validating set are similar in terms of clinical and demographic features. The optimal cut-off values for LNR were 0.09 and 0.33, and for LODDS were -2.09 and -0.65. CSS was significantly different by N, LNR and LODDS categories. The C-index of N stage was lower than that of LNR or LODDS. The AIC of N stage was higher than that of LNR or LODDS. Independent predictors included race, T stage, M stage and LNR (or LODDS) and they were incorporated in nomograms for 1-, 2- and 5-year CSS prediction. Calibration plots showed satisfied results of internal and external validity of the nomogram.

Conclusions: LNR and LODDS staging methods have better prognostic efficacy than traditional N staging method in CGA patients with node metastasis. Besides, the two values are promising substitute for N staging in nomogram development when other independent prognostic factors are incorporated.

Key words: Cardia; Adenocarcinoma; Stomach Neoplasms; Lymph Node Ratio; Neoplasm Staging

Strengths and limitations of this study

- This study used national cancer registry data for cardia gastric adenocarcinoma research;
- Novel staging methods based on the number of positive lymph node was established for prognostic prediction;
- Nomograms based on the new staging methods were constructed and validated;
- This study needs to be confirmed by other populations.

Patient consent form: The SEER database review is granted exemption from obtaining patients' consents.

Word count: 3191 (excluding its abstract, acknowledgments, tables, figure legends, and references) abstract 281.

Introduction

Gastric cancer (GC) generally includes 2 topographical categories: non-cardia GC that arises from more distant sites and cardia GC that arises in gastroesophageal junction (GEJ). In contrast to steady decline of non-cardia GC incidence, cardia GC occurs more frequently, particular in high-income countries (1, 2). This trend is associated with obesity, gastroesophageal reflux disease (GERD), and Barrett esophagus (2). In addition to different incidence trend, clinicalpathological feature and long-term survival vary between the two GC subtypes (3). For accurate prediction of survival, precise staging is required. The Tumor-Node-Metastasis (TNM) classification 7th edition by the American Joint Committee on Cancer (AJCC) recommends at least 15 lymph nodes (LN) collection for N staging (4, 5). However inadequate LN harvest frequently occurs due to many conditions, thus precise staging cannot be obtained sometimes. It has been demonstrated that LN ratio (LNR) could better estimate survival of GC patients after curative gastrectomy, regardless of the number of LN examined (6), and may be promising for aiding TNM staging system (7). Apart from that, log odds of positive LN (LODDS) outperformed N and LNR staging system when predicting survival of GC patients (8-10). Therefore the traditional N staging classification may be substituted with different methods, with even improved performance. Nevertheless little evidence evaluates the performance of the two LN staging systems aforementioned in cardia GC, since it has distinct clinical characteristics and epidemiology from overall GC.

Here we use nationwide cancer registry data to appraise the prognostic value of LNR and LODDS in patients with node positive cardia gastric adenocarcinoma (CGA), and, if possible, construct nomogram for survival prediction based on the new LN staging system.

Methods

Study design and Participants Selection

This study is a SEER register-based retrospective cohort study, which aimed to enroll patients with node positive cardia gastric adenocarcinoma (CGA), review crucial clinical characteristics and observe survival of this population. The source of SEER data is registered cancer cases from various locations throughout the United States. The permission of data access was obtained by sending application form and receiving confirmation mail with valid username (21268-Nov2019) and password.

We used SEER*Stat (version 8.3.8) to access to Incidence - SEER Research Data, 18 Registries, Nov 2019 Sub 2000-2017 (SEER 18 database) (11) for collection of node positive CGA patients. The inclusion criteria were as follows: 1) the International Classification of Disease for Oncology, Third Edition (ICD-O-3) for primary tumor site was C16.0 (cardia); 2) broad histological recode was 8140-8389: adenomas and adenocarcinomas; 3) diagnostic confirmation was positive histology; 4) surgery was performed; 5) diagnosed during 2010-2015; 6) the definite number of regional positive nodes was clear and not zero. We excluded cases with unknown race, T stage information, tumor size and grade. As shown in Figure 1, the final cohort enrolled 1 038 patients with node positive CGA, of whom 857 were male and 181 were female. Three hundred and thirty eight (32.56%) were over 70 years old. Eight hundred and ninety six (86.32%) were white, 64 were black and 78 were other races. Next 70% of the entire patients were randomly assigned to

training set (N = 723) and the rest was assigned to validating set (N = 315).

Technical Information

The main outcome was cancer specific survival (CSS), which was referred to as death specifically due to CGA and the period between first diagnosis and death. In addition, we extracted the following variables for analysis: sex, race, age, AJCC 7th TNM stage information, tumor size, grade, number of regional nodes examined and number of regional nodes positive. The stage information was further corrected according to AJCC 8th criteria. LNR and LODDS were calculated as previously reported (12). Briefly, LNR was defined as the ratio of the number of positive nodes divided by the total number of examined nodes. LODDS was calculated using the formula: log(NPLN+0.50)/(NDLN−NPLN+0.50), in which 0.50 was added to both the numerator and denominator to avoid an infinite number.

The optimal thresholds for cutting LNR and LODDS into trichotomous variables were determined by X-tile software (version 3.6.1) (13), which were based on the maximal log-rank chi-square value that represented the greatest group difference of CSS probability. LNR and LODDS were cut into 3 levels because they are proposed as the alternative indicators for N stage in node positive GC that included N1, N2 and N3.

Statistics

The distributions of baseline features between training set and validating set were described and compared by chi-square test. Survival curves, median survival and CSS rates were generated using the Kaplan-Meier method. Outcome difference between groups was analyzed by the log-rank test. After testing proportional hazard assumption, multivariable Cox regression model was used to establish prognostic model for CSS. The prognostic power was evaluated using Akaike Information Criterion (AIC) and Harrell concordance index (C-index). A predictive model with lower AIC indicated better model fit, while with higher C-index indicated better discriminative ability. A value of C-index of 0.5 indicates no predictive power, and an index of 1.0 indicates complete differentiation. Cox stepwise regression analysis was also performed to construct nomogram for prediction of 1-, 2-, and 5-year CSS. Validation of nomogram was performed by internal and external calibration plots (14). Bootstraps with 1 000 resample were used for validation activities. Receiver operator characteristic (ROC) curves and areas under the ROC curves (AUCs) were calculated to evaluate how accurately the CSS was predicted by different models. Decision curve analysis (DCA) was performed to determine the clinical application of different model: the proportion of true positive results minus the proportion of false positive results, and then, the relative risks of false positive and false negative results were weighted to obtain the net benefits of decision-making. All statistical analyses were performed using R software (version 3.5.3). A two-tailed P value of less than 0.05 was considered statistically significant.

Patient and Public Involvement

The development of the research question and outcome measures were not informed by patients' priorities, experience, and preferences. The patients were involved during the retrospective review of public database where cases were diagnosed during 2010-2015. Patients were not involved in the recruitment to and conduct of the study. The findings of the study will be disseminated by

online article to all study participants whose identity kept confidential during the whole research.

Ethics approval statement

The Ethics committee(s) and IRB name: the Ethics Committee of Anhui Medical College; Reason for exemption: The observational nature of the study

Results

Table 1 summarized the demographic and clinical feature of the participants. Six hundred and twenty eight patients (60.50%) were diagnosed with a tumor less than 5cm. Six hundred and forty patients (61.66%) were with grade III or IV. The numbers of patients with T1, T2, T3 and T4 respectively were 94, 125, 717 and 102. The numbers of patients with N1, N2 and N3 respectively were 479, 330 and 229. Seventy five patients (7.23%) were with distant metastasis at presentation. The median CSS was 27 months. The 1-, 2- and 5-year CSS rates were 76.8%, 53.0% and 29.2%, respectively. There was no statistical difference of baseline characteristics between training set and validating set. The detailed information of the two sets was also presented in Table 1.

Table 1. Baseline information of the included patients with node positive CGA, N(%).

Groups	Training set (N = 723)	Validating set (N = 315)	P-value
Sex			
Male	596 (82.43)	261 (82.86)	0.939
Female	127 (17.57)	54 (17.14)	
Age			
<70	490 (67.77)	210 (66.67)	0.781
≥70	233 (32.23)	105 (33.33)	
Race			
White	628 (86.86)	268 (85.08)	
Black	40 (5.53)	24 (7.62)	0.437
Others	55 (7.61)	23 (7.30)	
Tumor size			
<5cm	442 (61.13)	186 (59.05)	0.573
≥5cm	281 (38.87)	129 (40.95)	
Grade			
I-II	279 (38.59)	119 (37.78)	0.859
III-IV	444 (61.41)	196 (62.22)	
T stage			
T1a	17 (2.35)	4 (1.27)	
T1b	53 (7.33)	20 (6.35)	
T2	83 (11.48)	42 (13.33)	0.224
T3	501 (69.29)	216 (68.57)	
T4a	49 (6.78)	28 (8.89)	
T4b	20 (2.77)	5 (1.59)	
N stage			
N1	332 (45.92)	147 (46.67)	0.921

N2	229 (31.67)	101 (32.06)	
N3	162 (22.41)	67 (21.27)	
M stage			
M0	678 (93.78)	285 (90.48)	0.079
M1	45 (6.22)	30 (9.52)	
Low nodes yield			
Yes	532 (73.58)	243 (77.14)	0.300
No	191 (26.42)	72 (22.86)	
No. of nodes harvest	17 (12, 25)	16 (11, 24)	0.400
No. of positive nodes	3 (1, 6)	3 (1, 6)	1.000
Median survival (months)	28 (25, 32)	25 (21, 32)	0.361
CSS rate (%)			
1-year	77.0 (74.0, 80.2)	76.3 (71.6, 81.2)	
2-year	53.7 (50.1, 57.5)	51.4 (46.0, 57.5)	
5-year	30.3 (26.7, 34.5)	26.4 (20.9, 33.4)	

Abbreviation: CGA, cardia gastric adenocarcinoma; CSS, cancer-specific survival

According to X-tile software results, the optimal cut-off values for LNR were 0.09 and 0.33, and for LODDS were -2.09 and -0.65. Thus patients were separated into low (R1), medium (R2) or high LNR (R3) group, or low (L1), medium (L2) or high LODDS (L3) group. For model optimization, LNR and LODDS were also categorized into trichotomous factors using cut-off values of P_{25} and P_{75}. The discrimination ability of the model based on interquartile was lower (Suppl. Table 1), so this model was not further analyzed. Next we illustrated the survival curves of the patients according to N, LNR or LODDS staging system. As shown in Figure 2 training set section, CSS was significantly different by all the three staging systems (all the log-rank P values < 0.0001); however the 95% CIs of N2 and N3 survival curve initially separated and partly overlapped afterwards. The inferior discriminative ability of N system was further supported by AIC and C-index. As shown in Table 2, the C-index of N stage was lower than that of LNR or LODDS. Similarly, the AIC of N stage was higher than that of LNR or LODDS. The clinical characteristics with statistical significance for CSS were further incorporated in the Cox regression model as potential confounders (Suppl. Table 2), and all the variables met proportional hazard assumption (Suppl. Figure 1). The prognostic value of adjusted model was better than crude mode generally. In addition, the value of LNR system seemed to be worse than LODDS system; however the difference was not noticeable, so we considered both of the systems into nomogram construction.

Table 2. Prognostic values of variables for patients with node positive CGA (N = 1 038).

Variables	Crude model			Adjusted model		
	HR (95% CI)	C-index	AIC	HR (95% CI)	C-index	AIC
Training set (N = 723)						
N stage		0.582	5403		0.632	5365
N1	1 (ref)			1 (ref)		
N2	1.53 (1.24, 1.91)			1.42 (1.14, 1.77)		

N3	2.15 (1.70, 2.71)			2.03 (1.60, 2.59)		
LNR*		0.607	5376		0.643	5350
R1	1 (ref)			1 (ref)		
R2	1.88 (1.44, 2.44)			1.74 (1.33, 2.29)		
R3	3.02 (2.30, 3.97)			2.63 (1.97, 3.50)		
LODDS*		0.609	5373		0.644	5346
L1	1 (ref)			1 (ref)		
L2	1.93 (1.48, 2.51)			1.80 (1.36, 2.37)		
L3	3.13 (2.38, 4.13)			2.77 (2.07, 3.70)		
Validating set (N = 315)						
N stage		0.596	1957		0.675	1931
N1	1 (ref)			1 (ref)		
N2	1.81 (1.31, 2.51)			1.75 (1.25, 2.46)		
N3	2.18 (1.51, 3.15)			2.23 (1.50, 3.30)		
LNR*		0.646	1927		0.691	1913
R1	1 (ref)			1 (ref)		
R2	2.20 (1.47, 3.30)			1.91 (1.26, 2.90)		
R3	4.16 (2.76, 6.28)			3.58 (2.30, 5.56)		
LODDS*		0.647	1927		0.789	1914
L1	1 (ref)			1 (ref)		
L2	2.07 (1.39, 3.09)			2.08 (1.38, 3.14)		
L3	4.22 (2.79, 6.39)			4.10 (2.65, 6.34)		

Abbreviations: CGA, cardia gastric adenocarcinoma; HR, hazard ratio; CI, confidence interval; AIC, Akaike information criterion; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes.

Adjusted model considered race, tumor size, grade, T stage and M stage.

* cut-off values for LNR were 0.09 and 0.33, and for LODDS were -2.09 and -0.65.

Stepwise Cox regression analysis showed race, tumor grade, low nodes yield, T stage, M stage and LNR (or LODDS) were independent predictors, so these factors were included in nomograms. For both LNR and LODDS, the total score was 40, and higher score suggested lower survival (Figure 3 and Suppl. Figure 2). Next calibration plot was used to assess the internal and external validity of the nomogram (Figure 3 and Suppl. Figure 2). Since the cross-spot line was generally close to the grey reference line, we concluded the predicted CSS was well correlated with the actual situation. In addition, ROC curves indicated that the AUC of the model based on N stage was lower than that of the model based on the nomogram of LNR or LODDS (Suppl. Figure 3). DCA plot also showed that the nomogram model was superior to traditional model (Suppl. Figure 3).

Discussion

The present study analyzes national cancer registry databases and demonstrates that survival of patients with node-positive CGA is well predicted when the traditional N staging method is substituted with LNR or LODDS system. This finding both exists in training and validating sets. In training set, the survival curves separate clearly when patient grouping is implemented by LNR or LODDS method, which is not achieved by traditional N staging system. Adjusted model that

simultaneously considers staging, clinical and demographic features outperforms crude model that only takes staging into account. Therefore multiple independent survival factors are incorporated in nomogram construction, which suggests older age at diagnosis, white, higher grade, greater tumor infiltration, higher proportion of positive LN, and metastasis as risk factors. The nomograms perform steadily in 1-, 2- and 5-year CSS prediction as the validation plots show.

Previous studies have demonstrated the superiority of LNR or LODDS for prognostic prediction in GC after surgical resection (8-10, 15-17). However the GC patients are not further separated and investigated according to primary tumor site, since there is much difference between cardia and non-cardia GC in terms of tumor features, etiological factors, and biological behaviors (3). In AJCC cancer staging 7th edition, tumors involving EGJ was categorized as esophagus cancer (5), which was however argued by the viewpoint that GC staging system has a better ability to predict survival of EGJ tumor (18, 19). In the latest 8th edition (20), a tumor that has its epicenter within 2 cm of EGJ and involves the EGJ (Siewert type I/II) is classified as esophageal cancer. Other situation, including a tumor with epicenter more than 2 cm from EGJ or a tumor located with 2 cm of EGJ but does not involve EGJ, is classified as stomach cancer. The superiority of the new system is confirmed by a retrospective observational study from two high-volume institutions in China, regardless of Siewert type (21). In terms of Siewert type II junctional adenocarcinoma, a marginal advantage of the esophagus cancer system is found in discriminating survival rates after 3 and 5 years, however the advantage of GC system lies in division of the N3 category into N3a and N3b, so the authors concludes neither the esophageal nor the stomach staging system is flawless in predicting survival in Siewert type II junctional cancer (22). Above all, CGA is probably a special entity that has a different biological property compared with genuine gastric and genuine esophageal cancer. To the best of our knowledge, the present study first reveals a superior performance of prognostic prediction based on LNR or LODDS in node positive CGA patients. Unfortunately we are unable to consider Siewert type due to unavailable information from SEER database; therefore we encourage further studies to pay special attention on tumor location.

LNR and LODDS have been proved to be the strongest indictors of survival in gastric adenocarcinoma when LN harvest is inadequate (16, 17). It is demonstrated that in general, more LN resection is associated with better survival, which may be the result of either improved N classification or a therapeutic effect of lymphadenectomy. For esophageal cancer, worldwide data shows that yielding 10 nodes for pT1, 20 for pT2, and 30 or more for pT3/T4 is recommended for maximum 5-year survival (23). For GC, greater LN harvest also shows improved survival (24). It is suggested that at least 16 nodes be assessed pathologically and evaluation of more than 30 nodes is desirable (25). Overall it is encouraged to harvest as many LN as possible, balancing the extent of LN resection necessary for accurate N staging and maximum survival without unnecessarily increasing the morbidity of radical lymphadenectomy. Nevertheless, many conditions would lead to insufficient LN harvest. It is estimated that only one fifth GC patients have sufficient LN examined in Iran (26), while more than 15 LNs are examined in 64% of patients in the US (25). The LNR and LODDS staging methods do not require adequate number of LN assessment. In the present study, low nodes yield is a risk factor for poor survival in univariate analysis; however it loses significance in LNR or LODDS based multivariate model, which indicates that it probably exerts little impact with consideration of LNR or LODDS. In fact, the

new node category method is stable when nodal assessment is insufficient during surgery not only for GC (8, 15-17) but also for colorectal cancer (27), esophageal cancer (28), oral squamous cell carcinoma (29), gallbladder cancer (30), etc.

The association between LNR and survival is an exciting aspect of cardia GC that is currently emerging and may be clinically meaningful. The higher ratio of positive LN indicates worse outcome in cardia GC. Patients are at 2-3 folds higher risk of cancer specific death if the ratio is over 33%. The ratio of 9-33% also indicates a double risk. This effect is independent of other crucial clinical characteristics, thus providing a useful tool for surgeons to predict the prognosis, and to be taken as evidence for the surgeon to tend towards truly radical, i.e., complete lymph node clearance rather than limited clearance (31). In addition, LNR minimizes the "stage migration" phenomenon that can be observed using the current N staging system (32).

One limitation of this study is that some important factors that are associated with survival are not considered in the model due to unavailable data source. For example, ECOG/KPS score is commonly taken into account in survival analysis due to its remarkable relationship with general status and prognosis. Unfortunately the SEER 18 database does not record the score at diagnosis, so the impact of it is not considered in this analysis. Treatment mode is also associated with clinical outcome. This study enrolled patients who received gastric resection; however other information about chemo- or radiotherapy is not available in SEER 18 database. Randomized clinical trial demonstrates that compared with surgery alone, preoperative administration of carboplatin and paclitaxel with concurrent radiotherapy significantly improved overall survival among patients with esophageal or GEJ cancer (HR = 0.657) (33). The NCCN clinical practice guidelines for GEJ cancer recommend preoperative chemoradiation or perioperative chemotherapy due to substantial survival benefit compared with surgery alone (34). To overcome this limitation, a database that provides with fully detailed medical records is needed for analysis. In addition, consideration of the potential factors aforementioned would greatly improve prognostic power of survival prediction model. Another limitation is that our results are based on training set and confirmed by validating set; however the features of the two groups are similar. So this finding needs to be proved among populations with distinct features.

In conclusion, LNR and LODDS staging methods have better prognostic efficacy than traditional N staging method in CGA patients with regional node metastasis. Besides, the two values are promising substitute for N staging in nomogram development when other independent prognostic factors are incorporated.

a. **Contributorship statement:** WXQ work conception, data interpretation, critical review for important content, final approval of the manuscript and agreement to be accountable for all aspects of the work; BM administrative work, funding, critical review for important content, final approval of the manuscript and agreement to be accountable for all aspects of the work; ZC acquisition, analysis of data, drafting the work, final approval of the manuscript and agreement to be accountable for all aspects of the work.
b. Competing interests: None.
c. **Funding:** This work was supported by [Major Project of Natural Science of Anhui Provincial Department of Education] grant number [KJ2019ZD73] and [Domestic Visiting Study Project for Outstanding Young Talents in Colleges and Universities in Anhui Province] grant number

[2021].

d. Data sharing statement: Dataset available from the SEER website (https://seer.cancer.gov/data-software/).

Acknowledgement: We thank all patient advisers for their special contribution.

References:

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. [Journal Article]. 2018 2018-11-01;68(6):394-424.
2. Abdi E, Latifi-Navid S, Zahri S, Yazdanbod A, Pourfarzi F. Risk factors predisposing to cardia gastric adenocarcinoma: Insights and new perspectives. Cancer Med. [Journal Article; Research Support, Non-U.S. Gov't; Review]. 2019 2019-10-01;8(13):6114-26.
3. Amini N, Spolverato G, Kim Y, Squires MH, Poultsides GA, Fields R, et al. Clinicopathological features and prognosis of gastric cardia adenocarcinoma: a multi-institutional US study. J SURG ONCOL. [Comparative Study; Journal Article; Multicenter Study]. 2015 2015-03-01;111(3):285-92.
4. Sobin L, Gospodarowicz M, Wittekind C. TNM classification of malignant tumours. 7th edition.: Wiley-Blackwell; 2009.
5. Edge S, Byrd D, Compton C, Fritz A, Greene F, Trotti A. AJCC cancer staging manual. 7th edition.: Springer; 2009.
6. Hou Y, Wang X, Chen J. Prognostic significance of metastatic lymph node ratio: the lymph node ratio could be a prognostic indicator for patients with gastric cancer. WORLD J SURG ONCOL. [Journal Article]. 2018 2018-10-04;16(1):198.
7. Zhu J, Xue Z, Zhang S, Guo X, Zhai L, Shang S, et al. Integrated analysis of the prognostic role of the lymph node ratio in node-positive gastric cancer: A meta-analysis. INT J SURG. [Journal Article; Meta-Analysis; Review]. 2018 2018-09-01;57:76-83.
8. Sun Z, Xu Y, Li DM, Wang ZN, Zhu GL, Huang BJ, et al. Log odds of positive lymph nodes: a novel prognostic indicator superior to the number-based and the ratio-based N category for gastric cancer patients with R0 resection. CANCER-AM CANCER SOC. [Journal Article; Research Support, Non-U.S. Gov't]. 2010 2010-06-01;116(11):2571-80.
9. Zhao E, Zhou C, Chen S. Prognostic nomogram based on log odds of positive lymph nodes for gastric carcinoma patients after surgical resection. FUTURE ONCOL. [Journal Article]. 2019 2019-12-01;15(36):4207-22.
10. Gu P, Deng J, Sun Z, Wang Z, Wang W, Liang H, et al. Superiority of log odds of positive lymph nodes (LODDS) for prognostic prediction after gastric cancer surgery: a multi-institutional analysis of 7620 patients in China. SURG TODAY. [Journal Article]. 2021 2021-01-01;51(1):101-10.
11. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence - SEER Research Data, 18 Registries, Nov 2019 Sub (2000-2017) - Linked To County Attributes - Time Dependent (1990-2017) Income/Rurality, 1969-2018 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, released April 2020, based on the November 2019 submission..
12. Yu Y, Zhang P, Yao R, Wang J, Wang P, Xue X, et al. Prognostic value of log odds of positive lymph nodes in node-positive lung squamous cell carcinoma patients after surgery: a SEER

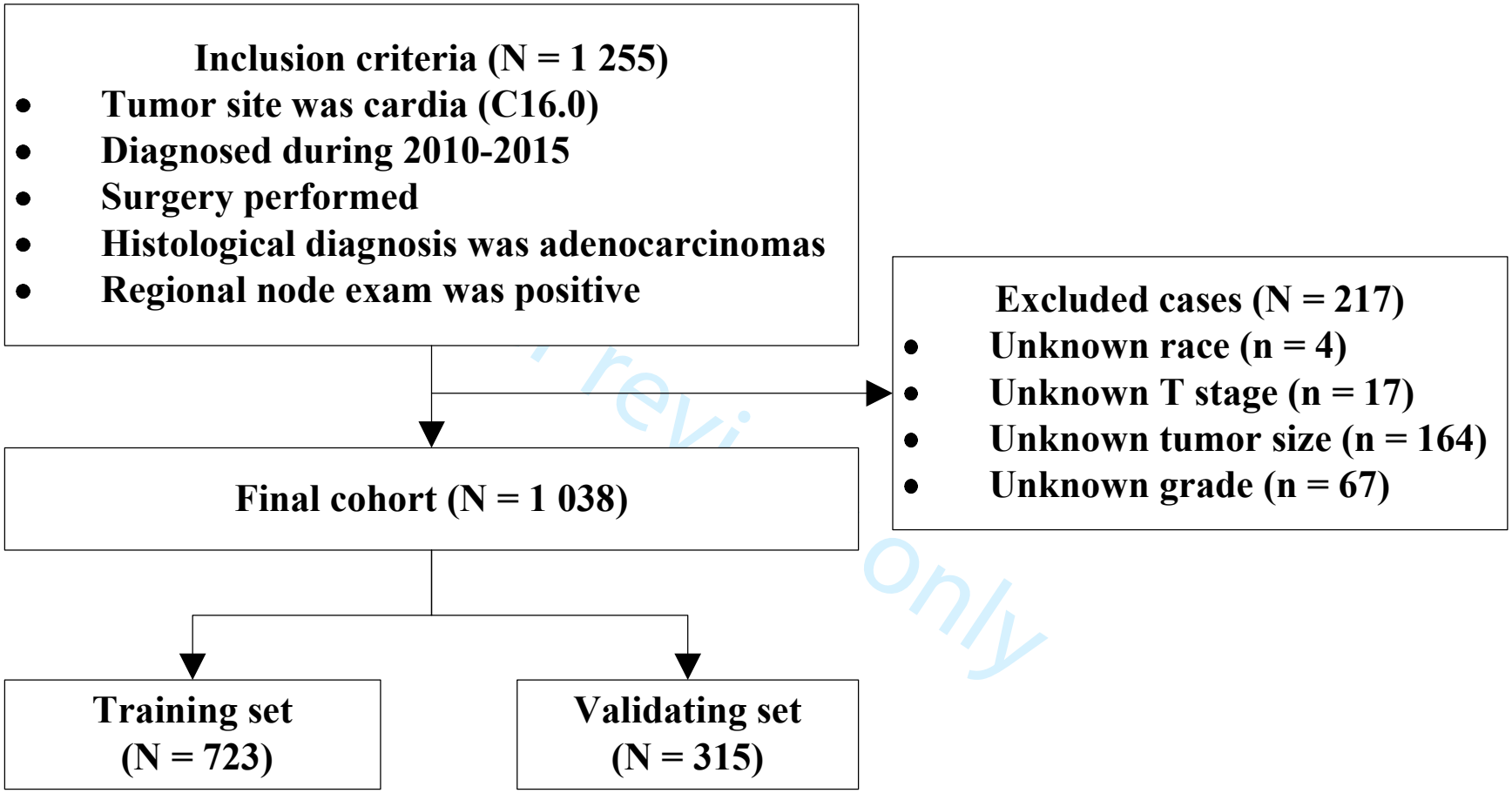
population-based study. Transl Lung Cancer Res. [Journal Article]. 2020 2020-08-01;9(4):1285-301.
13. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. CLIN CANCER RES. [Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.]. 2004 2004-11-01;10(21):7252-9.
14. Iasonos A, Schrag D, Raj GV, Panageas KS. How to build and interpret a nomogram for cancer prognosis. J CLIN ONCOL. [Journal Article; Review]. 2008 2008-03-10;26(8):1364-70.
15. Jian-Hui C, Shi-Rong C, Hui W, Si-le C, Jian-Bo X, Er-Tao Z, et al. Prognostic value of three different lymph node staging systems in the survival of patients with gastric cancer following D2 lymphadenectomy. Tumour Biol. [Journal Article]. 2016 2016-08-01;37(8):11105-13.
16. Aurello P, Petrucciani N, Nigri GR, La Torre M, Magistri P, Tierno S, et al. Log odds of positive lymph nodes (LODDS): what are their role in the prognostic assessment of gastric adenocarcinoma? J GASTROINTEST SURG. [Comparative Study; Journal Article]. 2014 2014-07-01;18(7):1254-60.
17. Kilic MO, Gundogdu SB, Ozden S, Saylam B, Tez M. The prognostic value of different node staging systems in patients with </=15 lymph nodes following surgery for gastric adenocarcinoma. ACTA CHIR BELG. [Journal Article]. 2018 2018-02-01;118(1):1-6.
18. Suh YS, Han DS, Kong SH, Lee HJ, Kim YT, Kim WH, et al. Should adenocarcinoma of the esophagogastric junction be classified as esophageal cancer? A comparative analysis according to the seventh AJCC TNM classification. ANN SURG. [Comparative Study; Journal Article; Research Support, Non-U.S. Gov't]. 2012 2012-05-01;255(5):908-15.
19. Huang Q, Shi J, Feng A, Fan X, Zhang L, Mashimo H, et al. Gastric cardiac carcinomas involving the esophagus are more adequately staged as gastric cancers by the 7th edition of the American Joint Commission on Cancer Staging System. Mod Pathol. [Journal Article; Research Support, Non-U.S. Gov't; Validation Study]. 2011 2011-01-01;24(1):138-46.
20. Amin M, Edge S, Greene F, Byrd D, Brookland R. AJCC cancer staging manual. 8th edition.: Springer; 2016.
21. Liu K, Feng F, Chen XZ, Zhou XY, Zhang JY, Chen XL, et al. Comparison between gastric and esophageal classification system among adenocarcinomas of esophagogastric junction according to AJCC 8th edition: a retrospective observational study from two high-volume institutions in China. GASTRIC CANCER. [Comparative Study; Journal Article; Observational Study]. 2019 2019-05-01;22(3):506-17.
22. Karstens KF, Ghadban T, Sawez S, Konczalla L, Woestemeier A, Bachmann K, et al. Comparison of the 8th UICC staging system for esophageal and gastric cancers in Siewert type II junctional adenocarcinomas. Eur J Surg Oncol. [Comparative Study; Journal Article]. 2020 2020-04-01;46(4 Pt A):638-43.
23. Rizk NP, Ishwaran H, Rice TW, Chen LQ, Schipper PH, Kesler KA, et al. Optimum lymphadenectomy for esophageal cancer. ANN SURG. [Journal Article; Research Support, Non-U.S. Gov't]. 2010 2010-01-01;251(1):46-50.
24. Macalindong SS, Kim KH, Nam BH, Ryu KW, Kubo N, Kim JY, et al. Effect of total number of harvested lymph nodes on survival outcomes after curative resection for gastric adenocarcinoma: findings from an eastern high-volume gastric cancer center. BMC CANCER. [Journal Article; Research Support, Non-U.S. Gov't]. 2018 2018-01-12;18(1):73.
25. Schoenleber SJ, Schnelldorfer T, Wood CM, Qin R, Sarr MG, Donohue JH. Factors influencing lymph node recovery from the operative specimen after gastrectomy for gastric adenocarcinoma. J

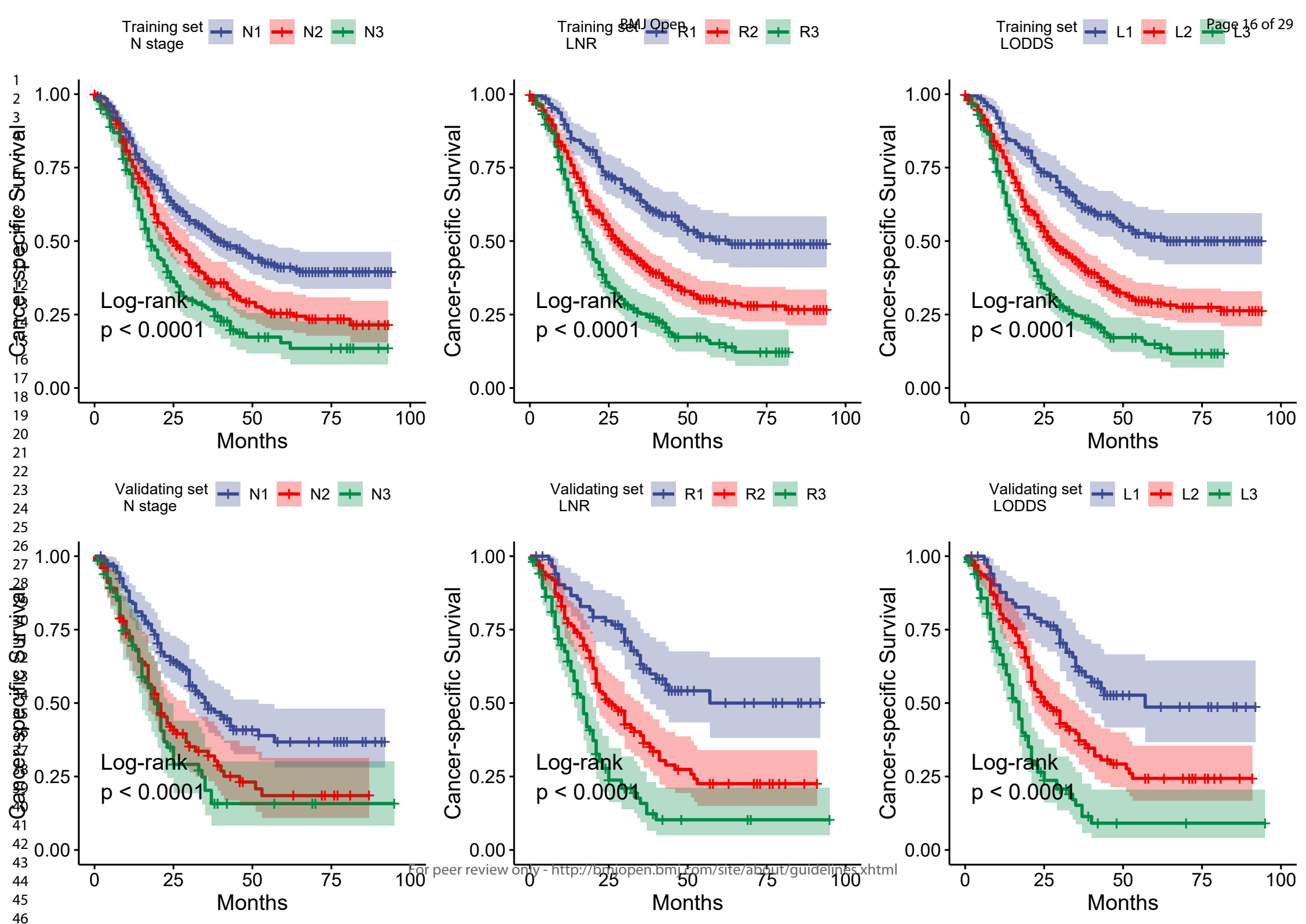
GASTROINTEST SURG. [Journal Article]. 2009 2009-07-01;13(7):1233-7.
26. Khanjani N, Mirzaei S, Nasrolahi H, Hamedi SH, Mosalaei A, Omidvari S, et al. Insufficient lymph node assessment in gastric adenocarcinoma. J Egypt Natl Canc Inst. [Journal Article]. 2019 2019-10-22;31(1):2.
27. Pei JP, Zhang CD, Fan YC, Dai DQ. Comparison of Different Lymph Node Staging Systems in Patients With Resectable Colorectal Cancer. FRONT ONCOL. [Journal Article]. 2018 2018-01-20;8:671.
28. Cao J, Yuan P, Ma H, Ye P, Wang Y, Yuan X, et al. Log Odds of Positive Lymph Nodes Predicts Survival in Patients After Resection for Esophageal Cancer. ANN THORAC SURG. [Journal Article; Multicenter Study]. 2016 2016-08-01;102(2):424-32.
29. Jin W, Zhu Z, Wu Y, Ding X, Wu H, Song X, et al. Prognostic value of log odds of positive lymph nodes in patients with resectable oral squamous cell carcinoma. ORAL ONCOL. [Journal Article; Research Support, Non-U.S. Gov't]. 2020 2020-09-01;108:104709.
30. Li J, Sun Y, Zhao B, Tang C, Fan D, Jiang W, et al. Lymph Node Ratio-Based Staging System for Gallbladder Cancer With Fewer Than Six Lymph Nodes Examined. FRONT ONCOL. [Journal Article]. 2020 2020-01-20;10:542005.
31. Spillane AJ, Cheung BL, Winstanley J, Thompson JF. Lymph node ratio provides prognostic information in addition to american joint committee on cancer N stage in patients with melanoma, even if quality of surgery is standardized. ANN SURG. [Journal Article]. 2011 2011-01-01;253(1):109-15.
32. Rosa F, Tortorelli AP, Alfieri S, Doglietto GB. Lymph node ratio for gastric cancer: useful instrument or just an expedient to retrieve fewer lymph nodes? ANN SURG. [Letter; Comment]. 2014 2014-04-01;259(4):e65.
33. van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge HM, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med. [Journal Article; Multicenter Study; Randomized Controlled Trial; Research Support, Non-U.S. Gov't]. 2012 2012-05-31;366(22):2074-84.
34. Ajani JA, D'Amico TA, Bentrem DJ, Chao J, Corvera C, Das P, et al. Esophageal and Esophagogastric Junction Cancers, Version 2.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. [Journal Article]. 2019 2019-07-01;17(7):855-83.

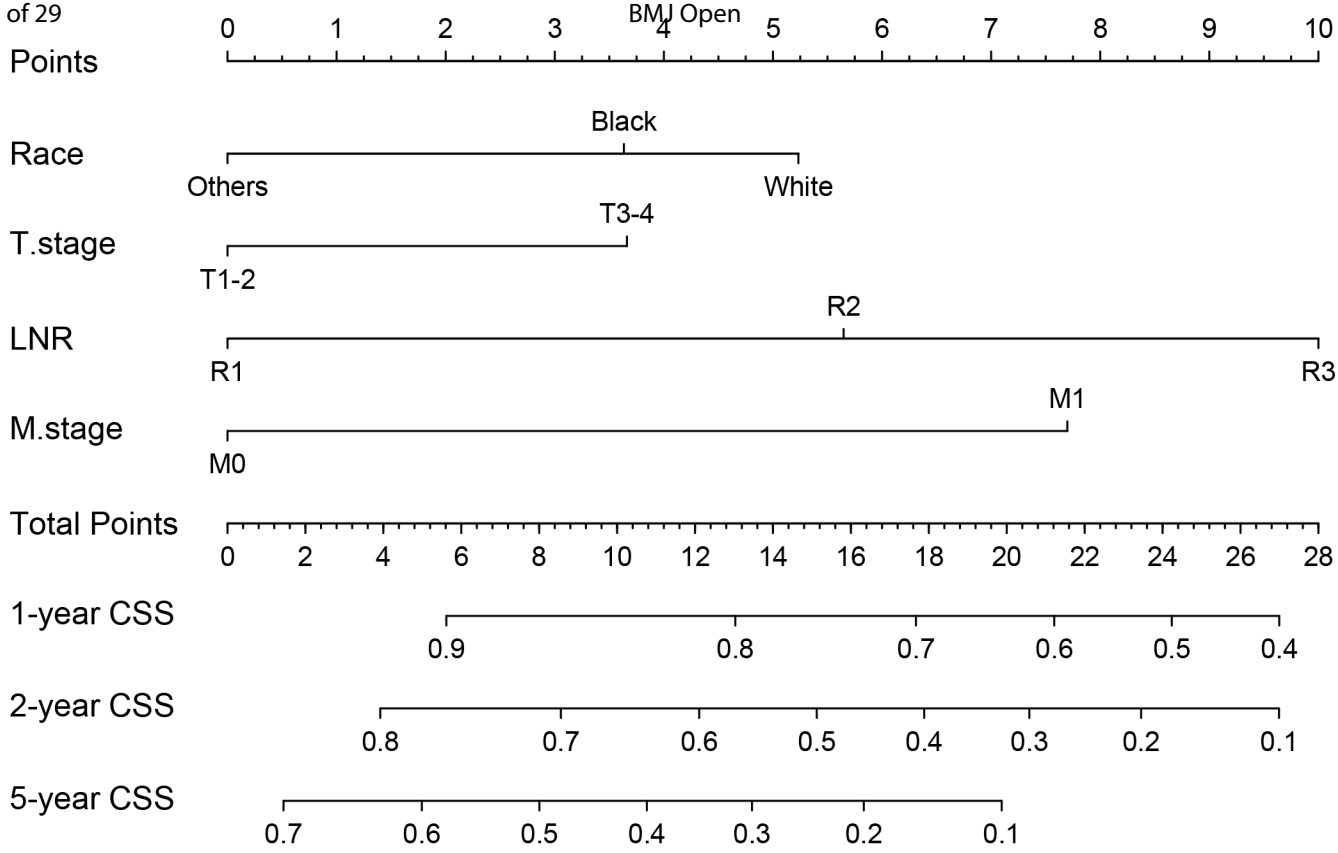
Illustrations

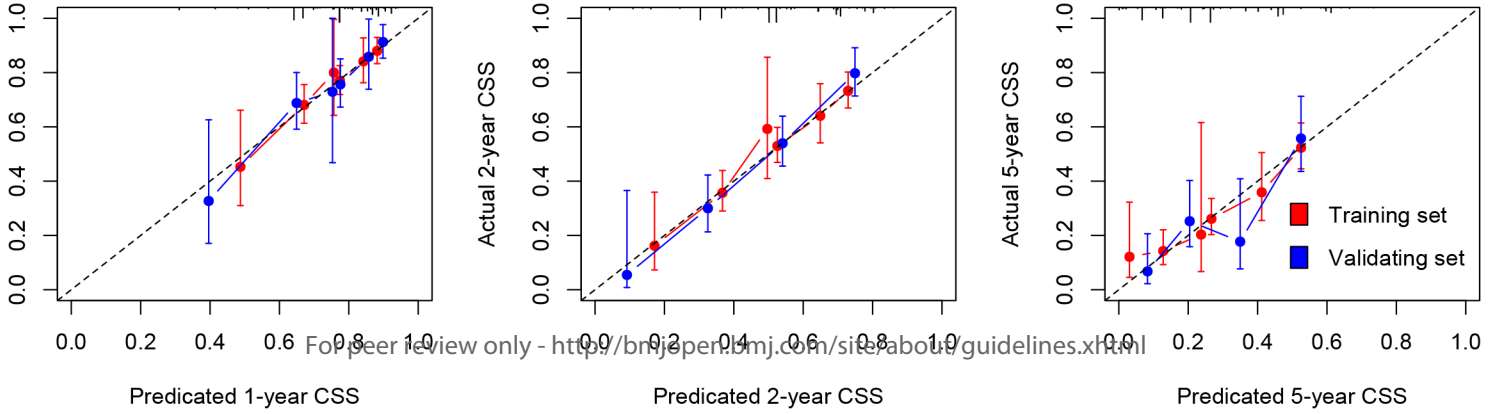
Figure 1. Flow diagram of patient selection and grouping.
Figure 2. Survival curves of training and validating sets by different staging systems.
Figure 3. Construction of nomogram based on Tumor-Lymph node ratio-Metastasis stating system and calibration plots for the nomogram.

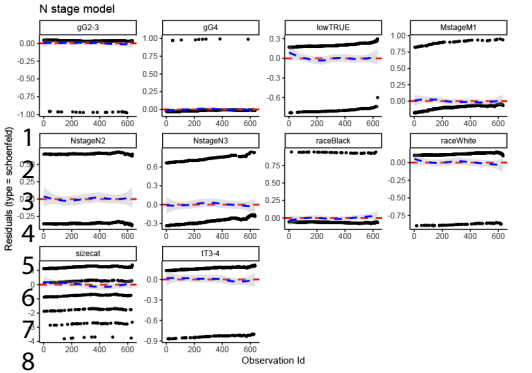
Suppl. Figure 1. Plots of Schoenfeld, Martingale, and Deviance residuals for proportional hazard assumption test in models that incorporate N stage, lymph node ratio and log odds of positive lymph nodes.
Suppl. Figure 2. Construction of nomogram based on Tumor-Log odds of positive lymph nodes -Metastasis stating system and calibration plots for the nomogram.
Suppl. Figure 3. Receiver operator characteristic (ROC) curves and decision curve analysis (DCA) plots for comparison of the prediction powers of the different models.

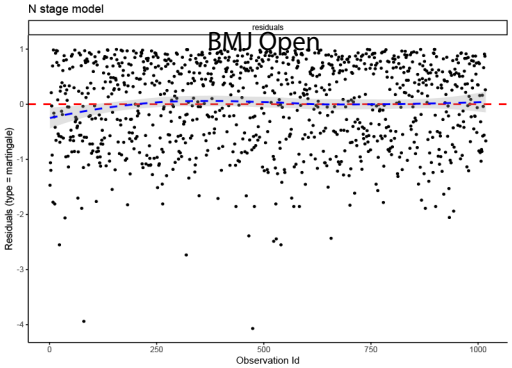


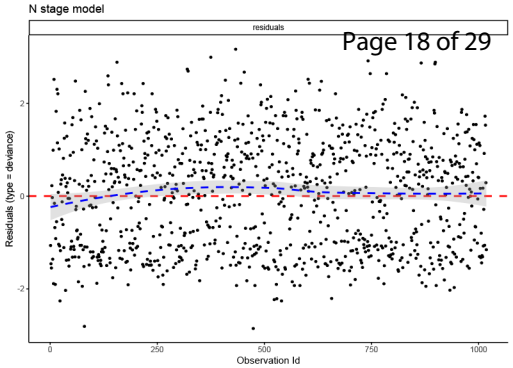


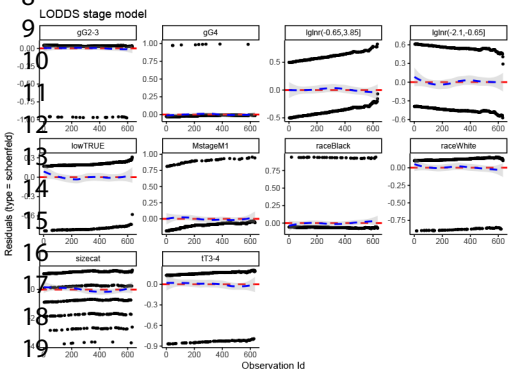


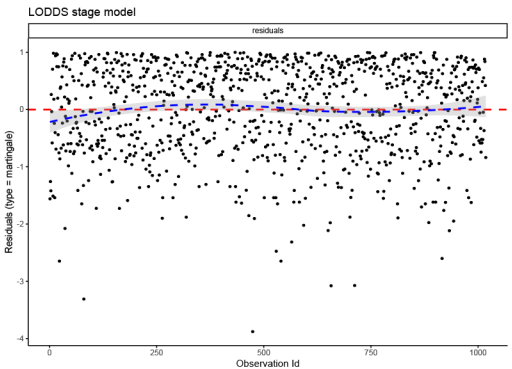


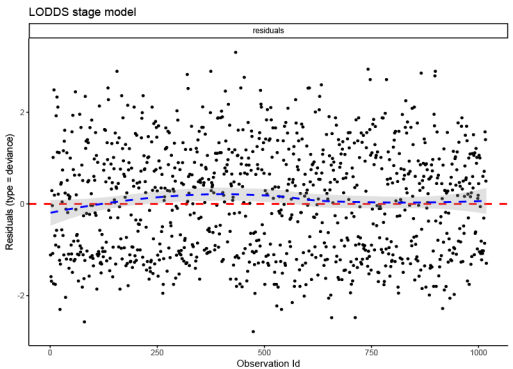


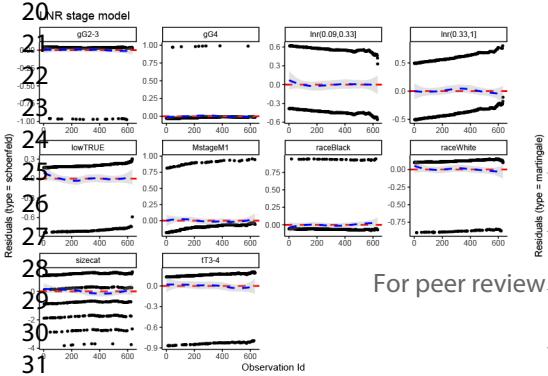


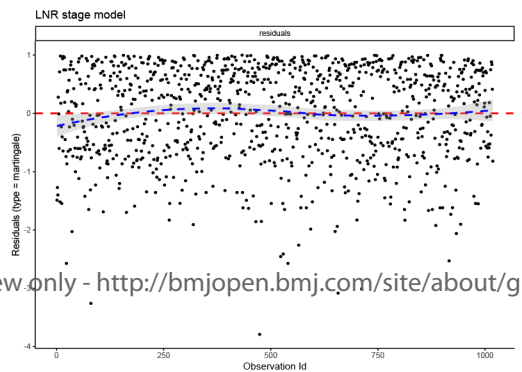


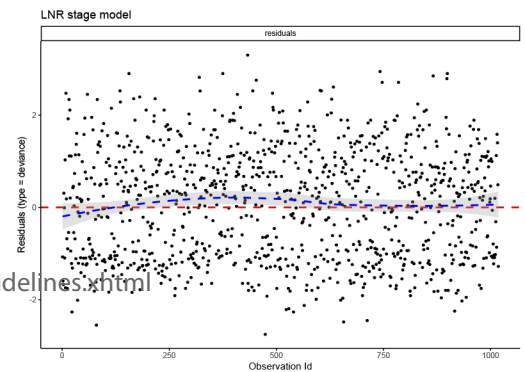


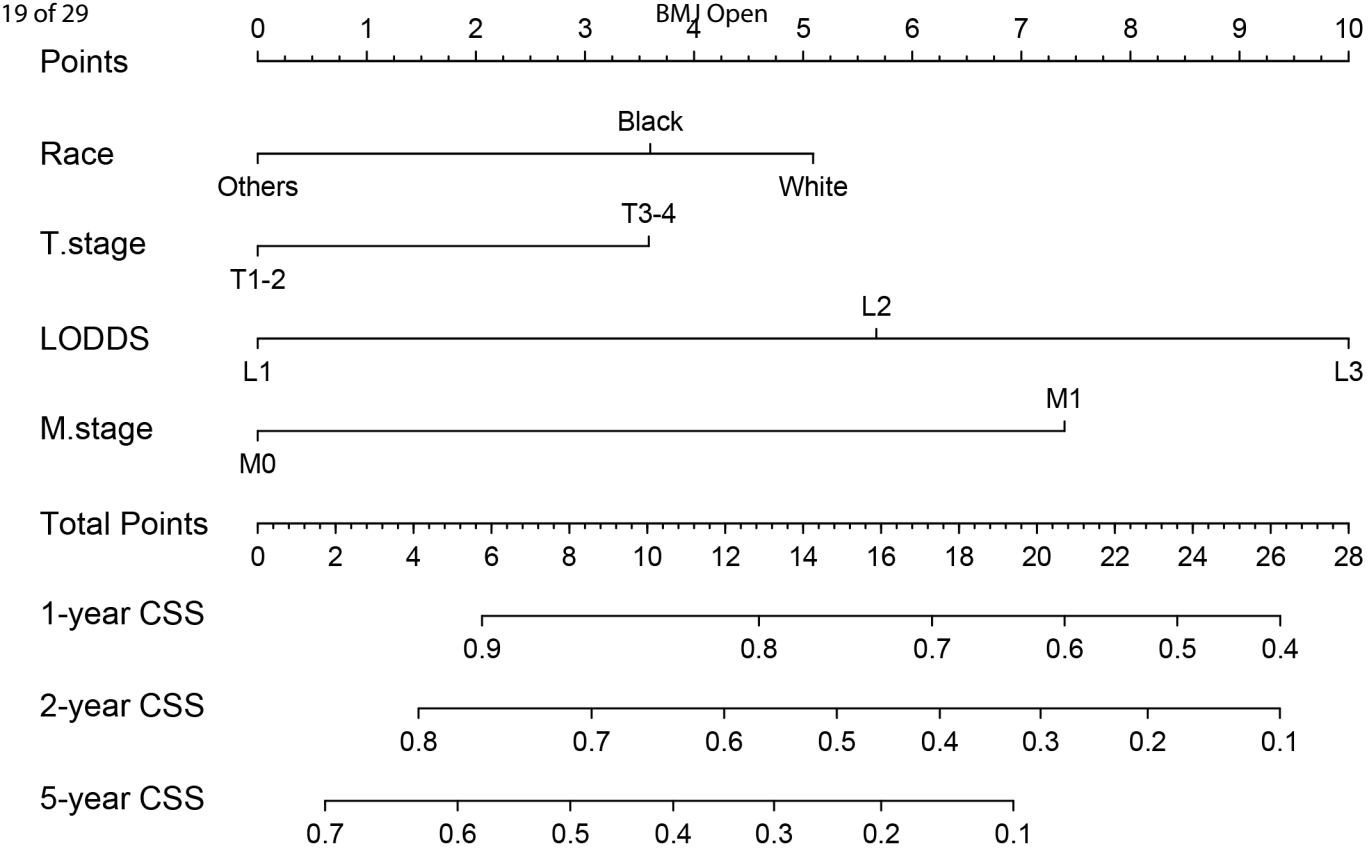


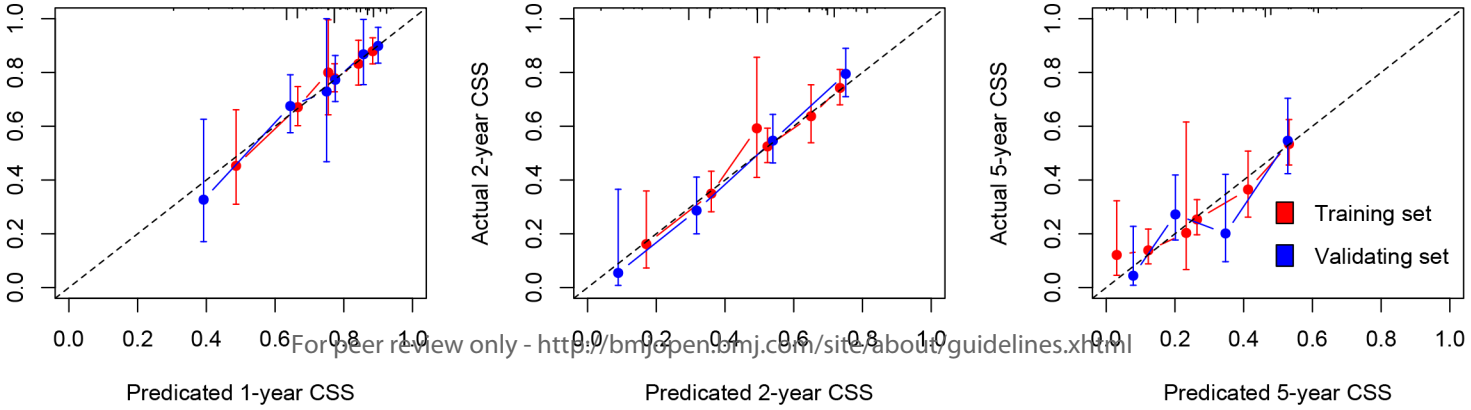


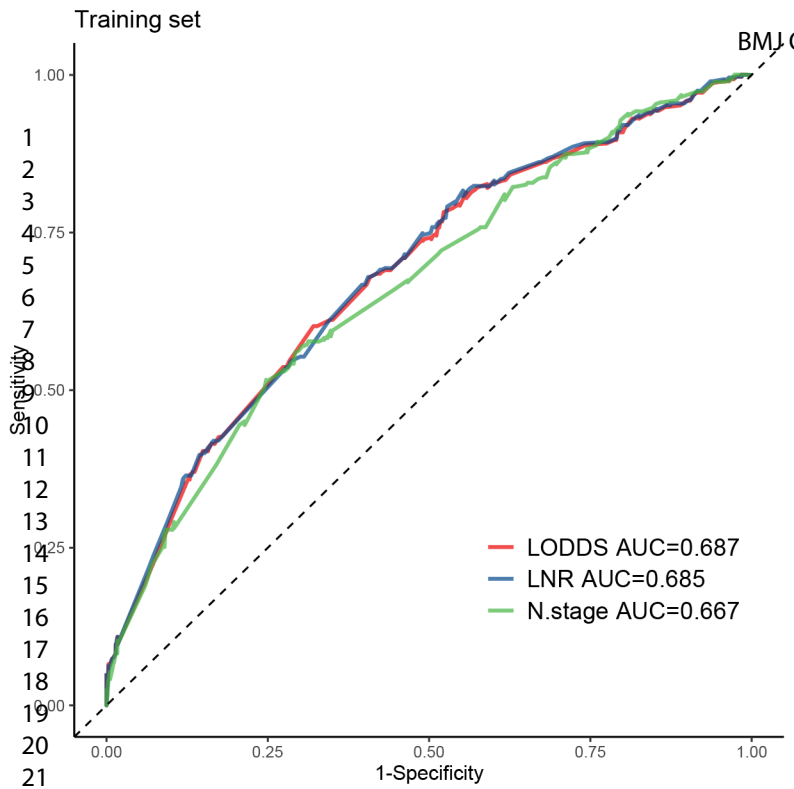


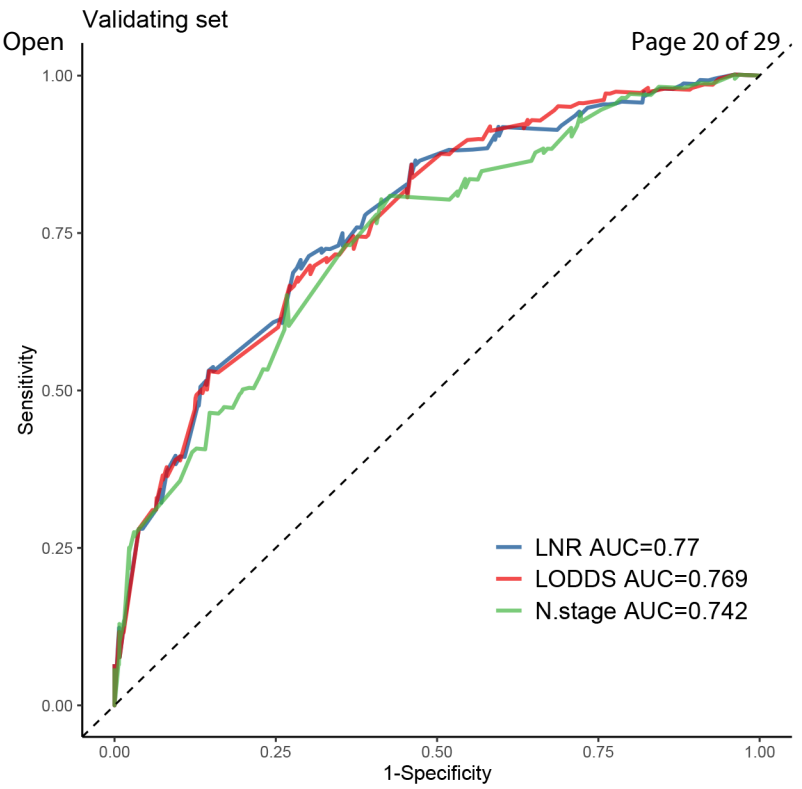


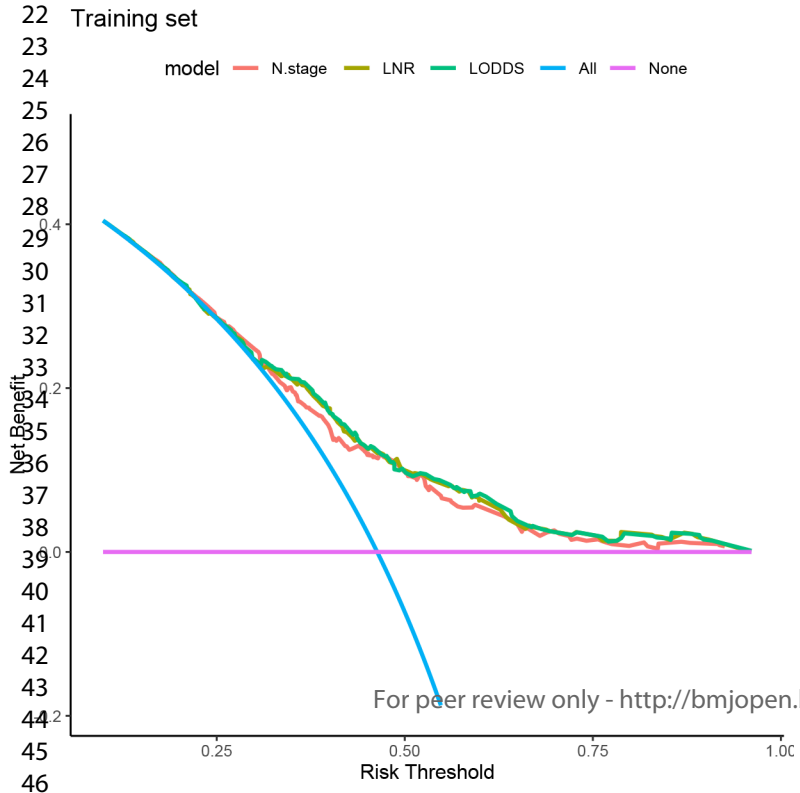


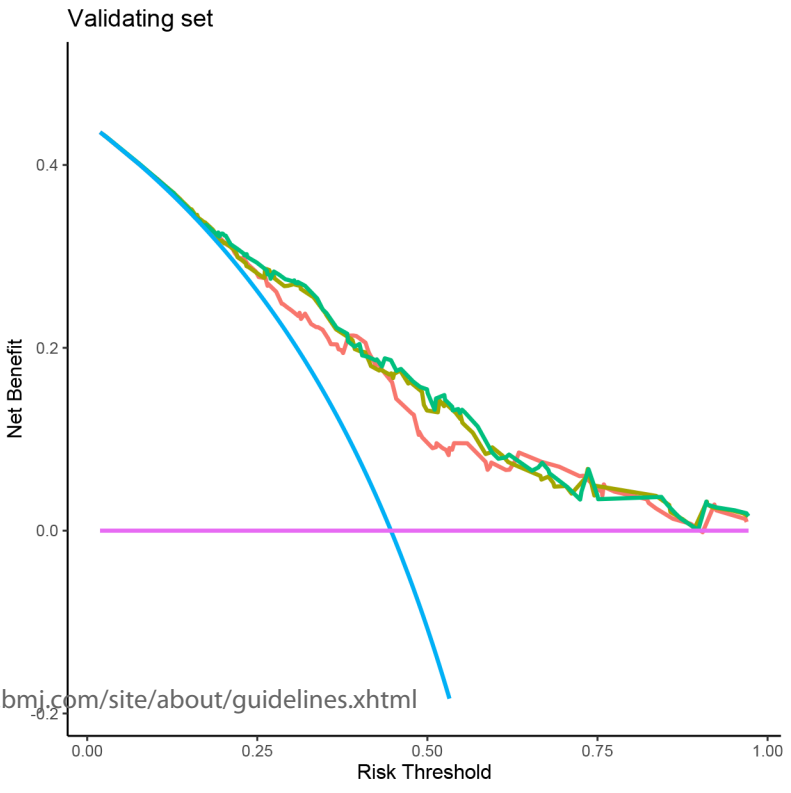












Supplementary Table 1. Comparison of the discrimination ability of different models based on different cutoff values.

	Crude model			Adjusted model	
	HR (95% CI)	C-index	AIC	HR (95% CI)	C-index
LNR					
Cutoff_1		0.607	5376	0.643	5350
<0.09	1 (ref)			1 (ref)	
0.09~0.33	1.88 (1.44, 2.44)			1.74 (1.33, 2.29)	
>0.33	3.02 (2.30, 3.97)			2.63 (1.97, 3.50)	
Cutoff_2		0.605	5378	0.641	5355
<0.09	1 (ref)			1 (ref)	
0.09~0.40	1.97 (1.52, 2.54)			1.85 (1.38, 2.54)	
>0.40	3.16 (2.38, 4.21)			2.72 (2.02, 3.67)	
LODDS					
Cutoff_1		0.609	5373	0.644	5346
<-2.09	1 (ref)			1 (ref)	
-2.09~-0.65	1.93 (1.48, 2.51)			1.80 (1.36, 2.37)	
>-0.65	3.13 (2.38, 4.13)			2.77 (2.07, 3.70)	
Cutoff_2		0.605	5378	0.640	5352
<-2.10	1 (ref)			1 (ref)	
-2.09~-0.37	2.00 (1.54, 2.59)			1.86 (1.42, 2.44)	
>-0.37	3.26 (2.45, 4.33)			2.83 (2.10, 3.81)	

Cutoff_1 was generated by minimal p-value method via X-tile software, cutoff_2 was generated using P25 and P75.

Supplementary Table 2. Univariate analysis of the potential confounders.

Variables	HR (95% CI)	P-value
Age (increased by 10ys)	1.06 (0.98 – 1.14)	0.065
Tumor size (increased by 1cm)	1.13 (1.06 – 1.19)	<0.001
Sex		
Male	1	
Female	1.09 (0.89 – 1.34)	0.386
Race		
Others	1	
Black	1.42 (0.91 – 2.21)	0.118
White	1.57 (1.13 – 2.19)	0.007
Grade		
G1	1	
G2-3	2.08 (1.20 – 3.60)	0.009
G4	3.08 (1.32 – 7.22)	0.009
T		
T1-2	1	
T3-4	1.70 (1.38 – 2.10)	<0.001
N		
N1	1	
N2	1.61 (1.34 – 1.93)	<0.001
N3	2.15 (1.77 – 2.62)	<0.001
M		
M0	1	
M1	2.61 (2.00 – 3.39)	<0.001
Low nodes yield		
No	1	
Yes	1.64 (1.35 – 1.99)	<0.001

Editor's Comments to Author (if any):

- Please revise your title so that it includes your study design. This is the preferred format for the journal.

Response: The study design is included in the title. It is a register-based retrospective cohort study.

- Please revise the abstract >> design section. A "database review" is not an appropriate description of your study.

Response: This section has been revised as “a register-based retrospective cohort study”.

- Please revise the Patient and Public Involvement statement. This section should be included as a sub-heading in the methods section of all manuscripts. It should provide a brief description of any patient involvement in study design or conduct of the study, as well as any plans to disseminate the results to study participants. If patients and or public were not involved please state this.

The Patient and Public Involvement statement should NOT contain details of participant recruitment, patient consent or ethics approval. This information should be included elsewhere in your methods section. Please see our blog for further information regarding PPI: http://blogs.bmj.com/bmjopen/2018/03/23/new-requirements-for-patient-and-public-involvement-statements-in-bmj-open/

Response: The PPI statement has been revised according to the information from blog.

“The development of the research question and outcome measures were not informed by patients’ priorities, experience, and preferences. The patients were involved during the retrospective review of public database where cases were diagnosed during 2010-2015. Patients were not involved in the recruitment to and conduct of the study. The findings of the study will be disseminated by online article to all study participants whose identity kept confidential during the whole research.”

- Please work on improving the reporting of the methods. For example, what was the study's design? What are the settings? More information is needed on the data source used. Was it an anonymised dataset? What permissions were obtained? Did this study require approval from your ethics committee? If not then please explain why not.

Response: The reporting of the methods has been revised at the beginning of the Methods section.

“This study is a SEER register-based retrospective cohort study, which aimed to enroll patients with node positive cardia gastric adenocarcinoma (CGA), review crucial clinical characteristics and observe survival of this population. The source of SEER data is registered cancer cases from various locations throughout the United States. The permission of data access was obtained by sending application form and receiving confirmation mail with valid username (21268-Nov2019) and password. This study was granted exemption from requiring informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the Anhui Medical College.”

- Along with your revised manuscript, please provide a completed copy of the STROBE checklist (http://www.strobe-statement.org/).

Response: We provide a STROBE checklist and each item is linked to the line number of the revised manuscript.

##Reviewer: 1
Dr. Rui Zhong, Southwest Medical University

Comments to the Author:
1.The continuous factors evaluated, LNR, age, tumour size, LODDS were all categorized and then included in the model. Categorization results in substantial loss of statistical power and reduced interpretability. For instance, by selecting the cutoff of age at 65, the interpretation is that a 64 year old is the same as a 30 year old, but the 64 year old is different than a 65 year old. This makes no biological sense. Further, although the authors state that the 'best optimal cutoff' was selected (which is a form of data dredging), it is questioned that the best optimal cutoff would result in cutoffs as they did (i.e. age=65, tumour size=25, etc). The best option would be to leave the continuous variables as continuous for modeling purposes.
Response: Thank you for your suggestion. According to the coding rule of SEER database, age and tumor size are not always in numeric format (e.g. year of 85+, or tumor size of >990 mm), nevertheless you kindly remind us that stratification by cutoff values will result in substantial loss of statistical power. Therefore the revised manuscript transforms age and tumor size into categorized variables that contain much more strata. Age is transformed as a variable of 9 levels, with the lowest of 0-10 and the highest of 80+ (interval of 10 years). However univariate analysis shows that age is not associated with survival (see Suppl. Table 1.), so it is not included in the final model. Likewise tumor size is transformed as a variable of 6 levels, with the lowest of less than 1cm and the highest of over 5cm (interval of 1cm).
LNR and LODDS are still kept as trichotomous factors because they are used as alternative indicators for AJCC N stage.

2.What selection process was used to select factors for inclusion in the multivariable model? Please explain whether a single factor regression analysis was performed before the variables entered the multivariate COX regression. In addition, please explain how this process addresses the potential effects of confounding or collinearity.
Response: In previous manuscript, we selected factors due to clinical significance (for example, higher stage or large tumor size indicates unfavorable outcome). Inspired by your question, we consider both univariate model results and clinical significance in the revised manuscript, and finally include tumor size, race, grade, T stage, M stage and low nodes yield as adjusted variables for stepwise Cox regression model. The results of univariate analyses are listed in the supplementary table 1. So the confounding effect is addressed by multivariable analysis that incorporates with potential confounders.

3.Please consider augmenting the discussion of the findings concerning LNR. LNR association with survival is an exciting aspect of cardia gastric cancer that is currently emerging and may be clinically meaningful.
Response: Thank you for your suggestion. In the previous paper, we focused on the advantage of LNR when LN harvest is inadequate. In the revised paper, we further discuss the clinical meaning of LNR. We are very grateful for the comment that helps us improve this study.

4.Why did the author use the x-titile software for the cutoff value to choose the third quartile

instead of the binary or interquartile range?

Response: We transformed LNR and LODDS into trichotomous variables and selected two cut-off points that represented the greatest group difference of CSS probability according to the minimum p-value method. Thus LNR was re-coded as R1, R2 and R3; LODDS was re-coded as L1, L2 and L3, which was similar with the trichotomous AJCC N staging (N1, N2 and N3). Since only node-positive patients were enrolled, no N0 patients existed here. We address this issue in the revised manuscript in order to make it clear and understandable for readers. Thank you for your question.

5.As the author said whether LNR or LODDS based staging system outperforms TNM 8th edition needs to be further investigated. We want to know whether all the stagings can be corrected to the eighth edition based on the existing fields of the seer databases.

Response: Inspired by your suggestion, all the stagings are now corrected to the 8th edition. Accordingly, the results have been modified. Thank you.

6.Given the importance of the Cox PH model for the development of the nomogram, it would appropriate to include validation that the assumptions of a Cox PH model are met. Please include plots (in the main text or a supplemental figure) of the Schoenfeld, Martingale, and Deviance residuals.

Response: The plots for PH model validation are included in the supplementary figure 1. Plots of Schoenfeld, Martingale, and Deviance residuals for models that incorporate N stage, LNR and LODDS are all presented. The tests show that PH assumption is met in all models (P>0.05). Thank you for the advice.

7.ECOG/Karnofsky performance scores not utilized. Please comment on why these were not utilized as they serve as significant reference points for PC treatment. If possible, this would be a great thing to include in this analysis or the analyses suggested above.

Response: Unfortunately, variables that reflect general status are not available in SEER database, so ECOG or KPS was not utilized. We address this issue as a limitation in the Discussion Section of the revised paper. Thank you for the comment.

"…One limitation of this study is that some important factors that are associated with survival are not considered in the model due to unavailable data source. For example, ECOG/KPS score is commonly taken into account in survival analysis due to its remarkable relationship with general status and prognosis. Unfortunately the SEER 18 database does not record the score at diagnosis, so the impact of it is not considered in this analysis……To overcome this limitation, a database that provides with fully detailed medical records is needed for analysis."

8.altered cutoff values would be an essential factor to analyze during the further optimization of this model. This needs to be further explained in the discussion.

Response: Thank you for your suggestion. In order to cut LNR and LODDS into trichotomous factor, the previous study selected two points by using the minimal p-value method via X-tile software. Unlike one cutoff point selection, ROC and maximally selected rank statistics cannot be applied in two-point selection. Therefore the revised manuscript attempts to generate trichotomous factors using P25/P75, construct regression model and compare discrimination ability with X-tile

based cutoff values. As a result, the X-tile based values have higher power and are finally included in further analysis. We address this issue in the revised paper and present the process in Suppl. Table 2. Although the previous results remain, this step is very crucial for optimizing the models and improving the study reliability. We deeply appreciate this comment.

"…For model optimization, LNR and LODDS were also categorized into trichotomous factors using cut-off values of P25 and P75. The discrimination ability of the model based on interquartile was lower (Suppl. Table 2), so this model was not further analyzed…."

9.Please explain why the N stages do not appear in this nomogram. For example, they do not show significance in the multivariate COX regression?

Response: In the present study, we attempt to construct a new alternative indicator for N stage, because the current N stage classification may not perform well in cardia gastric cancer, so we presented the nomograms that cooperated with LNR or LODDS, other than N staging. In other words, N stages DO appear in the nomogram, but in the form of LNR or LODDS. In addition, the other reviewer suggested us to pick one plot to avoid confusion and unclear message; we only show one nomogram that cooperates with LNR in the main text, and show the other plot in the supplementary file. We hope the revision is acceptable.

10.We believe that the nomogram established by the author should be compared with traditional models, such as ROC curve and Decision Curve Analysis.

Response: In the revised paper, ROC and DCA curve are made to compare the prognostic powers between the new nomogram models based on LNR and LODDS and the traditional model based on N stage. The results indicate that the new nomogram models are better. For details, please see the supplementary figure 3. We really thank you for the suggestion that further confirms our results.

##Reviewer: 2
Dr. Rasa Zarnegar , Weill Cornell Medical College

Comments to the Author:
I think this is a nice paper with 2 nomograms for the determination for CGA.

I think there are some revisions that would make this paper better. The SEER DB has access to total number of nodes analyzed and the number of positive nodes. It is important to use the current guidelines 8th Edition for this analysis even though the data was from prior to implementation of the 8th. The concept should still hold and validate the rigor of the study based on current guidelines.
Response: All the 7th stagings are now corrected to the 8th edition. Thank you for your brilliant suggestion.

2. Raw data on the patient population No of nodes harvested and total positive is required to determine the frequency of low yield in the study design.
Response: In the revised paper, we describe the number of nodes harvested and total positive, and the frequency of low nodes yield (please see Table 1). The frequency of low yield is also in the multivariable model because it is associated with survival in univariate analysis (Suppl. Table 2).

3. There should be discussion and data on neoadjuvant therapy as this likely impact survival and the number of patient that received therapy.
Response: Neoadjuvant therapy is likely to influence survival; unfortunately the database, Incidence - SEER Research Data, 18 Registries, Nov 2019 Sub 2000-2017 (SEER 18 database), does not provides with information about chemotherapy, therefore it is unavailable for this study. We treat it as a limitation and discuss the impact of neoadjuvant therapy in the Discussion Section. *"…Treatment mode is also associated with clinical outcome. This study enrolled patients who received gastric resection; however other information about chemo- or radiotherapy is not available in SEER 18 database. Randomized clinical trial demonstrates that compared with surgery alone, preoperative administration of carboplatin and paclitaxel with concurrent radiotherapy significantly improved overall survival among patients with esophageal or GEJ cancer (HR = 0.657) (31). The NCCN clinical practice guidelines for GEJ cancer recommend preoperative chemoradiation or perioperative chemotherapy due to substantial survival benefit compared with surgery alone (32). To overcome this limitation, a database that provides with fully detailed medical records is needed for analysis…."*

4. I suggest picking one nomogram. I think its important to send a clear message and by presenting 2 nomograms the authors are creating confusion and an unclear message. I think by being more focused on one approach would allow for improved implementation. The authors may want to compare whichever they select with conventional lymphadenectomy is so desired.
Response: Thanks for the advice. We pick the nomogram that incorporates with LNR because the calculation of LNR is easier than LODDS, which is more convenient in clinical practice. The nomogram and calibration curves based on LODDS are shown in supplementary figure 2. In addition, for more clear presentation, we put the calibration curves of training (red) and validating

set (blue) into one plot.

STROBE Statement—checklist of items that should be included in reports of observational studies

All line numbers are based on the manuscript with tracked change.

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **title page**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **page2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **page 4**
Objectives	3	State specific objectives, including any prespecified hypotheses **page 4**
Methods		
Study design	4	Present key elements of study design early in the paper **page 4**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **page 4-5**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **page 4** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls **NA** *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants **NA**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed **NA** *Case-control study*—For matched studies, give matching criteria and the number of controls per case **NA**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **page 5**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group**page 5**
Bias	9	Describe any efforts to address potential sources of bias **page 5**
Study size	10	Explain how the study size was arrived at **NA**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **page 5-6**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **page 5-6**
		(*b*) Describe any methods used to examine subgroups and interactions **page 4-6**
		(*c*) Explain how missing data were addressed **page 4**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed **NA** *Case-control study*—If applicable, explain how matching of cases and controls was addressed **NA** *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy **NA**
		(*e*) Describe any sensitivity analyses **NA**
Results		

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **page 4**
		(b) Give reasons for non-participation at each stage **page 4**
		(c) Consider use of a flow diagram **figure 1, page 4**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **page 6**
		(b) Indicate number of participants with missing data for each variable of interest **figure 1, page 4**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **Table 1 page 6-7**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time **page 6**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure **NA**
		Cross-sectional study—Report numbers of outcome events or summary measures **NA**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **table 1, page 7-9**
		(*b*) Report category boundaries when continuous variables were categorized **page 7**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **NA**
Discussion		
Key results	18	Summarise key results with reference to study objectives **page 9**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **page 10-11**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **page 9-11**
Generalisability	21	Discuss the generalisability (external validity) of the study results **page 11**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **page 11**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Prognostic value of different lymph node staging methods for node-positive cardia gastric cancer: a register-based retrospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-050378.R2
Article Type:	Original research
Date Submitted by the Author:	29-Jul-2021
Complete List of Authors:	Wang, Xiao-Qing; Anhui Medical College Bao, Min; Anhui Medical College Zhang, Cheng; First Affiliated Hospital of Anhui Medical University
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Gastroenterology and hepatology
Keywords:	Gastrointestinal tumours < ONCOLOGY, Gastrointestinal tumours < GASTROENTEROLOGY, SURGERY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title page

Article title: Prognostic value of different lymph node staging methods for node-positive cardia gastric cancer: a register-based retrospective cohort study

Author information: Xiao-Qing Wang 1, Min Bao 1, Cheng Zhang 2

1 Anhui Medical College, Hefei, Anhui, China PR

2 Anhui Provincial Cancer Institute, the First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China PR

Corresponding to: Cheng Zhang, ahmuzc@sina.cn

Disclaimer: The views expressed in the submitted article are our own and not an official position of the institution or funder.

Word count: 2962 (excluding its abstract, acknowledgments, tables, figure legends, and references)

Number of figures and tables: This draft included 3 figures and 2 tables.

Disclosure of relationships and activities: None.

ABSTRACT

Objective: To investigate the prognostic efficacy of lymph node ratio (LNR) and log odds of positive lymph nodes (LODDS) in node-positive cardia gastric adenocarcinoma (CGA).

Design: A registry-based retrospective cohort study.

Setting: Patients diagnosed with node-positive CGA in the Surveillance, Epidemiology, and End Results (SEER) database from 2010 to 2015.

Participants: A total of 1038 patients were enrolled and randomly assigned (7:3) to the training set (N = 723) or validating set (N = 315).

Primary outcome measure: Cancer-specific survival (CSS).

Results: The baseline characteristics of the training and validation sets were similar. Based on the optimal cut-off values, LNR was classified into low (<0.09), medium (0.09~0.33), and high (>0.33) groups; LODDS was also classified into low (<-2.09), medium (-2.09~-0.65), and high (>-0.65) groups. CSS was significantly different across LNR and LODDS subgroups. The Harrell concordance index of the N stage was lower than that of the LNR or LODDS. The Akaike information criterion of the N stage was higher than that of the LNR or LODDS. Independent predictors included race, T stage, M stage, and LNR (or LODDS), and they were incorporated into nomograms for 1-, 2-, and 5-year CSS prediction. Calibration plots showed satisfactory results for internal and external validity of the nomogram.

Conclusions: LNR and LODDS staging methods have better prognostic efficacy than the traditional N staging method in CGA with node metastasis. Moreover, the two values are promising substitutes for N staging in nomogram development when other independent prognostic factors are incorporated.

KEY WORDS

Cardia; Adenocarcinoma; Stomach Neoplasms; Lymph Node Ratio; Neoplasm Staging

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study used the national cancer registry data for cardia gastric adenocarcinoma research;
- Novel staging methods based on the number of positive lymph nodes have been established for prognostic prediction.
- Nomograms based on the new staging methods were constructed and validated;
- The validity of the outcomes of the study needs to be confirmed in other populations.

Patient consent form: The SEER database review is granted exemption from obtaining patients' consents.

Word count: 2962 (excluding its abstract, acknowledgments, tables, figure legends, and references)

INTRODUCTION

Gastric cancer (GC) generally includes two topographical categories: non-cardia GC that occurs at a more distal part of the stomach and GC of the cardia that occurs at the gastroesophageal junction (GEJ). In contrast to the steady decline in the incidence of non-cardia GC, GC of the cardia occurs more frequently, particularly in high-income countries (1, 2). This trend is associated with obesity, gastroesophageal reflux disease, and Barrett esophagus (2). In addition to the difference in the incidence trend, the clinic pathological features and long-term survival vary between the two GC subtypes (3). Precise staging is necessary for the accurate prediction of survival. The Tumor-Node-Metastasis (TNM) classification 7th edition of the American Joint Committee on Cancer (AJCC) recommends harvesting of at least 15 lymph nodes (LN) for N staging (4, 5). However, inadequate LN harvest is frequent because of various reasons; thus, precise staging is difficult. It has been demonstrated that the LN ratio (LNR) could provide a better estimate of the survival of patients with GC after curative gastrectomy, regardless of the number of LNs examined (6), and might be a promising aid along with the TNM staging system (7). Furthermore, in previous reports, the log odds of positive LN (LODDS) outperformed the N and LNR staging systems in predicting the survival of patients with GC (8-10). Therefore, the traditional N staging classification might be substituted with different methods with improved performance. Nevertheless, few studies have evaluated the performance of the two LN staging systems in GC of the cardia, which has distinct clinical characteristics and epidemiology than other types of GC.

Here, we used the data of a nationwide cancer registry to evaluate the prognostic value of LNR and LODDS in patients with node-positive cardia gastric adenocarcinoma (CGA), and, if possible, construct a nomogram for the prediction of survival based on the new LN staging system.

METHODS

Study design and Participant Selection

This study was a Surveillance, Epidemiology, and End Results (SEER) registry-based retrospective cohort study, which aimed to enroll patients with node-positive CGA, review their critical clinical characteristics, and observe the survival of this population. The source of the SEER data is registered cases of cancer from various locations throughout the United States. Permission for data access was obtained by sending an application form and receiving confirmation mail with a valid username (21268-Nov2019) and password.

A SEER*Stat (version 8.3.8) was used to access the Incidence-SEER Research Data, 18 Registries, Nov 2019 Sub 2000-2017 (SEER 18 database) (11) and to obtain data of node-positive patients with CGA. The inclusion criteria were as follows: 1) the International Classification of Disease for Oncology, Third Edition (ICD-O-3) code for the primary tumor site was C16.0 (cardia); 2) broad histological recode was 8140-8389 adenomas and adenocarcinomas; 3) diagnostic confirmation was by positive histology; 4) surgery was performed; 5) diagnosis was during 2010-2015; and 6) the definite number of positive regional nodes was known and was not zero. Cases with unknown race, T stage information, tumor size, or tumor grade were excluded. As shown in Figure 1, the final cohort comprised 1038 patients with node-positive CGA, of whom 857 were male, and 181 were female. A total of 338 (32.56%) patients were above 70 years of age. Eight hundred and ninety-six (86.32%) patients were White, 64 were Black, and 78 were of other races. Of the total

cohort, 70% of the patients were randomly assigned to the training set (N = 723), and the remaining were assigned to the validation set (N = 315).

Technical Information

The main outcome was cancer-specific survival (CSS), which was defined as the period between the first diagnosis and death specifically due to CGA. In addition, we extracted the following variables for analysis: sex, race, age, AJCC 7th TNM stage information, tumor size, tumor grade, number of regional nodes examined, and number of regional nodes that were positive. The information about the stage of cancer was further corrected according to the AJCC 8th criteria. LNR and LODDS were calculated as previously reported (12). Briefly, LNR was defined as the ratio of the number of positive nodes divided by the total number of examined nodes. LODDS was calculated using the formula: log(NPLN+0.50)/(NDLN−NPLN+0.50), in which 0.50 was added to both the numerator and denominator to avoid an infinite number.

The optimal thresholds for dividing LNR and LODDS into trichotomous variables were determined using the X-tile software (version 3.6.1) (13), which were based on the maximal log-rank chi-square value that represented the greatest group difference in CSS probability. LNR and LODDS were classified into three levels because they are proposed as alternative indicators for N stage in node-positive GC, including N1, N2, and N3.

Statistics

The distributions of baseline characteristics between the training and validation sets were described and compared using chi-square test. Survival curves, median survival, and CSS rates were generated using Kaplan-Meier method. Outcome differences between the groups were analyzed using log-rank test. After testing proportional hazard assumption, a multivariable Cox regression model was used to establish a CSS prognostic model. The prognostic power was evaluated using Akaike information criterion (AIC) and Harrell concordance index (C-index). A predictive model with a lower AIC indicated a better model fit, while a higher C-index indicated a better discriminative ability. A C-index value of 0.5 indicated no predictive power, and an index of 1.0 indicated complete differentiation. Cox stepwise regression analysis was also performed to construct a nomogram for the prediction of 1-, 2-, and 5-year CSS. Validation of the nomogram was performed using internal and external calibration plots (14). Bootstraps with 1000 resamples were used for the validation activities. Receiver operator characteristic (ROC) curves and areas under the ROC curves (AUCs) were calculated to evaluate the accuracy of CSS prediction using different models. Decision curve analysis (DCA) was performed to determine the clinical application of different models: the proportion of true positive results minus the proportion of false positive results, and the relative risks of false-positive and false-negative results were weighted to obtain the net benefits of decision-making. All statistical analyses were performed using R software (version 3.5.3). A two-tailed P value of less than 0.05 was considered statistically significant.

Patient and Public Involvement

Due to the retrospective and observational nature of the study, the research question and outcome measures were not developed and influenced by patients' priorities, experiences, and preferences. Patients were not involved in the design, recruitment, and conduct of this study. Patients were not

asked to assess the burden of the intervention and time required to participate in the research. The findings of the study will be disseminated online and are freely available for public.

Ethics approval statement

The Ethics Committee of Anhui Medical College exempted the requirement for ethics approval because of the observational nature of the study.

RESULTS

Table 1 summarizes the demographic and clinical features of the participants. In all, 628 patients (60.50%) were diagnosed with a tumor less than 5cm. Six hundred and forty patients (61.66%) were diagnosed with grade III or IV cancer. The number of patients with T1, T2, T3, and T4 stage was 94, 125, 717, and 102, respectively. The number of patients with N1, N2, and N3 stage was 479, 330, and 229, respectively. Seventy-five patients (7.23%) had distant metastasis at presentation. The median CSS was 27 months. The rate of 1, 2, and 5-year CSS was 76.8%, 53.0%, and 29.2%, respectively. There was no statistical difference in the baseline characteristics between the training and validating set. The detailed information about the two sets is also presented in Table 1.

Table 1. Baseline information of the included patients with node positive CGA, N(%).

Groups	Training set (N = 723)	Validating set (N = 315)	P-value
Sex			
Male	596 (82.43)	261 (82.86)	0.939
Female	127 (17.57)	54 (17.14)	
Age			
<70	490 (67.77)	210 (66.67)	0.781
≥70	233 (32.23)	105 (33.33)	
Race			
White	628 (86.86)	268 (85.08)	
Black	40 (5.53)	24 (7.62)	0.437
Others	55 (7.61)	23 (7.30)	
Tumor size			
<5cm	442 (61.13)	186 (59.05)	0.573
≥5cm	281 (38.87)	129 (40.95)	
Grade			
I-II	279 (38.59)	119 (37.78)	0.859
III-IV	444 (61.41)	196 (62.22)	
T stage			
T1a	17 (2.35)	4 (1.27)	
T1b	53 (7.33)	20 (6.35)	
T2	83 (11.48)	42 (13.33)	0.224
T3	501 (69.29)	216 (68.57)	
T4a	49 (6.78)	28 (8.89)	
T4b	20 (2.77)	5 (1.59)	

N stage			
N1	332 (45.92)	147 (46.67)	
N2	229 (31.67)	101 (32.06)	0.921
N3	162 (22.41)	67 (21.27)	
M stage			
M0	678 (93.78)	285 (90.48)	0.079
M1	45 (6.22)	30 (9.52)	
Low nodes yield			
Yes	532 (73.58)	243 (77.14)	0.300
No	191 (26.42)	72 (22.86)	
No. of nodes harvest	17 (12, 25)	16 (11, 24)	0.400
No. of positive nodes	3 (1, 6)	3 (1, 6)	1.000
Median survival (months)	28 (25, 32)	25 (21, 32)	0.361
CSS rate (%)			
1-year	77.0 (74.0, 80.2)	76.3 (71.6, 81.2)	
2-year	53.7 (50.1, 57.5)	51.4 (46.0, 57.5)	
5-year	30.3 (26.7, 34.5)	26.4 (20.9, 33.4)	

Abbreviation: CGA, cardia gastric adenocarcinoma; CSS, cancer-specific survival

According to X-tile software results, the optimal cut-off values for LNR were 0.09 and 0.33, and for LODDS were -2.09 and -0.65. Thus, patients were classified into the low (<0.09, R1), medium (0.09~0.33, R2), or high LNR (>0.33, R3) groups; or low (<-2.09, L1), medium (-2.09~-0.65, L2), or high LODDS (>-0.65, L3) groups. For model optimization, LNR and LODDS were also categorized into trichotomous factors using the cut-off values of P_{25} and P_{75}. The discrimination ability of the model based on the interquartiles was poor (Suppl. Table 1); hence, this model was not analyzed further. Next, we created the survival curves of the patients according to the N staging, LNR, or LODDS staging system. As shown in Figure 2 in the training set, CSS was significantly different between all the three staging systems (all the log-rank P values < 0.0001); however the 95% confidence intervals (CIs) of N2 and N3 survival curve were initially divergent and partly overlapped afterward. The inferior discriminative ability of the N system was further reinforced by the AIC and C-index. As shown in Table 2, the C-index of the N stage was lower than that of LNR or LODDS. Similarly, the AIC of the N stage was higher than that of the LNR or LODDS. The clinical characteristics with statistical significance for CSS were further incorporated in the Cox regression model as potential confounders (Suppl. Table 2), and all the variables met the proportional hazard assumption (Suppl. Figure 1, all the P values> 0.05). The prognostic value of the adjusted model was generally better than that of the crude model. In addition, the prognostic value of the LNR system seemed to be poorer than that of the LODDS system; however, the difference was not significant; hence, we incorporated both the systems for nomogram construction.

Table 2. Prognostic values of variables for patients with node positive CGA (N = 1 038).

Variables	Crude model			Adjusted model		
	HR (95% CI)	C-index	AIC	HR (95% CI)	C-index	AIC

Training set (N = 723)						
N stage		0.582	5403		0.632	5365
N1	1 (ref)			1 (ref)		
N2	1.53(1.24, 1.91)			1.42(1.14, 1.77)		
N3	2.15 (1.70, 2.71)			2.03(1.60, 2.59)		
LNR		0.607	5376		0.643	5350
R1	1 (ref)			1 (ref)		
R2	1.88 (1.44, 2.44)			1.74 (1.33, 2.29)		
R3	3.02 (2.30, 3.97)			2.63 (1.97, 3.50)		
LODDS		0.609	5373		0.644	5346
L1	1 (ref)			1 (ref)		
L2	1.93 (1.48, 2.51)			1.80 (1.36, 2.37)		
L3	3.13 (2.38, 4.13)			2.77 (2.07, 3.70)		
Validating set (N = 315)						
N stage		0.596	1957		0.675	1931
N1	1 (ref)			1 (ref)		
N2	1.81 (1.31, 2.51)			1.75 (1.25, 2.46)		
N3	2.18 (1.51, 3.15)			2.23 (1.50, 3.30)		
LNR		0.646	1927		0.691	1913
R1	1 (ref)			1 (ref)		
R2	2.20 (1.47, 3.30)			1.91(1.26, 2.90)		
R3	4.16 (2.76, 6.28)			3.58 (2.30, 5.56)		
LODDS		0.647	1927		0.789	1914
L1	1 (ref)			1 (ref)		
L2	2.07 (1.39, 3.09)			2.08 (1.38, 3.14)		
L3	4.22 (2.79, 6.39)			4.10 (2.65, 6.34)		

Abbreviations: CGA, cardia gastric adenocarcinoma; HR, hazard ratio; CI, confidence interval; AIC, Akaike information criterion; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes.

Adjusted model considered race, tumor size, grade, T stage and M stage.

Stepwise Cox regression analysis showed race, T stage, M stage, and LNR (or LODDS) were independent predictors; hence, these factors were included in the nomograms. For both LNR and LODDS, the total score was 40, and a higher score suggested lower survival (Figure 3 and Suppl. Figure 2). Next, the calibration plot was used to assess the internal and external validity of the nomogram (Figure 3 and Suppl. Figure 2). Since the cross-spot line was generally close to the grey reference line, we concluded that the predicted CSS was well correlated with the actual state. In addition, ROC curves indicated that the AUC of the model based on N stage was lower than that of the model based on the nomogram of LNR or LODDS (Suppl. Figure 3). However the DCA plot does not show advantage of the nomogram (Suppl. Figure 3).

DISCUSSION

The present study analyzed the databases of the national cancer registry and demonstrated that survival of patients with node-positive CGA could be well predicted when the traditional N staging method is substituted with an LNR or LODDS system. This outcome was seen both in

training and validation set. In the training set, the survival curves clearly separated when the patient grouping was implemented following the LNR or LODDS method, which was not achieved by the traditional N staging system. An adjusted model that simultaneously considered the staging, clinical, and demographic features, outperformed the crude model that only considers staging. Therefore, multiple independent survival factors were incorporated in the nomogram construction, which suggested White, deeper infiltration of the tumor, higher proportion of positive LN, and metastasis as risk factors. The nomograms performed consistently across the 1, 2, and 5-year prediction of the CSS as seen in the validation plots.

Previous studies have demonstrated the superiority of LNR or LODDS for prognostic prediction in GC after surgical resection (8-10, 15-17). However, the patients were not further classified according to the primary tumor site; this is a critical limitation since there is a significant difference between cardia and non-cardia GC in terms of tumor features, etiological factors, and biological behaviors (3). In the AJCC cancer staging 7th edition, tumors involving GEJ were categorized as esophageal cancer (5). This was debatable because the GC staging system has a better ability to predict survival of a GEJ tumor (18, 19). In the latest 8th edition (20), a tumor that has its epicenter within 2 cm of the GEJ and involves the GEJ (Siewert type I/II) is classified as esophageal cancer. Other types of GCs, including a tumor with an epicenter more than 2 cm from the GEJ or a tumor located with 2 cm of the GEJ but not involving the GEJ, are classified as GC. The superiority of the new system was confirmed by a retrospective observational study from two institutions in China that have a high volume of cases of GC, regardless of the Siewert type (21). In terms of the Siewert type II junctional adenocarcinoma, a marginal superiority of the esophageal cancer was found in discriminating survival rates after three and five years. However the advantage of the GC system lies in the division of the N3 category into N3a and N3b. Hence, the authors concluded that neither the esophageal nor the stomach staging system is accurate in predicting survival in Siewert type II junctional cancer (22). Moreover, CGA is probably a special entity that has different biological characteristics compared to distinct gastric or esophageal cancer. To the best of our knowledge, the present study is the first to demonstrate a superior prognostic prediction based on LNR or LODDS in patients with node-positive CGA. Unfortunately, we were unable to consider the Siewert type due to a lack of information in the SEER database; hence, further studies are necessary with a special focus on tumor location.

LNR and LODDS have been proven to be the strongest indicators of survival in gastric adenocarcinoma when LN harvest is inadequate (16, 17). It has been demonstrated that, in general, more extensive LN resection is associated with better survival, which might be due to either improved N classification or a therapeutic effect of lymphadenectomy. For esophageal cancer, the worldwide data shows that harvesting 10 nodes for pT1, 20 for pT2, and 30 or more for pT3/T4 is desirable for reaching maximum 5-year survival (23). For GC, a higher LN harvest also shows improved survival (24). It is suggested that at least 16 nodes be evaluated pathologically and evaluation of more than 30 nodes is desirable (25). Overall, it is encouraged to harvest as many LNs as possible; balancing the extent of LN resection necessary for accurate N staging and maximum survival without unnecessarily increasing the morbidity caused by radical lymphadenectomy. Nevertheless, many conditions can lead to inadequate LN harvest. It is estimated that only one-fifth of the patients with GC have an adequate number of LN examined in Iran (26), while more than 15 LNs are examined in 64% of the patients in the US (25). The LNR

and LODDS staging methods do not require an adequate number of LNs to be evaluated. In the present study, a low LN yield was found to be a risk factor for poor survival in univariate analysis; however, it was not significant in the LNR or LODDS based multivariate model, which indicates that LN harvest has little impact on prediction of survival based on LNR or LODDS. In fact, the new node category method is consistent when nodal assessment is inadequate during surgery not only for GC (8, 15-17) but also for colorectal cancer (27), esophageal cancer (28), oral squamous cell carcinoma (29), gallbladder cancer (30), and others.

The association between LNR and survival is a promising aspect of cardia GC that is currently emerging and might be clinically relevant. A higher ratio of positive LN indicates a worse outcome in cardia GC. Patients are at 2-3 times higher risk of cancer-specific death if the ratio is over 33%. The ratio of 9-33% also indicates a twofold risk. This effect is independent of other crucial clinical characteristics; thus, it is a useful tool for surgeons to predict the prognosis. This is also evidence supporting truly radical surgery, i.e., complete lymph node resection rather than limited resection (31). In addition, LNR minimizes the "stage migration" phenomenon that occurs with the current N staging system (32).

One limitation of this study is that some important factors associated with survival have not been considered in the model due to unavailable data. For example, the Eastern Cooperative Oncology Group or Karnofsky Performance Status score is commonly considered in the survival analysis due to its remarkable relationship with the general status and prognosis. Unfortunately, the SEER 18 database does not record the score at diagnosis; hence, its impact is not considered in this analysis. The treatment modality is also associated with clinical outcomes. This study enrolled patients who underwent gastric resection; however other information about chemo- or radiotherapy is not available in the SEER 18 database. A previous randomized clinical trial demonstrated that compared with surgery alone, preoperative administration of carboplatin and paclitaxel with concurrent radiotherapy significantly improved the overall survival among patients with esophageal or GEJ cancer (HR = 0.657) (33). The National Comprehensive Cancer Network clinical practice guidelines for GEJ cancer recommend preoperative chemoradiation or perioperative chemotherapy due to substantial survival benefits compared with surgery alone (34). To overcome this limitation, a database that provides fully detailed medical records is necessary for analysis. Moreover, the inclusion of these factors would greatly improve the prognostic power of the survival prediction model. Another limitation is that our results are based on the training set and confirmed by the validation set; however, the baseline characteristics of the two groups are similar. Hence, these results need to be validated among populations with different characteristics. The third limitation is clinical usability. The DCA result is proposed for assessing the potential clinical impact of risk models for recommending treatment or intervention, and the suggested clinical usability of the nomogram may be poorer than that of other models. In this regard, although this model may have some merits regarding outcome prediction, its use for guiding clinical decisions should be further studied.

In conclusion, staging methods based on LNR and LODDS have better prognostic ability than the traditional N staging method in patients of CGA with regional lymph node metastasis. Moreover, the two values are promising substitutes for N staging in nomogram development when other independent prognostic factors are incorporated.

Contributorship statement: WXQ work conception, data interpretation, critical review for important content, final approval of the manuscript and agreement to be accountable for all aspects of the work; BM administrative work, funding, critical review for important content, final approval of the manuscript and agreement to be accountable for all aspects of the work; ZC acquisition, analysis of data, drafting the work, final approval of the manuscript and agreement to be accountable for all aspects of the work.

Competing interests: None.

Funding: This work was supported by [Domestic Visiting Study Project for Outstanding Young Talents in Colleges and Universities in Anhui Province] grant number [gxgnfx2021183] and [Major Project of Natural Science of Anhui Provincial Department of Education] grant number [KJ2019ZD73].

Data sharing statement: Dataset available from the SEER website (https://seer.cancer.gov/data-software/).

Acknowledgement: We thank all patient advisers for their special contribution.

References:

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. [Journal Article]. 2018 2018-11-01;68(6):394-424.
2. Abdi E, Latifi-Navid S, Zahri S, Yazdanbod A, Pourfarzi F. Risk factors predisposing to cardia gastric adenocarcinoma: Insights and new perspectives. Cancer Med. [Journal Article; Research Support, Non-U.S. Gov't; Review]. 2019 2019-10-01;8(13):6114-26.
3. Amini N, Spolverato G, Kim Y, Squires MH, Poultsides GA, Fields R, et al. Clinicopathological features and prognosis of gastric cardia adenocarcinoma: a multi-institutional US study. J SURG ONCOL. [Comparative Study; Journal Article; Multicenter Study]. 2015 2015-03-01;111(3):285-92.
4. Sobin L, Gospodarowicz M, Wittekind C. TNM classification of malignant tumours. 7th edition.: Wiley-Blackwell; 2009.
5. Edge S, Byrd D, Compton C, Fritz A, Greene F, Trotti A. AJCC cancer staging manual. 7th edition.: Springer; 2009.
6. Hou Y, Wang X, Chen J. Prognostic significance of metastatic lymph node ratio: the lymph node ratio could be a prognostic indicator for patients with gastric cancer. WORLD J SURG ONCOL. [Journal Article]. 2018 2018-10-04;16(1):198.
7. Zhu J, Xue Z, Zhang S, Guo X, Zhai L, Shang S, et al. Integrated analysis of the prognostic role of the lymph node ratio in node-positive gastric cancer: A meta-analysis. INT J SURG. [Journal Article; Meta-Analysis; Review]. 2018 2018-09-01;57:76-83.
8. Sun Z, Xu Y, Li DM, Wang ZN, Zhu GL, Huang BJ, et al. Log odds of positive lymph nodes: a novel prognostic indicator superior to the number-based and the ratio-based N category for gastric cancer patients with R0 resection. CANCER-AM CANCER SOC. [Journal Article; Research Support, Non-U.S. Gov't]. 2010 2010-06-01;116(11):2571-80.
9. Zhao E, Zhou C, Chen S. Prognostic nomogram based on log odds of positive lymph nodes for gastric carcinoma patients after surgical resection. FUTURE ONCOL. [Journal Article]. 2019 2019-12-01;15(36):4207-22.
10. Gu P, Deng J, Sun Z, Wang Z, Wang W, Liang H, et al. Superiority of log odds of positive lymph

nodes (LODDS) for prognostic prediction after gastric cancer surgery: a multi-institutional analysis of 7620 patients in China. SURG TODAY. [Journal Article]. 2021 2021-01-01;51(1):101-10.

11. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence - SEER Research Data, 18 Registries, Nov 2019 Sub (2000-2017) - Linked To County Attributes - Time Dependent (1990-2017) Income/Rurality, 1969-2018 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, released April 2020, based on the November 2019 submission..

12. Yu Y, Zhang P, Yao R, Wang J, Wang P, Xue X, et al. Prognostic value of log odds of positive lymph nodes in node-positive lung squamous cell carcinoma patients after surgery: a SEER population-based study. Transl Lung Cancer Res. [Journal Article]. 2020 2020-08-01;9(4):1285-301.

13. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. CLIN CANCER RES. [Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.]. 2004 2004-11-01;10(21):7252-9.

14. Iasonos A, Schrag D, Raj GV, Panageas KS. How to build and interpret a nomogram for cancer prognosis. J CLIN ONCOL. [Journal Article; Review]. 2008 2008-03-10;26(8):1364-70.

15. Jian-Hui C, Shi-Rong C, Hui W, Si-le C, Jian-Bo X, Er-Tao Z, et al. Prognostic value of three different lymph node staging systems in the survival of patients with gastric cancer following D2 lymphadenectomy. Tumour Biol. [Journal Article]. 2016 2016-08-01;37(8):11105-13.

16. Aurello P, Petrucciani N, Nigri GR, La Torre M, Magistri P, Tierno S, et al. Log odds of positive lymph nodes (LODDS): what are their role in the prognostic assessment of gastric adenocarcinoma? J GASTROINTEST SURG. [Comparative Study; Journal Article]. 2014 2014-07-01;18(7):1254-60.

17. Kilic MO, Gundogdu SB, Ozden S, Saylam B, Tez M. The prognostic value of different node staging systems in patients with </=15 lymph nodes following surgery for gastric adenocarcinoma. ACTA CHIR BELG. [Journal Article]. 2018 2018-02-01;118(1):1-6.

18. Suh YS, Han DS, Kong SH, Lee HJ, Kim YT, Kim WH, et al. Should adenocarcinoma of the esophagogastric junction be classified as esophageal cancer? A comparative analysis according to the seventh AJCC TNM classification. ANN SURG. [Comparative Study; Journal Article; Research Support, Non-U.S. Gov't]. 2012 2012-05-01;255(5):908-15.

19. Huang Q, Shi J, Feng A, Fan X, Zhang L, Mashimo H, et al. Gastric cardiac carcinomas involving the esophagus are more adequately staged as gastric cancers by the 7th edition of the American Joint Commission on Cancer Staging System. Mod Pathol. [Journal Article; Research Support, Non-U.S. Gov't; Validation Study]. 2011 2011-01-01;24(1):138-46.

20. Amin M, Edge S, Greene F, Byrd D, Brookland R. AJCC cancer staging manual. 8th edition.: Springer; 2016.

21. Liu K, Feng F, Chen XZ, Zhou XY, Zhang JY, Chen XL, et al. Comparison between gastric and esophageal classification system among adenocarcinomas of esophagogastric junction according to AJCC 8th edition: a retrospective observational study from two high-volume institutions in China. GASTRIC CANCER. [Comparative Study; Journal Article; Observational Study]. 2019 2019-05-01;22(3):506-17.

22. Karstens KF, Ghadban T, Sawez S, Konczalla L, Woestemeier A, Bachmann K, et al. Comparison of the 8th UICC staging system for esophageal and gastric cancers in Siewert type II junctional adenocarcinomas. Eur J Surg Oncol. [Comparative Study; Journal Article]. 2020 2020-04-01;46(4 Pt A):638-43.

23. Rizk NP, Ishwaran H, Rice TW, Chen LQ, Schipper PH, Kesler KA, et al. Optimum lymphadenectomy for esophageal cancer. ANN SURG. [Journal Article; Research Support, Non-U.S. Gov't]. 2010 2010-01-01;251(1):46-50.

24. Macalindong SS, Kim KH, Nam BH, Ryu KW, Kubo N, Kim JY, et al. Effect of total number of harvested lymph nodes on survival outcomes after curative resection for gastric adenocarcinoma: findings from an eastern high-volume gastric cancer center. BMC CANCER. [Journal Article; Research Support, Non-U.S. Gov't]. 2018 2018-01-12;18(1):73.

25. Schoenleber SJ, Schnelldorfer T, Wood CM, Qin R, Sarr MG, Donohue JH. Factors influencing lymph node recovery from the operative specimen after gastrectomy for gastric adenocarcinoma. J GASTROINTEST SURG. [Journal Article]. 2009 2009-07-01;13(7):1233-7.

26. Khanjani N, Mirzaei S, Nasrolahi H, Hamedi SH, Mosalaei A, Omidvari S, et al. Insufficient lymph node assessment in gastric adenocarcinoma. J Egypt Natl Canc Inst. [Journal Article]. 2019 2019-10-22;31(1):2.

27. Pei JP, Zhang CD, Fan YC, Dai DQ. Comparison of Different Lymph Node Staging Systems in Patients With Resectable Colorectal Cancer. FRONT ONCOL. [Journal Article]. 2018 2018-01-20;8:671.

28. Cao J, Yuan P, Ma H, Ye P, Wang Y, Yuan X, et al. Log Odds of Positive Lymph Nodes Predicts Survival in Patients After Resection for Esophageal Cancer. ANN THORAC SURG. [Journal Article; Multicenter Study]. 2016 2016-08-01;102(2):424-32.

29. Jin W, Zhu Z, Wu Y, Ding X, Wu H, Song X, et al. Prognostic value of log odds of positive lymph nodes in patients with resectable oral squamous cell carcinoma. ORAL ONCOL. [Journal Article; Research Support, Non-U.S. Gov't]. 2020 2020-09-01;108:104709.

30. Li J, Sun Y, Zhao B, Tang C, Fan D, Jiang W, et al. Lymph Node Ratio-Based Staging System for Gallbladder Cancer With Fewer Than Six Lymph Nodes Examined. FRONT ONCOL. [Journal Article]. 2020 2020-01-20;10:542005.

31. Spillane AJ, Cheung BL, Winstanley J, Thompson JF. Lymph node ratio provides prognostic information in addition to american joint committee on cancer N stage in patients with melanoma, even if quality of surgery is standardized. ANN SURG. [Journal Article]. 2011 2011-01-01;253(1):109-15.

32. Rosa F, Tortorelli AP, Alfieri S, Doglietto GB. Lymph node ratio for gastric cancer: useful instrument or just an expedient to retrieve fewer lymph nodes? ANN SURG. [Letter; Comment]. 2014 2014-04-01;259(4):e65.

33. van Hagen P, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge HM, Wijnhoven BP, et al. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med. [Journal Article; Multicenter Study; Randomized Controlled Trial; Research Support, Non-U.S. Gov't]. 2012 2012-05-31;366(22):2074-84.

34. Ajani JA, D'Amico TA, Bentrem DJ, Chao J, Corvera C, Das P, et al. Esophageal and Esophagogastric Junction Cancers, Version 2.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. [Journal Article]. 2019 2019-07-01;17(7):855-83.

FIGURE LEGENDS

Figure 1. Flow diagram of the patient selection and grouping.

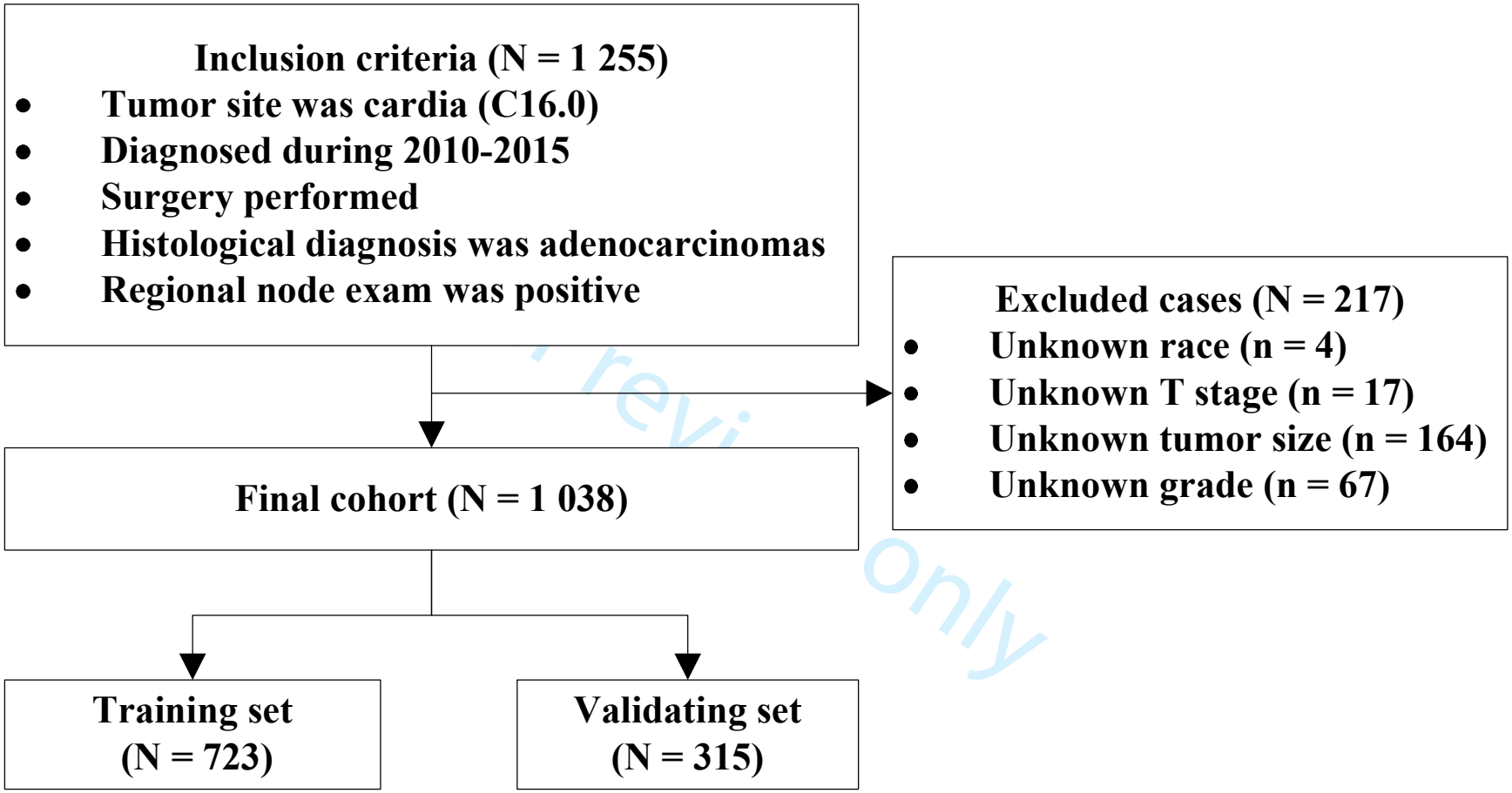
Figure 2. Survival curves of the training and validating sets by different staging systems.

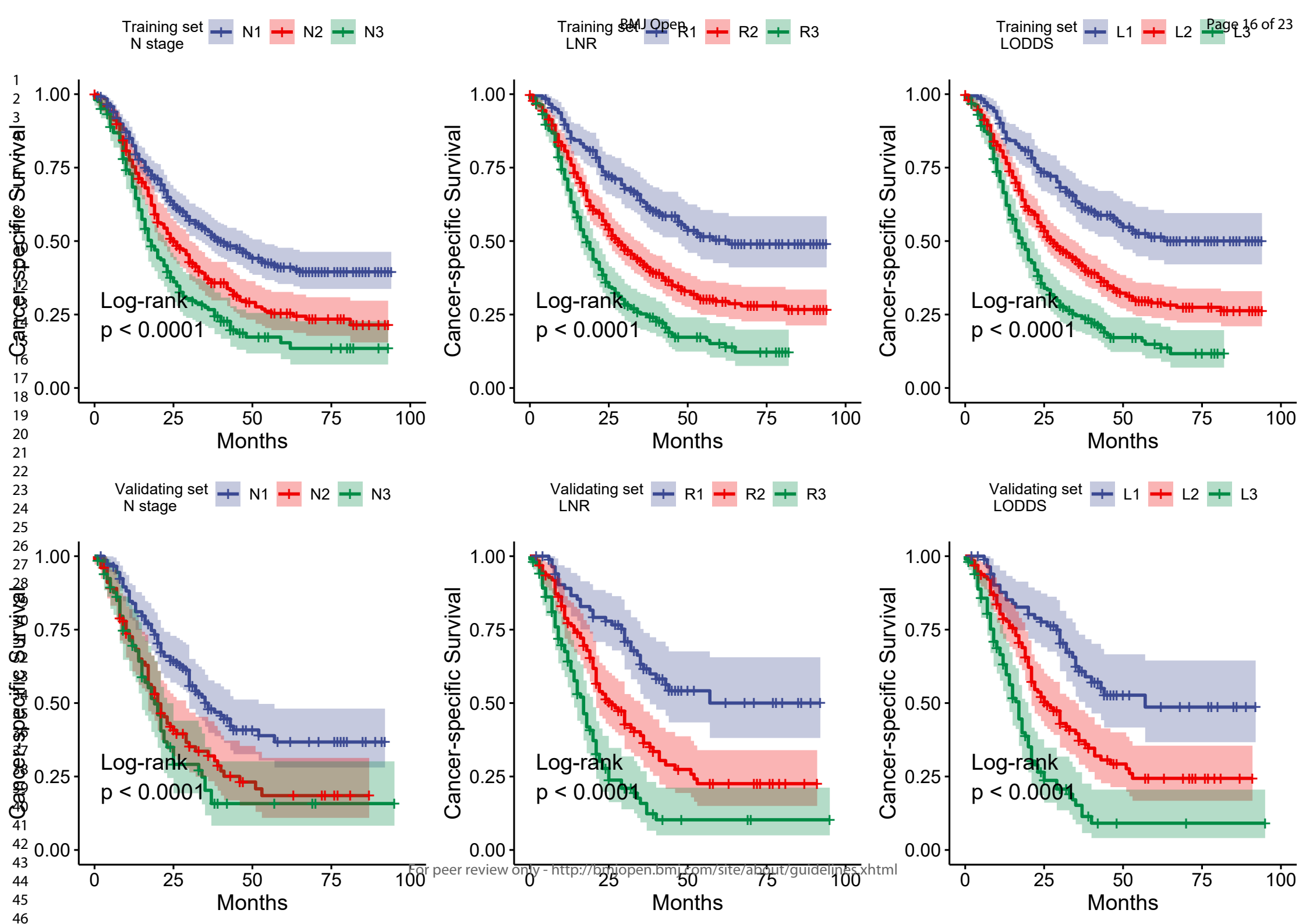
Figure 3. Construction of nomogram based on Tumor-Lymph node ratio-Metastasis stating system and calibration plots for the nomogram.

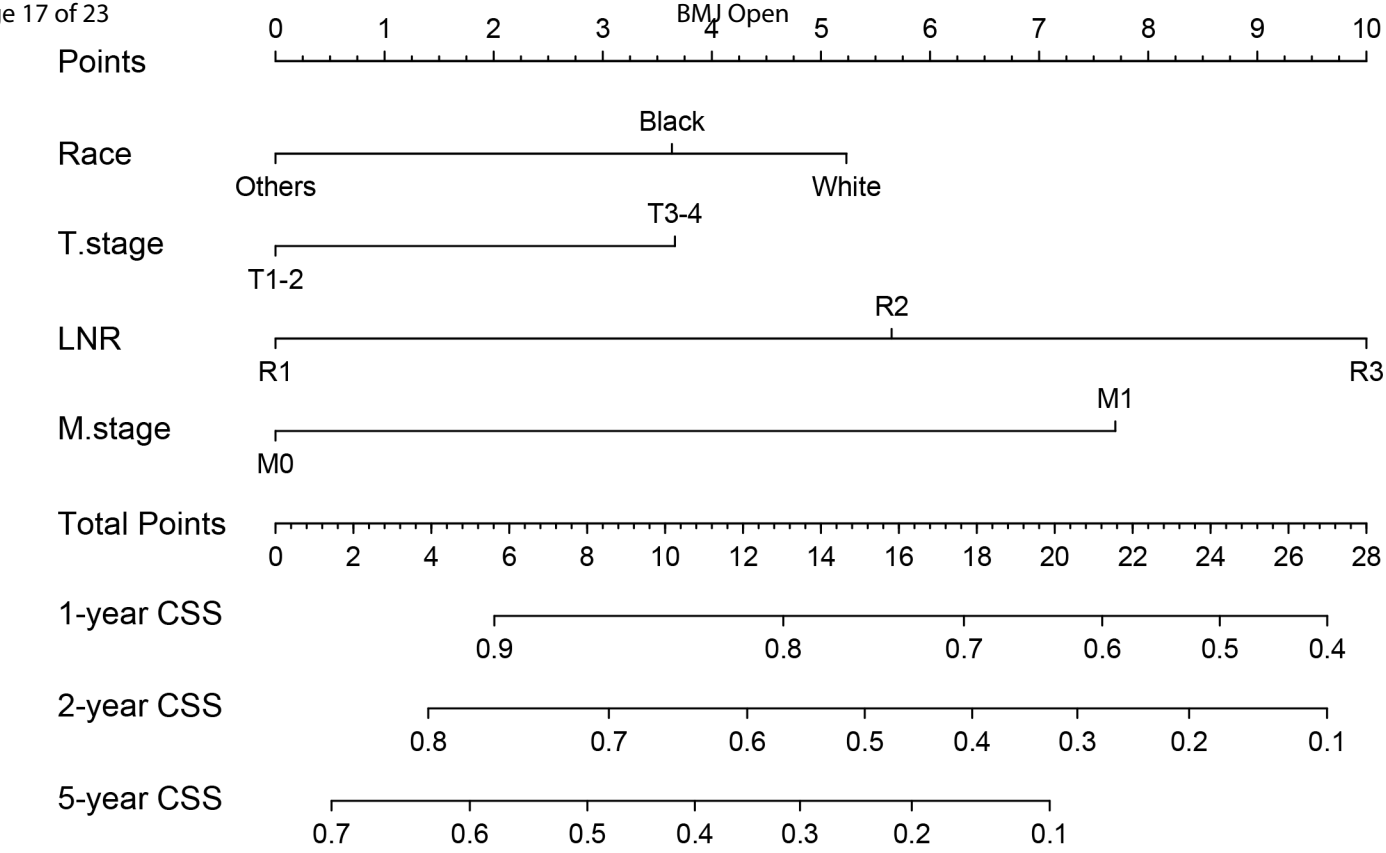
Suppl. Figure 1.Kaplan Meier plots for proportional hazard assumption test in models that were based on N stage, lymph node ratio, and log odds of positive lymph nodes.

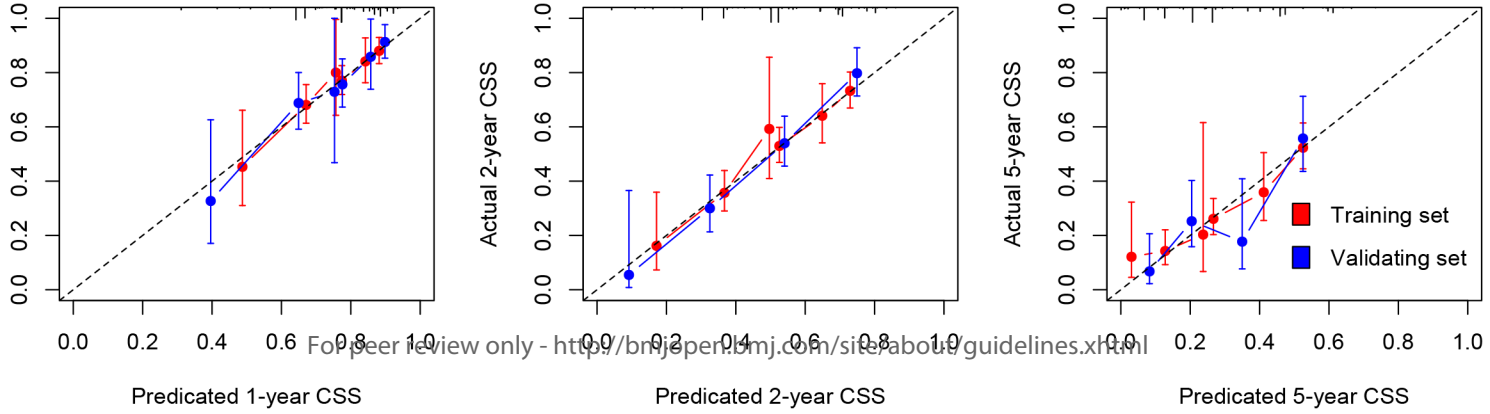
Suppl. Figure 2. Construction of nomogram based on Tumor-Log odds of positive lymph nodes -Metastasis stating system and calibration plots for the nomogram.

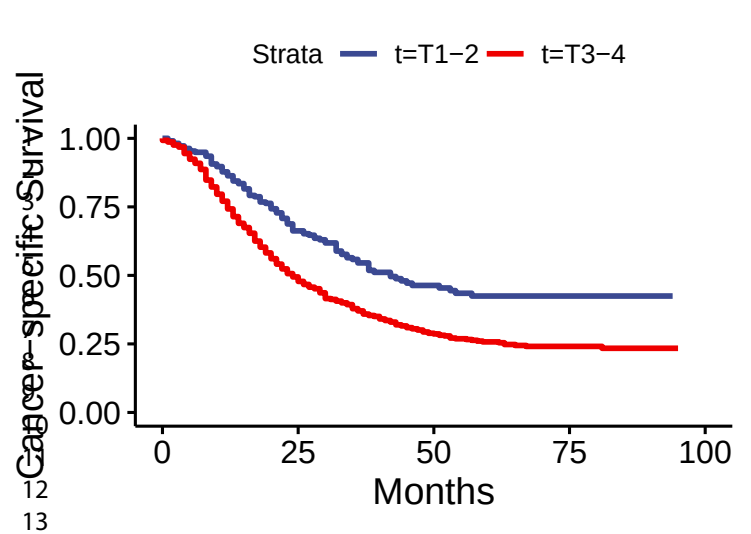
Suppl. Figure 3. Receiver operator characteristic curves and decision curve analysis plots for comparison of the prediction powers of the different models.

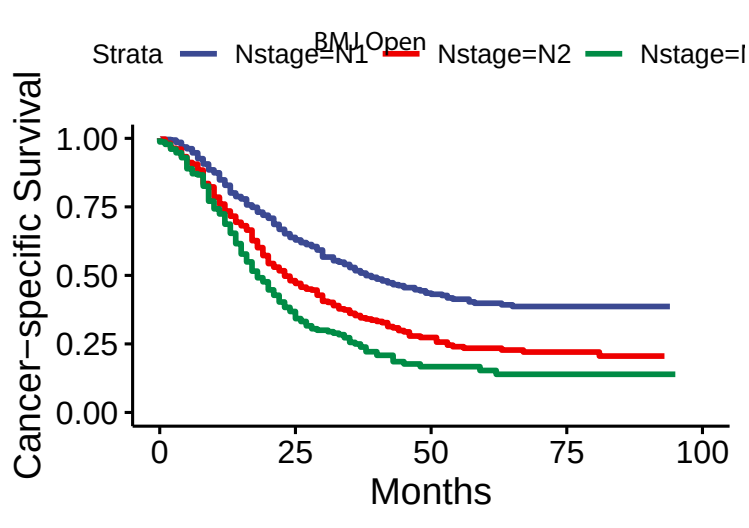


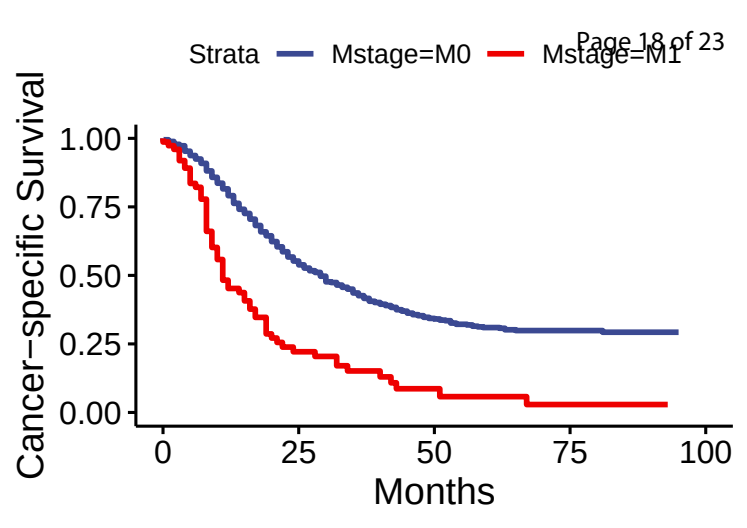


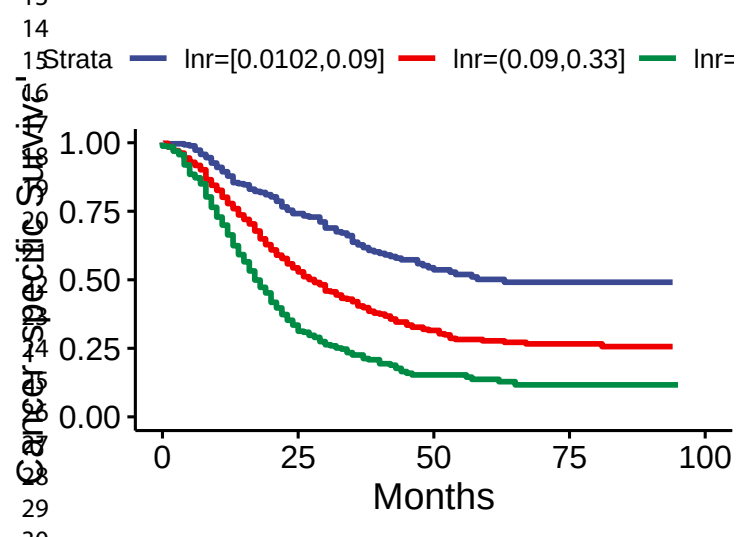


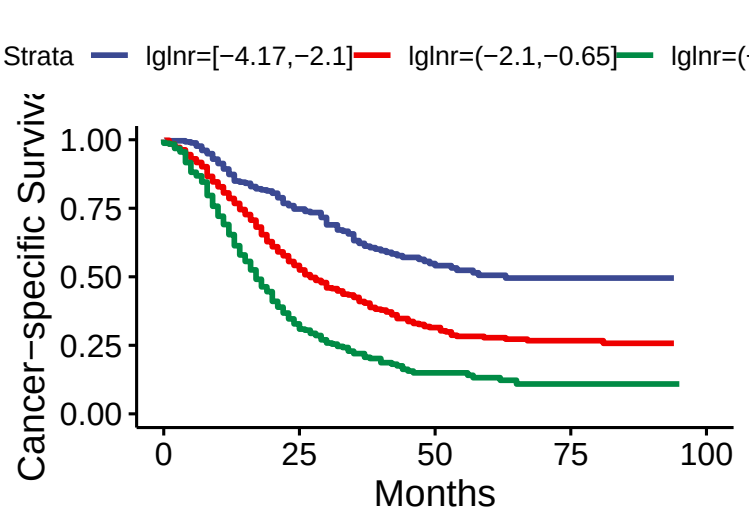


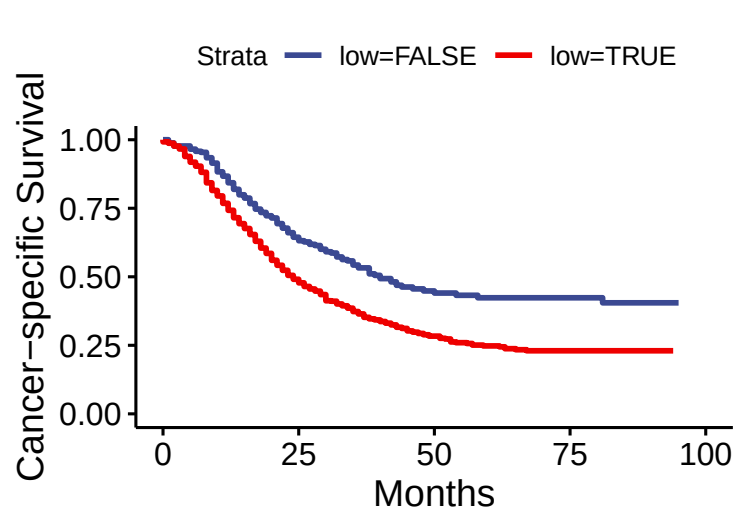


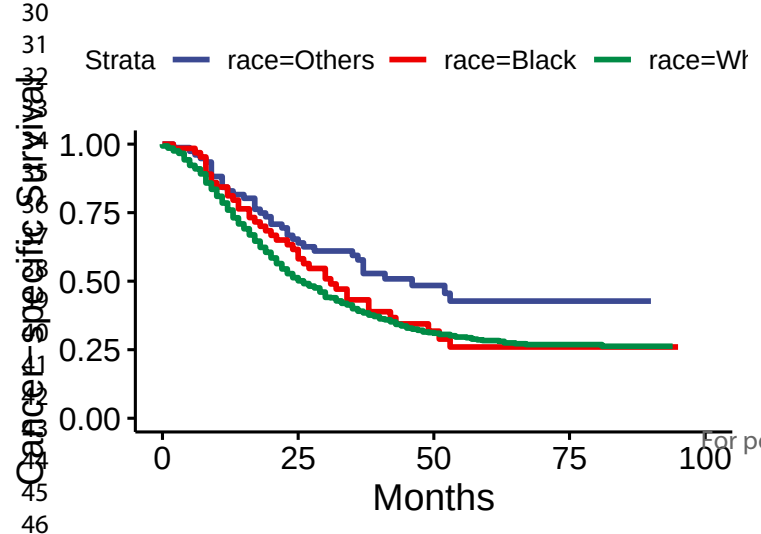


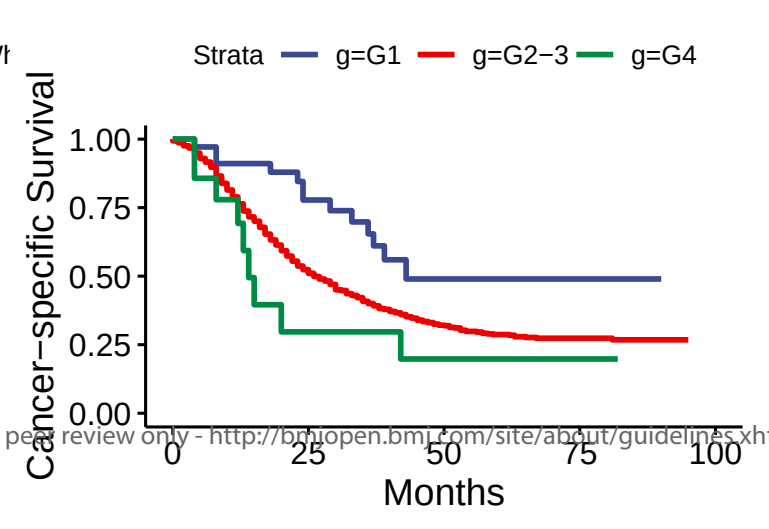


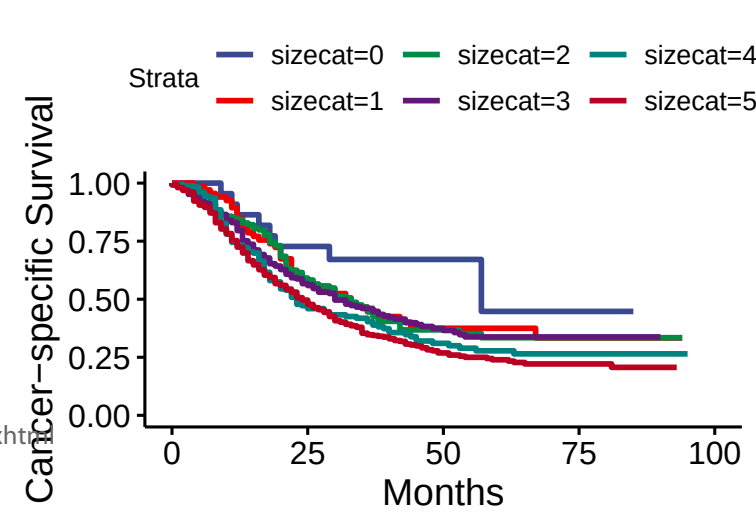


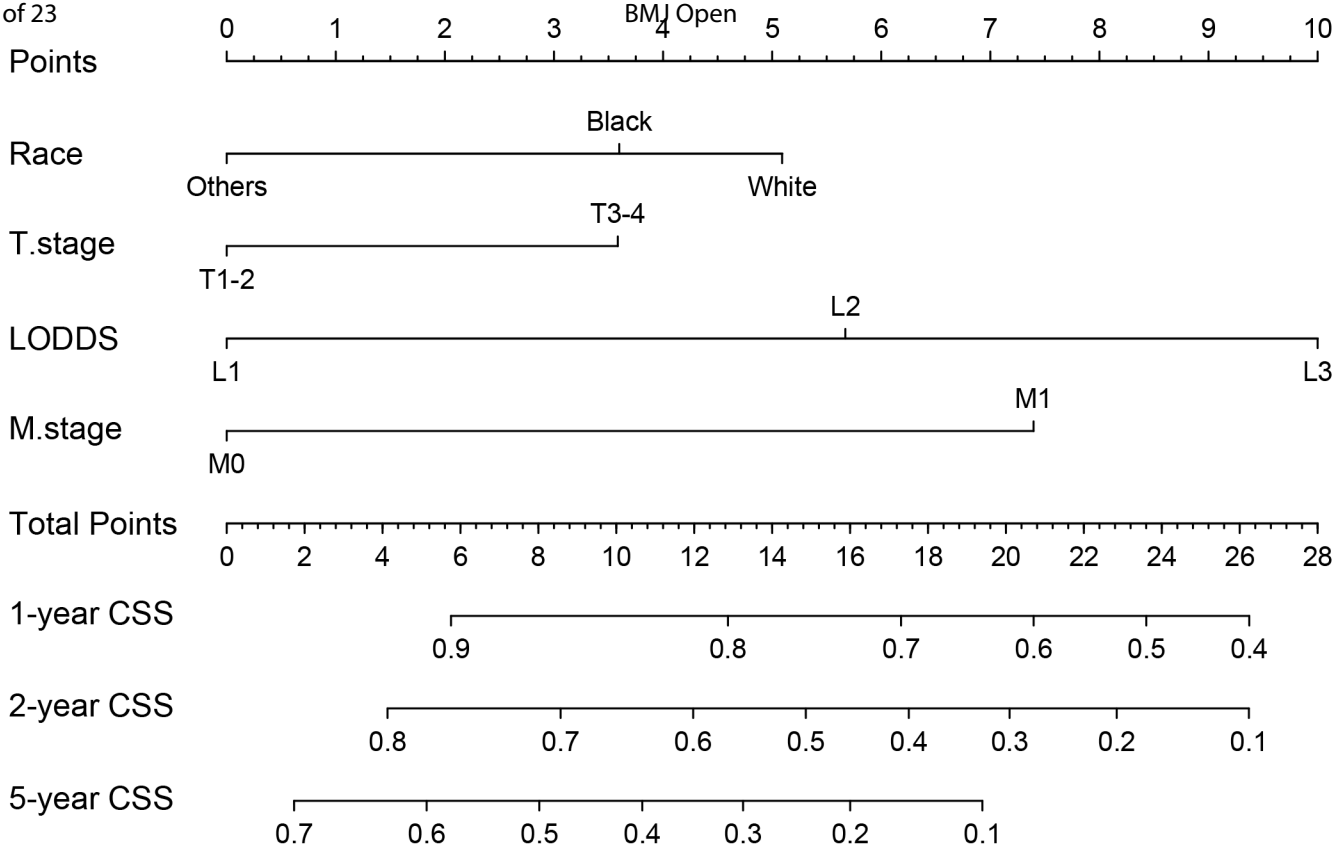


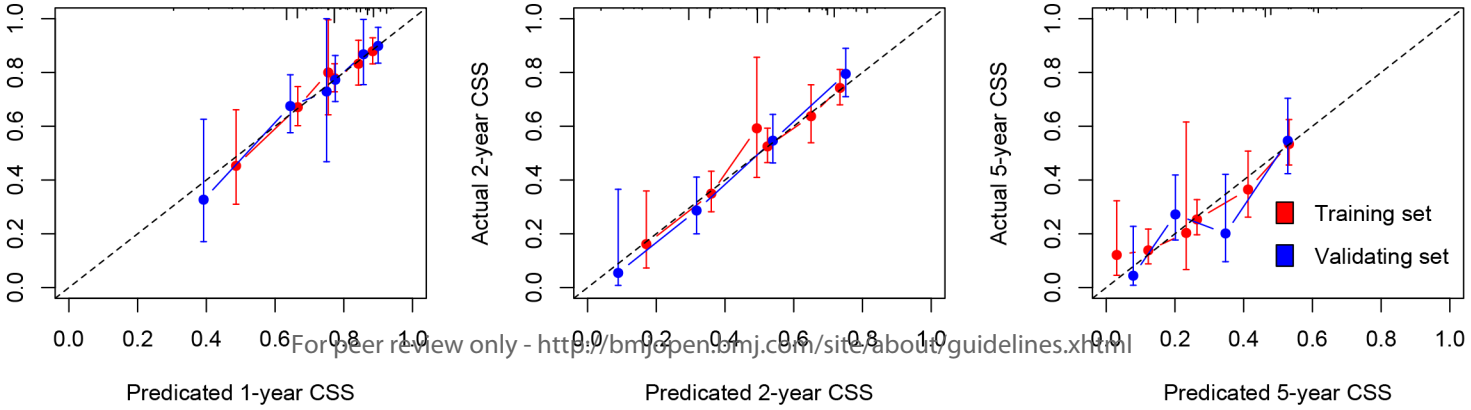


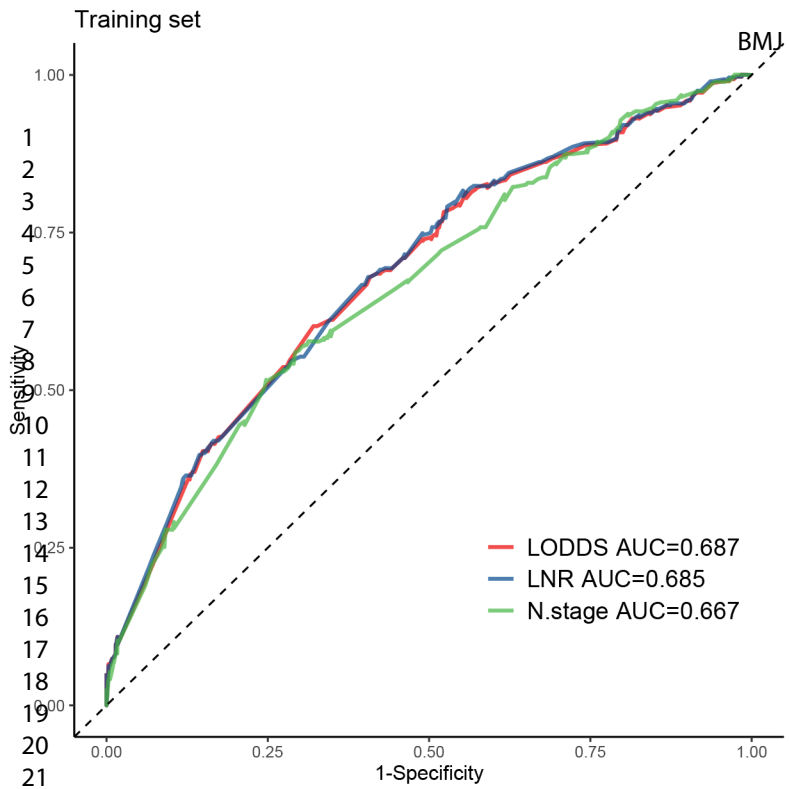


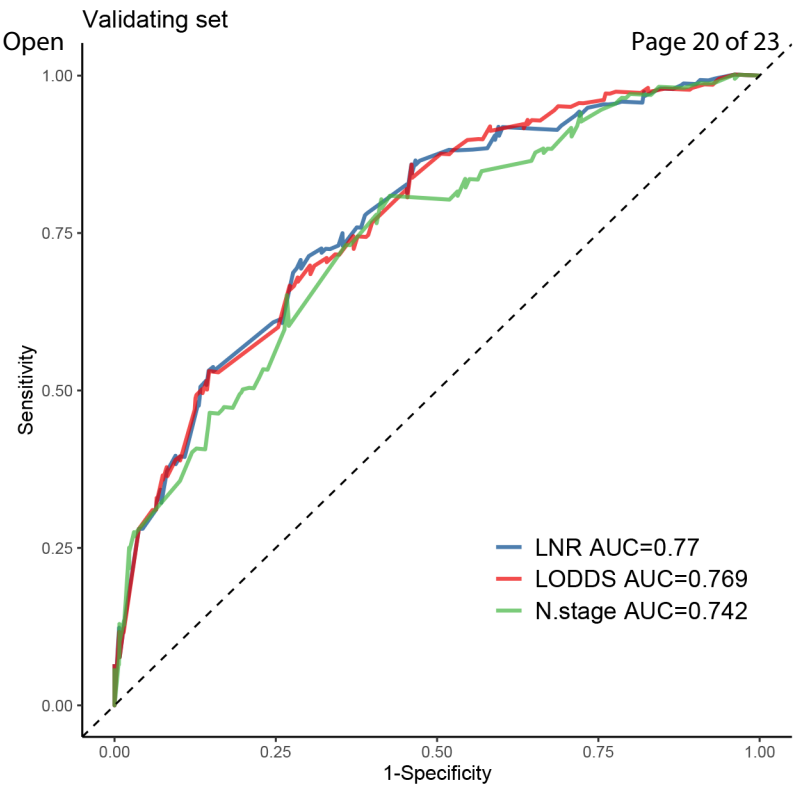


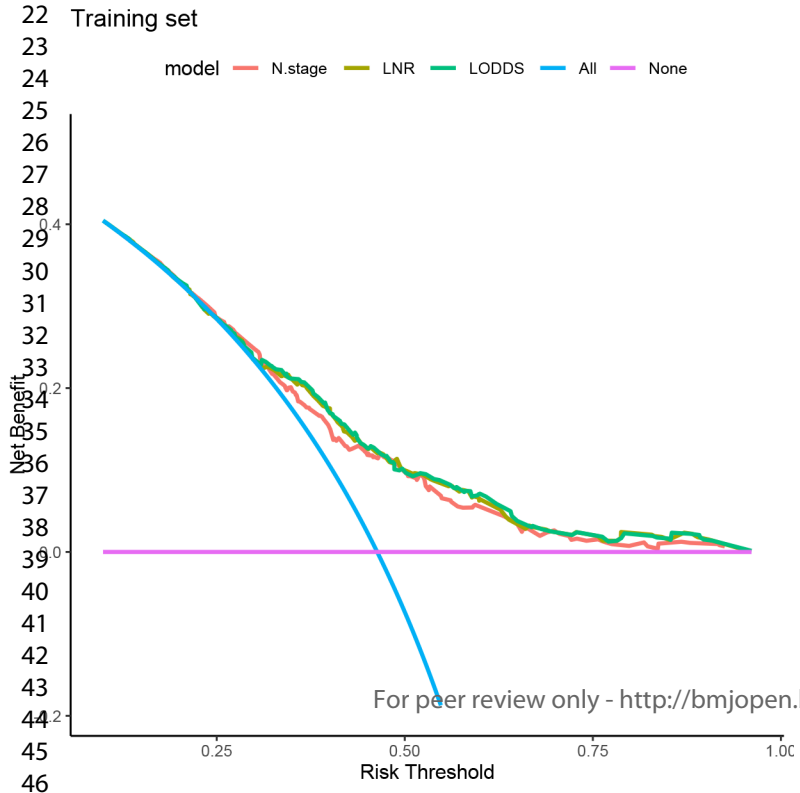


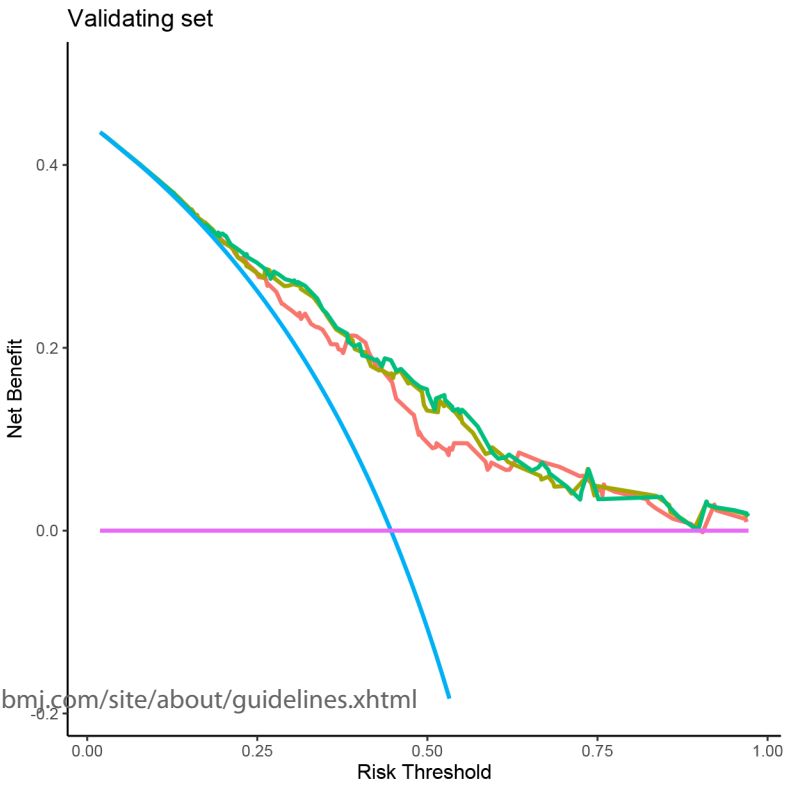












Supplementary Table 1. Comparison of the discrimination ability of different models based on different cutoff values.

	Crude model			Adjusted model	
	HR (95% CI)	C-index	AIC	HR (95% CI)	C-index
LNR					
Cutoff_1		0.607	5376	0.643	5350
<0.09	1 (ref)			1 (ref)	
0.09~0.33	1.88 (1.44, 2.44)			1.74 (1.33, 2.29)	
>0.33	3.02 (2.30, 3.97)			2.63 (1.97, 3.50)	
Cutoff_2		0.605	5378	0.641	5355
<0.09	1 (ref)			1 (ref)	
0.09~0.40	1.97 (1.52, 2.54)			1.85 (1.38, 2.54)	
>0.40	3.16 (2.38, 4.21)			2.72 (2.02, 3.67)	
LODDS					
Cutoff_1		0.609	5373	0.644	5346
<-2.09	1 (ref)			1 (ref)	
-2.09~-0.65	1.93 (1.48, 2.51)			1.80 (1.36, 2.37)	
>-0.65	3.13 (2.38, 4.13)			2.77 (2.07, 3.70)	
Cutoff_2		0.605	5378	0.640	5352
<-2.10	1 (ref)			1 (ref)	
-2.09~-0.37	2.00 (1.54, 2.59)			1.86 (1.42, 2.44)	
>-0.37	3.26 (2.45, 4.33)			2.83 (2.10, 3.81)	

Cutoff_1 was generated by minimal p-value method via X-tile software, cutoff_2 was generated using P25 and P75.

Supplementary Table 2. Univariate analysis of the potential confounders.

Variables	HR (95% CI)	P-value
Age (increased by 10ys)	1.06 (0.98 – 1.14)	0.065
Tumor size (increased by 1cm)	1.13 (1.06 – 1.19)	<0.001
Sex		
Male	1	
Female	1.09 (0.89 – 1.34)	0.386
Race		
Others	1	
Black	1.42 (0.91 – 2.21)	0.118
White	1.57 (1.13 – 2.19)	0.007
Grade		
G1	1	
G2-3	2.08 (1.20 – 3.60)	0.009
G4	3.08 (1.32 – 7.22)	0.009
T		
T1-2	1	
T3-4	1.70 (1.38 – 2.10)	<0.001
N		
N1	1	
N2	1.61 (1.34 – 1.93)	<0.001
N3	2.15 (1.77 – 2.62)	<0.001
M		
M0	1	
M1	2.61 (2.00 – 3.39)	<0.001
Low nodes yield		
No	1	
Yes	1.64 (1.35 – 1.99)	<0.001

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **title page**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **page2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **page 3**
Objectives	3	State specific objectives, including any prespecified hypotheses **page3**
Methods		
Study design	4	Present key elements of study design early in the paper **page 3**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **page 3-4**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **page 4** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls **NA** *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants **NA**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed **NA** *Case-control study*—For matched studies, give matching criteria and the number of controls per case **NA**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **page 4**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **page 3-4**
Bias	9	Describe any efforts to address potential sources of bias **page 4**
Study size	10	Explain how the study size was arrived at **NA**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **page 4**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **page 4**
		(*b*) Describe any methods used to examine subgroups and interactions **page 4**
		(*c*) Explain how missing data were addressed **page 4**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed **NA** *Case-control study*—If applicable, explain how matching of cases and controls was addressed **NA** *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy **NA**
		(*e*) Describe any sensitivity analyses **NA**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible,

		examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **page 5**
		(b) Give reasons for non-participation at each stage **page 5**
		(c) Consider use of a flow diagram **figure 1, page 3**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **page 5-6**
		(b) Indicate number of participants with missing data for each variable of interest **figure 1, page 3**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **Table 1 page 5-6**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time **page 5**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure **NA**
		Cross-sectional study—Report numbers of outcome events or summary measures **NA**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **table 1, page 5-6**
		(*b*) Report category boundaries when continuous variables were categorized **page 6**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **NA**
Discussion		
Key results	18	Summarise key results with reference to study objectives **page 7-8**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **page 9**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **page 8-9**
Generalisability	21	Discuss the generalisability (external validity) of the study results **page 9**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **page 10**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.