Retrospective Cohort Study

INTERNATIONAL JOURNAL OF SURGERY

OPEN

Finding the minimum number of retrieved lymph nodes and negative lymph nodes in gastric cancer surgery: a real-world study

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Background: Lymph node retrieval deficiency can lead to understagement and postoperative cancer recurrence, it is crucial to establish the standard number of retrieved lymph nodes (rLNs) and negative lymph nodes (nLNs) for patients undergoing gastrectomy.

Methods: Patients who has gastric adenocarcinoma and underwent either radical subtotal gastrectomy (RSG) or radical total gastrectomy (RTG) between 2000 and 2022 were retrospectively included. The authors utilized restricted cubic spline (RCS) analysis to determine the ideal threshold for rLNs and nLNs. Survival analysis was conducted using Kaplan–Meier (KM) curves, log-rank tests and forest plots. Propensity score matching (PSM) was utilized to balance parameters between two groups. The median follow-up time for this study was 3095 days.

Results: Our study found that there are significant tumor characteristic differences between RSG and RTG. For patients with N0–N3a stage undergoing RSG, retrieving greater than or equal to 24 lymph nodes intraoperatively were associated with better prognosis both before and after PSM [overall survival (OS): $P<0.001$, $P=0.019$]; whereas for N3b stage, at least 32 rLNs were required (OS: $P=0.006$, $P=0.023$). Similarly, for patients with N0–N3a stage undergoing RTG, retrieving greater than or equal to 27 lymph nodes intraoperatively were associated with better prognosis both before and after PSM (OS: $P<0.001$, $P=0.047$); whereas for N3b stage, at least 34 rLNs were required (OS: $P<0.001$, $P=0.003$). Additionally, for patients undergoing RSG, having greater than or equal to 21 nLNs (OS: $P<0.001$, $P=0.013$), and for those undergoing RTG, having greater than or equal to 22 nLNs (OS: $P<0.001$, $P<0.001$), were also associated with better prognosis both before and after PSM.

Conclusions: For patients receiving RSG, rLNs should reach 24 when lymph nodes are limited, and 32 when lymph node metastasis is more extensive, with a minimum number of nLNs ideally reaching 21. Similarly, for patients receiving RTG, rLNs should reach 27 when lymph nodes are limited, 34 when lymph node metastasis is more extensive, and a minimum number of nLNs ideally reaching 22.

Keywords: negative lymph nodes, radical subtotal gastrectomy, radical total gastrectomy, retrieved lymph nodes

Introduction

The high incidence and mortality rates of gastric cancer globally pose a significant burden on healthcare systems[1]. Accurate staging of gastric cancer is crucial for guiding treatment decisions and predicting prognosis[2]. For surgically resectable gastric cancer patients without distant metastasis, N staging is the most critical factor in determining treatment and prognosis, followed

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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International Journal of Surgery (2024) 110:5574–5584

Received 27 March 2024; Accepted 7 May 2024

Supplemental Digital Content is available for this article. Direct URL citations are provided in the HTML and PDF versions of this article on the journal's website, www.lww.com/international-journal-of-surgery.

Published online 16 May 2024

http://dx.doi.org/10.1097/JS9.0000000000001626

by T staging[3]. While T staging can be assessed through intraoperative pathological examination precisely, N staging depends on the number of detected positive lymph nodes, which is closely related to the total number of lymph nodes sampled during intraoperative biopsy. Insufficient total detection of lymph nodes may result in the omission of some positive lymph nodes, thereby leading to the phenomenon of stage migration[4]. A study on gastric cancer resection in 2002 revealed that examining fewer than 10 lymph nodes is unreliable for staging, and after surpassing 15 lymph nodes, the proportion of positive tumors stabilizes. The proposal to use the number of retrieved lymph nodes (rLNs) as an adjusting variable in survival studies was made[5]. In patients undergoing curative gastric cancer resection, the status and quantity of rLNs provide accurate prognostic information[6]. However, there has been ongoing debate in the academic community regarding the minimum number of lymph nodes for achieving this effect.

The sixth edition of the American Joint Committee on Cancer (AJCC) guidelines for gastric cancer treatment stipulated that at least 15 lymph nodes should be examined during curative gastric cancer resection to achieve a more accurate N staging. The seventh and eighth editions revised this number to 16. However, it is evident that whether examining 15 or 16 lymph nodes, it is notably insufficient for N3b stage patients, and the evaluation of N staging for other N stages is also easily understood to be too low. Therefore, in recent years, several studies have explored the minimum number of rLNs for gastric cancer patients. Research by Yu-Yin Liu suggests that detecting at least 25 lymph nodes in patients undergoing curative distal gastrectomy significantly improves prognosis[7]. A large-scale data study combining the Surveillance, Epidemiology, and End Results (SEER) databases, along with the Yonsei University Gastric Cancer Database, found that for gastric adenocarcinoma, lymph node dissection and detection of at least 29 lymph nodes are associated with the greatest survival advantage[8]. Moreover, a multicenter clinical study in Japan suggested that at least 40 lymph nodes need to be examined for stage III gastric cancer patients to benefit from a better prognosis[9]. However, is more always better when it comes to the number of rLNs? The results of Shiela S Macalindong's study indicate that in moderately staged gastric cancer patients, prognosis increases with an increasing number of rLNs, but when exceeding 45, patients' overall survival (OS) and disease-free survival (DFS) are shortened[10]. This may be due to a higher likelihood of positive lymph nodes in patients with more than 45 detected, leading to a poorer prognosis.

In summary, multiple studies indicate that a minimum of 16 lymph nodes retrieved during curative gastric cancer resection is insufficient in clinical practice, and the optimal number varies among studies. Due to the selection of radical subtotal gastrectomy (RSG) and radical total gastrectomy (RTG) based on different tumor locations and sizes, the pathways and extent of lymph node metastasis are likely to vary. Therefore, we believe the minimum rLNs required also need to be analyzed separately according to the surgical procedure. However, there is a paucity of research did so. Similarly, studies on the number of negative lymph nodes (nLNs) are also scarce. Therefore, by utilizing gastric cancer data from our cancer center spanning from 2000 to 2022, this study established the minimum number of rLNs and nLNs during RSG and RTG.

HIGHLIGHTS

- This is a study with a large sample size seeking the minimum number of retrieved lymph nodes (rLNs) and negative lymph nodes (nLNs) during radical subtotal gastrectomy and total gastrectomy for curative purposes.
- For patients receiving radical subtotal gastrectomy, rLNs should reach 24 when lymph nodes are limited, and 32 when lymph node metastasis is more extensive, with a minimum number of nLNs ideally reaching 21.
- For patients receiving radical total gastrectomy, rLNs should reach 27 when lymph nodes are limited, 34 when lymph node metastasis is more extensive, and a minimum number of nLNs ideally reaching 22.

Materials and methods

Study design and patient population

This retrospective cohort single-center study was registered at the Research Registry. This study adheres to the ethical principles outlined in the Helsinki Declaration. Additionally, this study complies with the Strengthening The Reporting Of Cohort Studies in Surgery (STROCSS, Supplemental Digital Content 1, http://links.lww.com/JS9/C594) guidelines[11]. Ethical approval was obtained from the ethics committee of our cancer center (No. 050432-4-2307E). And we have obtained written informed consent from all patients.

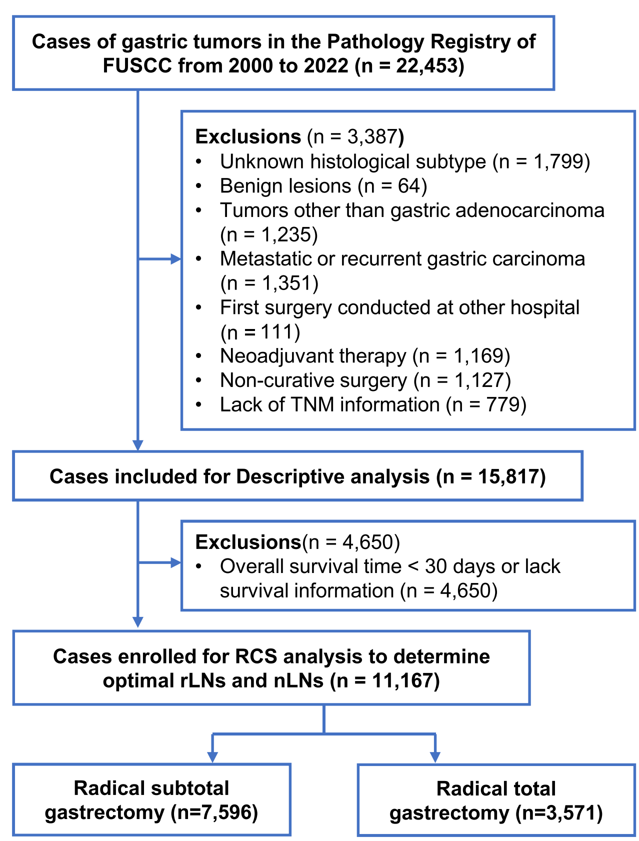

Figure 1. Flow chart of patient selection process. nLNs, negative lymph nodes; RCS, restricted cubic spline; rLNs, retrieved lymph nodes.

Table 1

Patient characteristics.

Characteristics[a]	GC (n = 15 817)
Age, mean (range)	59.35 (15–92)
Sex, n (%)	
Female	4814 (30.44)
Male	11 003 (69.56)
T stage, n (%)	
T1	4679 (29.58)
T2	2201 (13.92)
T3	2515 (15.90)
T4	6422 (40.60)
N stage, n (%)	
N0	6916 (43.73)
N1	2613 (16.52)
N2	2648 (16.74)
N3a	2564 (16.21)
N3b	1075 (6.80)
TNM stage, n (%)	
I	5438 (34.38)
II	3741 (23.65)
III	6638 (41.97)
BMI, Kg/m^2, n (%)	
< 18.5	924 (5.84)
≥ 18.5, <25	9290 (58.73)
≥ 25, <30	3086 (19.51)
≥ 30	248 (1.57)
Unknown	2269 (14.35)
AFP	
> 8 ng/ml	605 (3.83)
≤ 8 ng/ml	13 151 (83.15)
Unknown	2061 (13.02)
CEA	
> 5 ng/ml	2064 (13.05)
≤ 5 ng/ml	11 707 (74.01)
Unknown	2046 (12.94)
Primary tumor site	
Upper	3377 (21.35)
Middle	2995 (18.94)
Lower	7914 (50.03)
Diffuse	1531 (9.68)
Extent of gastrectomy	
Subtotal	11 153 (70.51)
Total	4664 (29.49)
Tumor size	
> 4 cm	5288 (33.43)
≤ 4 cm	10 461 (66.14)
Unknown	67 (0.43)
Vascular invasion	
Yes	7252 (45.85)
No	8521 (53.87)
Unknown	44 (0.28)
Lymphatic invasion	
Yes	6432 (40.67)
No	9365 (59.21)
Unknown	20 (0.12)
Perineural invasion	
Yes	7031 (44.45)
No	8743 (55.28)
Unknown	43 (0.27)
Grade	
Well	473 (2.99)
Moderate	7558 (47.78)
Poor	7260 (45.90)
Diffuse	11 (0.07)
Unknown	515 (3.26)

GC, gastric cancer; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen.

[a]Continuous data were showed as mean (ranges) and categoric data as number (%).

We retrospectively analyzed 22 453 patients who underwent surgical treatment in the Department of Gastric Surgery at our cancer center from 1 January 2000 to 31 December 2022. Inclusion criteria were as follows: (1) Patients with curative surgery for gastric cancer; (2) Patients with histological type being adenocarcinoma; (3) Patients with complete staging information; (4) Patients with information on the number of lymph node retrieved. Exclusion criteria were as follows: (1) Patients with benign gastric lesions; (2) Patients concomitant other cancer types; (3) Patients with metastatic or recurrent gastric cancer; (4) Patients with initial surgery performed at other hospital; (5) Patients underwent preoperative neoadjuvant therapy; (6) Patients with distant metastasis. According to the inclusion and exclusion criteria, a total of 15 817 patients were included in the initial study. The patients selection process is depicted in Figure 1.

The main study variables included: age, T stage, N stage, TNM stage, number of rLNs, number of positive lymph nodes, number of nLNs, BMI, alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), primary tumor site, extent of gastrectomy, tumor size, vascular invasion, lymphatic invasion, perineural invasion, and grade. BMI is calculated by dividing weight (in kilograms) by the square of height (in meters). The number of nLNs is derived by subtracting the number of positive lymph nodes from the total number of rLNs. Curative surgery for gastric cancer included RTG, radical distal gastrectomy, and radical proximal gastrectomy. Radical distal gastrectomy and radical proximal gastrectomy were grouped together as RSG.

Endpoints and follow-up

Follow-up was conducted by trained professionals via telephone interviews, with a cut-off date of 31 October 2023. The primary study endpoint was OS, defined as the time from the date of surgery to either death from gastric cancer or termination of follow-up. The secondary endpoint of the study is DFS, defined as the time from the date of surgery to the occurrence of recurrence or metastasis, or until the last follow-up date. The follow-up period ranges from 313 to 8695 days. The median follow-up time for this study was 2682 days, with an average follow-up time of 3095 days. By the end of follow-up, 4650 patients were unable to obtain survival status, resulting in a loss to follow-up rate of 29.3%. 3588 patients experienced tumor-related death during the follow-up period, with a 5-year survival rate of 51.4%. Patients with postoperative survival time less than 30 days and those lost to follow-up were excluded from subsequent analysis, remaining 11 167 patients.

Statistical analyses

All analyses and plots were performed using SPSS (IBM Corp, version 27.0.1.0), Origin (version 2018, 9.50), R (version 4.3.1), GraphPad Prism (version 9.5.730), along with Storm Statistical Platform. The R package "rms" was used for Restricted cubic spline (RCS) analysis, which determined three key points to establish cut-off values for rLNs and nLNs in achieving better OS. Patients were then divided into two groups based on the thresholds obtained: the adequate group and the limited group. Propensity score matching (PSM) was used to balance other potential prognostic variables between the two groups, with 1:1 without replacement matching and standard mean difference (SMD) less than 0.1. Forest plot visualization were generated using SangerBox. Survival analysis was conducted using Kaplan–Meier (KM) curves and compared between groups using the log-rank test for univariate comparisons. All statistical tests were two-sided, with a significance level of 0.05.

Results

Patient characteristics

This study included a total of 15 817 patients. Their demographics and pathological characteristics are shown in Table 1. Patients' age ranged from 21 to 92 years, with a median age of 61 years. Tumor distribution included the upper part (21.35%), middle part (18.94%), lower part (50.03%), and diffused distribution (9.68%). In terms of tumor differentiation, moderately differentiated tumors (47.78%) and poorly differentiated tumors (45.90%) constituted the vast majority. We subsequently compared the clinical and pathological characteristics between patients receiving RSG and RTG (Table 2), revealing significant differences in T stage ($P<0.001$), N stage ($P<0.001$), TNM stage ($P<0.001$), tumor location ($P<0.001$), and tumor size ($P<0.001$) etc. This confirms that patients undergoing RTG exhibit more advanced features compared to those undergoing RSG, necessitating separate analyses of rLNs for each group.

Data trends

In our study spanning from 2000 to 2022, a consistent increase in the number of rLNs was observed for both RSG and RTG procedures (Supplementary Fig. 1A-B, Supplemental Digital Content 2, http://links.lww.com/JS9/C595). In the past decade, among 10 747 patients with gastric adenocarcinoma, 10 233 (95.22%) achieved intraoperative adherence to the AJCC recommended standards. The overall data indicates that the average rLNs in our cancer center over 23 years is 25.88 with a median of 24. For patients undergoing RSG, the average rLNs is 24.4 with a median of 23, while for those undergoing RTG, the average rLNs is 29.42 with a median of 28 (Fig. 2A-B). As depicted in Figure 2C-D, there is a varying decline in the detection rates of N0, N1, and N2 stages with an increase in the number of rLNs, while the detection rate for N3 stage steadily increases. Supplementary Fig. 1C-D, Supplemental Digital Content 2, http://links.lww.com/JS9/C595 provides a more intuitive representation of the linear relationship between rLNs and the detection rates of lymph node positivity and N3 percentage, with this relationship being more pronounced in RTG.

Table 2

The statistical test of clinical pathological parameters between the radical subtotal gastrectomy (RSG) group and the radical total gastrectomy (RTG) group.

Characteristics[a]	RSG (n = 7596)	RTG (n = 3571)	P
Age, mean (range)	59.47 (21–92)	61.39 (22–91)	< 0.001
Sex, n (%)			< 0.001
Female	2420 (31.86)	993 (27.81)	
Male	5176 (68.14)	2578 (72.19)	
T stage, n (%)			< 0.001
T1	2688 (35.39)	581 (16.27)	
T2	1118 (14.72)	333 (9.33)	
T3	1105 (14.55)	908 (25.43)	
T4	2685 (35.35)	1749 (48.98)	
N stage, n (%)			< 0.001
N0	3560 (46.87)	1131 (31.67)	
N1	1242 (16.35)	562 (15.74)	
N2	1265 (16.66)	649 (18.17)	
N3a	1133 (14.92)	756 (21.17)	
N3b	396 (5.21)	473 (13.25)	
TNM stage, n (%)			< 0.001
I	3010 (39.63)	704 (19.71)	
II	1758 (23.14)	845 (23.66)	
III	2828 (37.23)	2,022 (56.62)	
BMI, Kg/m^2, n (%)			< 0.001
< 18.5	460 (6.06)	241 (6.75)	
≥ 18.5, <25	4708 (61.98)	2263 (63.37)	
≥ 25, <30	1520 (20.01)	841 (23.55)	
≥ 30	122 (1.61)	69 (1.93)	
Unknown	786 (10.35)	157 (4.40)	
AFP, n (%)			< 0.001
> 8 ng/ml	292 (3.84)	149 (4.17)	
≤ 8 ng/ml	6367 (83.82)	3104 (86.92)	
Unknown	937 (12.34)	318 (8.91)	
CEA, n (%)			< 0.001
> 5 ng/ml	905 (11.91)	680 (19.04)	
≤ 5 ng/ml	5759 (75.82)	2577 (72.16)	
Unknown	932 (12.27)	314 (8.79)	
Primary tumor site, n (%)			< 0.001
Upper	1115 (14.68)	1201 (33.63)	
Middle	761 (10.02)	1403 (39.29)	
Lower	5372 (70.72)	210 (5.88)	
Diffuse	348 (4.58)	757 (21.20)	
Tumor size, n (%)			< 0.001
> 4 cm	2063 (27.16)	1745 (48.87)	
≤ 4 cm	5498 (72.38)	1814 (50.80)	
Unknown	35 (0.46)	12 (0.34)	
Vascular invasion, n (%)			< 0.001
Yes	3328 (43.81)	2211 (61.92)	
No	4245 (55.88)	1352 (37.86)	
Unknown	23 (0.30)	8 (0.22%)	
Lymphatic invasion, n (%)			< 0.001
Yes	3014 (39.68)	2164 (60.60)	
No	4574 (60.22)	1402 (39.26)	
Unknown	8 (0.11)	5 (0.14)	
Perineural invasion, n (%)			< 0.001
Yes	3057 (40.24)	2205 (61.75)	
No	4521 (59.52)	1360 (38.08)	
Unknown	18 (0.24)	6 (0.17)	
Grade, n (%)			< 0.001
Well	265 (3.49)	81 (2.27)	
Moderate	3677 (48.41)	1642 (45.98)	
Poor	3443 (45.33)	1766 (49.45)	
Diffuse	5 (0.07)	3 (0.08)	
Unknown	206 (2.71)	79 (2.21)	

AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; RSG, radical subtotal gastrectomy; RTG, radical total gastrectomy.

[a]Continuous data were showed as mean (ranges) and categoric data as number (%).

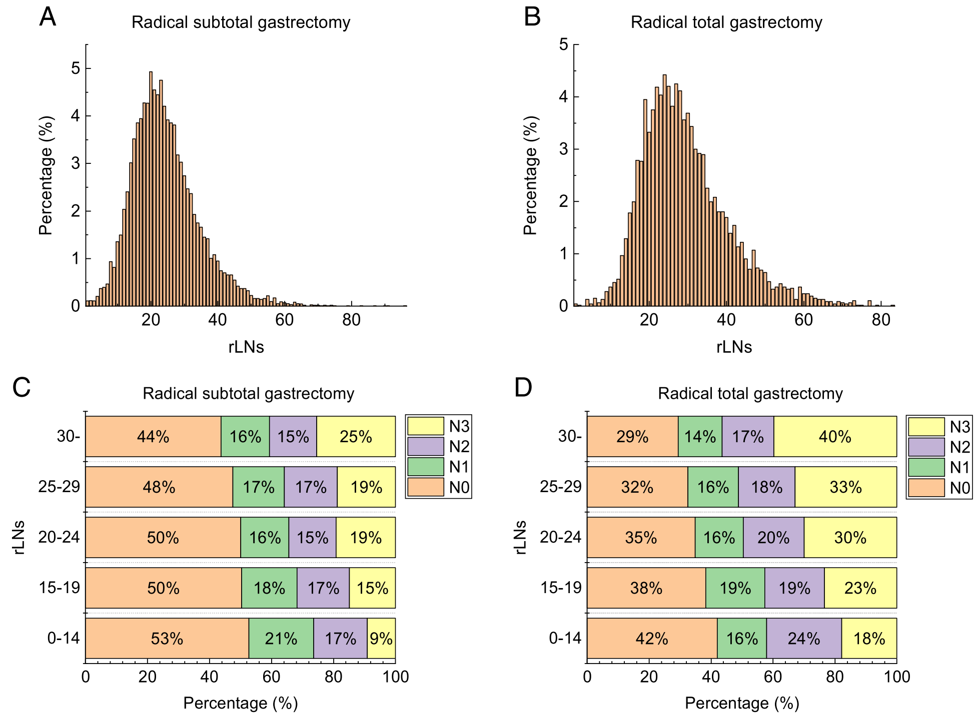

Figure 2. Trends related to the retrieved lymph nodes (rLNs) among patients undergoing radical subtotal gastrectomy and patients undergoing radical total gastrectomy at our cancer center between 2000 and 2022. (A) Distribution of rLNs of radical subtotal gastrectomy; (B). Distribution of rLNs of radical total gastrectomy; (C). Detection rates of N0, N1, N2, and N3 within different rLNs ranges in radical subtotal gastrectomy; (D). Detection rates of N0, N1, N2, and N3 within different rLNs ranges in radical total gastrectomy.

Determination of minimum number of rLNs

Due to the differing tumor status in RSG and RTG, the minimum number of rLNs for achieving the best survival prognosis also differs. In RSG, RCS analysis was performed based on OS for N0, N1, N2, N3a, and N3b, revealing that the minimum rLNs for patients in N0–N3a stages is around 24, while for N3b stage patients, the minimum rLNs is 32 (Fig. 3A-E). KM curves were plotted, and log-rank tests were conducted for patients in N0–N3a stages based on whether rLNs reached 24, showing that patients with sufficient rLNs have a significant OS and DFS advantage ($P<0.001$, $P<0.001$) (Supplementary Fig. 2A-B, Supplemental Digital Content 3, http://links.lww.com/JS9/C596). After PSM was conducted to eliminate confounding factors (Supplementary Table 1, Supplemental Digital Content 4, http://links.lww.com/JS9/C597), the data still indicated a significant survival advantage for the group with sufficient rLNs ($P=0.019$, $P=0.005$) (Fig. 3F, Supplementary Fig. 2C, Supplemental Digital Content 3, http://links.lww.com/JS9/C596). Similarly, for N3b stage patients, log-rank tests revealed a significant survival advantage for patients with rLNs greater than or equal to 32, both before and after PSM (OS: $P=0.006$, $P=0.023$; DFS: $P=0.006$, $P=0.020$) (Supplementary Table 2, Supplemental Digital Content 5, http://links.lww.com/JS9/C598, Fig. 3G, Supplementary Fig. 2D-F, Supplemental Digital Content 3, http://links.lww.com/JS9/C596).

Similarly, in RTG, RCS analysis based on OS for N0, N1, N2, N3a, and N3b indicated that the minimum number of rLNs for patients in N0–N3a stages is 27, while for N3b stage patients, the minimum rLNs is 34 (Fig. 4A-E). Log-rank tests for patients in N0–N3a stages based on whether rLNs reached 27 showed a significant survival advantage for patients with sufficient rLNs (OS and DFS: $P<0.001$) (Supplementary Fig. 3A-B, Supplemental Digital Content 6, http://links.lww.com/JS9/C599). After PSM (Supplementary Table 3, Supplemental Digital Content 7, http://links.lww.com/JS9/C600), the data still indicated the same trend (OS: $P=0.047$; DFS: $P=0.027$) (Fig. 4F, Supplementary Fig. 3C, Supplemental Digital Content 6, http://links.lww.com/JS9/C599). Similarly, for N3b stage patients, KM curves and log-rank tests revealed a significant survival advantage for patients with rLNs greater than or equal 34, both before and after PSM (OS: $P<0.001$, $P=0.003$; DFS: $P<0.001$, $P<0.001$) (Supplementary Table 4, Supplemental Digital Content 8, http://links.lww.com/JS9/C601, Fig. 4G, Supplementary Fig. 3D-F, Supplemental Digital Content 6, http://links.lww.com/JS9/C599).

Determination of the minimum number of nLNs

Considering the clinical significance of nLNs, we conducted separate analyses for patients undergoing RSG and RTG. As shown in Supplementary Fig. 4A-B, Supplemental Digital Content 9, http://links.lww.com/JS9/C602, over 23 years, our cancer center's nLNs for patients underwent RSG has increased annually, with an average of 21.9 and a median of 21. RCS analysis indicated that a minimum of 21 nLNs can lead to better survival benefits (Fig. 5A). The forest plot illustrates the prognostic risk of nLNs adequate group (nLNs $\geq$ 21) compared to nLNs limited group (nLNs < 21) across different T stages, N stages, and TNM stages (Fig. 5B). Log-rank tests before and after

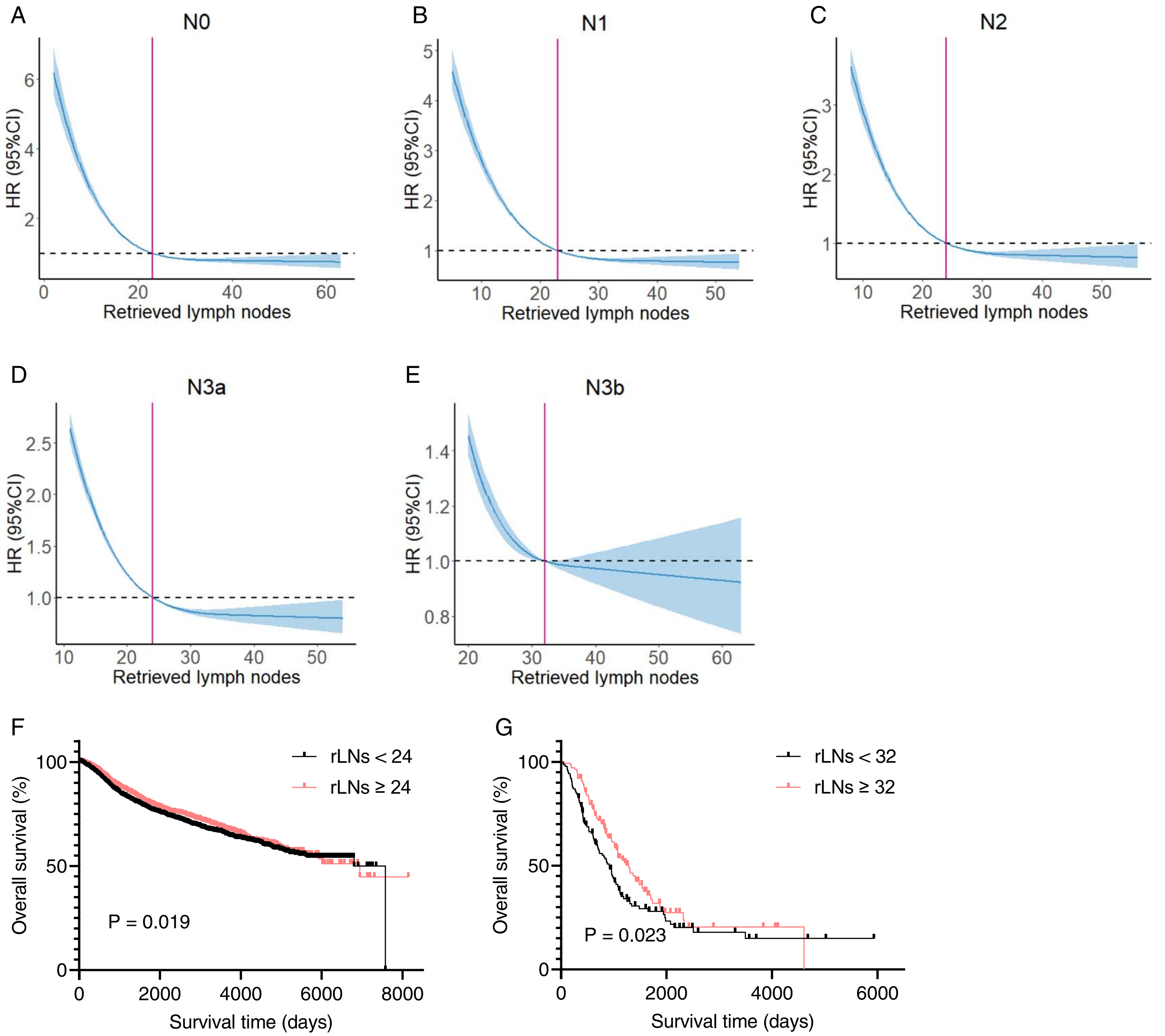

Figure 3. Identification and verification of the minimum number of retrieved lymph nodes (rLNs) in patients undergoing radical subtotal gastrectomy. (A–E) Restricted cubic spline analysis of rLNs using OS of patients in N0, N1, N2, N3, N3a, and N3b stages, respectively; (F) Kaplan–Meier curves comparing OS via log-rank test after PSM in patients with N0–N3a stages between the rLNs < 24 group and rLNs ≥ 24 group; (G) Kaplan–Meier curves comparing OS via log-rank test after PSM in patients with N3b stage between the rLNs < 32 group and rLNs ≥ 32 group. HR, hazard ratio; OS, overall survival; PSM, propensity score matching.

PSM confirmed the significant survival benefits compared to nLNs less than 21 (OS: $P<0.001$, $P=0.013$; DFS: $P<0.001$, $P=0.013$) (Supplementary Table 5, Supplemental Digital Content 10, http://links.lww.com/JS9/C603, Fig. 5C, Supplementary Fig. 4C-E, Supplemental Digital Content 9, http://links.lww.com/JS9/C602).

Subsequently, a similar analysis was performed for patients undergoing RTG. The results showed a stable increase in nLNs, with an average of 23.5 and a median of 22 (Supplementary Fig. 5A-B, Supplemental Digital Content 11, http://links.lww.com/JS9/C604). RCS analysis determined that a minimum of 22 nLNs is associated with better patient survival (Fig. 6A). The forest plot illustrates the prognostic risk of nLNs adequate group (nLNs ≥ 22) compared to nLNs limited group (nLNs < 22) across different T stages, N stages, and TNM stages (Fig. 6B). KM curves before and after PSM both confirmed the reliability of this cut-off point (OS: $P<0.001$, $P<0.001$; DFS: $P<0.001$, $P<0.001$) (Supplementary Table 6, Supplemental Digital Content 12, http://links.lww.com/JS9/C605, Fig. 6C, Supplementary Fig. 5C-E, Supplemental Digital Content 11, http://links.lww.com/JS9/C604).

Discussion

China has the highest incidence of gastric cancer worldwide, and due to inadequate health awareness and screening

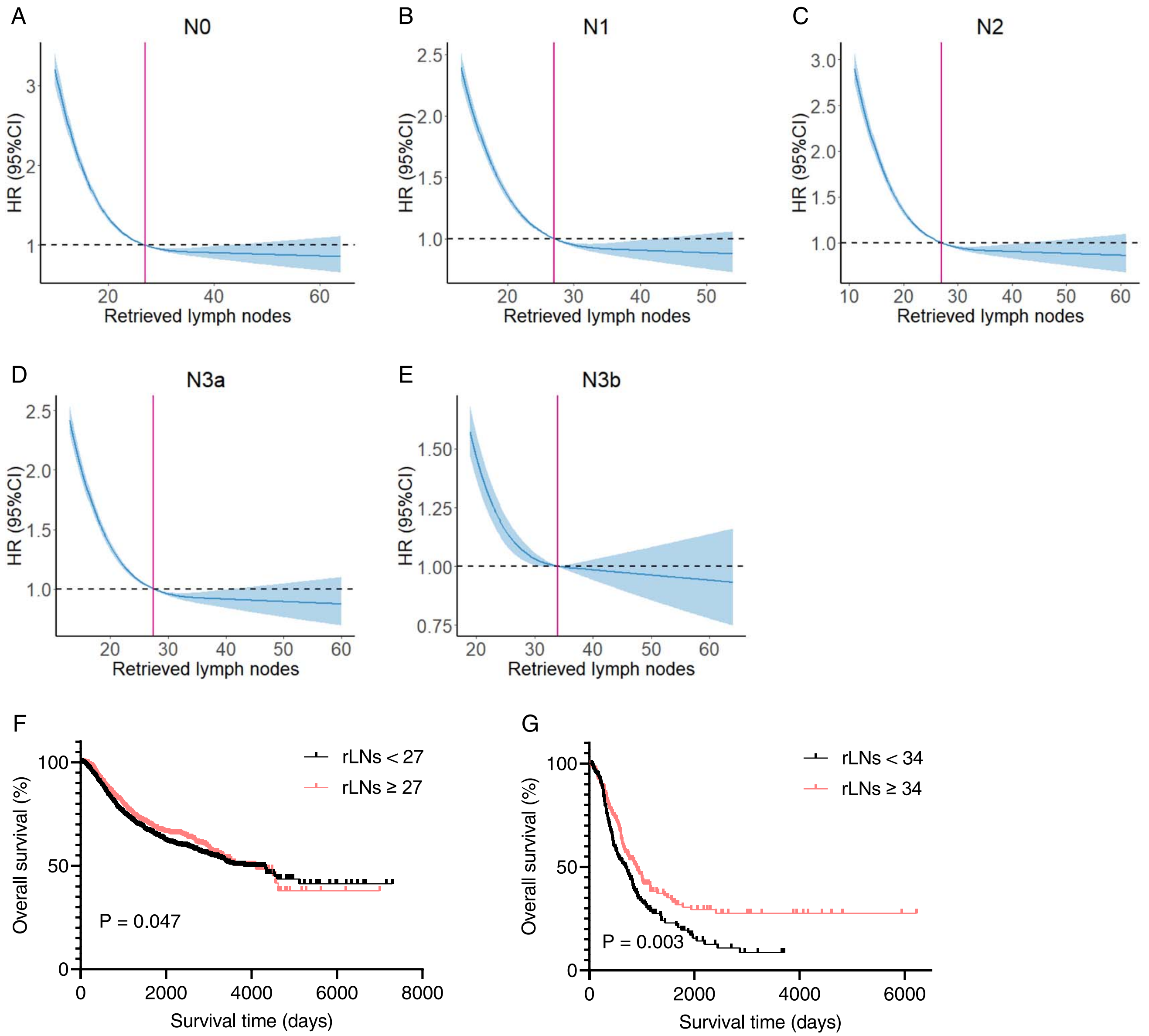

Figure 4. Identification and verification of the minimum number of retrieved lymph nodes (rLNs) in patients undergoing radical total gastrectomy. (A–E) Restricted cubic spline analysis of rLNs using OS of patients in N0, N1, N2, N3, N3a, and N3b stages, respectively; (F, G) Kaplan–Meier curves comparing OS via log-rank test after PSM in patients with N0–N3a stages between the rLNs < 27 group and rLNs ≥ 27 group; (H, I) Kaplan–Meier curves comparing OS via log-rank test after PSM in patients with N3b stage between the rLNs < 34 group and rLNs ≥ 34 group. HR, hazard ratio; OS, overall survival; PSM, propensity score matching.

implementation among residents, early detection rates of gastric cancer in China are relatively low[12]. Consequently, most diagnosed cases present at advanced stages. For patients with advanced-stage gastric cancer, surgical treatment remains the primary approach, with curative surgery being the optimal choice. Standardizing the procedures of gastric cancer surgery is crucial. Additionally, the risk of postoperative lymphatic metastasis in operable gastric cancer patients is higher than hematogenous metastasis. Therefore, for patients deemed suitable for curative surgery after preoperative assessment, achieving a safe range of lymph node dissection is imperative to prevent the omission of metastatic lymph nodes, which could lead to future recurrence and metastasis.

A 2015 study identified the extent of lymph node dissection as a quality assessment indicator for gastric cancer centers, revealing that a considerable proportion of cases did not meet the guideline requirements[13]. Data from our cancer center spanning from 2000 to 2022 demonstrate that over 85% of patients achieved the recommended lymph node dissection quantity according to the guidelines, with this proportion exceeding 95% in the last decade. We also observed that with an increase in the number of rLNs within a certain range, there was an escalation in the proportion of positive lymph nodes and N3 stage, indicating that limited rLNs can lead to errors in N staging assessment. Studies categorized cases with insufficient rLNs in N staging as N + 1 stage, revealing a more significant distinction in prognosis among

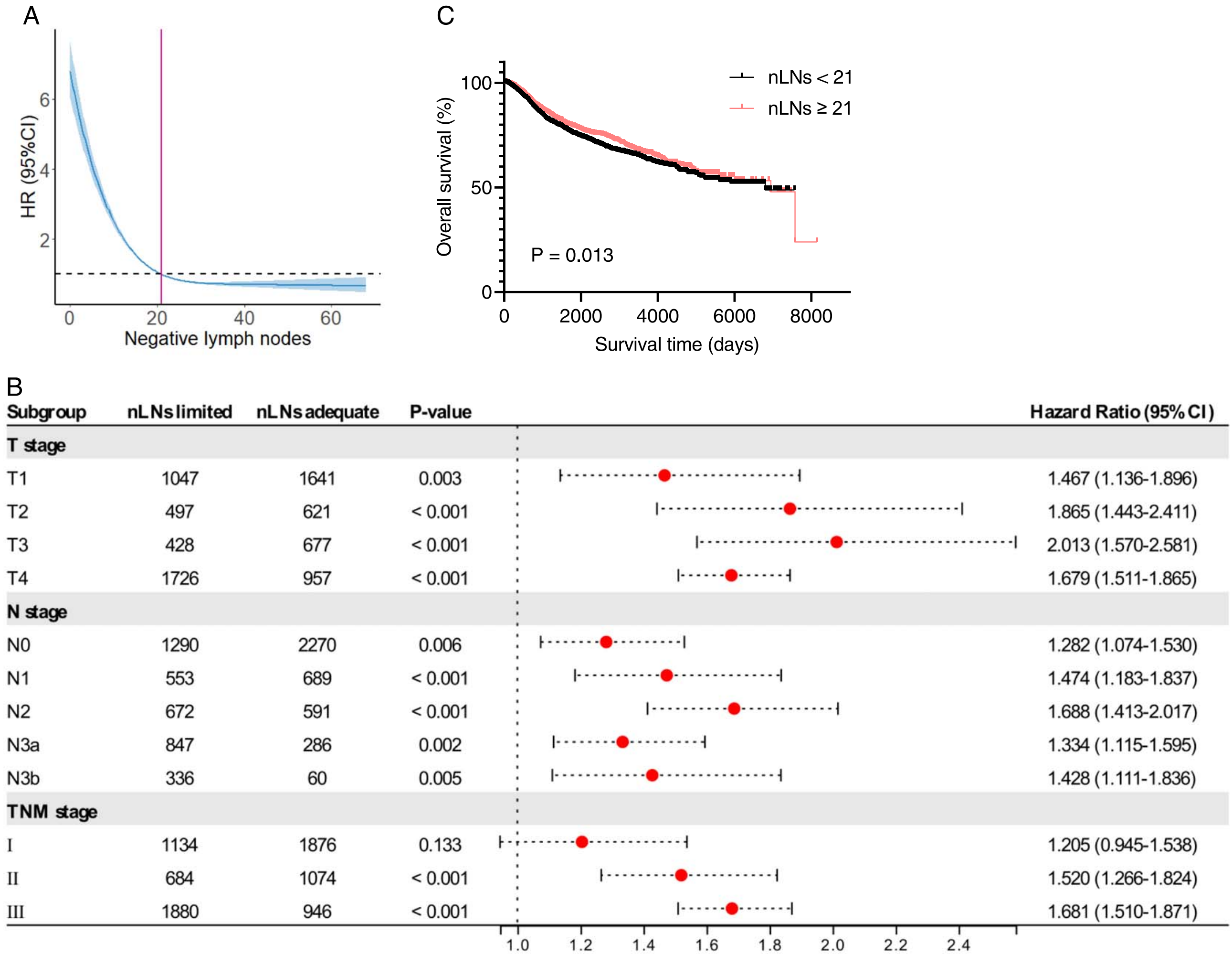

Figure 5. Identification and verification of the minimum number of negative lymph nodes (nLNs) in patients undergoing radical subtotal gastrectomy. (A) Restricted cubic spline analysis of nLNs using OS of patients; (B) Forest plot illustrates the prognostic risk of nLNs adequate group (nLNs ≥ 21) compared to nLNs limited group (nLNs < 21) across different T stages, N stages, and TNM stages; (C). Kaplan–Meier curves comparing OS via log-rank test after propensity score matching in patients with nLNs < 21 group and nLNs ≥ 21 group. HR, hazard ratio; OS, overall survival.

different N stages and TNM stages adjusted, thus affirming the necessity of thorough lymph node detection[14,15]. Overall, establishing appropriate rLNs thresholds can not only standardize the extent of surgery but also facilitate clinical practitioners in providing more targeted postoperative treatment and prognosis monitoring for patients with limited rLNs.

While there is a consensus that the minimum of 16 lymph nodes recommended by the 8th edition of AJCC is inadequate clinically, different research institutions have arrived at varying conclusions regarding the minimum number of rLNs. The research methods and entry points have also varied. In a study on lymph node-negative gastric cancer patients, subgroup analysis found that for T2–T4 stage patients, a higher survival rate was observed with more than 25 rLNs[16]. Corresponding to lymph node negativity, Bochao Zhao's team concluded that for advanced gastric cancer patients with lymph node metastasis, at least 25 lymph nodes needed to be dissected for curative aim[17]. Considering that different T stages of gastric cancer can affect the extent of lymph node metastasis, several studies have discussed the optimal rLNs based on T staging. Jia Yun Shen and colleagues conducted a subgroup analysis of rLNs in T3 stage patients, revealing that rLNs did not affect the prognosis of pT3N0 and pT3N1 groups but were independent prognostic factors in pT3N2 and pT3N3 groups[18]. Furthermore, research has explored the minimum number of rLNs in specific TNM stages of patients. For instance, Shogo Hayashi's team determined the optimal lymph node cut-off point for stage III gastric cancer patients as 40 through receiver operating characteristic (ROC) curve analysis[9]. Combining the above studies reveals a consistent finding across various research perspectives, whether based on T staging, N staging, or TNM staging: the retrieval of more than 20–30 lymph nodes is a common outcome in most studies. In light of these results, our study findings align with mainstream research to some extent but offer more specific insights.

We contend that the decision on how many lymph nodes to dissect primarily hinges on the extent of lymph node metastasis, namely the N stage. Thus, in this study, we employed RCS analysis to investigate rLNs that different N stages need. Furthermore, patients undergoing RTG usually exhibit a more

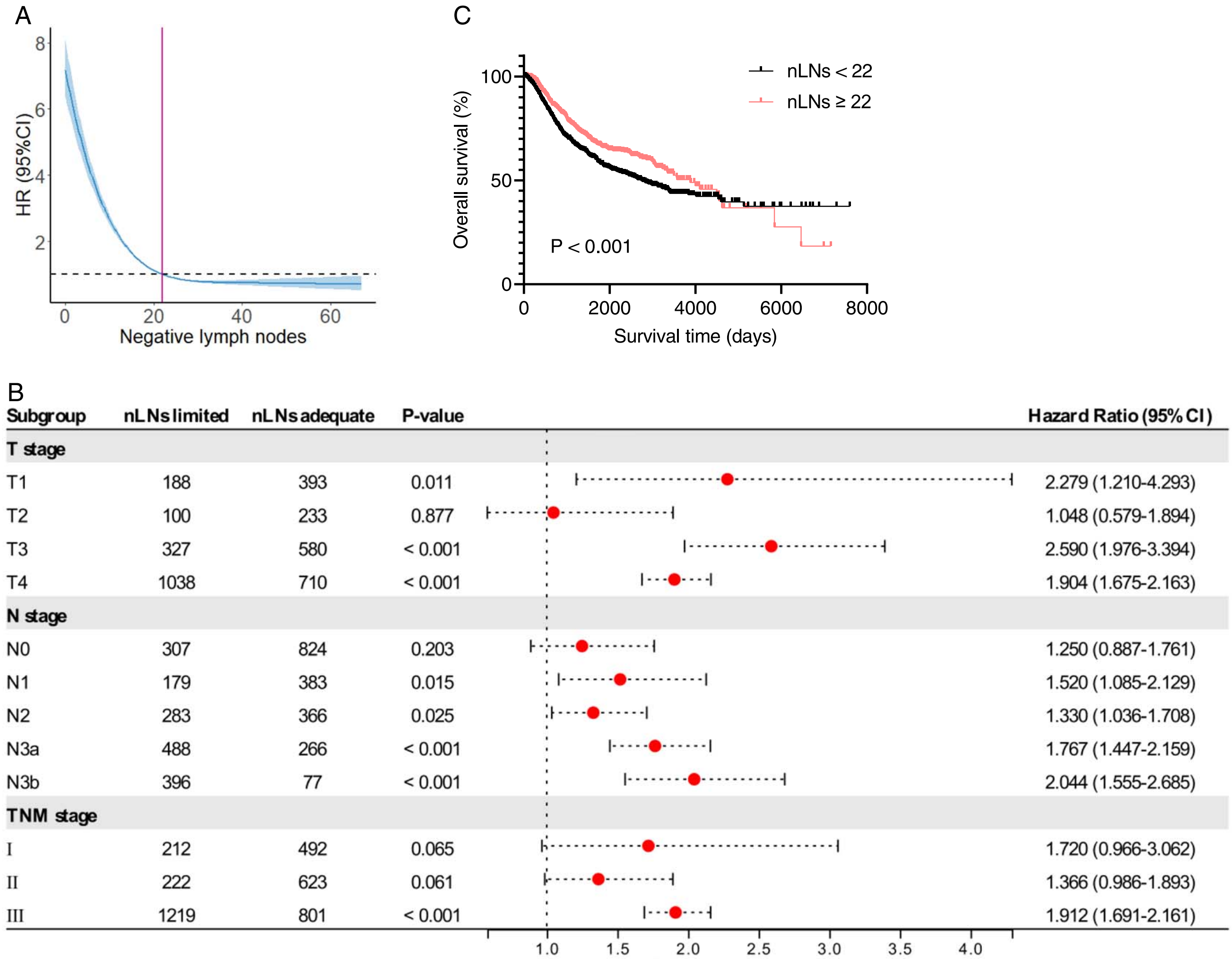

Figure 6. Identification and verification of the minimum number of negative lymph nodes (nLNs) in patients undergoing radical total gastrectomy. (A) Restricted cubic spline analysis of nLNs using OS of patients; (B) Forest plot illustrates the prognostic risk of nLNs adequate group (nLNs ≥ 22) compared to nLNs limited group (nLNs < 22) across different T stages, N stages, and TNM stages; (C) Kaplan–Meier curves comparing OS via log-rank test after propensity score matching in patients with nLNs < 22 group and nLNs ≥ 22 group. HR, hazard ratio; OS, overall survival.

severe disease condition. Consequently, the scope of lymph node metastasis and the extent of lymph node dissection should vary accordingly. Therefore, we conducted separate studies on RSG and RTG in this research. Our study utilized retrospective analysis of a large sample dataset comprising over 20 000 cases from our cancer center to explore the minimum number of rLNs. Based on our findings, we recommend that for patients undergoing RSG with N0–N3a stage (preoperative expected minimal lymph node metastasis), at least 24 lymph nodes should be dissected, while for N3b stage (preoperative expected more extensive lymph node metastasis), at least 32 rLNs is needed. For patients undergoing RTG with N0–N3a stage, at least 27 lymph nodes should be examined, and for N3b stage, at least 34 rLNs is needed. Compared to the 8th edition of the Gastric Cancer AJCC guidelines, which recommend intraoperative clearance of at least 16 lymph nodes, our results have more precisely determined the minimum number of lymph nodes to be cleared based on individual patient conditions. This approach is expected to significantly enhance patient outcomes.

When it comes to preoperative assessment of lymph node status, endoscopic ultrasound assessment can accurately diagnose lymph node metastasis in gastric cancer[19,20], thus recommending its use to preoperatively examine patients and subsequently select an appropriate number of rLNs accordingly to our research. Furthermore, studies have been conducted to determine metastatic lymph nodes in gastric cancer intraoperatively using autofluorescence microspectroscopy and multispectral imaging autofluorescence microscopy[21]. It is believed that combining these findings with our research results can maximally assist in achieving intraoperative radical lymph node dissection for patients, thereby reducing postoperative recurrence and metastasis.

Despite some gastric cancer patients being classified as having no lymph node metastasis or limited metastasis, there may exist a degree of micrometastasis that is more prone to being overlooked, thereby leading to postoperative recurrence and metastasis[22]. The retrieval of nLNs is crucial for eliminating potential micrometastases. Therefore, we also explored the numerical values of nLNs corresponding to favorable prognoses. Through our research, we recommend that for patients undergoing RSG, at least 21 nLNs should be retrieved, and for patients undergoing RTG, at least 22 nLNs is needed. Thus, our study provides guidance on both the total number of rLNs during surgery and the minimum number of nLNs. We believe that the combination of these factors will undoubtedly bring long-term survival benefits to patients with gastric cancer.

The number of rLNs, metastatic lymph nodes, and nLNs are all crucial factors for evaluating the prognosis of gastric cancer patients and guiding subsequent treatments. Some derived data have been reported to potentially hold even greater significance. Deng *et al.*[23] retrospectively analyzed the clinicopathological characteristics and overall survival of 299 gastric cancer patients with positive nodal metastasis after surgical treatment, finding that the ratio between negative and positive lymph nodes was more suitable for predicting overall survival. The same team published a study in 2013 indicated that the ratio between the number of metastatic lymph nodes and rLNs (LNR) has a superior prognostic value[24]. Above all of that, another study investigated the prognostic superiority of the log odds of positive lymph nodes (LODDS) compared to RML and rLNs in stage III colon cancer patients, revealing that LODDS may confound the prognostic association previously attributed to LNR and rLNs[25]. It is evident that there is still no consensus on which lymph node indicators is most effective. In the future, we will utilize existing data to provide our answer to this question.

However, our study has its limitations. In recent years, laparoscopic and robotic gastric cancer surgeries have become increasingly popular due to their faster postoperative recovery in clinical practice[26,27]. Yet, it remains unclear whether there is a need to differentiate them from traditional open surgeries in terms of rLNs. This aspect was not addressed in our study, but we are committed to exploring this issue in future research. Furthermore, while some previous studies have determined the optimal rLNs based on T staging and TNM staging separately, we also hope to delve deeper into this direction from the perspective of our data.

Conclusions

Our study recommends that patients with fewer preoperative identified lymph node metastases undergo intraoperative clearance of at least 24 lymph nodes during RSG, while those with more preoperatively identified lymph node metastases should have at least 32 lymph nodes cleared. For patients preparing for RTG, those with fewer preoperative identified lymph node metastases should have at least 27 lymph nodes retrieved intraoperatively, whereas those with more preoperatively identified lymph node metastases should have at least 34 lymph nodes examined. Ideally, negative lymph node counts for both procedures should reach 21 and 22, respectively.

Ethical approval

Ethical approval was obtained from the ethics committee of Fudan University Shanghai Cancer Center (No. 050432-4-2307E)

Consent

The written informed consent was obtained from all patients.

Source of funding

This work was supported by National Natural Science Foundation of China (82273370, 82202899, 82172702, 82373381, 82172803), Natural Science Foundation of Shanghai (22ZR1413000, 21ZR1414900), Shanghai Science and technology development fund (19MC1911000), Shanghai Municipal Key Clinical Specialty (shslczdzk01301) and Shanghai Science and Technology Innovation Action Plan Basic research project subproject (20JC1419303).

Author contribution

Y.L.: formal analysis, software, writing—original draft. C.Z.: investigation, methodology. L.G.: investigation, formal analysis. Q.Z.: data curation, software. J.C.: methodology, validation. X. W.: supervision, writing—review and editing. X.W.: validation, writing—review and editing. M.X.: conceptualization, funding acquisition, resources. J.C. : conceptualization, project administration, resources. W.S.: funding acquisition, resources. F.L.: supervision, funding acquisition, writing – review & editing.

Conflicts of interest disclosure

None.

Research Registration Unique Identifying Number (UIN)

Name of the registry: https://www.researchregistry.com/

Unique Identifying number or registration ID: Unique Identifying number: researchregistry10140

Hyperlink to your specific registration (must be publicly accessible and will be checked): https://researchregistry.knack.com/research-registry#userresearchregistry/registerresearchdetails/66040ccbb33e860028ebc5bf/

Guarantor

Midie Xu, Jie Chen.

Data availability statement

Datasets generated during the current study are available upon reasonable request.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Acknowledgements

The authors appreciate the contributions made by the patients in this study.

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