Significance of accurate hilar and intrapulmonary lymph nodes examination in stage IA-IIA non-small cell lung cancer, a retrospective cohort study

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Abstract

**Background** The examination of lymph node plays an important part in the nodal staging of non-small cell lung cancer (NSCLC). Till present, on the role of hilar and intrapulmonary (N1) station lymph node (LN) examined is not fully appreciated. In this study, we aimed to confirm the significance of N1 lymph node examined in the long-term survival for stage IA-IIA NSCLC patients and find the minimum number of lymph nodes.

**Methods** The data of patients who underwent radical lobectomy and confirmed as lymph node non-metastatsized from January 2008 to March 2018 were retrospectively screened. Pathology records were reviewed for the number of lymph nodes examined. Kaplan-Meier method and Cox regression model were used to identify survival and prognostic factors.

**Results** The median number of resected N1 LNs was 8. The number of patients with 0-2 N1 LNs, 3-5 N1 LNs, 6-8 N1 LNs, 9-11 N1 LNs and more than 11 N1 LNs examined was 181, 425, 477, 414 and 531, respectively. Gender (P=0.004), age (P<0.001), tumor size (P=0.004), differentiation degree (P=0.001) and the number of N1 LNs examined (P=0.008) were the independent prognostic factors of overall survival. Gender (P=0.006), age (P=0.031), tumor size (P=0.001), differentiation degree (P=0.001), vascular invasion (P=0.034) and the number of N1 LNs examined (P=0.007) were the independent prognostic factors of disease-free survival.

**Conclusion** Increasing the number of N1 LNs examination could improve the long-term survival of T1-2N0 NSCLC patients. At least six LNs should be examined in surgical and pathological management.

**Background** Lung cancer remains the most common type of cancer worldwide(1). For patients with stage IA-IIA non-small cell lung cancer (NSCLC), lobectomy with systemic LN dissection or systematic LN sampling remains the standard therapy.(2, 3) The 5-year overall survival rate for patients with stage IA-IIA is about 60%-92%(4).

Despite radical resection, patients still have a high risk of recurrence. Chris Kelsey et al, reported that the 5-year actuarial risk of disease recurrence was 36% in patients with stage I-II NSCLC.(5)
Inadequate lymph node (LN) examination may be an important risk factor for recurrence. The examination of LN plays an important role in the accurate node staging and node-positive patient needs adjuvant therapy to reduce their risk of recurrence. There still is no consensus regarding the optimal number of perioperative LN to be retrieved for pathological examination. Some studies evinced that a minimum of 10 LNs should be examined to guarantee an accurate N stage. (8) (9) Wenhua Liang et al, mentioned at least 16 LNs should be removed at surgical procedure. (10) As for mediastinal (N2) station LNs, the debate mainly concentrated on the selection between systemic mediastinal LN dissection and selective mediastinal LN sampling. Several researchers believe that mediastinal LN dissection should be routinely performed during surgeries. (7, 11) On the contrary, some studies revealed that selective mediastinal LN sampling had similar impact on surgical outcome compared with systemic mediastinal LN dissection for early NSCLC. (12-14) Currently, few studies have focused on the examination of the intrapulmonary or hilar (N1) station LNs. To assess the effect of N1 lymph node examination on the survival of stage IA-IIA NSCLC patients, we retrospectively screened 3014 NSCLC patients from our center and to find the minimum number of N1 examination.

Methods

Patients

This study was approved by the Institutional Review Board of Sun Yat-sen University Cancer Center (SYSUCC). The data of patients who underwent radical surgery at the thoracic surgery department of SYSUCC between January 2008 and March 2018 were reviewed. Inclusion criteria included: (1) confirmed pathological diagnosis of NSCLC (2) pathologically staged as IA-IIA (T1a-2BN0M0); (3) confirmed negative surgical margin (R0); (4) the overall survival (OS) and diseases-free survival (DFS) time were > 1 month. Exclusion criteria included: (1) underwent neoadjuvant therapy (2) had multiple primary cancers; (3) received sublobectomy. All patients were restaged according to the TNM staging criteria of the 8th National Comprehensive Cancer Network (NCCN) staging system.

Follow-up

In this study, the dissection of N1 and N2 LN stations were both performed by the surgeons and reconfirmed by pathologists. The information about the number of lymph nodes examined and other pathological factors were reviewed from pathology reports. Follow-ups were performed every
3 months in the first two years, every 6 months until 5 years and once a year thereafter. Surgeons would prescribe between chest radiography, ultrasonography or computed tomography scan as needed during follow-up. The study endpoint was OS and DFS, which were defined as the time from surgery to death and the time from surgery to first locoregional or distant recurrence or to death, respectively.

Statistical analysis
All statistical analyses were performed using the SPSS software, version 22.0 (SPSS, Inc., Chicago, IL). Analysis of Variance (ANOVA) was performed to compare quantitative data and Pearson’s χ² or Mann-Whitney U test were used to compare categorical data between five groups. The OS and DFS were estimated by using the method of Kaplan-Meier and compared using the log-rank test. Univariate and multivariate Cox proportional hazards regression analysis was used to identify prognostic factors for survival. Variables assessed in this study included: age, gender, tumor size, smoking history, 8th TNM stage, histology, differentiation degree, visceral pleura invasion, vascular invasion, adjuvant chemotherapy, number of N2 LNs examined, number of N1 LNs examined, thoracotomy or video-assisted thoracoscopic surgery (VATS). The number of N2 LNs examined was treated as rank variable categorized into 5 groups, namely, 0 to 2, 3 to 5, 6 to 8, 9 to 11, 12 to 15 and more than 15. The number of N1 LNs examined was also treated as rank variable categorized into 5 groups, namely, 0 to 2, 3 to 5, 6 to 8, 9 to 11 and more than 11. Variables whose P value were < 0.1 in univariate analyses were included in multivariate analyses. P values < 0.05 were considered statistically significant and all hypotheses were two-sided.

Results
Patient characteristics
A total of 2028 cases were eligible for analysis. The case characteristics are shown in Table 1. The median age of the investigated 1207 male and 812 female patients was 61 (range, 20 to 83 years). The main pathological type was adenocarcinoma (n = 1567). 266 patients received adjuvant chemotherapy. More than half of the patients (n = 1101) underwent minimally invasive VATS and 927 patients underwent traditional thoracotomy. The median number of N2 LNs examined was 11 (range, 0 to 104). The median number of N1 LNs examination was 8 (range, 0 to 38). The number of patients
with 0–2, 3–5, 6–8, 9–11 and > 11 examined N1 LNs was 181 (8.9%), 425 (21.0%), 477 (23.5%), 414 (20.4%) and 531 (26.2%), respectively.

### Table 1

| Characteristic | 0–2 N1 LN subgroups | 3–5 n = 425 | 6–8 n = 477 | 9–11 n = 414 | ≥ 12 n = 531 | Overall n = 2028 | P value |
|---------------|---------------------|-------------|-------------|-------------|--------------|----------------|--------|
| Gender | | | | | | | 0.289 |
| Male | 103 (56.9) | 246 (57.9) | 276 (57.9) | 245 (59.2) | 337 (63.5) | 1207 (59.4) | |
| Female | 78 (43.1) | 179 (42.1) | 201 (42.1) | 169 (40.8) | 194 (36.5) | 821 (40.5) | |
| Age (year) | 60.9 ± 9.6 | 60.3 ± 9.5 | 59.5 ± 9.8 | 59.6 ± 9.3 | 59.8 ± 9.0 | 59.9 ± 9.4 | 0.460 |
| Tumor size (cm) | 2.4 ± 1.0 | 2.5 ± 1.0 | 2.5 ± 1.0 | 2.6 ± 1.0 | 2.6 ± 1.0 | 2.5 ± 1.0 | 0.027 |
| Smoking history | | | | | | | 0.008 |
| No | 115 (63.5) | 238 (56.0) | 268 (56.2) | 224 (54.1) | 259 (48.8) | 1104 (54.4) | |
| Yes or ever | 66 (36.5) | 187 (44.0) | 209 (43.8) | 190 (45.9) | 272 (51.2) | 924 (45.6) | |
| 8th TNM stage | | | | | | | 0.537 |
| IA | 86 (47.5) | 222 (52.2) | 240 (50.3) | 194 (46.9) | 256 (48.2) | 998 (49.2) | |
| IB-IIIA | 95 (52.5) | 203 (47.8) | 237 (49.7) | 220 (53.1) | 275 (51.8) | 1030 (50.8) | |
| Histology | | | | | | | 0.582 |
| Adenocarcinoma | 145 (80.1) | 329 (77.4) | 365 (76.5) | 312 (75.4) | 416 (78.3) | 1567 (77.3) | |
| Squamous cell carcinoma | 19 (10.5) | 66 (15.5) | 74 (15.5) | 68 (16.4) | 83 (15.6) | 310 (15.3) | |
| Others | 17 (9.4) | 30 (7.1) | 38 (8.0) | 34 (8.2) | 32 (6.0) | 151 (7.4) | |
| Differentiation degree | | | | | | | 0.054 |
| Well | 26 (14.4) | 48 (11.3) | 28 (5.9) | 38 (9.2) | 56 (10.5) | 196 (9.7) | |
| Moderate | 98 (54.4) | 224 (52.8) | 275 (57.9) | 246 (59.6) | 264 (49.7) | 1107 (54.7) | |
| Poor | 57 (31.2) | 153 (35.9) | 174 (36.2) | 130 (31.2) | 211 (39.7) | 725 (35.6) | |
| Visceral pleura invasion | | | | | | | 0.605 |
| Negative | 119 (65.7) | 306 (72.0) | 329 (69.0) | 288 (69.6) | 375 (70.6) | 1417 (69.9) | |
| Positive | 62 (34.3) | 119 (28.0) | 148 (31.0) | 126 (30.4) | 156 (29.4) | 611 (30.1) | |
| Vascular invasion | | | | | | | 0.843 |
| Negative | 168 (92.8) | 399 (93.9) | 442 (92.7) | 391 (94.4) | 496 (93.4) | 1896 (93.5) | |
| Positive | 13 (7.2) | 26 (6.1) | 35 (7.3) | 23 (5.6) | 35 (6.6) | 132 (6.5) | |
| Number of N2 LNs examined | | | | | | | < 0.001 |
| 0–2 | 24 (13.3) | 24 (5.6) | 29 (6.1) | 13 (3.1) | 21 (4.0) | 111 (5.5) | |
| 3–5 | 29 (16.0) | 62 (14.6) | 66 (13.8) | 34 (13.0) | 33 (10.0) | 264 (13.0) | |
| 6–8 | 40 (22.1) | 79 (18.6) | 85 (17.4) | 68 (16.4) | 78 (14.7) | 348 (17.2) | |
| 9–11 | 25 (13.8) | 56 (13.2) | 83 (17.4) | 60 (14.5) | 76 (14.3) | 300 (14.8) | |
| ≥ 12–14 | 20 (11.0) | 72 (16.9) | 69 (14.5) | 65 (15.7) | 72 (13.6) | 298 (14.7) | |
| ≥ 15 | 43 (23.8) | 132 (31.1) | 147 (30.8) | 154 (37.2) | 231 (43.5) | 707 (34.9) | |
| Adjuvant chemotherapy | | | | | | | 0.582 |
| No | 160 (88.4) | 363 (85.4) | 413 (86.6) | 368 (88.9) | 458 (84.2) | 1762 (86.9) | |
| Yes or ever | 21 (11.6) | 62 (14.6) | 64 (13.4) | 46 (11.1) | 73 (15.8) | 266 (13.1) | |
| Thoracotomy or VATS | | | | | | | 0.176 |
| Thoracotomy | 68 (37.6) | 193 (45.4) | 230 (48.2) | 194 (46.9) | 242 (45.6) | 927 (45.7) | |
| VATS | 113 (62.4) | 232 (54.6) | 247 (51.8) | 220 (53.1) | 289 (54.4) | 1101 (54.3) | |

**Survival Analysis**

The 5-year OS rate of patients with 0–2, 3–5, 6–8, 9–11 and > 11 examined N1 LNs was 73.8%, 85.4%, 89.4%, 84.0% and 87.8%, respectively. The 5-year DFS rate of patients with 0–2, 3–5, 6–8, 9–11 and > 11 examined N1 LNs was 60.1%, 74.6%, 75.1%, 76.4% and 77.3%, respectively. As shown in Fig. 1,
patients with 0–2, 3–5, 6–8, 9–11 and > 11 examined N1 LNs had apparently different OS (log-rank P = 0.045, Fig. 1A) and DFS (log-rank P = 0.045, Fig. 1B).

As shown in Table 2, age, gender, tumor size, smoking history, 8th TNM stage, differentiation degree, vascular invasion and number of N1 LNs examined were statistically significant in univariate analysis of OS and visceral pleura invasion also had a P value less than 0.1. In multivariate analysis, advanced age (P < 0.001; HR 1.042; 95%CI 1.026–1.059), larger tumor size (P = 0.004; HR 1.280; 95%CI 1.081–1.516) and differentiation degree (P = 0.001; HR 1.350; 95%CI 1.064–1.714) were negatively correlating with OS. Male (P = 0.004; HR 0.535; 95%CI 0.351–0.814) and the number of N1 LNs examined (P = 0.007) were positively correlated with OS. Patients with > 11 N1 LNs (P < 0.001; HR 0.427; 95%CI 0.266–0.688) and 6–8 N1 LNs (P = 0.002; HR 0.484; 95%CI 0.304–0.770) examined were the two groups with the lowest HR value. All factors except adjuvant chemotherapy, number of N2 LNs examined and thoracotomy or VATS had a P value less than 0.1 in univariate analysis of DFS and were enrolled in multivariate analysis (Table 3). In multivariate analysis, advanced age (P = 0.031; HR 1.012; 95%CI 1.001–1.023), larger tumor size (P = 0.001; HR 1.237; 95%CI 1.094–1.399), differentiation degree (P = 0.001; HR 1.331; 95%CI 1.119–1.584) and vascular invasion (P = 0.034; HR 1.506; 95%CI 1.031–2.200) were negatively correlating with DFS. Male (P = 0.006; HR 0.663; 95%CI 0.495–0.888) and the number of N1 LNs examined were positively correlated with DFS. Patients with > 11 N1 LNs (P = 0.001; HR 0.542; 95%CI 0.384–0.766) and 9–11 N1 LNs (P = 0.002; HR 0.570; 95%CI 0.399–0.813) examined were the two groups with the lowest HR value.
| Factors                        | Univariate Analysis | Multivariate Analysis |
|-------------------------------|---------------------|-----------------------|
|                               | HR (95% CI)         | P value               |
|                               |                     |                       |
| Gender                        | 0.393 (0.283–0.546) | < 0.001               |
|                               | 0.535 (0.351–0.814) | 0.004                 |
| Age (year)                    | 1.049 (1.033–1.066) | < 0.001               |
|                               | 1.042 (1.026–1.059) | < 0.001               |
| Tumor size (cm)               | 1.461 (1.288–1.657) | < 0.001               |
|                               | 1.280 (1.081–1.516) | 0.004                 |
| Smoking history               | 2.057 (1.558–2.742) | < 0.001               |
|                               | 1.171 (0.773–1.614) | 0.577                 |
| 8th TNM stage                 | 1.818 (1.364–2.425) | < 0.001               |
|                               | 1.205 (0.767–1.892) | 0.419                 |
| Visceral pleura invasion      | 1.281 (0.973–1.687) | 0.077                 |
|                               | 1.154 (0.800–1.663) | 0.443                 |
| Histology                     | Ref                 | Ref                   |
| Squamous cell carcinoma       | Ref                 | Ref                   |
| Adenocarcinoma                | 0.629 (0.459–0.864) | 0.004                 |
|                               | 1.097 (0.711–1.651) | 0.606                 |
| Others                        | 0.946 (0.581–1.512) | 0.825                 |
|                               | 1.276 (0.776–2.128) | 0.349                 |
| Differentiation degree        | 1.683 (1.341–2.112) | < 0.001               |
|                               | 1.350 (1.064–1.714) | 0.014                 |
| Vascular invasion             | 1.899 (1.119–3.222) | 0.017                 |
|                               | 1.411 (0.817–2.437) | 0.216                 |
| Number of N2 LNs examined     |                     |                       |
| 0–2                           | Ref                 | Ref                   |
| 3–5                           | 0.427 (0.244–0.783) | 0.006                 |
| 6–8                           | 0.629 (0.368–1.073) | 0.089                 |
| 9–11                          | 0.516 (0.292–0.913) | 0.023                 |
| 12–14                         | 0.648 (0.372–1.129) | 0.126                 |
| > 14                          | 0.563 (0.342–0.929) | 0.024                 |
| Adjuvant chemotherapy         | 0.925 (0.625–1.369) | 0.695                 |
| Thoracotomy or VATS           | 0.864 (0.655–1.141) | 0.304                 |
| Number of N1 LNs examined     |                     |                       |
| 0–2                           | Ref                 | Ref                   |
| 3–5                           | 0.702 (0.451–1.091) | 0.116                 |
| 6–8                           | 0.533 (0.336–0.844) | 0.007                 |
| 9–11                          | 0.630 (0.398–0.997) | 0.049                 |
| > 11                          | 0.527 (0.329–1.845) | 0.008                 |
|                               | 0.427 (0.266–0.688) | < 0.001               |
Table 3

| Factors                             | Univariate Analysis |          | Multivariate Analysis |          |
|-------------------------------------|---------------------|----------|-----------------------|----------|
|                                     | HR (95%CI)          | P value  | HR (95%CI)            | P value  |
| Gender                              | 0.570 (0.458–0.709) | < 0.001  | 0.663 (0.495–0.888)   | 0.006    |
| Age (year)                          | 1.018 (1.007–1.029) | 0.002    | 1.012 (1.001–1.023)   | 0.031    |
| Tumor size (cm)                     | 1.402 (1.278–1.538) | < 0.001  | 1.237 (1.094–1.399)   | 0.001    |
| Smoking history                     | 1.537 (1.258–1.877) | < 0.001  | 1.033 (0.787–1.357)   | 0.814    |
| 8th TNM stage                       | 1.938 (1.570–2.393) | < 0.001  | 1.380 (0.993–1.918)   | 0.055    |
| Visceral pleura invasion            | 1.333 (1.088–1.631) | 0.005    | 1.048 (0.799–1.373)   | 0.736    |
| Histology                           | Ref                 |          | Ref                   |          |
| Squamous cell carcinoma             | 0.782 (0.611–0.999) | 0.049    | 1.239 (0.938–1.635)   | 0.131    |
| Adenocarcinoma                      | 0.946 (0.666–1.343) | 0.905    | 1.199 (0.802–1.791)   | 0.376    |
| Others                              | 1.559 (1.323–1.838) | < 0.001  | 1.331 (1.119–1.584)   | 0.001    |
| Differentiation degree              | 1.982 (1.378–2.852) | < 0.001  | 1.506 (1.031–2.200)   | 0.034    |
| Vascular invasion                   | Ref                 |          | Ref                   |          |
| Number of N2 LNs examined           | Ref                 |          | Ref                   |          |
|                                    | 0-2                 |          |                       |          |
|                                     | 0.627 (0.391–1.005) | 0.052    |                       |          |
|                                     | 6-8                 | 0.841 (0.545–1.297) | 0.433    |                       |          |
|                                     | 9-11                | 0.737 (0.470–1.156) | 0.183    |                       |          |
|                                     | 12-14               | 0.730 (0.463–1.151) | 0.175    |                       |          |
|                                     | > 14                | 0.759 (0.505–1.142) | 0.186    |                       |          |
| Adjuvant chemotherapy               | 1.127 (0.858–1.481) | 0.500    |                       |          |
| Thoracotomy or VATS                 | 0.933 (0.763–1.141) | 0.304    |                       |          |
| Number of N1 stations examined      | Ref                 |          | Ref                   |          |
|                                    | 0-2                 |          |                       |          |
|                                     | 0.654 (0.464–0.922) | 0.015    | 0.626 (0.443–0.883)   | 0.008    |
|                                     | 6-8                 | 0.680 (0.486–0.949) | 0.024    | 0.646 (0.461–0.904)   | 0.011    |
|                                     | 9-11                | 0.595 (0.418–0.847) | 0.004    | 0.570 (0.399–0.813)   | 0.002    |
|                                     | >11                 | 0.627 (0.445–0.884) | 0.008    | 0.542 (0.384–0.766)   | 0.001    |

Discussion

In the treatment of NSCLC patients, accurate node staging is important for prognostic prediction and treatment strategy. Clinical N staging based solely on CT and PET is not accurate enough for early NSCLC. Postoperative thorough pathological N examination is optimal N staging. The number of LN examined is an intuitive indicator of examination thoroughness. In this study, we found that the number of N1 LNs examined was the independent prognostic factor of OS and DFS for stage IA-IIA patients.

There are some potential explanations for the survival advantage brought by the larger number of N1 LN examined. An increasing number of N1 LNs examined would lead to a greater probability of discovering metastasized LNs in the hilar and lung, leading to stage migration; considered to be the main role in the improvement of OS and DFS in patients with large number of N1 LNs examined.

Inadequate LNs examination may result in some metastatic lymph nodes not being detected and patients would be wrongly staged as IA or IB. Actually, this part of patients should be staged as IIB
and received adjuvant therapy. Resecting micrometastases and the effect of immunologic microenvironment may be also related with survival advantage brought by the larger number of N1 LN examined. (16–19) In this study, patients received sublobectomy were excluded which means all intrapulmonary lymph nodes are removed along with the lobes. Resecting micrometastases did not have significant impact on survival advantage brought by the larger number of N1 LN examined. Several researchers have emphasized that a larger number of LNs examined could increase the accuracy of N staging and enhance prognosis. Pezzi et al retrospectively analyzed 98,970 patients from The National Cancer Data Base (NCDB) and found that the amounts of LNs examination apparently affect the long-term survival and at least 10 LNs should be examined in surgical management. (17) Ou SH et al, retrospectively investigated the data of 2545 patients and confirmed that the number of LN examination was the favorable prognostic factor for stage IA patients and suggested that the removal of 11–15 LNs could improve the patients prognosis. (20) However, the above two studies did not separately analyze the effects of N1 and N2 lymph nodes on survival. In our pilot study, the number of LNs examination has been identified as an independent prognostic factor for OS (P = 0.005). In this study, the survival advantage from the increase in the number of LNs examined should be attributed to the increase in the number of N1 LNs examined, which might be associated with the following reason. Patients in this study received a high-quality mediastinal lymph node dissection. The number of patients with at least 1, 2 and 3 N2 stations dissected was 2044 (98.2%), 1916 (91.5%) and 1509 (71.8%), respectively and the median number of N2 LNs examined was 11 in this study. The median number of N2 LNs examined was 12 in American College of Surgeons Oncology Group (ACOSOG) Z0030 Trial which had a superb quality of N2 LNs examination. (21) There is no apparent difference in median number of N2 LNs examined between this study and the ACOSOG Z0030 Trial. As the number of N2 LNs examined increases, the survival advantage would decrease when the quality of N2 LNs examination increasing to high level. The number of N2 LNs examined lost statistical significance in Cox regression model. But this result cannot deny the vital role of N2 LNs examination in the node staging. Both N1 and N2 LNs examination are important for accurate node staging.
Some researchers have focused on the importance of the N1 lymph node examined. Mert Saynak et al, reported that T1N0 patients with inadequate N1 LN examined had similar local recurrence-free survival compared with T1N1 patients.(22) The ACOSOG Z0030 trial also found a tendency that the greater the number of intrapulmonary LN examined, the better the patients survival outcomes would be.(23) John Varlotto et al, demonstrated that a minimum of 11 to 16 lymph nodes should be examined when only examining N1 lymph nodes(24). Similar to the above study, patients with more than 11 N1 LNs examined had the lowest HR value in multivariate analysis of OS and DFS, signifying that at least 12 N1 LNs should be examined in order to achieve an optimal OS and DFS. However, it is difficult to accomplish this goal in clinical practice. Only 26.2% of patients accepted more than 11 N1 LNs examined in this study. Patients with 6–8 N1 LNs examined had the second lowest HR value in multivariate analysis of OS. At least 6 N1 LNs examined was a realistic goal in clinical practice.

Therefore, we recommend at least 6 N1 LNs examined in surgical and pathological management. However, the examination of N1 lymph node has not received enough attention. One of the manifestations was that the quality of LN examination exists noteworthy variability during surgical and pathological management.(25, 26) Another manifestations is that incomplete intrapulmonary lymph node retrieval in pathological examination. One previous study revealed that a median of six additional LNs were discovered after rechecking remnant lung specimens and the median number of N1 LNs examined was only 3 in the community-based Memphis Metropolitan Area Quality of Surgical Resection cohort.(27) Although with superb quality of N2 LNs examination, the median number of N1 LNs examined was 5 in the ACOSOG Z0030 trial.(21) In this study, the median number of resected N1 LNs was eight. The pattern of LN examination that N1 LNs were dissected by the surgeons and reconfirmed by pathologists contributed to this result.

There are some limitations exist in this study. First, this was a single-center retrospective study and associated biases may have been inevitable. Second, external validation was not performed to validate the findings. In addition, data of this study did not find the survival advantage from the increase in the number of N2 LNs examined and cannot answer how many N2 LNs should be examined in surgical and pathological management. Therefore, further validation from multicenter
database is needed and meanwhile, the findings from this study should be cautiously interpreted.

Conclusions
Increasing the number of N1 LNs examined could improve the OS and DFS of T1-2N0 NSCLC patients.

At least six N1 LNs should be examined in surgical and pathological

List Of Abbreviations
NSCLC: non-small cell lung cancer
LDCT: low-dose computed tomography
NCCN: National Comprehensive Cancer Network
LN: lymph node
OS: overall survival
DFS: disease-free survival
ACOSOG: American College of Surgeons Oncology Group

Declarations
Ethics approval and consent to participate
This study was approved by the Institutional Review Board of Sun Yat-sen University Cancer Center.

Consent for publication
All patients enrolled in the study signed the consent for publication

Availability of data and material
The key raw data have been deposited into the Research Data Deposit (http://www.researchdata.org.cn), with the Approval and the datasets used in this study are publicly available.

Competing interests
The authors declare that they have no competing interests

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Authors' contributions
JYW and WYZ designed the study. FFD, YZZ and WYZ drafted the manuscript, QHY, SQD, TC and JLC
collected and interpreted the data. WYZ carried out the statistical analysis. FFD and YZZ critically revised the manuscript. All authors read and approved the final manuscript.

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Figures
Figure 1

(A) OS for patients with different N1 LNs examined patients. (B) DFS for patients with different N1 LNs examined patients.
