Prognostic impact of at least 12 lymph nodes after neoadjuvant therapy in rectal cancer: A meta-analysis

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Abstract

BACKGROUND
The number of dissected lymph nodes (LNs) in rectal cancer after neoadjuvant therapy has a controversial effect on the prognosis.

AIM
To investigate the prognostic impact of the number of LN dissected in rectal cancer patients after neoadjuvant therapy.

METHODS
We performed a systematic review and searched PubMed, Embase (Ovid), MEDLINE (Ovid), Web of Science, and Cochrane Library from January 1, 2000 until January 1, 2020. Two reviewers examined all the publications independently and extracted the relevant data. Articles were eligible for inclusion if they compared the number of LNs in rectal cancer specimens resected after neoadjuvant treatment (LNs ≥ 12 vs LNs < 12). The primary endpoints were the overall survival (OS) and disease-free survival (DFS).

RESULTS
Nine articles were included in the meta-analyses. Statistical analysis revealed a statistically significant difference in OS [hazard ratio (HR) = 0.76, 95% confidence interval (CI): 0.66-0.88, \( P = 0.122 \), \( P = 0.336 \)], DFS (HR = 0.76, 95% CI: 0.63-0.92, \( P = 0.584 \), \( P = 0.013 \)), and distant recurrence (DR) (HR = 0.67, 95% CI: 0.48-0.93, \( P = 0.663 \), \( P = 0.352 \)) between the LNs ≥ 12 and LNs < 12 groups, but local recurrence (HR = 0.67, 95% CI: 0.38-1.16, \( P = 0.0 \), \( P = 0.348 \)) showed no statistical difference. Moreover, subgroup analysis of LN negative patients revealed a statistically significant difference in DFS (HR = 0.67, 95% CI: 0.52-0.88, \( P = 0 \), \( P = 0.565 \)) between the LNs ≥ 12 and LNs < 12 groups.

CONCLUSION
Although neoadjuvant therapy reduces LN production in rectal cancer, our data indicate that dissecting at least 12 LNs after neoadjuvant therapy may improve the patients’ OS, DFS, and DR.

**Key Words:** Rectal cancer; Neoadjuvant therapy; Lymph node; Prognostic; Overall survival; Meta-analysis

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**Core Tip:** After neoadjuvant treatment of rectal cancer, the lymph node (LN) output is significantly reduced. There is no consensus on the relationship between the number of LNs resected and the prognosis of rectal cancer after neoadjuvant treatment. This is the first meta-analysis to compare the impact of the number of LNs on the prognosis of rectal cancer after neoadjuvant treatment. We studied the effects of resection of at least 12 LNs and less than 12 LNs after neoadjuvant treatment of rectal cancer on overall survival, disease-free survival, distant recurrence, and local recurrence.

**INTRODUCTION**

Colorectal cancer is ranked third and second among global cancer morbidity and mortality, respectively. The incidence rate of colorectal cancer is third in men and fourth in women[1]. In 2018, 43030 new cases of rectal cancer were diagnosed in the United States[2].

According to both the International Union Against Cancer (UICC) and American Joint Commission on Cancer (AJCC), a minimum of 12 lymph nodes (LNs) should be obtained from surgical specimens to stage a colorectal cancer12. Today, the standard of care for patients with locally advanced rectal cancer is neoadjuvant chemoradiation therapy (CRT), followed by total mesorectal excision (TME)3. However, many studies have reported a significant decrease in the number of LNs retrieved from patients with rectal cancer who have received preoperative chemoradiation5-9. For example, a meta-analysis reported an average reduction of 3.9 LNs in neoadjuvant CRT compared with no neoadjuvant CRT13. Therefore, it remains controversial whether 12 or more resected LNs should be recommended by the UICC or AJCC after many neoadjuvant treatments for rectal cancer.

In the past 20 years, neoadjuvant therapy has been widely applied in rectal cancer. An increasing number of scholars have focused on the influence of the number of resected LNs after neoadjuvant therapy on the prognosis of rectal cancer. Presently, resecting more than 12 LNs or fewer than 12 LNs after neoadjuvant treatment for rectal cancer is controversial for prognosis.

Given the prognostic impact of the number of LNs, we performed a first series of meta-analyses to compare the prognostic impact of surgical resection of greater than 12 vs fewer than 12 LNs in patients with rectal cancer after neoadjuvant treatment.

**MATERIALS AND METHODS**

**Search strategy and inclusion criteria**

For this systematic review, we adhered to the Meta-analysis of Observational Studies guidelines[14] and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement[15]. A systematic search was performed based on the following databases: PubMed, Embase (Ovid), MEDLINE (Ovid), Web of Science, and Cochrane Library from January 1, 2000 to January 1, 2020. We used “rectal cancer”, “neoadjuvant....
therapy”, “preoperative radiotherapy”, “preoperative chemotherapy”, “lymph nodes” and corresponding free words to search the literature in the above databases. Regardless of the type of study, the articles were eligible for inclusion if they compared the number of LNs in rectal cancer specimens resected after neoadjuvant treatment (LNs ≥ 12 vs LNs < 12).

First, all the identified titles and abstracts were examined by two independent reviewers (Tan L and Liu ZL). Next, the same two reviewers independently examined the full text of potentially relevant articles. In the event of disagreement, a third reviewer (Ma Z) was consulted and the relevant articles were discussed until a consensus was reached.

Data extraction and quality assessment
The following relevant information was extracted from all the included publications: First author, year of publication, country, number of patients, age, tumor grade, neoadjuvant therapy, surgery, years of follow-up, and outcome type. The main outcomes were the overall survival (OS) and disease-free survival (DFS) differences between the LNs ≥ 12 and LNs < 12 groups in patients after neoadjuvant therapy. The secondary outcomes were the distant recurrence (DR) and local recurrence (LR) differences between the LNs ≥ 12 and LNs < 12 groups in patients after neoadjuvant therapy. Therefore, if available, the following data were extracted: Hazard ratios (HRs), 95% confidence intervals (CIs), and P values of OS, DFS, DR, and LR. When the literature did not report HRs, only OS and DFS Kaplan-Meier curves and Engauge Digitizer (version 10.8) were used to determine the survival rate at the corresponding time points on the curve, followed by the HR calculation table[1]. We took the cutoff point if the HR reported in the literature was LNs < 12 vs LNs ≥ 12. All the data were independently extracted by two authors (Tan L and Liu ZL) and compared for consistency.

The quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS), with a maximum of nine points per study. Publication bias was assessed by visual inspection of the symmetry of the funnel plot. Since we considered that DFS heterogeneity was derived from patients with positive LNs, we performed subgroup analysis of the DFS of LN negative patients based on LNs ≥ 12 vs LNs < 12.

Statistical analysis
We used the Stata (version 15.3) Meta package for meta-analysis[5]. Binary outcome data are reported as HRs with 95% CIs using the Mantel–Haenszel method. Weighted mean differences were calculated for the effect size of continuous variables. Heterogeneity was assessed using I² statistics, with values above 50% considered considerable heterogeneity. An a priori decision to use the random-effects model was made to account for the assumed considerable heterogeneity between the studies.

RESULTS
After removing duplicates, our computer-aided search yielded 11871 publications from PubMed, Medline (Ovid), Embase (Ovid), Web of Science, and Cochrane Library (Figure 1). In total, nine publications and 4494 patients with rectal cancer were eligible for inclusion. Table 1 indicates the characteristics of the included studies. Seven[17,18,21,23] of the nine studies (3840 patients) reported the main endpoint of OS; five[17,19,21,24] of the nine studies (1811 patients) reported the endpoint of DFS; three[17,23,29] of nine studies (953 patients) reported the endpoint of DR; two[17,29] of the nine studies (716 patients) reported the endpoint of LR. The NOS scores of the nine studies ranged from eight to nine (Figure 2). The literature collected was considered to be qualified.

Primary endpoints
OS for LNs ≥ 12 vs LNs < 12: Seven of the nine included studies reported OS data based on at least 12 LNs vs fewer than 12 LNs; the HRs and 95% CIs of these studies and the summary HRs are shown in Figure 3A. The total summary estimated HR was 0.76 (95% CI: 0.66-0.88, P = 0.336). Heterogeneity tests showed that the trials did not have heterogeneity (I² = 12.2%, P = 0.336).

DFS for LNs ≥ 12 vs LNs < 12: Among the nine studies collected, five reported DFS data based on at least 12 LNs and fewer than 12 LNs; the 95% CIs and HRs for each study and the summary HRs are shown in Figure 3B. The total summary estimated HR was 0.76 (95% CI: 0.63-0.92, P = 0.013). Heterogeneity tests showed that the trials had
### Table 1 Characteristics of studies included in the meta-analysis

| Ref.                  | Country       | n      | Age (yr)       | Stage | Treatment                                      | Surgery       | Year of follow-up | Outcome         |
|----------------------|---------------|--------|----------------|-------|-----------------------------------------------|---------------|------------------|-----------------|
| Wang et al[17], 2019 | China         | 495    | 50: 160; > 50: 335 | I-IV  | Neoadjuvant treatment (RT 45–55 Gy + capecitabine) | AR, APR, Hartmann | No report        | OS, DFS, DR    |
| Lykke et al[18], 2015| Denmark       | 2123   | 60-75          | I-IV  | Neoadjuvant treatment                         | TME           | No report        | OS              |
| de Campos-Lobato et al[23], 2013 | United States | 237    | 57 (49-66)     | II-III | Neoadjuvant treatment                         | LAR, APR      | 55 (36-77) mo    | OS, DR, LR     |
| Kim et al[20], 2015  | South Korea   | 302    | 39-73          | I-IV  | Neoadjuvant treatment (IV 5-FU leucovorin or oral 5-FU based) | LAR, APR, CAA | 57 mo            | OS, DFS         |
| Doll et al[22], 2009 | Germany       | 102    | 18-75          | I-IV  | Neoadjuvant diachemotherapy (RT 45 Gy + 5-FU)   | (L)AR, Miles  | No reports       | OS              |
| La Torre et al[19], 2013 | Italy       | 123    | 67.9 (27-91)   | I-IV  | Neoadjuvant diachemotherapy (RT 45 Gy + 5-FU)   | LAR, APR      | 50 (9-120) mo    | OS, DFS         |
| Kim et al[24], 2015  | South Korea   | 433    | 62 ± 11.1      | I-IV  | Perioperative chemoradiation (45.0-50.4 Gy + 5-FU and leucovorin) | TME           | 41.2 mo          | DFS             |
| Han et al[21], 2016  | South Korea   | 458    | 60 (22-99)     | I-III | Neoadjuvant treatment (RT 45-50.4 Gy + 5-FU)   | TME           | 52 mo            | OS, DFS         |
| Klos et al[25], 2010 | United States | 221    | 53 ± 13        |       | Neoadjuvant treatment (RT 45.0-50.4 Gy + 5-FU) | TME           | 36 (21.6-63.6) mo| LR, DR          |

OS: Overall survival; DFS: Disease-free survival; DR: Distant recurrence; LR: Local recurrence; RT: Radiotherapy; 5-FU: 5-Fluorouracil; TME: Total mesolectal excision; APR: Abdominoperineal resection; LAR: Low anterior resection; CAA: Coloanal anastomosis; AR: Anterior resection.

significant heterogeneity ($I^2 = 68.4\%, P = 0.013$).

**Secondary endpoints**

**DR for LNs ≥ 12 vs LNs < 12:** Three of the nine included studies reported DR data based on LN ≥ 12 vs LN < 12; the 95% CIs and HRs for each study and the summary HRs are shown in Figure 3C. The total summary estimated HR was 0.67 (95% CI: 0.48-0.93, $P = 0.237$). Heterogeneity tests showed that the trials had no significant heterogeneity ($I^2 = 30.5\%, P = 0.237$).

**LR for LNs ≥ 12 vs LNs < 12:** Two of the nine included studies reported LR data based on LN ≥ 12 vs LN < 12. The total summary estimated HR was 0.67 (95% CI: 0.38-1.16, $P = 0.348$), with no statistical significance.

**Sensitivity analysis**

Sensitivity analysis showed that any deletion of a set of data had no effect on the results (Figure 4). We considered heterogeneity in patients with LN positivity. So, we conducted subgroup analysis of LN negative patients; the HRs and 95% CIs for each study and the summary HRs are shown in Figure 5. The total summary estimated HR was 0.67 (95% CI: 0.52-0.88), and no heterogeneity was found.

**Publication bias**

Publication bias was assessed by visual examination of the symmetry of the funnel plot. Our funnel plot showed no publication bias (Figure 6).

**DISCUSSION**

The AJCC and College of American Pathologists recommend examination of a minimum of 12 LNs to stage rectal cancer accurately. Sampling of 12 LNs may not be achievable in patients who have received neoadjuvant chemoradiation therapy[4,26]. Therefore, it remains controversial whether 12 LNs can be used as an accurate staging index for rectal cancer patients who have received preoperative neoadjuvant...
chemoradiation therapy. The mean number of LNs retrieved from rectal cancers treated with neoadjuvant therapy is significantly lower than that from rectal cancers treated by surgery alone\[8,9\]. The number of LNs needed to stage neoadjuvant-treated cases accurately is unknown. In patients receiving neoadjuvant radiotherapy and chemotherapy, the number of LN dissections needs to reach 12. Is this requirement suitable as a risk factor to evaluate the prognosis of patients with rectal tumors? Is it possible that the number of evaluated LN dissections is fewer than 12 because neoadjuvant radiotherapy and chemotherapy will lead to a decrease in the number of LN dissections? To confirm whether the number of LN dissections to judge the prognosis of rectal cancer patients who received neoadjuvant radiotherapy and chemotherapy is still applicable to 12, we performed this meta-analysis study.
Figure 3 Forest plots for the meta-analyses. A: Overall survival; B: Disease-free survival; C: Distant recurrence. HR: Hazard ratio; CI: Confidence interval; OS: Overall survival; DFS: Disease-free survival; DR: Distant recurrence.
The main finding of the present study is that, among patients with rectal cancer, dissecting at least 12 LNs after neoadjuvant treatment improved OS and DR compared with dissecting fewer than 12 LNs. In this study, we confirmed that at least 12 LNs should be dissected in rectal cancer patients after neoadjuvant radiotherapy and chemotherapy to evaluate the prognosis of patients well, and the number of LNs dissected was closely related to the improvement in the survival rate of rectal cancer patients. Additionally, for subgroup analysis, the DFS of LN negative patients with greater than 12 LNs dissected was better than that of patients with fewer than 12 LNs dissected. These data suggest that surgical resection of at least 12 LNs after neoadjuvant treatment of rectal cancer improves prognosis.

Most scholars agree that the LN yield affects the prognosis of rectal cancer. Presently, they have studied the effect of resection of 12 LNs on the prognosis. At the same time, many scholars have studied the effect of different numbers of LNs resected on the prognosis of patients with neoadjuvant therapy for rectal cancer. We summarize the literature on the effect of different LN numbers on the prognosis in the last 10 years (Table 2). Yeo et al.\(^\text{[31]}\) showed that at least 8.5 LNs removed from rectal cancer surgery after neoadjuvant therapy could significantly improve the 5-year OS. La Torre et al.\(^\text{[32]}\), Tsai et al.\(^\text{[33]}\), and Han et al.\(^\text{[34]}\) found that at least 6, 7, and 8 LNs resected after neoadjuvant treatment could improve the prognosis. Pitto et al.\(^\text{[35]}\) found that at least 10 to 20 LNs resected after neoadjuvant radiotherapy improved the 5-year OS compared with fewer than 9 and more than 20. The above studies indicated that the small number of LNs dissected after neoadjuvant therapy is not a sign of a good tumor response to neoadjuvant therapy, and a relatively large number of LNs is still needed to be dissected to ensure a good prognosis.

The prognostic impact of resecting more than 12 LNs and fewer than 12 LNs after neoadjuvant treatment for rectal cancer is controversial. For example, Dev et al.\(^\text{[36]}\) found that resecting fewer than 12 LNs in rectal cancer patients undergoing neoadjuvant radiotherapy should be considered a better prognostic factor, but Wang et al.\(^\text{[37]}\) and Lykke et al.\(^\text{[38]}\) believed that resecting at least 12 LNs is an independent and favorable prognostic factor for rectal cancer after neoadjuvant therapy. Moreover, Khan et al.\(^\text{[39]}\) and La Torre et al.\(^\text{[40]}\) believed that at least 12 LNs dissected after neoadjuvant treatment of rectal cancer do not affect the prognosis. Our meta-analysis combining the available data showed that resection of at least 12 LNs after neoadjuvant therapy improves the prognosis.

The LN harvest is influenced by several factors, including the patient's anatomic and pathologic workup, surgical dissection technique, and use of methylene blue and neoadjuvant treatment\(^\text{[12-30]}\). Pathological techniques are considered a factor that affects the LN yield due to improper specimen analysis and processing. Factors associated with patients, such as advanced age and obesity, are associated with lower LN yields\(^\text{[12,17,28]}\). Standard TME should be performed to help achieve optimal tumor resection. The injection of methylene blue solution into the inferior mesenteric artery is an effective and simple way to increase the LN harvest in the histopathological examination of the TME of rectal specimens\(^\text{[39]}\), especially those receiving neoadjuvant therapy\(^\text{[40-42]}\). Presently, the use of neoadjuvant radiotherapy and chemotherapy is the standard treatment for rectal cancer in many European countries, leading to fewer LN tests\(^\text{[43]}\). If 12 LNs are considered the number needed for the accurate staging of stage II tumors, only 20% of cases treated with neoadjuvant therapy had adequate LN sampling\(^\text{[4]}\). To date, the number of dissected LNs needed to stage neoadjuvant-treated cases accurately is unknown. Additionally, the clinical significance of this information is unknown in the neoadjuvant setting because postoperative therapy is indicated in all patients who receive preoperative therapy regardless of the surgical pathology results. Therefore, technical measures are needed to improve the postoperative LN detection rate in patients with rectal cancer after neoadjuvant radiotherapy and chemotherapy. For example, standard TME in combination with the injection of methylene blue into the inferior mesenteric artery can be used to increase LN yield after neoadjuvant therapy. At the same time, the application of nano-carbon lymphatic tracer technology can also effectively improve the detection rate of postoperative LNs in patients with rectal cancer.

In recent decades, the therapeutic effect of rectal cancer has made great progress with the development of laparoscopic technology and medical devices. Murphy et al.\(^\text{[44]}\) found that the 5-year relative survival of rectal cancer improved significantly from 1992-1996 to 2010-2014. The emergence of neoadjuvant therapy, especially neoadjuvant radiotherapy and chemotherapy, significantly reduced the local recurrence rate and tumor staging of patients\(^\text{[45,46]}\). Neoadjuvant radiotherapy and chemotherapy have been regarded as the standard treatments for locally advanced rectal cancer, and the side effects of neoadjuvant radiotherapy and chemotherapy
Table 2 Prognosis of different lymph node yield after neoadjuvant therapy for rectal cancer

| Ref.          | n   | Treatment                                      | Number of LNs compared | OS (HR or percent) | DFS (HR or percent) |
|---------------|-----|-----------------------------------------------|------------------------|--------------------|---------------------|
| Yeo et al[27], 2020 | 94  | Neoadjuvant CRT (RT 45 Gy + capecitabine)     | LNs ≥ 8.5 vs LNs < 8.5 | HR: 0.31 (95%CI: 0.15-0.64, P < 0.001) | -                   |
|               |     |                                               | LNs ≥ 16.5 vs LNs < 16.5 | -                  | HR: 0.46 (95%CI: 0.17-1.27, P = 0.13) |
| La Torre et al[19], 2013 | 123 | Neoadjuvant CRT (RT 45 Gy + 5-FU)             | LNs ≥ 6 vs LNs < 6     | 5-yr OS: 84% vs 75% (P = 0.03) | 5-yr DFS: 83% vs 75% (P = 0.03) |
| Tsai et al[28], 2011 | 372 | Neoadjuvant CRT (RT 45 Gy + 5-FU and/or capecitabine) | LNs ≥ 7 vs LNs ≤ 7   | 5-yr OS: 86.9% vs 81% (P = 0.067)  | -                   |
| Han et al[21], 2016 | 458 | Neoadjuvant CRT (RT 45–50.4 Gy + 5-FU)       | LNs ≥ 8 vs LNs < 8     | HR: 0.5 (95%CI: 0.2-0.9, P = 0.002) | HR: 0.6 (95%CI: 0.4-1.1, P = 0.042) |
| Pitta et al[29], 2020 | 104 | Neoadjuvant RT (RT 45 Gy + capecitabine)     | LNs: 10-20 vs LNs ≤ 9 and ≥ 20 | -                  | HR: 0.313 (95%CI: 0.1-0.99, P = 0.049) |

OS: Overall survival; DFS: Disease-free survival; LNs: Lymph nodes; HR: Hazard ratio; CRT: Chemoradiation therapy; RT: Radiotherapy; 5-FU: 5-Fluorouracil; CI: Confidence interval.

Figure 4 Sensitivity map for the meta-analysis of disease-free survival. CI: Confidence interval.

cannot be ignored, such as chronic sexual dysfunction[47] and diarrhea[48,49]. Some patients with high-risk diseases may need more intensive treatment, while others may have severe side effects due to the use of current protocols[50]. The criteria for the inclusion of patients with rectal cancer to undergo neoadjuvant radiotherapy and chemotherapy need to be further optimized, and multidisciplinary team discussion is warranted to determine whether a patient should receive neoadjuvant therapy for rectal cancer.

This meta-analysis was mostly limited by its inclusion of cohort study data only; no randomized controlled study was included. Cohort studies are prone to introduce bias, and two of these studies did have OS results. The HR data in four studies could not be extracted directly and were calculated from Kaplan-Meier curves, a calculation process that may cause errors. Additionally, this study only analyzed the prognosis of patients in the LNs ≥ 12 and LNs < 12 groups after neoadjuvant therapy. Insufficient data existed to analyze the effect of other LN numbers on the prognosis. Differences in surgical treatment reported in the literature, as well as different surgical procedures, may influence the LN yield, which may lead to bias.
Figure 5 Forest plot for the meta-analysis of disease-free survival in the subgroup of lymph node negative patients. HR: Hazard ratio; CI: Confidence interval; DFS: Disease-free survival.

CONCLUSION

Although neoadjuvant therapy reduces the production of LNs in rectal cancer, our data indicate that dissecting at least 12 LNs after neoadjuvant therapy may improve the patients’ OS, DFS, and DR.
ARTICLE HIGHLIGHTS

Research background
Neoadjuvant therapy significantly reduces the number of yielded lymph nodes (LNs) for rectal cancer, and the number of dissected LNs in rectal cancer after neoadjuvant therapy has a controversial effect on the prognosis.

Research motivation
Studies have shown that the number of LNs after rectal cancer is significantly reduced after neoadjuvant therapy. Some scholars have found that less than 12 LNs in rectal cancer patients receiving neoadjuvant radiotherapy should be considered as a better prognostic factor. However, others believe that dissecting at least 12 LNs is an independent and favorable prognostic factors for rectal cancer after neoadjuvant therapy. Therefore, it is necessary to conduct a meta-analysis to systematically and comprehensively study the influence of the number of LNs retrieved after neoadjuvant treatment on the survival outcome of patients with rectal cancer.

Research objectives
To evaluate the effect of LN production in rectal cancer after neoadjuvant treatment on survival through meta-analysis.

Research methods
The meta-analysis methods were adopted to realize the objectives.

Research results
Nine articles were included in the meta-analyses. Statistical analysis revealed a statistically significant difference in overall survival (OS) [hazard ratio (HR) = 0.76,
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