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Lymph node regression grading of locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy

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Abstract

Neoadjuvant chemoradiotherapy (nCRT) and total rectal mesenteric excision are the main standards of treatment for locally advanced rectal cancer (LARC). Lymph node regression grade (LRG) is an indicator of prognosis and response to preoperative nCRT based on postsurgical metastatic lymph node pathology. Common histopathological findings in metastatic lymph nodes after nCRT include necrosis, hemorrhage, nodular fibrosis, foamy histiocytes, cystic cell reactions, areas of hyalinosis, residual cancer cells, and pools of mucin. A number of LRG systems designed to classify the amount of lymph node regression after nCRT is mainly concerned with the relationship between residual cancer cells and regressive fibrosis and with estimating the number of lymph nodes existing with residual cancer cells. LRG offers significant prognostic information, and in most cases, LRG after nCRT correlates with patient outcomes. In this review, we describe the systematic classification of LRG after nCRT, patient prognosis, the correlation with tumor regression grade, and the typical histopathological findings of lymph nodes. This work may serve as a reference to help predict the clinical complete response and determine lymph node regression in patients based on preservation strategies, allowing for the formulation of more accurate treatment strategies for LARC patients, which has important clinical significance and scientific value.

Key Words: Lymph node regression grade; Histopathological; Rectal cancer; Chemoradiotherapy; Treatment response; Neoadjuvant therapy

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Core Tip: Studies on lymph node regression grading after neoadjuvant chemoradiotherapy (nCRT) for rectal cancer are limited but serve clinicians for assessing the lymph node response to treatment based on the efficacy of the primary

tumor after preoperative nCRT, providing guidance in formulating more accurate surgical or therapeutic strategies for the next stage of patient management and in determining patient prognosis. We discuss its histopathology, prognosis, correlation with tumor regression grading, and clinical applications and prospects.

INTRODUCTION

Neoadjuvant chemoradiotherapy (nCRT) and total rectal mesenteric excision (TME) are the main standards of treatment for locally advanced rectal cancer (LARC)[1-5]. The response of lymph nodes (LNs) to neoadjuvant therapy is reflective of the possibility of regression, similar to the main tumor body. LN regression grade (LRG) is based on postsurgical metastatic LN pathology and is an indicator of the response to preoperative nCRT and patient prognosis[6,7]. The status of tumor-draining LNs (TDLN) has been considered the most significant indicator of prognosis in patients with LARC, and the number of LN metastases is currently the only measure of ypN staging[8-12]. Several studies have demonstrated that nCRT decreases the detection of positive LNs and the total number of positive LNs, thereby affecting the accuracy of the patient's ypN stage[13-16]. In addition, the majority of studies and applications focused on tumor regression have centered on the primary tumor, while the impact of LRG on tumor regression and prognosis has not been fully explored. nCRT treatment based on well-predicted and assessed regression is beneficial for individualized clinical decision making and multidisciplinary diagnosis and treatment.

In the following study, we present the characteristics and histopathological findings of LNs observed as a result of nCRT, summarize the concepts for LRG, introduce some LRG staging systems for rectal cancer, describe the patient prognosis and the relationship with tumor regression grade (TRG), explore the limitations and critical issues, and discuss the clinical impact of LRG on rectal cancer.

LITERATURE SEARCH

The main purpose of the present review is to identify the latest studies relating to LRG after neoadjuvant radiotherapy in patients with LARC and to compare their main elements. We performed a database search on PubMed and selected papers published in English between January 2000 and January 2022. PubMed was last accessed on 2 February 2022. The following keywords and terms were used. ("rectal OR rectum") AND ("carcinoma OR neoplasm OR malignant OR malignancy OR cancer") AND ("lymph node grade OR LRG OR lymph node grading") AND ("chemoradiotherapy OR therapy OR chemotherapy OR radiotherapy") AND ((2000/1/1[PDAT]: 2022/1/31[PDAT])), to retrieve relevant articles. All articles are in English. Meta-analyses, reviews, and other articles containing nonoriginal data were excluded from our review. All articles retrieved were selected and screened by three independent authors. Related data on the articles were retrieved by a standardized data collection method. A flow chart of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses is shown in Figure 1.

HISTOPATHOLOGICAL DISCOVERIES FOLLOWING NCRT

The primary purpose of the pathologic procedure was the macrosurvey of the resected tumor and LN specimens[17]. Operative specimens were detached from the anterior wall with a fixation for 24 h in 40 g/L formaldehyde. External surfaces of the specimen were stained with black ink for the easy identification of surgical margins. Serial sections of the entire tumor and attached mesentery were performed at 3- to 4-mm intervals vertically along the longitudinal axis of the rectum. To assess the LNs around the rectum, the interrectal fat was removed after tumor sampling. All LNs were identified by palpation and removed using scissors and a scalpel, followed by histological examination[18].

Based on the histology, tumor regression after nCRT essentially constitutes subacute to subchronic inflammation that follows the cytotoxic effects occurring weeks before. In the majority of cases, the tumor was removed sometime after completing the final cycle of preoperative chemotherapy[17].

At the cellular level, in the case of complete LN regression, the malignant cells were eradicated through cytotoxic therapy and/or subsequently by the inflammatory response, and the LNs were displaced by fibrous tissue. In contrast, there was a high probability of an abundance of residual tumor cells in the LNs, such as small single cells or tumor cell clusters. Microscopic analysis of metastatic disease was performed on all dissected LNs[19]. The following modes of tumor regression could be observed: Necrosis, hemorrhage, nodular fibrosis, foamy histiocytes, cystic cell reaction, areas of hyalinosis, residual cancer cells, and pools of mucin (Figure 2)[20,21]. Fernández-Aceñero *et al*[21] analyzed the potential prognostic effects of those response modes, such as cystic cell reaction and mucus pool, on disease-free survival (DFS) and disease-specific survival (DSS) and found no significant correlation between survival and response. In addition, several other LN markers have prognostic significance. For instance, mounting evidence suggests that extracapsular LN involvement is one prognostic contributor to recurrence and poor prognosis in malignancies of the gastrointestinal tract[22,23]. The presence or absence of fibrosis is usually used to differentiate nonmetastatic LNs from metastatic LNs that have completely regressed[19].

However, histopathological assessments have several limitations. First, the number of patients with stage ypN0 disease downgraded to only microscopic LN involvement is difficult to assess. Second, patients receiving nCRT had fewer LNs retrieved than those who underwent only radical surgery. After nCRT, fibrosis in the metastatic LNs is not as pronounced as in the primary tumor. Normal lymphocytes still occupied most LNs, and only fibrosis occurred around metastatic tumor cells. However, the changes in normal lymphocytes after radiotherapy were uncertain, with most showing no response and some fibrosis, making it much more difficult for pathologists to distinguish normal LNs from completely regressed LNs, especially when only a small number of metastatic tumor cells were present. Therefore, only some LRG1 patients were in complete remission after nCRT, while others had normal LNs, so pathologists could not assess whether the small fibrotic tissue lesion was normal LN or a metastatic LN before treatment. Finally, pathologists cannot distinguish patients with fibrosis-free LNs from

those with residual ypN0 tumors as complete responders and non-responders. Nevertheless, we ought to recognize that a complete response is not a safe assumption among patients with clinical LN+ on magnetic resonance imaging with no pathological abnormalities. Does the absence of fibrosis among the LN imply that no tumor cells were present before nCRT was performed, or does the presence of fibrosis among the LN imply that tumor cells were once present? These questions should be investigated in future studies.

CLASSIFICATION OF LRG

Numerous publications have shown that TRG is significantly relevant to the assessment of patient outcomes[13,24] and is an essential prognostic indicator for patients with LARC[25-27]. LRG, like TRG, is an assessment of local metastatic LN treatment response indicators for nCRT based on postoperative patient histopathology[9,28,29]. When classifying the degree of LN regression, the following two aspects should be assessed: the relationship between residual cancer cells and regressive fibrosis, the basis of which is usually described, and the number of LNs with residual cancer cells, which is usually expressed as a percentage (%) (Figure 3).

Relevant studies have documented that residual tumor cells may still be present in local LNs despite complete regression of the primary disease[30]. In some studies[31], this occurred in up to 17% of cases, especially when a watch-and-wait strategy after nCRT was chosen, likely leading to recurrence and treatment failure. Therefore, pathologic evaluation of LNs in patients undergoing surgery after nCRT can contribute to an accurate determination of the clinical stage of the tumor and the metastatic LN response to nCRT (Table 1).

Caricato et al[18]

In 2007, Caricato *et al*[18] retrospectively analyzed colorectal LNs in 35 patients undergoing preoperative CRT with LARC and reported, for the first time, the tissue effects of preoperative CRT on colorectal LNs and defined the grade of LN regression as

follows: LRG1 for the absence of histologically identifiable residual cancer and fibrosis extending through the different areas of the LN; LRG2 for near-complete pathologic response (pCR); LRG3 for the presence of residual cancer cells with evident fibrosis; LRG4 for poor response; and LRG5 for nodal metastasis with the absence of regressive changes. It was also concluded that LRG was significantly correlated with TRG in primary tumors. However, this study had a small sample size, and no follow-up was performed clinically, so the prognosis of patients with LRG was not investigated further.

***Mirbagheri et al*[28]**

In 2014, Mirbagheri *et al*[28] retrospectively analyzed clinical data from 190 patients who had LARC and received nCRT and found that LRG, similar to the TRG standard, could be used as an influencing factor for tumor recurrence. They also proposed a TRG-like LRG scoring system as follows for LRG0 for normal LNs; LRG1 for 100% fibrosis, no residual cancer; LRG2 for 75%-100% fibrosis, 0-25% cancer; LRG3 for 50%-75% fibrosis, 25%-50% cancer; LRG4 for 25%-50% fibrosis, 50%-75% cancer; and LRG5 for 0-25% fibrosis, 75%-100% cancer (Figure 4). Their study results indicated that: (1) LVI (P = 0.029), tumors in the middle of the rectum and higher TRG scores were correlated with higher LRG scores; and (2) LN regression was a major factor in the prediction of tumor recurrence, and lower LN regression scores were associated with an enhanced survival curve. Mirbagheri *et al*[28] also proposed not only the LRG score but, for the first time, LRG maximum (LRG-max) and LRG-sum (LRG-sum). Subsequent analysis of these parameters indicated significant associations with tumor prognosis. Further research has provided additional evidence supporting a significant association between these parameters and tumor prognosis.

LRG-max: Since the number of LNs varies in each specimen and different regression scores may be calculated for different LNs depending on their treatment response, total scores were determined according to the worst score for each patient (specimen). For

example, if one specimen contains two LNs whose scores were 2 and 3, the LRG-max would be 3.

LRG-sum: This reflects the overall tumor burden of the specimen for all LNs. For example, if one specimen contains two LNs whose scores were 2 and 3, the LRG-sum would be 5.

***Beppu et al*[32]**

In 2015, Beppu *et al*[32] retrospectively analyzed clinical data from 178 patients suffering from LARC who were treated with nCRT preoperatively, investigated the requirement of chemoradiotherapy for positive LNs that had completely regressed, and proposed the following LRG score set: LRG 1 for minor regression, fibrosis outgrown by cancer or no fibrosis with extensive residual cancer; LRG 2 for good regression, residual cancer outgrown by fibrosis; and LRG 3 for total regression, no cancer cells, single cells or small groups of cancer cells. The results showed that the primary tumor response to chemoradiotherapy was related to a positive nodal response. In contrast, for patients with a TRG of 3, the LRG score was associated with positive node size. The conclusion was also drawn that for the complete regression of positive nodes, the requirements were: (1) Degeneration of the primary tumor, with a TRG of 3; and (2) a diameter of < 6 mm for positive nodes.

The following year, Beppu *et al*'s group performed subgroup analyses with 229 patients receiving preoperative nCRT in T3 rectal cancer and showed that total positive node regression following preoperative chemoradiotherapy is the only factor independently associated with favorable overall survival[33]. Therefore, it was concluded that positive nodes showing complete regression after preoperative chemoradiotherapy could improve the prognosis of rectal cancer patients with positive LNs before treatment.

***Lee et al*[34]**

In 2019, Lee *et al*[34] evaluated postoperative LNs in 389 patients with rectal cancer treated with nCRT and then received radical resection. Lee defined the degree of regression of metastatic LNs after nCRT according to tumor cell percentage and degree of fibrosis and proposed a system for grading pathological LRG (pLRG) as follows: pLRG0 is a LN with normal nodal architecture, and without evidence of cancer cells or fibrosis, pLRG1 is a LN with 100% fibrosis, pLRG2 is a LN with < 25% cancer cells, pLRG3 has scattered glandular elements with fibrosis, pLRG4 is a LN with > 50% cancer cells, and pLRG5 is a complete replacement with cancer cells. The results showed that: (1) The LRG-sum distribution correlated significantly with the TRG in primary tumors; and (2) In the multivariate analysis, LRG-sum was the factor most related to RFS among the LN-related variables, in addition to ypT staging. According to the findings from this study, LRG was an influential factor for tumor prognosis in patients with rectal cancer following nCRT and surgical resection. It was shown that LRG was associated with a completely regressed primary tumor; accordingly, predicting LN regression based upon completely regressed primary tumors was beneficial, especially in patients considering a nonsurgical approach after nCRT.

***Sun et al*[35]**

In 2020, Sun *et al*[35] retrospectively analyzed the clinical data of 257 LARC patients receiving nCRT and proposed the following LRG scoring system: LRG 0, normal LN architecture without evidence of regression or cancer cells; LRG 1, 100% fibrosis; LRG 2, < 25% remaining cancer cells; LRG 3, 25–50% scattered glandular elements with fibrosis; LRG 4, > 50% viable cancer cells; and LRG 5, complete replacement with cancer cells. Sun *et al*[35] suggested that, to some extent, LRG was associated with the primary tumor response. In addition, it may help predict clinical complete remission (the cCR) and determine LN regression in patients based on preservation strategies (*e.g.*, local excision or an approach of "watch and wait"[36,37]. Furthermore, higher LRG scores were correlated with higher TRG, later ypN and ypT staging, and poorer DFS and OS.

***Cui et al*[38]**

In 2020, Cui *et al*[38] retrospectively analyzed the clinical data of 358 patients with LARC who received nCRT and proposed the following set of LRG scores: LRG0, negative LN; LRG1, complete regression with no residual tumor cells; LRG2, rare residual tumor cells; LRG3, fibrosis outgrown by residual tumor cells; LRG4, residual tumor cell outgrown by fibrosis; and LRG5, absence of regression with no fibrosis. The results showed that in the univariate analysis, the factors that correlated with DFS were ypN, ypT, the number of negative LNs (NLN), LN ratio (LNR), TRG, m-TTRG (modifying ypT stage by combining ypT and TRG), LRG-sum, LRG-max, M-NLRG (modifying ypN stage by combining LNR and LRG-max) and the LRG ratio (average of LRG-sum). M-NLRG and M-TTRG were significantly related to DFS in the multivariate Cox regression analysis. It was concluded that LRG significantly contributes to the prognosis in rectal cancer patients receiving nCRT and can improve the ypTNM staging system. A modified ypTNM staging system combining TRG, LRG-max and LNR could enhance DFS prediction for various subgroups of patients.

CORRELATION BETWEEN LRG AND TRG

The relationship between primary tumors and LRG is still controversial among studies[39,40]. Most of these differences could be accounted for by different treatment plans, varied diagnostic standards for LRG, small sample sizes and patient heterogeneity.

Several studies[18,21,34] have reported that LRG was significantly correlated with TRG in primary tumors. Lee *et al*[34] evaluated postoperative LNs in 389 patients with rectal cancer treated with nCRT and concluded that LRG-sum distribution correlated significantly with the TRG in primary tumors ($P < 0.001$). LRG was associated with a completely regressed primary tumor. Accordingly, predicting LN regression based upon completely regressed primary tumors is beneficial, especially for patients considering a nonsurgical approach after nCRT. There are also studies[28,35] that suggest that higher TRG scores are correlated with higher LRG scores. Sun *et al*[35]

retrospectively analyzed the clinical data of 257 LARC patients who were receiving nCRT and found that in the TRG 1, 2 and 3 groups, LRG scores were significantly increased. Higher scores of LRG were also found to be associated with more advanced stages of ypT and ypN. Considering these results, Sun *et al*[35] suggested that, to some extent, LRG may help predict the clinical complete response (the cCR) and determine LN regression in patients based on preservation strategies (*e.g.*, local excision or an approach of "watch and wait"). Additional studies have suggested that LRG is associated with TRG only under specific conditions, and the study by Beppu *et al*[32] concluded that: (1) Primary tumor radiosensitivity was associated with positive LNs; and that (2) LRG scores were associated with positive LN size only if the primary tumor had TRG 3 response.

Others[31] have argued that primary tumor TRG does not predict the LN presence of residual lesions. In 2006, Hughes *et al*[31] examined a total of 211 clinical-stage T3-T4 patients receiving preoperative CRT treatment outcomes and treatment details and concluded that primary tumor pathologic complete response failed to predict the circumrectal LN response, and the extent of the primary tumor response was a predictor of LN response.

Nevertheless, it is significant to note that different diagnostic standards for LRG were used in these previous studies, including the subgrouping of patients, which introduces some heterogeneity. Therefore, no conclusions concerning the association between TRG and LRG can be drawn at this time, and future large-scale research is needed with more homogeneous population groups to clarify this relationship.

PROGNOSTIC SIGNIFICANCE OF LRG

Most studies[33,34,41] have suggested that LRG is a factor in the prognosis of rectal cancer patients receiving radical resection after nCRT. The study by Beppu *et al*[32] concluded that patients with completely regressed LNs typically had the best outcome. Beppu *et al*'s, Lee *et al*'s, Cui *et al*'s subgroup review of 229 patients receiving preoperative nCRT in T3 rectal cancer showed that total positive node regression following preoperative

chemoradiotherapy is the only factor independently related to favorable overall survival[32,34,38]. While complete LN regression has been consistently correlated with improved DFS and OS as well as reduced local and distal recurrence risk, the impact of partial and subtotal LN regression [which is expected to be the main advantage of LRG *vs* TNM and American Joint Committee on Cancer (AJCC) grade] remains poorly understood. Studies from Mirbagheri *et al*[28] and Sun *et al*[35] concluded that a higher LRG was correlated with poorer DFS and OS. Mirbagheri *et al*[28] used multivariate Cox proportional hazard regression analysis and did not find that the LRG score was a factor for mortality, but it was an important predictor of relapse. However, the assumption that patients who had LN complete regression (LRG1) might fare better than LRG0 patients was not adequately tested, considering the small sample size of LRG1 patients. Tominaga *et al*[41] retrospectively analyzed 421 rectal cancer patients receiving preoperative nCRT, and the results indicated that LRG1 is a significant and independent factor for predicting recurrence-free survival. However, their results indicated that patients with grade 1 LN regression had similar local recurrence rates (LR) and 5-year recurrence-free survival rates as patients with LRG 0. However, in 120 patients with grade 2-5 LN regression, the 5-year recurrence-free survival rate and the LR resembled those of patients with LRG0, and the LR and the 5-year recurrence-free survival rate were poor irrespective of LRG (LR of 8.4%-14.0% and recurrence-free survival rate of 38.1%-61.1%). In addition, a large number of studies[28,34,35] have concluded that LRG-max and/or LRG-sum are significantly associated with prognosis. Lee *et al*[34] evaluated postoperative LNs in 389 patients with rectal cancer treated with nCRT and then received radical resection. In the multivariate analysis, LRG-sum was the most related contributor to RFS in LN-related variables alongside ypT staging. In 2020, Cui *et al*[38] suggested that in the univariate analysis, the contributors correlated with DFS were LRG-sum, LRG-max, M-NLRG and the LRG ratio.

However, in 2016, Fernández-Aceñero *et al*[21] retrospectively analyzed 106 rectal cancer patients receiving treatment at a single institution and concluded that there was

no remarkable correlation between any factors or DSS and the LN tumor regression model in terms of prognosis.

In summary, we consider LRG to be an independent predictor of DFS for patients with LARC receiving nCRT and radical surgery. Since LN regression is highly correlated with other significant variables (*e.g.*, LVI and TRG), this characteristic might lose its statistical significance in some computational models, explaining the failure of certain studies to show that LRG has independent prognostic value relative to these other parameters[28].

CRITICAL ISSUES OF LRG

Necessity of LRGs

With the increasing development of comprehensive therapy for rectal cancer, the National Comprehensive Cancer Network has suggested that the therapy criteria for LARC are nCRT and TME[42-46], whose application has brought tremendous prognostic improvement for LARC patients with lower LR[47-50] as well as better anal preservation for patients with low rectal cancer[51,52]. A subset of LARC patients treated with nCRT can achieve complete tumor regression and are thus candidates for nonsurgical treatment[53]. NCRT leads to different degrees of tumor regression, with some patients achieving pCR for the primary tumor[27,54-56]. The LR was low in this patient group, and the tumor-free survival and overall rates were high[27,57,58]. Furthermore, numerous studies have demonstrated that TRG is significantly correlated with patient outcomes[13,24] and is an important prognostic factor for patients with LARC. LRG, like TRG, reflects the response of locally metastatic LNs to nCRT treatment based on postoperative patient histopathology[9,28]. In relevant studies, it is fully documented that residual tumor cells may still be present in local LNs despite the complete regression of primary tumors[30]. Currently, no single histopathological feature of colorectal cancer can reliably predict LN metastasis[59]. Some studies have demonstrated that different responses may exist between primary tumors and mesenteric LNs of the rectum[60]. Despite complete tumor regression, LN involvement may still occur. This was found in

up to 17% of cases in some studies[31], especially when a watch-and-wait strategy was chosen after nCRT, likely leading to recurrence and treatment failure. Therefore, the pathologic evaluation of LNs in patients treated with surgery after nCRT could help to accurately determine the clinical staging of tumors and the response of metastatic LNs to nCRT.

The status of TDLN was the most significant factor in the prognosis of patients who have rectal cancer[61-63]. The number of metastatic LNs is currently the only basis for ypN staging, and several studies have demonstrated that nCRT leads to a decrease in the total number of LNs detected and the number of positive LNs[64,65]. Thus, the accuracy of staging ypN can be affected[13,14].

Several studies[66] have shown that current AJCC staging systems cannot accurately evaluate patient prognosis following nCRT because nCRT decreases the tumor stage and leads to varying degrees of treatment response. However, others argue that good prediction and assessment of regression during nCRT treatment and multidisciplinary consultation can allow for more individualized clinical decision making and treatment. The vast majority of studies on tumor response to therapy have focused on the primary tumor, while the effect of LRG on tumor treatment response and prognosis has not yet been fully appreciated.

TRG: The assessment of nCRT treatment regression in clinical practice relies mainly on postsurgical pathological examination results. Tumors were also graded by TRG according to the relative proportions of resident tumor cells in pathological specimens and the degree of fibrosis after treatment. Mandard *et al*[24] proposed the following: TRG1 for the absence of residual cancer and fibrosis - complete regression; TRG2 for the presence of rare residual cancer; TRG3 for an increase in the number of residual cancer cells but predominantly fibrosis; TRG4 for residual cancer outgrowing fibrosis; and TRG5 for the absence of regressive changes. Dworak *et al*[25] proposed a TRG staging system in 1997, which classified regression into stages 0 to 4 based on better to worse tumor regression. The seventh edition of the 2010 AJCC Cancer Stage Manual, put

forward by the American Joint Committee on Cancer, reads as follows[29,67]: TRG0 for no viable cells present – complete; TRG1 for small groups of cancer cells/moderate-single cells – minimal; TRG2 for residual cancer outgrown by fibrosis; and TRG3 for no tumor-killing or poor/minimal killing, extensive residual cancer (Figure 5). Siddiqui *et al*[68] showed a strong association between patient prognosis and postoperative TRG grade, and they defined Dworak grades 3 and 4 and Mandard grades 1 and 2 as a better prognosis and Dworak grades 0 to 2 and Mandard grades 3 to 5 as a worse prognosis.

Limitations of ypN staging

Currently, the AJCC 8th edition staging system, based solely upon the number of positive LNs for ypN staging, still follows the same ypN staging criteria for patients receiving nCRT and those undergoing surgery alone. Of the currently available TNM staging systems, ypN staging is classified according to the absolute number of positive LNs (PLNs). The guideline is based on little evidence and is largely derived from the historic view that evaluating a smaller number of nodes results in understaging[69,70]. In addition, although it has been determined that increases in nodal harvest are related to improved survival, generally accepted staging theories explaining this relationship are unsupported by the evidence, and several authors have suggested that the higher number of LNs may indicate immune competence in individual patients instead of an improved means of detecting metastatic nodes[71,72]. A large population study in the United States showed that less than 50% of patients achieved the recommended number of LNs[73,74]. Thus, there are two main reasons why the AJCC guidelines have been questioned. First, recommendations for staging guidelines and treatment of rectal cancer depend heavily on data collected from colon cancer patients who are thought to be appropriate for rectal cancer[75,76]. Moreover, LNs found in rectal specimens were smaller in number and size than those found in colonic specimens[70,77]. Second, LNs detected after nCRT was significantly decreased[78,79]. Due to the increasing use of preoperative treatment of rectal cancer, pathology reports demonstrating low counts of LNs are increasingly being received by colorectal surgeons.

This ypN staging system only focuses on the numbers of metastatic LNs regardless of the tumor load in LNs following nCRT. The relevant literature suggests that LN regression should also be considered when assessing LN status. The main reasons for this may be twofold. First, the current ypN staging ignores the influence of LN treatment response on prognosis. A similar number of LN-positive patients might have a different number of LN metastases and a different metastatic load before treatment. The degrees of LN metastatic tumor regression following nCRT may reflect the different biological behaviors of tumors in different individuals, leading to different prognoses. Second, a decrease in the detection of positive LNs and the total number of positive LNs following nCRT can result in a bias in ypN staging based on using the number of positive LNs as grouping criteria[80,81].

One meta-analysis[82] demonstrated that patients receiving nCRT had a mean decrease of 3.9 total LNs detected and 0.7 PLN. Patients treated with neoadjuvant radiotherapy had 2.1 fewer total LNs detected. Ceelen *et al*[83] retrospectively analyzed 4037 patients who have rectal cancer registered in the Belgian Rectal Cancer Registry (Project for Rectal Cancer, PROCARE) between 2006 and 2012 who received nCRT and demonstrated a 12.3% reduction in the total number of detected LNs after short-range radiotherapy and a 31.3% reduction after long-range radiotherapy or long-range simultaneous radiotherapy. For each 1 Gy increase in the radiation dose, the number of detected LNs decreased by 0.21%[84]. Each additional LN detected was related to a 2.7% reduction in the risk of death in patients undergoing surgery alone, a 1.5% reduction in the risk of death in patients with short-range preoperative radiotherapy, and no reduced risk of death in patients with long-range simultaneous preoperative radiotherapy. Data from the publicly available SEER database[85,86] also revealed no significant difference between the two groups in terms of tumor-specific survival rates when the TLN cutoff number was 12, so the criterion of at least 12 LNs may not apply to patients receiving nCRT.

In summary, nCRT can reduce LN retrieval, decrease the N stage, and encourage downstaging of the primary tumor[87] and pN stage migration, leading to staging bias.

This bias could affect the ypN staging system and decrease the accuracy in assessing patient prognosis after nCRT for rectal cancer[88,89]. Therefore, the current ypN staging grouping in TNM staging is probably not applicable to patients receiving nCRT.

DISCUSSION AND OUTLOOK FOR THE FUTURE

The evaluation and grading of LN regression are feasible for rectal cancer patients following nCRT by the histopathological examination of specimens excised after treatment. Thus, the implementation of LRG in histopathology reports for rectal cancer patients undergoing neoadjuvant radiotherapy is strongly recommended. LRG may even have more prognostic value than currently used staging systems (*e.g.*, TNM stage), primarily derived from untreated or unspecified tumor data. Suppose an apparently regressing LN also shows evidence of residual tumor. In that case, that LN is designated as a positive LN (ypN+), despite the good prognostic value for LN regression.

Lee *et al*[34] evaluated postoperative LNs in 389 patients with rectal cancer treated with nCRT followed by radical resection. In the multivariate analysis, LRG-sum was the most related contributor to RFS in LN-related variables alongside ypT staging. In 2020, Cui *et al*[38] In the univariate analysis, the factors that correlated with DFS were LRG-sum, LRG-max, M-NLRG and the LRG ratio.

However, considering a large number of LRG systems, the main focus of international and interdisciplinary committees should be to determine a consensus that can be applied to LRG reports. Critical concerns such as interobserver variability can also be resolved by individual and institutional training. Efforts should be made by both pathologists and clinicians alike to standardize specimen handling and LRG reporting. Although LRG can be used as a morphologic "biomarker," evidence for clinical trials could not be produced from studies with larger cohorts. The primary purpose of clinical trials should never be to compare different LRG systems but rather to scrutinize the histology and identify a standardized reporting method for LRG, which may further enhance the evidence of the value of LRG for the management of nCRT-treated LARC patients.

Recommendations for the standardized macroscopic and histopathological examination of LNs from rectal cancer excision specimens following nCRT are as follows: We prefer a 5-tier grading system and use the Mirbagheri system[28] in our daily work, which is very similar to the 4-tier modified Dworak TRG system[90]. A reproducible and easy-to-apply grading system for predicting clinical outcomes at a systematic level (comparing adequacy of various therapies) and for the individual patient (assessing their response to treatment, guiding further management, insight into prognosis) are useful. We consider this to be a good option. Based on this concept, additional data from evidence-based studies on the prognostic impact of LRG have confirmed that it is a strong prognostic morphological "biomarker" for guiding clinical decisions, modifying postoperative adjuvant therapy, improving operative strategies and monitoring intensities, and providing potential endpoints and alternative markers of prognosis for research programs and patients within clinical trials, which have yet to be presented.

Moreover, in addition to traditional radiotherapy, chemotherapy and surgery, some new oncological treatment methods have emerged recently, such as *Her-2, MSI,* and *BRAF* targeting for rectal cancer or the recently introduced immune checkpoint inhibitors[91]. Although immunotherapy has made considerable advances for a range of cancers, including non-small-cell lung cancer[92], the advances have not yet been extended to most rectal cancer patients[93]. The majority of rectal cancers are microsatellite stable, where immunotherapies targeting cytotoxic T lymphocyte-associated protein 4, programmed death-1 and programmed death-ligand 1 are currently recommended only for patients with high *MSI-H*[55,94]. Despite this, evidence suggests that it is important for the immune system to combat rectal cancer, as several studies have demonstrated that pretreatment densities of tumor-infiltrating lymphocytes predict better oncologic outcomes[95-97]. Furthermore, increasing numbers of preclinical models demonstrate that current chemotherapy and radiotherapy protocols can activate and synergize the immune system using immunotherapy[98-100]. Nevertheless, there is poor knowledge of the tissue alterations resulting from such

emerging therapeutic strategies. Careful histopathological examination of posttreatment tissues and LNs could offer significant insight into the impact of these new agents and resistance mechanisms. Such research is expected to clarify the value of both TRG and LRG and additional detailed histological discoveries equivalent to those reported in the research originally used to introduce TRG into pathology.

CONCLUSION

In summary, LRG should be recognized as an indicator of the response to nCRT and considered a determinant of prognosis for rectal cancer patients and should be included in pathology reports. With further and more extensive evidence-based validation, LRG may become a strong prognostic morphological "biomarker" that can be used to guide clinical decisions, modify postoperative adjuvant therapy, and improve operative strategies and monitoring radiation intensities, as well as provide potential endpoints and alternative markers of prognosis for research programs and patients in clinical trials.

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