Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Lymph node regression grading of locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy" (ID: 76232). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer's comments are as flowing:

Responds to the reviewer's comments:

Reviewer #1:

1. Response to comment: (Why the primary tumor is completely dissolved, but the LN is partially regressed)

Response: Through the suggestions of the reviewer we have explained here. Line 460-470, currently, no single histopathological feature of colorectal cancer can reliably predict lymph node metastasis. Some studies have demonstrated that different responses may exist between primary tumors and mesenteric lymph nodes of the rectum. Despite complete tumor regression, lymph node involvement may still occur. This was found in up to 17% of cases in some studies, especially when a watch-and-wait strategy was chosen after nCRT, likely leading to recurrence and treatment failure. Therefore, the pathologic evaluation of lymph nodes in patients treated with surgery after nCRT could help to accurately

determine the clinical staging of tumors and the response of metastatic lymph nodes to nCRT.

2. Response to comment: (Does normal LN develop fibrosis after treatment?)

Response: We have re-written this part according to the Reviewer’s suggestion. Line 170-178, after neoadjuvant chemoradiotherapy, fibrosis in the metastatic lymph nodes is not as pronounced as in the primary tumor. Normal lymphocytes still occupied most lymph nodes, 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 lymph nodes from completely regressed lymph nodes, especially when only a small number of metastatic tumor cells were present.

3. Response to comment: (Any molecule or factor that can predict the regression of LN after treatment)

Response: Microscopic analysis of metastatic disease was performed on all dissected LNs. Line 153-155, several 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. Special thanks to you for your good comments.

Reviewer #2:

1. Response to comment: (Literature extraction process)

Response: We have made correction according to the Reviewer’s comments. The

main purpose of the present review is to identify the latest studies relating to LRG after neoadjuvant radiotherapy in patients with locally advanced rectal cancer 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 (PRISMA) is shown in Figure 1.

2. Response to comment: (Image copyright)

Response: We apologize for the negligence to mark the copyright. All images are now marked with the original icon. Figure3 of the manuscript was hand-drawn by first author (Lei He), and the rest of the pathology images were extracted by pathologist (Ping Zheng), we did not and will not use other authors' images without permission. Special thanks to you for your good comments.

Responds to the editor’ s comments:

Science editor:

1. Response to comment: (Literature extraction process and flow chart)

We have made correction according to the Reviewer’ s comments. The main purpose of the present review is to identify the latest studies relating to LRG after neoadjuvant radiotherapy in patients with locally advanced rectal cancer 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 (PRISMA) is shown in Figure 1. Special thanks to you for your good comments.

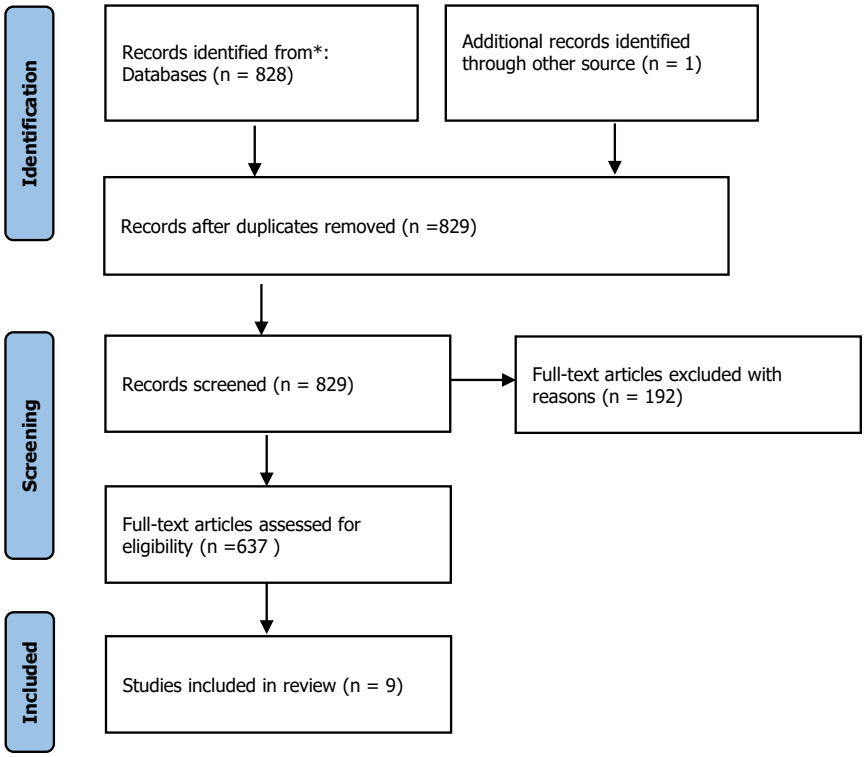

Figure 1 PRISMA 2020 flow diagram.

2. Response to comment: (Add more references)

We thank you for reminding us this important point. We have already added these related references in the manuscript as Reference [3]-[5], [9]-[12], [15]-[16], [36]-[37], [44]-[46], [48]-[50], [52], [54-60], [62]-[65], [69]-[79]. Special thanks to you for your good comments.

Other changes:

1. Line 517-537, the statements of "PLN=0 (ypN0); PLN=1-3 (ypN1); and PLN⩾4 (ypN2). This ypN staging system focuses on the numbers of metastatic LNs only regardless of the tumor load in LNs following nCRT. The relevant literature suggests that lymph node regression should also be considered when assessing lymph node status. The main reasons for this may be twofold: first, the current ypN staging ignores the influence of lymph node treatment

response on prognosis. A similar number of lymph node-positive patients might have a different number of lymph node metastases and a different metastatic load before treatment. The degrees of lymph node 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 lymph nodes and the total number of positive lymph nodes following nCRT can result in a bias in ypN staging based on using the number of positive lymph nodes as grouping criteria. [53 – 56]" were corrected as "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. 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 lymph nodes may indicate immune competence in individual patients instead of an improved means of detecting metastatic nodes. A large population study in the United States showed that less than 50% of patients achieved the recommended number of lymph nodes. 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. Moreover, lymph nodes found in rectal specimens were smaller

in number and size than those found in colonic specimens. Second, lymph nodes detected after nCRT was significantly decreased. Due to the increasing use of preoperative treatment of rectal cancer, pathology reports demonstrating low counts of lymph nodes are increasingly being received by colorectal surgeons."

2. Line 1220-1222, "PRISMA 2020 Checklist statement: The authors have read the PRISMA 2020 Checklist, and the manuscript was prepared and revised according to the PRISMA 2020 Checklist." was added.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions。

Kind regards.

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