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ORIGINAL ARTICLE

# To what extent should the intestinal be resected proximally after radiotherapy: hint from a pathological view

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## Abstract

**Background:** Neoadjuvant chemoradiotherapy (nCRT) is associated with post-operative anastomotic complications in rectal-cancer patients. Anastomosis involving at least one non-irradiated margin reportedly significantly reduces the risk of post-operative anastomotic complications in radiation enteritis. However, the exact scope of radiotherapy on the remaining sigmoid colon remains unknown.
**Methods:** We evaluated the radiation damage of proximally resected colorectal segments in 44 patients with rectal cancer, who received nCRT followed by conventional resection (nCRT-C, $n = 21$) or proximally extended resection (nCRT-E, $n = 23$). The segments from another 13 patients undergoing neoadjuvant chemotherapy (nCT) were used as control. We dissected these samples at a distance of 2 cm between the two adjacent sections. Radiation damage in proximally resected colorectal segments was evaluated using the radiation injury score (RIS) and the concentration and distribution patterns of angiostatin.
**Results:** Compared to those in the nCT group, the nCRT group showed higher RIS, levels of angiostatin, and proportion of diffuse pattern of angiostatin. With increasing distance from the tumor site, these parameters all gradually decreased; and the differences came to be not significant at the site that is over 20 cm from the tumor. The nCRT-E group showed lower RIS (median: 2 vs 4, $P = 0.002$) and a greater proportion of non-diffuse angiostatin (87% vs 55%, $P = 0.039$) at the proximal margins compared with the nCRT-C group.

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**Conclusions:** The severity of the radiation damage of the proximal colon is inversely proportional to the proximal-resection margin length. Little damage was left on the proximal margin that was over 20 cm from the tumor. Removal of an initial length of ≥20 cm from the tumor may be beneficial for rectal-cancer patients after nCRT.

**Key words:** radiation injury; proximally extended resection; angiostatin; rectal cancer; neoadjuvant chemoradiotherapy

## Introduction

Neoadjuvant chemoradiotherapy (nCRT) followed by surgery is a standard treatment approach for locally advanced rectal cancer. Radiation has positive effects in treating mid-low rectal cancer, including tumor downstaging and a reduced risk of local recurrence, thus improving sphincter-preserving surgery [1]. Recent advances in radiation techniques have improved the targeting of the delivery of external beam radiation to tumors; however, collateral damage to the adjacent healthy colon and tissue can occur [2]. Our previous study and other studies suggested that pre-operative radiation increases anastomotic leakage [3–5].

Gelland *et al.* [6] found that, compared with anastomosis with both ends irradiated, using at least one healthy side reduced the anastomotic leakage rate from 50% to 7%. Consensus has been made on the surgical principle of radiation enteritis that, for safe anastomosis, at least one end should be non-irradiated. According to the radiotherapy plan for mid-low rectal cancer, the distal rectum was exposed to radiation and thus inevitably injured [2]. However, few studies have investigated radiation injury of the remaining sigmoid colon, which we conventionally use for proximal anastomosis in East Asia.

In China, surgeons conventionally use part of the rectosigmoid or sigmoid colon within 10 cm from the tumor edge as the proximal margin. In 2016, we started a prospective randomized clinical trial (RCT number NCT02649647). This trial aims to compare the clinical outcome between two resection approaches: the proximally extended resection and the conventional resection. The extended resection involves the proximal margin of the descending colon or sigmoid colon junction, located at an equivalent level to the common iliac bifurcation/fifth lumbar vertebra (L5), which we called the Tianhe procedure [7]. We considered this region to be free of irradiation because of its fixed location outside of the clinical target volume in anorectal cancer. Tissues from this clinical trial are ideal for determining the effect of pre-operative radiotherapy on the remaining sigmoid colon. Unexpected pelvic activity often varies, which makes it difficult to macroscopically recognize the specific position of non-irradiated sites. Whether the length of the resected sections produced by the new surgical procedure is sufficient for rectal-cancer patients after nCRT remains unknown.

In this study, using parameters including radiation injury score (RIS), angiostatin levels, and diffusion patterns, we aimed to microscopically investigate radiation injury of the remaining sigmoid colon and determine non-irradiated sites on the proximal colon.

## Patients and methods

### Patients and sample collection

This study was approved by the Institutional Review Board of the Sixth Affiliated Hospital, Sun Yat-sen University. All samples were collected with written informed consent from the patients and approval from the Institutional Review Board. Forty-four rectal-cancer patients who underwent sphincter-preserving surgery after neoadjuvant chemoradiotherapy (nCRT) were recruited between February 2016 and June 2018, according to the still ongoing RCT study (clinical trial number NCT02649647). Among these patients, 21 patients underwent conventional resection (nCRT-C) and the other 23 patients underwent proximally extended resections (nCRT-E). The extents of resection of these two procedures are shown in Figure 1A. Thirteen rectal-cancer patients who underwent neoadjuvant chemotherapy (nCT) during the same period were chosen as controls.

Fresh specimens obtained from the rectal-cancer patients were labeled and measured after excision. Frozen samples for protein extraction were dissected at a distance of 2 cm between two adjacent sections. The distal edge (labeled 'DE') was defined as a region 2 cm from the inferior border of the tumor, while the proximal edge (labeled 'PE') was defined as the surgical margin on the proximal colon. Sections labeled P1 were obtained with a distance of 2 cm from the superior border of the tumor, leaving 2 cm between sites P1 and P2, and so on (Figure 1B). Corresponding formalin-fixed samples were used for conventional paraffin embedding and sectioning.

Because fresh tissues shrink to some extent after being completely excised, we also calculated the fresh sample-shrinkage rate (Figure 1C) before formalin fixation for clinical-application purposes. Briefly, the length was measured at two time points: initially during surgery (recorded as the 'initial length'), with the cutting edges marked when the surgeon began separating the rectum, and then again after the rectum was completely resected (recorded as the 'excisional length'). Notably, due to tissue shrinkage after excision, and lengths of sigmoid colon among patients possibly varying to some extent, specific sections demonstrated in Figure 1B might be missing, resulting in non-matched numbers of DE/PE and P1–P6.

### Standard histopathological assessment

Sections with a thickness of 4 μm were sliced from formalin-fixed paraffin-embedded samples and placed on slides for hematoxylin and eosin (HE) and Masson's trichrome stains according to standard protocols. Histopathological assessment of each section was performed by calculating the RIS based on our previous study [8]. Since all patients enrolled in this study had undergone surgery at approximately 6–8 weeks after irradiation, the pathological changes corresponded to the acute or subacute phase and the basic parameters of the RIS included the following: mucosal ulcerations, mucosal telangiectasia, inflammation, epithelial atypia, submucosal fibrosis, edema, and vascular sclerosis. All specimens were subjected to blinded evaluation and values were assigned to each morphological feature and the overall RIS.

### Assessment of angiostatin levels and diffusion patterns

Angiostatin levels and specific diffusion patterns of angiostatin have been shown to be potentially related to the severity of

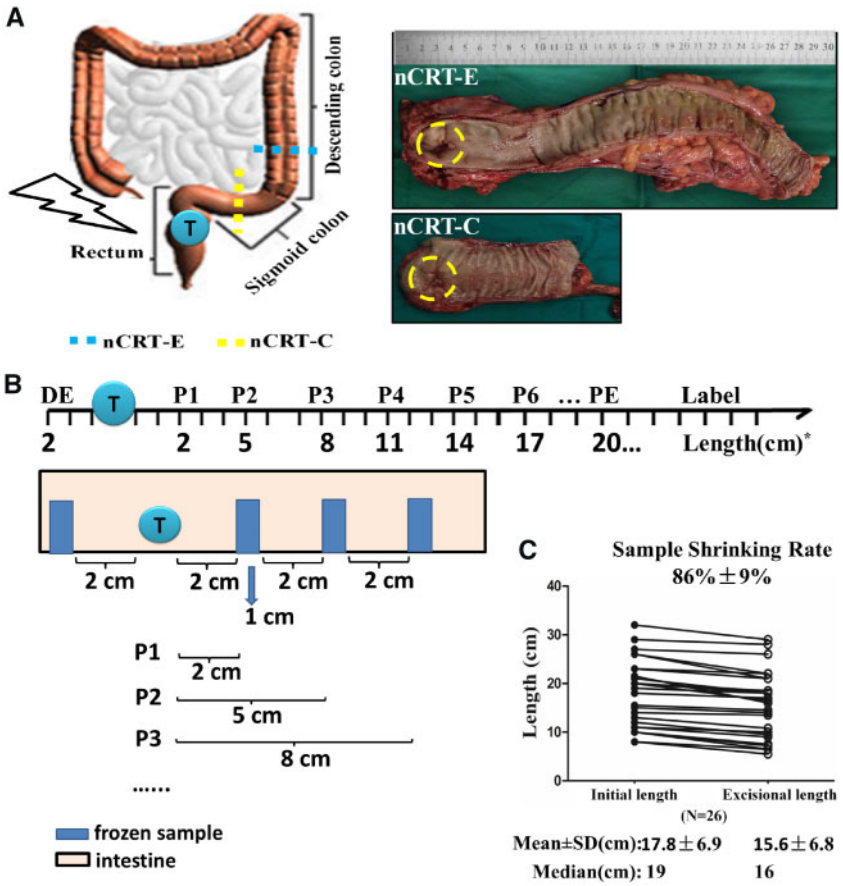


**Figure 1.** Surgical procedures and methods for sample measurement. (**A**) Conventional resection (nCRT-C) involves an excision of at least 10 cm of the intestine proximal to the tumor, with rectum–sigmoid colon anastomosis (yellow line, left). For proximally extended resection (nCRT-E), a modified technique with excision of the entire sigmoid colon and rectum-descending colon anastomosis was performed (blue line, left). The gross intestine in the two surgical procedures is shown (yellow circle indicates the tumor region, right). (B) The ruler shows notations (upper row) and the corresponding length (*excisional length, measured after fresh specimens were excised, bottom row). 'DE' denotes the distal surgical edge and 'PE' denotes the proximal surgical edge. The method for obtaining and measuring the fresh surgical segments is shown below the ruler. The sections were dissected at a distance of 2 cm between two adjacent sections. (C) Sample lengths were measured at two time points: one during surgery (initial length) and the other after the specimen was completely resected (excisional length). The scatter plot shows the change in the length of each sample; the mean shrinkage rate was 86% ± 9%.

radiation injury. Therefore, these two parameters were evaluated to distinguish radiation injury in tissues in this study. All experiments were performed according to the manufacturer's standard protocols, which we have previously described [8]. Briefly, anti-human angiostatin K1-3 polyclonal primary antibody (R30129, NSJ Bioreagents, San Diego, CA, USA) was used to evaluate the distributed patterns of angiostatin. An enzyme-linked immunosorbent assay (ELISA) kit (ELH-Angiostatin-1, RayBiotech, CA, USA) and Western blotting (Angiostatin-1, RayBiotech, CA, USA) were used to confirm the level and specificity of angiostatin in protein lysates extracted from fresh-frozen samples. For the ELISA assay, the level of angiostatin was calculated by plotting the measured optical density of the standard curve. For Western blotting, an anti-GAPDH antibody (60004–1-IG, Proteintech Group, USA) was used to confirm equal loading of the samples.

### Post-operative anastomotic complications

Post-operative anastomotic complications included anastomotic leakage and anastomotic stenosis. With reference to the proposal of the International Study Group of Rectal Cancer, the anastomotic leakage was evaluated by Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) scan [9], while anastomotic stenosis was diagnosed by colonoscopy and recorded during the follow-up period [5].

### Statistical analysis

All data were analysed by IBM SPSS Statistics 20.0 and GraphPad Prism 5.0. Differences between the groups were analysed using the Chi-square test or Fisher exact test for categorical or dichotomous outcomes. The median value of seven basic parameter scores and the overall RIS between the two surgical groups were compared using the Mann–Whitney *U* test. Statistical significance was defined by two-tailed *p*-values less than 0.05.

## Results

### Clinical data and margin characterization

Among the nCRT-E, nCRT-C, and nCT groups, no significant differences were observed in sex, age, initial tumor stage, tumor distance from the anal verge, length of distal resection margins, or number of metastatic nodes. In addition, the chemotherapy regimen and surgery interval time were comparable between

**Table 1.** Patients' clinical data and margin characterization

| Variable | nCRT-E ($n=23$) | nCRT-C ($n=21$) | nCT ($n=13$) |
|---|---|---|---|
| Gender | | | |
| Female | 5 (22) | 4 (19) | 1 (8) |
| Male | 18 (78) | 17 (81) | 12 (92) |
| Age at surgery, year | 53 (29–68) | 54 (32–71) | 58 (25–70) |
| Initial TNM stage | | | |
| II | 4 (17) | 2 (10) | 1 (8) |
| III | 19 (83) | 19 (90) | 12 (92) |
| Tumor distance from anal verge[c] | 5.4 (3.0–13.0) | 5.4 (2.9–8.0) | 6.3 (3.5–13.0) |
| Chemotherapy regimen | | | |
| Xeloda | 1(4) | 1 (5) | 0 (0) |
| Capecatadine | 1 (4) | 2 (10) | 0 (0) |
| FOLFOX | 21 (91) | 18 (86) | 9 (69) |
| FOLXIRI | 0 (0) | 0 (0) | 4 (31) |
| Radiotherapy dose | | | |
| 50 Gy | 21 (91) | 20 (95) | – |
| < 50 Gy | 2 (9) | 1 (5) | – |
| Interval to surgery, weeks | 7 (6–12)[a] | 8 (6–15)[a] | 3 (2-6) |
| Proximal-resection margin[d], cm | 18 (10–29)[ab] | 8 (5–16)[a] | 7 (1-18) |
| Length of the specimen[d], cm | 22 (14–35)[ab] | 14 (8–21)[a] | 11 (8-33) |
| Distal resection margin[d], cm | 2.0 (1.0–5.0) | 2.0 (0–4.0) | 1.5 (1.0–6.5) |
| Number of harvested lymph nodes | 9 (1–29) | 12 (4–30) | 15 (3–23) |
| Number of metastatic nodes | | | |
| 0 | 18 (78) | 17 (81) | 7 (54) |
| 1 | 3 (13) | 1 (5) | 2 (15) |
| 2–3 | 2 (9) | 2 (10) | 2 (15) |
| ≥4 | 0 (0) | 1 (5) | 2 (15) |

Values were presented as median (range) or *n* (%).
[a]nCRT-E or nCRT-C vs nCT-C, $P<0.05$.
[b]nCRT-E vs nCRT-C, $P<0.05$.
[c]Data obtained from CT or MRI.
[d]Excisional length (without formalin fixation).

the nCRT-E and nCRT-C groups. However, surgical-sample length and proximal-resection margin in the nCRT-E group were significantly greater than those in the other two groups (both $P<0.001$; Table 1).

## Status of the proximal segments after nCRT evaluated by RIS

Pathological examination of proximally resected segments showed low RIS and basic parameters for the entire proximal colorectal segment in the nCT group, while the nCRT group showed higher RIS at the near distance from the tumor region. With increasing distance from the tumor site, the RIS gradually decreased (Figure 2A). Representative images of the different segments between the two groups are shown in Figure 2B. We also compared the RIS of each proximal segment in the nCRT group to the average RIS of all segments in the nCT group. The results showed that proximal segment P1 through P5 of nCRT group displayed significantly higher RIS values than those in the nCT group (all $P<0.05$; Figure 2C). In the nCRT group, more than 50% of the P6 and P5 segments showed a RIS $\leq 2$ (Figure 2D).

## Angiostatin levels and distribution mode in the proximal segments after nCRT

Consistently with the results of the evaluation of RIS, the entire proximal colorectal segments in the nCT group maintained a steady low level of angiostatin, while the nCRT group showed clear up-regulation of angiostatin levels and a gradual decrease along the proximal colon segments (Figure 3A). Furthermore, the levels of angiostatin in segments P1 through P4 in the nCRT group were much higher than the average levels of angiostatin in the nCT group (all $P<0.005$; Figure 3B). The trend of angiostatin levels along the proximal colon was also confirmed by Western blotting (Figure 3C). In terms of the distribution patterns of angiostatin, the nCT group predominantly displayed a non-diffuse pattern of angiostatin in the entire proximal colorectal segment, while the nCRT group displayed a higher proportion of the diffuse pattern at site P1 (approximately 60%; representative images are showed in Figure 3D), which gradually decreased with distance from the tumor region. The proportion of the diffuse pattern was less than 20% at site P6 (Figure 3E).

## Comparison of radiation injury of surgical margins between the nCRT-C and nCRT-E groups

The comparison of radiation injury of surgical margins showed that the RIS of the proximal margins was markedly higher in the nCRT-C group than in the nCRT-E group ($P=0.002$; Table 2). Supplementary Figure 1 shows representative images of surgical margins in the nCRT-C group and in the nCRT-E group. Among the seven parameters used to assess radiation injury, the extent of epithelial atypia, mucosal ulceration, mucosal telangiectasias, and the grade of vascular sclerosis in the proximal margins were lower in the nCRT-E group than in the nCRT-C group. Additionally, a higher proportion of the non-diffuse pattern of angiostatin was observed at the proximal margins in the nCRT-E group compared with the nCRT-C group (83% vs 55%, $P=0.039$; Figure 3F).

In the nCRT-E group, the proximally extended resection margins were all located at an equivalent level to the common iliac bifurcation/L5 anatomically, but the length of the proximal margins varied individually and hence might not always reached the length of P6 (17 cm), which is about 20 cm before resection if considering the shrinkage rate of ~90% (Figure 1C). Therefore, we separated the nCRT-E group into two subgroups of patients: one subgroup had a proximal surgical margin located at site P6 (PE = P6 group) and the other subgroup had P6 segments not located at surgical margins (P6 group). No significant differences were observed in RIS and the concentration and distribution patterns of angiostatin between the two subgroups (all $P>0.05$; Table 3). This result suggests that the proximally extended resection should have resected most of the radio-injured margins, if not completely, no matter whether the proximal margins reach P6.

## Comparison of incidence of anastomotic leakage between the nCRT-C and nCRT-E groups

Follow-up data from the 44 registered patients in the PERN clinical trial showed that the primary anastomotic complication within 30 days after surgery was Anastomotic leakage (AL), but no anastomotic stenosis was observed. A lower incidence of AL was observed in the nCRT-E group compared to the nCRT-C group, but the difference did not reach statistical significance (13.0% vs 23.8%, $P=0.448$).

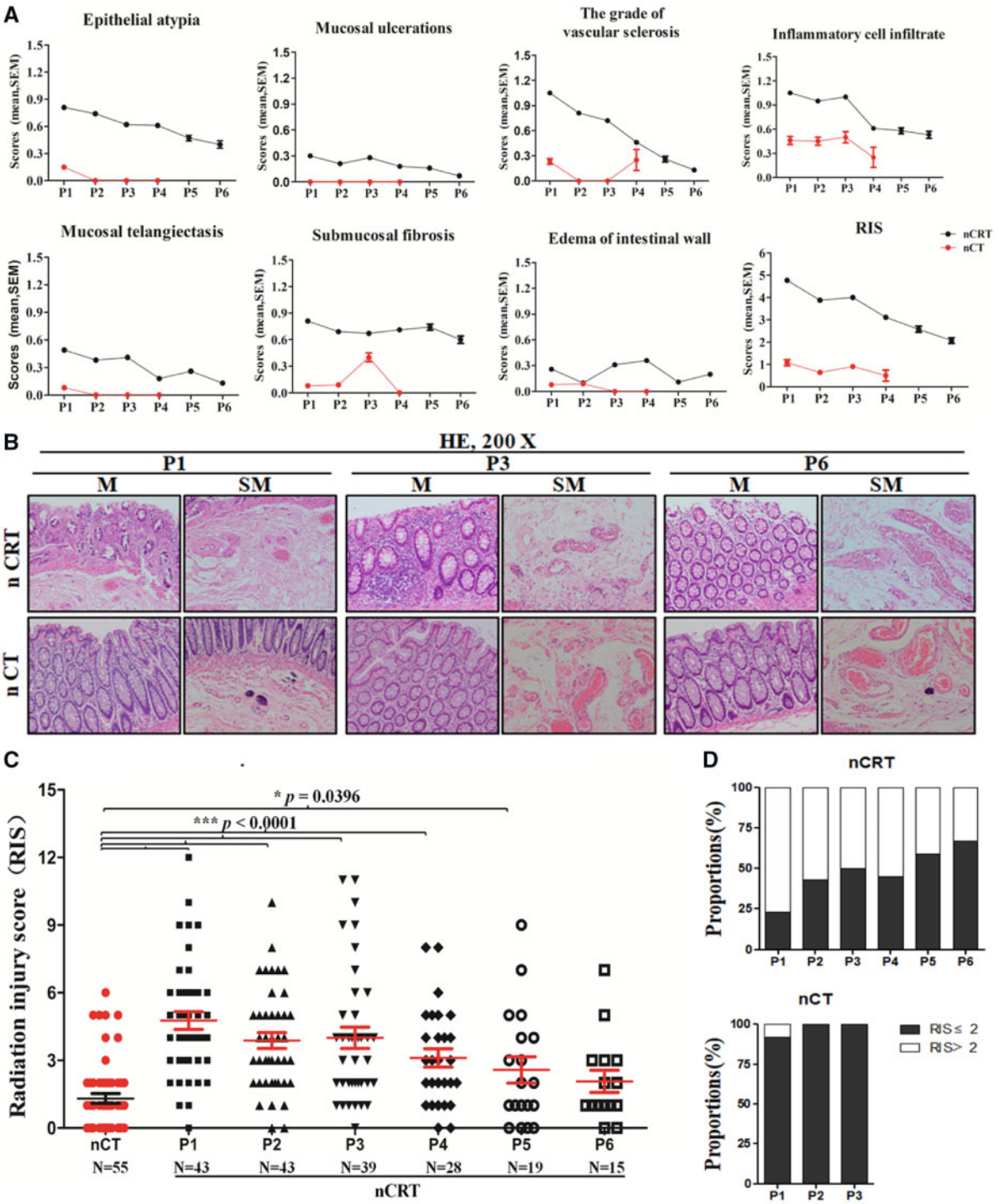


**Figure 2.** Histopathological examination of proximally resected segments. (A) The curves show the gradually decrease in the radiation injury score (RIS) in the proximal segments in the nCRT group, with only a low level of RIS change found in the nCT group. (B) Representative histopathological features of proximal segments in the nCRT and nCT groups (HE stain, 200× magnification). 'M' indicates the mucosal layer and 'SM' indicates the submucosal layer. Obvious histopathological changes were observed in the nCRT group (on the top panel): disorganized structure of the mucosal layer with epithelial atypia, vascular sclerosis, and submucosal fibrosis appearing on the P1 segment. Mucosal telangiectasia, inflammatory cell infiltration, and changes in gland structure and quantity were observed on section P3. Subsequently, the morphological variations of the segments became normal on section P6, as assessed by microscopy. However, no significant morphological changes in the proximal segments were observed in the nCT group. (C) Comparison of the RIS of each proximal colon segment in the nCRT group to the average RIS of all segments in the nCT group. The red scatter plot represents the average RIS of all segments in the nCT group. The RIS scores at proximal segments P1 through P5 of the nCRT group were significantly higher than those in the nCT group (all $P < 0.05$). However, no significant alterations were observed at segment P6 compared with the nCT group ($P = 0.075$). (D) The proportion of low RIS (RIS $\leq 2$) gradually increased along the proximal segments in the nCRT group, with a proportion of 67% (10/15) at P6 segments.

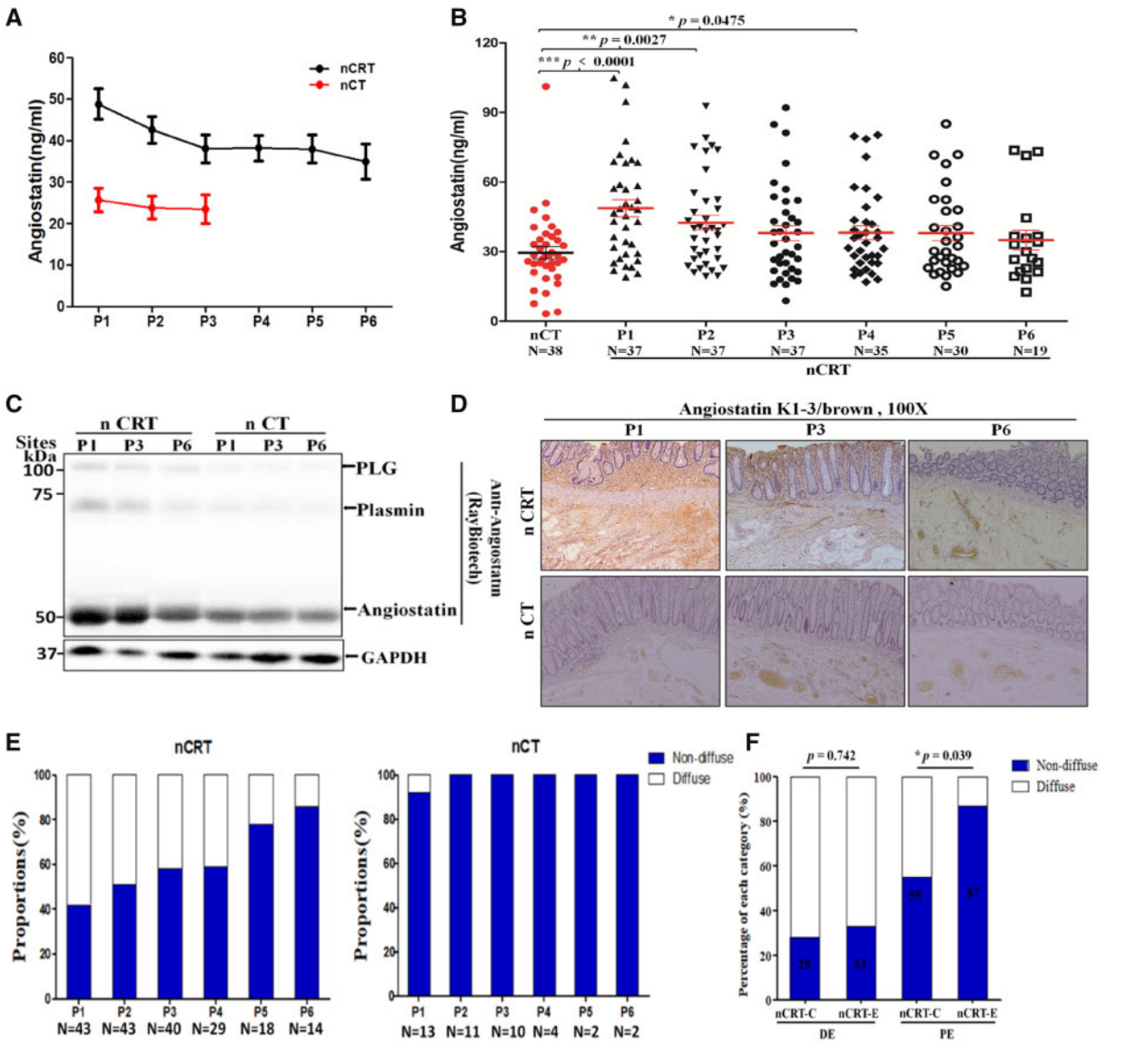


**Figure 3.** Assessment of angiostatin levels along proximal colon segments. (A) The angiostatin level remained steady throughout the entire proximal segments in the nCT group, while up-regulated angiostatin levels were present in all segments in the nCRT group, with a gradual decrease along the proximal segments. (B) The levels of angiostatin in the P1 through P4 segments in the nCRT group were much higher than the average level of angiostatin in the nCT group (all $P < 0.05$). No significance was observed at site P5 compared with the nCT group ($P = 0.102$). (C) Confirmation of angiostatin levels in different proximal colon segments by Western blotting (antibody from RayBiotech). (D) Representative figures showing the distribution patterns of angiostatin in different proximal segments in the two groups. In the nCRT group, strong angiostatin staining and dispersion in both the submucosal and mucosal layers were observed in P1 segments, with slightly weaker staining but no diffusion patterns found in P3 segments (100×). Compared with the P6 segments in the nCRT group, the entire proximal segment in the nCT group was mainly positive for angiostatin staining inside the vessels of the submucosa. (E) The proportion of diffusion patterns of angiostatin gradually decreased in proximal segments in the nCRT group. A diffusion pattern of angiostatin was observed only in 8% (1/13) of P1 segments in the nCT group, while the other proximal segments showed a non-diffuse pattern (normal pattern). (F) A significantly higher proportion of the non-diffuse pattern of angiostatin was observed at the proximal margins in the n-CRT-E group compared with the nCRT-C group (55% vs 87%, $P = 0.039$), whereas no significant difference was observed at the distal margins between the two groups (33% vs 27%, $P = 0.742$).

## Discussion

In the present study, by analysing surgically resected colon samples, we confirmed that pathological changes can be quantified. RIS was less than 2 points in this particular margin and angiostatin predominantly accumulated inside the vessel lumen of the submucosa, indicating relatively normal tissue from a pathological perspective. Extended resection of the proximal margin with an initial length of approximately 20 cm from the tumor site (P6) appears to be sufficient for rectal-cancer patients after nCRT.

nCRT has become a general recommendation for patients with locally advanced rectal cancer and is associated with decreased rates of local recurrence of rectal cancer [1, 10, 11]. Despite substantial improvements in radiotherapy technology, various complications related to radiation damage in adjacent tissues remain a concern. A number of reports have emphasized the significantly high incidence of post-operative anastomotic leakage and stenosis after nCRT [3, 4, 12]. Absolute requisites for secure anastomosis include clear margin, good vascularity, absence of tension, and healthy bowel [13]. This study aimed to differentiate healthy bowel for anastomosis

**Table 2.** Radiation injury scoring of surgical margins (individual items)

| Morphologic feature | Distal edge | | | Proximal edge | | |
|---|---|---|---|---|---|---|
| | nCRT-E ($n = 20$) | nCRT-C ($n = 18$) | *P*-value | nCRT-E ($n = 23$) | nCRT-C ($n = 20$) | *P*-value |
| Epithelial atypia | 1 (1–2) | 1 (0–2) | 0.768 | 0 (0–2) | 1 (0–2) | 0.005 |
| Mucosal ulcerations | 1 (0–2) | 1 (0–2) | 0.614 | 0 (0–1) | 1 (0–2) | 0.016 |
| Mucosal telangiectasia | 1 (0–2) | 2 (0–3) | 0.158 | 0 (0–1) | 0 (0–2) | 0.014 |
| Inflammatory cell infiltrate | 1 (0–2) | 1 (1–2) | 0.506 | 0 (0–2) | 1 (0–2) | 0.032 |
| Edema of intestinal wall | 0 (0–1) | 0 (0–1) | 0.605 | 0 (0–1) | 0 (0–1) | 0.816 |
| Submucosal fibrosis | 1 (0–2) | 1 (0–2) | 0.767 | 1 (0–2) | 1 (0–2) | 0.179 |
| Vascular sclerosis | 1 (0–3) | 2 (0–3) | 0.234 | 0 (0–2) | 1 (0–3) | 0.002 |
| Total score | 6 (3–11) | 9 (3–12) | 0.471 | 2 (0–7) | 4 (0–11) | 0.002 |

Values are presented as median (range) and compared by using the Mann–Whitney *U* test.

**Table 3.** Subgroup comparison of radiation injury at P6 segments

| Parameter | P6 = PE ($n = 8$) | P6 ($n = 7$) | *P*-value |
|---|---|---|---|
| Radiation injury score, median (range) | 1.5 (0–7) | 1 (0–5) | 0.367 |
| Angiostatin level, ng/mL, median (range) | 32.1 (21.2–73.7) | 33.0 (12.6–44.6) | 0.487 |
| Angiostatin pattern (Non-diffuse), *n* (%) | 5 (62.5) | 7 (100) | 0.999 |

away from irradiation injury. To determine the scope of the effects of pre-operative radiotherapy on the remaining proximal colon, we adopted the previously described RIS system to analyse radiation damage. The RIS system is a popular method for evaluating radiation injury in general [14–17]. We also used in parallel the expression level and distribution pattern of angiostatin as two parameters to evaluate the severity of radiation-induced damage [8]. Interestingly, we found that the changes in angiostatin were consistent with the changes in RIS. The irradiated area had a high RIS and high angiostatin level, which gradually decreased with distance from the irradiated tumor region. This finding can greatly aid the assessment of radiation injury by adding more relevant parameters that are potentially robust and reliable.

According to the clinical target volumes for radiotherapy in rectal cancer, the distal rectum is in a fixed position and inevitably injured by pre-operative radiotherapy [2]. Based on these parameters, we found that the RIS, angiostatin levels, and angiostatin diffusion patterns were markedly higher in the distal margins than in the proximal margins. In the segments affected by radiation, we observed disorganization of glands, mucosal ulceration, and an increased proportion of telangiectasia in the mucosal layer, while fibrotic vascular sclerosis appeared in the submucosal layer. Vascular stenosis is correlated with the hemodynamic effect, whereas high levels of angiostatin inhibit vessel formation and delay wound healing [18–20]. Our findings indicated insufficient blood supply and inappropriate bowel healing in the irradiated region. These features are consistent with numerous previous reports [14, 16, 21, 22].

For mid-low rectal-cancer surgery, the length of the distal margin is limited when considering sphincter preservation. However, the optimal length of the proximal margin remains controversial. In Western countries, many surgeons prefer the descending colon for anastomosis [10]; by contrast, many Chinese surgeons choose the sigmoid colon for anastomosis. Reasons for the use of the sigmoid colon include the following. (i) A 10-cm margin of the proximal edge of the tumor is considered adequate in terms of oncological safety. (ii) The blood supply to the sigmoid can be maintained after anastomosis. Komen *et al.* [23] compared colonic perfusion after 'high tie' to colonic perfusion after 'low tie' in patients undergoing rectal resection. They found that perfusion was better in the 'low-tie' group. (iii) Leupin *et al.* [24] examined the surgical resection specimens of patients with rectal cancer receiving short-term or long-term pre-operative irradiation therapy. The specimens were taken at least 10 cm (proximal side) from the tumor. They found that the pathological features of acute radiation colitis were absent or detected only occasionally in the long-term group. However, their findings were based only on a descriptive study and the lengths of the specimens were inconsistent. The present study and our previous study [25] suggest that obvious radiation-induced injuries exist in the conventional proximal surgical margins after nCRT. The present study also demonstrated that nCRT induces radiation injury on the proximal sigmoid colon in a gradient fashion; the severity of radiation damage is inversely proportional to the length of the proximal-resection margin.

Tumor regions receive a relatively high dose during radiotherapy. The longer the distance from the tumor, the more the dose falls off. Saunders *et al.* [26] found that Orientals have a lower incidence of sigmoid colon adhesions and decreased colonic mobility than Westerners. These findings might explain the observation that pre-operative radiation induces radiation injury on the proximal colon in a gradient fashion. Therefore, removal of the sigmoid colon with possible radiation damage at an initial length of approximately 20 cm from the tumor site is encouraged for rectal-cancer patients after nCRT. The American Society of Colon and Rectal Surgeons also recommends choosing the descending colon for anastomosis rather than the sigmoid colon, especially in a radiated pelvis [27].

Preliminary follow-up data showed that extended resection can potentially reduce the risk of anastomotic complications, although not statistically significantly. This result is similar to that of our previous retrospective study based on another clinical trial [28]. Thus, the long-term morbidity of complications

and the beneficial merits of extended resection should only be revealed when the clinical trial is enrolling more participants or even finished (NCT02649647).

In conclusion, our study provides new evidence that nCRT induces radiation injury on the proximal sigmoid colon in a gradient fashion; the severity of radiation damage is inversely proportional to the length of the proximal-resection margin and thus proximal removal of an initial length of approximately 20 cm from the tumor site is encouraged for rectal-cancer patients after nCRT.

## Supplementary data

Supplementary data is available at *Gastroenterology Report* online.

## Authors' contributions

D.C.C. and L.W. designed and conceived the study; P.H.W., Q.H.Z., T.H.M., X.Y.H., and H.M.W. performed the experiments and acquisition of data; P.H.W. and Q.H.Z. performed analysis and interpretation of data; Q.Y.Q., X.Y.H., and Y.Y.K. recruit patients and follow up on the ongoing clinical trial; P.H.W. and D.C.C. drafted the manuscript; L.W., D.C.C., Q.H.Zh., Q.Y.Q., Z.X.Y., and X.Y.H. revised the manuscript; all authors approved the final version of the manuscript.

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## Conflicts of interest

All authors declared no conflicts of interest.

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