Dear Editor and Reviewers,

Thank you for your comments and suggestions concerning our manuscript entitled "Feasibility of real-time in vivo distal resection margin selection guided by probe-based confocal laser endomicroscopy in transanal total mesorectal excision for low rectal cancer" (Manuscript NO.: 78818, Prospective Study). We have revised our manuscript according to the comments and suggestions. All responses are listed point-by-point below.

Reviewer #1: In the manuscript entitled "Feasibility of real-time in vivo distal resection margin selection guided by probe-based confocal laser endomicroscopy in transanal total mesorectal excision for low rectal cancer", the authors evaluated the feasibility of optical biopsy using probe-based confocal laser endomicroscopy to select the DRM during Transanal total mesorectal excision for low rectal cancer. Overall, the manuscript is well written and easy to read and understand.
Answer: Thank you for your comments.

However, the study is suffering without many important variables to support its conclusion.
Q1. The sample size was very small, and there were no proper follow-up details for these patients.
Answer: Thank you for your question. We calculated the appropriate sample size for our study. From January 2019 to June 2019, the average time for intraoperative diagnosis by frozen section was 25 ± 10 min in our hospital. We hypothesized that the average time of intraoperative diagnosis by pCLE would be 20 min, and 43 cases were determined. With this number of cases, the study would have 90% power to detect a difference between the two techniques to prove the superiority of pCLE (two-sided type I error=0.05). Therefore, we performed PCLE in 13 more cases and added these 13 more cases to this study.

Now we have a total of 43 cases. We have added this information to the METHOD section and RESULTS section as below:

METHOD section

Sample size calculation

From January 2019 to June 2019, the average time for intraoperative diagnosis by frozen section was 25 ± 10 min in our hospital. We hypothesized that the average time of intraoperative diagnosis by pCLE would be 20 min, and 43 cases were determined. With this number of cases, the study would have 90% power to detect a difference between the two techniques to prove the superiority of pCLE (two-sided type I error=0.05).

RESULTS section

Patient demographics and tumor characteristics

From January 2019 to January 2021, a total of 43 consecutive patients were enrolled according to the predefined inclusion and exclusion criteria.

Moreover, we updated the follow-up details for all patients, including the rate of anastomotic stenosis, tumor recurrence and metastasis after surgery. The median follow-up time was 24 (range, 22-46) months. We have added this information to the Results section and TABLE 4 as below:

RESULTS section

The median Wexner score was 4 (IQR = 3-6), as evaluated at six months after stoma closure. The median follow-up period was 24 (range, 22-46) months. One patient had anastomotic stenosis. Two patients had liver metastasis at 6 months and 13 months after surgery. One patient died one year after metastasis, and another died 18 months after metastasis. Notably, one patient had cancer recurrence 18 months after surgery.

Table 4: Surgical and functional outcomes

Variable

Operative duration: median (IQR), min	240 (202-265)
pCLE examination duration: median (IQR), min	17 (15-18)
Estimated blood loss: median (IQR), ml	27 (20-50)
DRM distance: median (IQR), mm	7.0 (5.0-10.0)
Anastomotic leakage, n (%)	2 (4.7)
Positive DRM, n (%)	0 (0)
Wexner score*, median (IQR)	5 (3-6)
Anastomotic stenosis, n (%)	1 (2.3)
Recurrence, n (%)	1 (2.3)
Metastasis, n (%)	2 (4.7)

pCLE, probe-based confocal laser endomicroscopy; *DRM,* distal resection margin.

*An incontinence score designed by Wexner et al., determined at 6 months after stoma closure.

Q2. Epithelial features should also be considered during pCLE evaluation of rectal cancer. Using this strategy, the colonic crypt architecture was classified into three types. However, the authors failed to give clear features of both epithelial and vascular structures to the accuracy of pCLE, which is very important to know the accuracy of the resection.

Answer: Thank you for your suggestion. It is very important to know the colonic crypt architecture and vessel architecture classification for pCLE. pCLE optical biopsy diagnostic criteria are defined according to the "Miami criteria" (*Wallace M, Lauwers GY, Chen Y, Dekker E, Fockens P, Sharma P, Meining A (2011) Miami classification for probe-based confocal laser endomicroscopy. Endoscopy 43:882-891*) and Kuiper T et al.'s diagnostic classification (*Kuiper T, van den Broek FJ, van Eeden S, Wallace MB, Buchner AM, Meining A, van Hee K, Fockens P, Dekker E (2011) New classification for probe-based confocal laser endomicroscopy in the colon.*

Endoscopy 43:1076-1081). Briefly, the diagnostic criteria include the crypt architecture and vessel architecture classification. The epithelial architecture was divided into three types. Normal mucosa was scored as crypt type 1 and presented normal regular luminal openings, size, and distribution of crypts covered by a homogeneous layer of epithelial cells, including goblet cells. Hyperplastic polyps and inflammatory lesions were scored as crypt type 2 and presented regular-shaped or star-shaped luminal crypt openings with normal or reduced goblet cells. Neoplastic lesions were scored as crypt Type 3, which included variable width of epithelial lining with tubular-shaped crypts and loss of goblet cells (striped dark epithelium) and irregular and decreased volume of lamina propria. For vessel architecture (*Kuiper T, van den Broek FJ, van Eeden S, Wallace MB, Buchner AM, Meining A, van Hee K, Fockens P, Dekker E (2011) New classification for probe-based confocal laser endomicroscopy in the colon. Endoscopy 43:1076-1081*), normal mucosa was scored as vessel Type 1, presented hexagonal, honeycomb appearance that presents a network of capillaries outlining the luminal openings of the crypts. Hyperplastic polyps and inflammatory lesions were scored as vessel Type 2, presented hexagonal, honeycomb appearance with mild (or no) increase in the number of capillaries or increased amounts of normal vessels without leakage. Neoplasia was scored as vessel type 3, presenting dilated and distorted vessels with elevated leakage and irregular architecture with little or no orientation to adjunct tissue. We analyzed the pCLE imaging features and made relative diagnoses according to the above categories. According to the above categories, we analyzed the tissue features in 86 pCLE videos and made relative diagnoses. The intraoperative real-time pCLE imaging correctly diagnosed 36 tumor lesions and 40 normal lesions in 40 pathological tumor lesions and 46 pathological normal lesions. We have added this information to the METHOD and RESULTS sections.

We added this information to the Methods and Results sections as below:

METHOD section

pCLE optical biopsy diagnostic criteria

The pCLE optical biopsy diagnostic criteria were according to the "Miami criteria"[13] and Kuiper T et al's diagnostic classification[14]. Briefly, the diagnostic criteria include the crypt architecture and vessel architecture classification. The crypt architecture was divided into three types. Normal mucosa was scored as crypt type 1 and presented normal regular luminal openings, size, and distribution of crypts covered by a homogeneous layer of epithelial cells, including goblet cells. Hyperplastic polyps and inflammatory lesions were scored as crypt Type 2 and presented regular-shaped or star-shaped luminal crypt openings with normal or reduced goblet cells. Neoplastic lesions were scored as crypt Type 3, which included variable width of epithelial lining with tubular-shaped crypts and loss of goblet cells (striped dark epithelium) and irregular and decreased volume of lamina propria. For vessel architecture, normal mucosa was scored as vessel Type 1 and presented a hexagonal, honeycomb appearance that presented a network of capillaries outlining the luminal openings of the crypts. Hyperplastic polyps and inflammatory lesions were scored as vessel Type 2, presented hexagonal, honeycomb appearance with mild (or no) increase in the number of capillaries or increased amounts of normal vessels without leakage. Neoplasia was scored as vessel type 3, presenting dilated and distorted vessels with elevated leakage and irregular architecture with little or no orientation to adjunct tissue. We analyzed the pCLE imaging features and made relative diagnoses according to the above categories.

RESULTS section

In total, 43 patients underwent pCLE examination, including 21 patients who underwent neoadjuvant chemoradiotherapy. Representative pCLE images and matched images of hematoxylin and eosin (H&E)-stained rectal tissues are shown in Figure 2. In normal rectal tissues, pCLE images presented normal round crypt structures with regular luminal openings, covered by a homogeneous single-cell-layered epithelium with dark goblet cells, and regular narrow vessels with hexagonal, honeycomb appearance surrounding crypts

(Figure 2 A). In rectal neoplastic tissues, pCLE images presented dark and irregularly thickened epithelium with decreased volume of lamina propria and dilated, distorted vessels with elevated leakage (Figure 2 C). We analyzed the tissue features in 86 pCLE videos and made relative diagnoses. The intraoperative real-time pCLE imaging correctly diagnosed 36 tumor lesions and 40 normal lesions in 40 pathological tumor lesions and 46 pathological normal lesions.

Q3. In methods, the authors need to discuss the preprodeure preparation details of the pCLE procedure, like probe usage.

Answer: Thank you for your suggestion. Before image acquisition, fluorescein sodium was injected intravenously. The fluorescent agent used was 10% fluorescein sodium (Baiyunshan Mingxing Pharmaceutical Company, Guangzhou, China). The fluorescein sodium (0.5 ml) hypersensitivity test was implemented 20 min before pCLE examination. Then, 2.5 ml of fluorescein sodium diluted with 2.5 ml of 0.9% sodium chloride was injected intravenously 5 min prior to pCLE imaging. After strict sterilization, one end of the probe was connected to the laser outlet of Cellvizio, and the other end was placed on the surgical table. Adequate exposure of the tumor lesion was achieved using a colorectal retractor (CooperSurgical Lone Star colorectal retractor, Beijing Xinya S&T Co., Ltd., Beijing, China). pCLE imaging was performed by the surgeon under direct vision by using the probe in direct contact with the tissues.

We have added this information to the Methods section as below:

METHOD section

Before image acquisition, fluorescein sodium was injected intravenously. The fluorescent agent used was 10% fluorescein sodium (Baiyunshan Mingxing Pharmaceutical Company, Guangzhou, China). The fluorescein sodium (0.5 ml) hypersensitivity test was implemented 20 min before pCLE examination. Then, 2.5 ml of fluorescein sodium diluted with 2.5 ml of 0.9% sodium chloride was injected intravenously 5 min prior to pCLE imaging. After strict sterilization,

one end of the probe was connected to the laser outlet of Cellvizio, and the other end was placed on the surgical table. Adequate exposure of the tumor lesion was achieved using a colorectal retractor (CooperSurgical Lone Star colorectal retractor, Beijing Xinya S&T Co., Ltd., Beijing, China), and pCLE imaging was performed by the surgeon under direct vision by using the probe in direct contact with the tissues (Figure 1).

Q4. pCLE-related details are lacking, the authors didn't include the details like what is the resolution, magnification, and at frame in the examination was performed.

Answer: Thank you for your suggestion. In our study, we used the ColoFlex UHD probe, a flexible mini-probe with a lateral resolution of 1 μm. The probe allows optical biopsies with 1000 times magnification. The pCLE imaging data were collected at a scan rate of 12 frames/s. We have added these pCLE-related details as below:

METHOD section

pCLE was performed using the Cellvizio Endomicroscopy System (Mauna Kea Technologies [MKT], Paris, France). The ColoFlex UHD probe, a flexible mini-probe with a lateral resolution of 1 μm, was used in our study. The pCLE imaging data were collected at a scan rate of 12 frames/s. The probe has a field of view of 240 μm and can image at a depth of 60 μm below the mucosal surface, and it allows optical biopsies with 1000 times magnification.

Q5. The pCLE classification system was not discussed, which is most important to assess the accuracy.

Answer: Thank you for your suggestion. The pCLE optical biopsy diagnostic criteria were according to the "Miami criteria" (*Wallace M, Lauwers GY, Chen Y, Dekker E, Fockens P, Sharma P, Meining A (2011) Miami classification for probe-based confocal laser endomicroscopy. Endoscopy 43:882-891*) and Kuiper T et al.'s diagnostic classification (*Kuiper T, van den Broek FJ, van Eeden S, Wallace MB,*

Buchner AM, Meining A, van Hee K, Fockens P, Dekker E (2011) New classification for probe-based confocal laser endomicroscopy in the colon. Endoscopy 43:1076-1081). Briefly, the diagnostic criteria include the crypt architecture and vessel architecture classification. The crypt architecture was divided into three types. Normal mucosa was scored as crypt type 1 and presented normal regular luminal openings, size, and distribution of crypts covered by a homogeneous layer of epithelial cells, including goblet cells. Hyperplastic polyps and inflammatory lesions were scored as crypt type 2 and presented regular-shaped or star-shaped luminal crypt openings with normal or reduced goblet cells. Neoplastic lesions were scored as crypt Type 3, which included variable width of epithelial lining with tubular-shaped crypts and loss of goblet cells (striped dark epithelium) and irregular and decreased volume of lamina propria. For vessel architecture (*Kuiper T, van den Broek FJ, van Eeden S, Wallace MB, Buchner AM, Meining A, van Hee K, Fockens P, Dekker E (2011) New classification for probe-based confocal laser endomicroscopy in the colon. Endoscopy 43:1076-1081*), normal mucosa was scored as vessel Type 1, presented hexagonal, honeycomb appearance that presents a network of capillaries outlining the luminal openings of the crypts. Hyperplastic polyps and inflammatory lesions were scored as vessel Type 2, presented hexagonal, honeycomb appearance with mild (or no) increase in the number of capillaries or increased amounts of normal vessels without leakage. Neoplasia was scored as vessel type 3, presenting dilated and distorted vessels with elevated leakage and irregular architecture with little or no orientation to adjunct tissue. We analyzed the pCLE imaging features and made relative diagnoses according to the above categories.

For the patients who received neoadjuvant chemotherapy, we adopted a pCLE scoring classification system created by Adriana Vaz Safatle-Ribeiro et al. (*Safatle-Ribeiro AV, Marques CFS, Pires C, Arraes L, Baba ER, Meirelles L, Kawaguti FS, da Costa Martins B, Lenz LT, de Lima MS, Gusmon-Oliveira CC, Ribeiro U, Jr., Maluf-Filho F, Nahas SC (2021) Diagnosis of Clinical Complete Response by Probe-*

Based Confocal Laser Endomicroscopy (pCLE) After Chemoradiation for Advanced Rectal Cancer. J Gastrointest Surg 25:357-368), assigning one point to the presence of each feature as follows: vascular features including fluorescein leakage and increased vessel/crypt ratio; epithelial features including dark irregular crypts, intratumoral budding, back-to-back glands, and cribriform pattern. Hence, in our study, patients with 0-1 points were diagnosed with complete response (no residual neoplasia), and those with 2-6 points were diagnosed with partial response (residual neoplasia). Consequently, we analyzed the diagnostic accuracy of pCLE in patients with neoadjuvant treatment according to the above classification. We analyzed the pCLE imaging features and made relative diagnoses according to the above categories. We have added this information to the Methods section as below:

METHOD section

pCLE optical biopsy diagnostic criteria

The pCLE optical biopsy diagnostic criteria were according to **the** "Miami criteria"[13] and Kuiper T et al's diagnostic classification[14]. Briefly, the diagnostic criteria include the crypt architecture and vessel architecture classification. The crypt architecture was divided into three types. Normal mucosa was scored as crypt type 1 and presented normal regular luminal openings, size, and distribution of crypts covered by a homogeneous layer of epithelial cells, including goblet cells. Hyperplastic polyps and inflammatory lesions were scored as crypt Type 2 and presented regular-shaped or star-shaped luminal crypt openings with normal or reduced goblet cells. Neoplastic lesions were scored as crypt Type 3, which included variable width of epithelial lining with tubular-shaped crypts and loss of goblet cells (striped dark epithelium) and irregular and decreased volume of lamina propria. For vessel architecture, normal mucosa was scored as vessel Type 1 and presented a hexagonal, honeycomb appearance that presented a network of capillaries outlining the luminal openings of the crypts. Hyperplastic polyps and inflammatory lesions were scored as vessel Type 2, presented hexagonal,

honeycomb appearance with mild (or no) increase in the number of capillaries or increased amounts of normal vessels without leakage. Neoplasia was scored as vessel type 3, presenting dilated and distorted vessels with elevated leakage and irregular architecture with little or no orientation to adjunct tissue. We analyzed the pCLE imaging features and made relative diagnoses according to the above categories.

For the patients who received neoadjuvant chemoradiotherapy, we adopted a pCLE scoring classification system created by Adriana Vaz Safatle-Ribeiro et al[15], assigning one point to the presence of each feature as follows: vascular features including fluorescein leakage and an increased vessel/crypt ratio; and epithelial features including dark irregular crypts, intratumoral budding, back-to-back glands, and a cribriform pattern. Hence, in our study, patients with 0–1 points were diagnosed with complete response (no residual neoplasia), and those with 2–6 points were diagnosed with partial response (residual neoplasia). Consequently, we analyzed the diagnostic accuracy of pCLE in patients with neoadjuvant chemoradiotherapy according to the above classification.

Q6. Based on the literature, combining pCLE with epithelial features and vascular scoring could improve the diagnosis of the presence of residual rectal neoplasia.

Answer: Thank you for your suggestion. For the patients who received neoadjuvant chemoradiotherapy, we adopted a pCLE scoring classification system created by Adriana Vaz Safatle-Ribeiro et al. (*Safatle-Ribeiro AV, Marques CFS, Pires C, Arraes L, Baba ER, Meirelles L, Kawaguti FS, da Costa Martins B, Lenz LT, de Lima MS, Gusmon-Oliveira CC, Ribeiro U, Jr., Maluf-Filho F, Nahas SC (2021) Diagnosis of Clinical Complete Response by Probe-Based Confocal Laser Endomicroscopy (pCLE) After Chemoradiation for Advanced Rectal Cancer. J Gastrointest Surg 25:357-368*), assigning one point to the presence of each feature as follows: vascular features including fluorescein leakage and increased

vessel/crypt ratio; epithelial features including dark irregular crypts, intratumoral budding, back-to-back glands, and cribriform pattern. Hence, in our study, patients with 0-1 points were diagnosed with complete response (no residual neoplasia), and those with 2-6 points were diagnosed with partial response (residual neoplasia). Consequently, we analyzed the diagnostic accuracy of pCLE in patients with neoadjuvant chemoradiotherapy according to the above classification. In this study, pCLE imaging correctly diagnosed 15 cases of residual neoplasia (scored in range 2-6 points) in 18 cases of pathological partial response. We have added this information to the Methods and Results section as below:

METHOD section

For the patients who received neoadjuvant chemoradiotherapy, we adopted a pCLE scoring classification system created by Adriana Vaz Safatle-Ribeiro et al[15], assigning one point to the presence of each feature as follows: vascular features including fluorescein leakage and an increased vessel/crypt ratio; and epithelial features including dark irregular crypts, intratumoral budding, back-to-back glands, and a cribriform pattern. Hence, in our study, patients with 0–1 points were diagnosed with complete response (no residual neoplasia), and those with 2–6 points were diagnosed with partial response (residual neoplasia). Consequently, we analyzed the diagnostic accuracy of pCLE in patients with neoadjuvant chemoradiotherapy according to the above classification.

RESULTS section

In 21 patients who underwent neoadjuvant chemoradiotherapy, 3 patients had a complete response, while 18 had a partial response after neoadjuvant chemoradiotherapy according to the pathological reports after surgery. Representative endoscopic images and corresponding pCLE images are shown Figure 3. All patients received endoscopic examination before surgery to evaluate the residual lesions. Seven patients' endoscopic reports showed a complete response, presenting a residual scar (Figure 3 A). Fourteen patients'

endoscopic reports showed a partial response, presenting a residual tumor lesion (Figure 3 C). In complete response rectal tissues (no neoplastic features), the typical pCLE images showed regular crypts with thickening epithelium and enlarged vessels with fibrotic stroma (Figure 3 B). The pCLE images of residual neoplasia showed atypical glands with dark and irregular crypts and enlarged twisty vessels (Figure 3 D). The pCLE imaging correctly diagnosed 15 cases of residual neoplasia (scored in range 2-6 points) in 18 cases of pathological partial response.

Q7. Intra- and interobserver agreements were not calculated in this study. Reliability assessment can be performed by means of intraclass correlation coefficients.

Answer: Thank you for your suggestion. The intraobserver agreement was calculated by means of intraclass correlation coefficients (ICCs). Based on the 95% confidence interval of the ICC estimate, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively. Cohen's kappa (κ) was calculated to assess the interobserver agreement of the two observers. The κ value was graded as follows: poor (0.01-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), and excellent (0.81-1.00). In our study, the mean ICC was 0.839 (95% CI = 0.763-0.892), which means that the intraobserver agreement was good. The interobserver agreement was substantial for the detection of rectal cancer, with a mean κ of 0.764 (standard error = 0.083). We have added this information to the Methods and Results section as below:

METHOD section

The intraobserver agreement was calculated by means of intraclass correlation coefficients (ICCs). Based on the 95% confident interval of the ICC estimate, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively. Cohen's kappa (κ) was calculated to assess the

interobserver agreement of the two observers. The κ value was graded as follows: poor (0.01–0.20); fair (0.21–0.40); moderate (0.41–0.60); substantial (0.61–0.80); and excellent (0.81–1.00). Statistical Package for the Social Sciences software (Release 22.0, SPSS, Inc., 2012) was applied for statistical analyses.

RESULTS section

In our study, the mean ICC was 0.839 (95% CI = 0.763-0.892), which means that the intraobserver agreement was good. The interobserver agreement was substantial for the detection of rectal cancer, with a mean κ of 0.764 (standard error = 0.083).

Q8. Pathology features of TME grades to assess the quality of TME specimens are lacking TaTME outcomes are based on the surgeons' experiences; hence, it would be better to include these details in the methods section.

Answer: Thank you for your suggestion. The TME specimen quality should be assessed based on the following features. Grade 1 represents low quality: incomplete mesorectum; mesorectum fascia defects deeper than 5 mm; conical gross specimen. Grade 2 represents moderate quality: relatively intact mesorectum; mesorectum fascia defects deeper than 5 mm; no visible muscularis propria with adequate resection margin; approximately conical gross specimen. Grade 3 represents high quality: intact mesorectum; no mesorectum fascia defects deeper than 5 mm; no visible muscularis propria; cylindrical specimen. A circumferential resection margin (CRM) was defined as positive when it was less than 1 mm, and a positive CRM or positive distant resection margin (DRM) was considered R1 resection. All TME specimens were evaluated by pathologists after surgery. There were 40 specimens defined as grade 3 and 3 specimens defined as grade 2. We have added these details to the Methods section and RESULTS section as below:

METHOD section

The TME specimen quality should be assessed based on the following features. Grade 1 represents low quality: incomplete mesorectum; mesorectum

fascia defects deeper than 5 mm; conical gross specimen. Grade 2 represents moderate quality: relatively intact mesorectum; mesorectum fascia defects deeper than 5 mm; no visible muscularis propria with adequate resection margin; approximately conical gross specimen. Grade 3 represents high quality: intact mesorectum; no mesorectum fascia defects deeper than 5 mm; no visible muscularis propria; cylindrical specimen. A circumferential resection margin (CRM) was defined as positive when it was less than 1 mm, and a positive CRM or positive distant resection margin (DRM) was considered R1 resection. All TME specimens were evaluated by pathologists after surgery.

RESULTS section

The surgical and functional outcomes are shown in Table 4. No positive DRMs were detected in our study. All TME specimens were evaluated by pathologists after surgery. There were 40 specimens defined as grade 3 and 3 specimens defined as grade 2.

Q9. pCLE probe-based confocal laser endomicroscopy features, whether it showed as no neoplastic features or had any residual compared to the biopsy report that needs to be clearly rewritten.

Answer: Thank you for your suggestion. Kuiper T et al raised a pCLE scoring classification system for patients after neoadjuvant therapy assigning one point to the presence of each feature as follows: vascular features including fluorescein leakage and increased vessel/crypt ratio; epithelial features including dark irregular crypts, intratumoral budding, back-to-back glands, and cribriform pattern. Hence, in our study, patients with 0-1 points were diagnosed with complete response (no residual neoplasia), and those with 2-6 points were diagnosed with partial response (residual neoplasia). Consequently, we analyzed the diagnostic accuracy of pCLE in patients with neoadjuvant treatment according to the above classification.

In our study, 21 patients underwent neoadjuvant chemoradiotherapy, 3 patients had complete response, and 18 had partial response after neoadjuvant

chemoradiotherapy according to the pathological reports after surgery. In complete response rectal tissues (no neoplastic features), the typical pCLE images showed regular crypts with thickening epithelium and enlarged vessels with fibrotic stroma (Figure 3 B). The pCLE images of residual neoplasia showed atypical glands with dark and irregular crypts and enlarged twisty vessels (Figure 3 D). The pCLE imaging correctly diagnosed 15 cases of residual neoplasia (scored in range 2-6 points) in 18 cases of pathological partial response. We added this information to the Methods and Results section as below:

METHOD section

For the patients who received neoadjuvant chemoradiotherapy, we adopted a pCLE scoring classification system created by Adriana Vaz Safatle-Ribeiro et al[15], assigning one point to the presence of each feature as follows: vascular features including fluorescein leakage and an increased vessel/crypt ratio; and epithelial features including dark irregular crypts, intratumoral budding, back-to-back glands, and a cribriform pattern. Hence, in our study, patients with 0–1 points were diagnosed with complete response (no residual neoplasia), and those with 2–6 points were diagnosed with partial response (residual neoplasia). Consequently, we analyzed the diagnostic accuracy of pCLE in patients with neoadjuvant chemoradiotherapy according to the above classification.

RESULTS section

In 21 patients who underwent neoadjuvant chemoradiotherapy, 3 patients had a complete response, while 18 had a partial response after neoadjuvant chemoradiotherapy according to the pathological reports after surgery. Representative endoscopic images and corresponding pCLE images are shown Figure 3. All patients received endoscopic examination before surgery to evaluate the residual lesions. Seven patients' endoscopic reports showed a complete response, presenting a residual scar (Figure 3 A). Fourteen patients' endoscopic reports showed a partial response, presenting a residual tumor

lesion (Figure 3 C). In complete response rectal tissues (no neoplastic features), the typical pCLE images showed regular crypts with thickening epithelium and enlarged vessels with fibrotic stroma (Figure 3 B). The pCLE images of residual neoplasia showed atypical glands with dark and irregular crypts and enlarged twisty vessels (Figure 3 D). The pCLE imaging correctly diagnosed 15 cases of residual neoplasia (scored in range 2-6 points) in 18 cases of pathological partial response.

Q10. Please include follow-up endoscopy detail and the results of pCLE in lesions with partial and complete clinical response.

Answer: Thank you for your suggestion. In 21 patients who underwent neoadjuvant chemoradiotherapy, 3 patients had a complete response, while 18 had a partial response after neoadjuvant chemoradiotherapy according to the pathological reports after surgery. Representative endoscopic images and corresponding pCLE images are shown Figure 3. All patients received endoscopic examination before surgery to evaluate the residual lesions. Seven patients' endoscopic reports showed a complete response, presenting a residual scar (Figure 3 A). Fourteen patients' endoscopic reports showed a partial response, presenting a residual tumor lesion (Figure 3 C). The endoscopic examination correctly diagnosed 13 cases of residual neoplasia in 18 cases of pathological partial response. In complete response rectal tissues, the typical pCLE images showed regular crypts with thickening epithelium and enlarged vessels with fibrotic stroma (Figure 3 B). The pCLE images of residual neoplasia showed atypical glands with dark and irregular crypts and enlarged twisty vessels (Figure 3 D). The pCLE imaging correctly diagnosed 15 cases of residual neoplasia in 18 cases of pathological partial response. We have added this information to the Results section as below:

RESULTS section

In 21 patients who underwent neoadjuvant chemoradiotherapy, 3 patients had a complete response, while 18 had a partial response after neoadjuvant

chemoradiotherapy according to the pathological reports after surgery. Representative endoscopic images and corresponding pCLE images are shown Figure 3. All patients received endoscopic examination before surgery to evaluate the residual lesions. Seven patients' endoscopic reports showed a complete response, presenting a residual scar (Figure 3 A). Fourteen patients' endoscopic reports showed a partial response, presenting a residual tumor lesion (Figure 3 C). In complete response rectal tissues (no neoplastic features), the typical pCLE images showed regular crypts with thickening epithelium and enlarged vessels with fibrotic stroma (Figure 3 B). The pCLE images of residual neoplasia showed atypical glands with dark and irregular crypts and enlarged twisty vessels (Figure 3 D). The pCLE imaging correctly diagnosed 15 cases of residual neoplasia (scored in range 2-6 points) in 18 cases of pathological partial response.

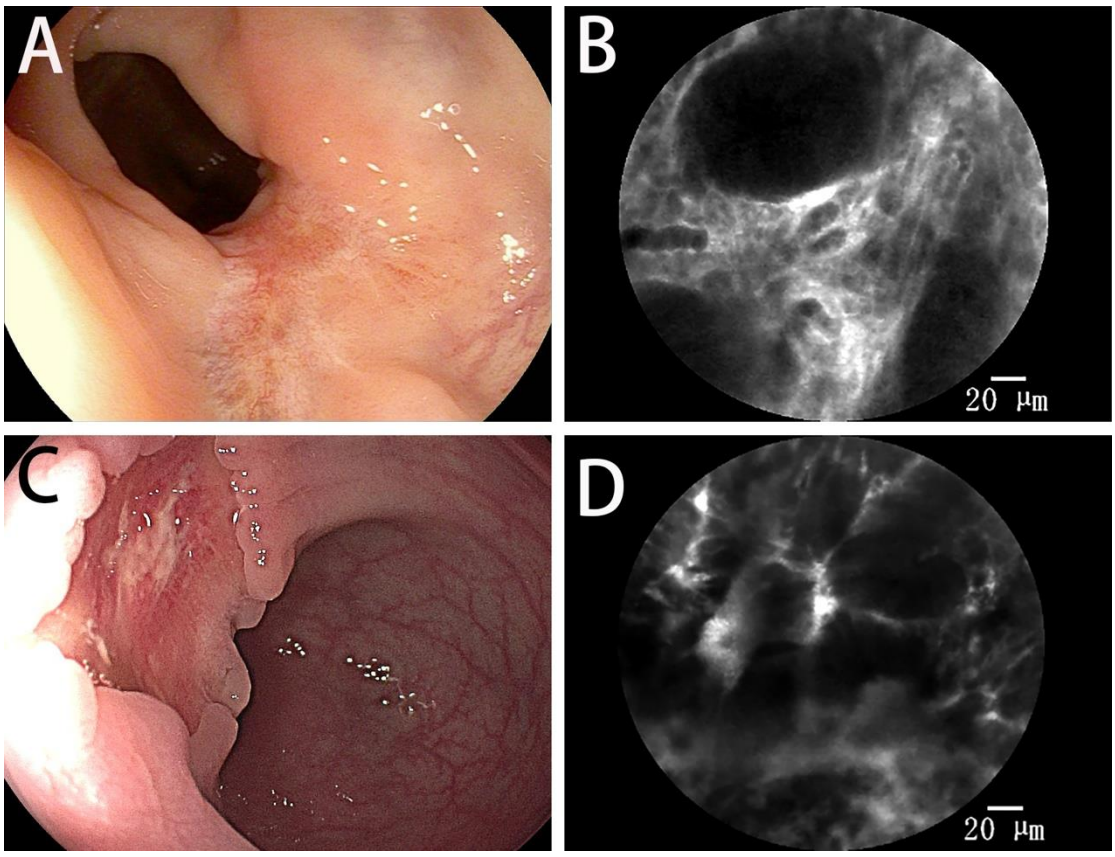

Figure 3 Endoscopic images and corresponding pCLE images of rectal tissues after neoadjuvant chemoradiotherapy. A. Endoscopic image of rectal tissue with complete response, presenting a residual scar; B. Corresponding

pCLE image showed regular crypts with thickening epithelium and enlarged vessels with fibrotic stroma. C. Endoscopic image of rectal tissue with partial response, presenting a residual tumor lesion; D. Corresponding pCLE image showed atypical glands with dark and irregular crypts and enlarged twisty vessels.

Reviewer #2

The authors assessed the feasibility of pCLE to select the DRM during TaTME for low rectal cancer. I am very interested in this report and personally think it worth for publication. However, I have one question. #1. You described "The probe has a field of view of 240 μm and can image at a depth of 60 μm below the mucosal surface." in the Materials and Methods section. Therefore, pCLE can only evaluate the mucosal layer. However, cancer cells sometimes crawl mainly submucosa rather than the mucosal layer, such as poorly differentiated adenocarcinomas. How do you think we should handle such cases?

Answer: Thank you for your comments. Most rectal cancer cells invade from the mucosal layer downward. However, cancer cells sometimes crawl mainly the submucosa rather than the mucosal layer, such as poorly differentiated adenocarcinomas, as you mentioned. Due to the limitation of current technology, the pCLE imaging depth is restricted to 60 μm. Therefore, in this study, the accuracy of pCLE optical biopsy was 88.37% rather than 100%. In this study, four cases of poorly differentiated adenocarcinomas were not detected by pCLE imaging. Therefore, in our experience, patients who have been diagnosed with poorly differentiated adenocarcinoma preoperatively should receive submucosal intraoperative frozen biopsy to ensure distal margin safety. We added this information to the discussion section as below:

DISCUSSION section

The limitation of this study was based on a single center, and the sample size was relatively small, which might limit the power of the study. Therefore, a

large-scale multicenter, prospective, randomized controlled trial needs to be performed. The cancer cells sometimes crawl mainly submucosa rather than the mucosal layer, such as poorly differentiated adenocarcinomas. Due to the limitation of current technology, the pCLE imaging depth is restricted to 60 μm. Therefore, in our experience, patients who have been diagnosed with poorly differentiated adenocarcinoma preoperatively should receive submucosal intraoperative frozen biopsy to ensure distal margin safety.

Thank you very much for your kind work. On behalf of my coauthors, we would like to express our great appreciation to the editor and reviewers. Thank you and best regards.

Revision reviewer

Specific comments: The authors have addressed my concerns and comments. It looks much better now. Good luck

Answer: Thank you for your comments.

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