Answering Reviewers letter

Dear reviewers and editors:

Thank you for your efficient work in procession of our manuscript entitled ”Intraoperative intraperitoneal chemotherapy increases the incidence of anastomotic leakage after anterior resection of rectal tumor” (Manuscript No:46022). We also really appreciate the dear reviewers for giving us precious advices, which are important for us to improve the quality of our work. Our study focused on intraoperative intraperitoneal chemotherapy for patients with locally advanced rectal carcinoma. This treatment method is newly developed and has been verified to achieve improved oncological prognosis in some reports. However, few reports have focused on its side effects. Our study investigated its impacts on postoperative anastomotic leakage after anterior resection of rectal carcinoma. We believe this is of great value to know the safety and feasibility of this therapy, which can provide some guidance for surgeons before they choose this modality for patients.

In our revised manuscript, patients were divided into chemotherapy group received intraoperative intraperatoneal chemotherapy and control group. We compared the incidence of AL in the two groups to explore the association between this newly treatment method and AL. So our research was a retrospective cohort study instead of case control study. Since the indication of intraoperative intraperitoneal chemotherapy was patients with locally advanced rectal carcinoma, 195 patients who were not this condition have been excluded.

In addition, we have carefully revised our paper based on the comments of reviewers, and the point-to-point responses to the reviewers’ comments are presented below:

Reviewer #1

Comment 1: In the page 5 of introduction section: in the sentences “Intraoperative intraperitoneal chemotherapy has been gradually adopted to treat rectal carcinoma patients in eastern countries in the past few years and this is not associated with neoadjuvant or adjuvant therapy[19, 20]”, the intraoperative intraperitoneal chemotherapy (from references of 19 and 20) is used for colorectal cancer patients. However, most patients will undergo neoadjuvant chemoradiotherapy followed by operation for anal preservation. In addition, preoperative chemoradiotherapy provides better local control and disease-free survival than postoperative chemoradiotherapy for rectal cancer patients in randomized trials. The authors should provide more information of aforementioned results in the introduction section. Furthermore, authors can describe whether the roles of intraoperative intraperitoneal chemotherapy are different in

patients with colon cancer and in those with rectal cancer.

Response: Indeed, preoperative chemoradiotherapy can achieve improved prognosis of tumor in patients with locally advanced rectal cancer. And this has been confirmed in many previous prospective randomized controlled trials. Intraoperative intraperitoneal chemotherapy is a new treatment modality aiming to further improve the clinical outcomes of patients by killing the intrapertitoneal residual tumor cells after operation. This therapy is not in contradiction with neoadjuvant therapy, patients who have already received preoperative chemoradiotherapy can still receive intraoperative intraperitoneal chemotherapy. More information about neoadjuvant therapy has been added to the introduction part followed the advice of reviewers. As for the different roles of this emerging therapy between colon cancer and rectal cancer, no obvious difference has been observed in clinical work so far. This treatment can be administrated in both colon cancer and rectal cancer patients with the same aims to eradicate the exfoliated tumor cells.

Comment 2: In the methods section, the authors should provide more information of grade B or C according to the proposal from the International Study Group of Rectal Cancer in 2010. In addition, the definition of AL is based both on the clinical symptoms and image findings? What is the criteria for AL in image findings?

Response: We really appreciate this advice, which makes our paper improved significantly. We have provided more information of grade B and C anastomotic leakage (AL) in the revised methods section. Grade B AL patients require only conservative treatment. Grade C AL patients require a secondary operation. As for the definition of AL, AL is generally defined as defect in anastomotic site. But AL can be diagnosed through clinical symptoms and image findings. The hydrops and pneumatosis in the pelvic cavity in CT images or anastomotic defect in endoscopy images can imply the existence of AL.

Comment 3: In the multivariate analyses of the “Results” section: considering the number of patients from each factors are not different (for example, male 418, lobaplatin only 16), how to calculate and analyze these issues should be addressed. In addition, how to define synchronous primary malignancy in your study?

Response: We really agree with this advice. In our revised manuscript, patients were divided into chemotherapy group received intraoperative intraperatoneal chemotherapy and control group. We compared the incidence of AL in the two groups to explore the association between this newly treatment method and AL. 32 variables were collected to evaluate the comparability of the two groups and most variables were comparable. For the variables that were imbalanced between the two groups, stratification analysis and multivariate logistic regression analysis were subsequently conducted to adjust for the confounder effects. As for synchronous primary malignancy, this is defined as simultaneous existence of two or more tumor lesions of separate origins in the rectum in our previous manuscript. However, in our revised manuscript, as we changed our study design, 195 patients who were not locally advance rectal carcinoma at the date of diagnosis were excluded. Therefore, only 8 patients with synchronous primary malignancy

remained in our research. So we didn't analyze this factors in our revised paper as the sample size was too small.

Comment 4: In the Discussion section: Since the number of use of lobaplatin in the intraoperative intraperitoneal chemotherapy is only 16 patients. It is difficult to conclude that the Intraoperative intraperitoneal chemotherapy can increases the incidence of anastomotic leakage can increases the incidence of anastomotic leakage after anterior resection of rectal tumor. At least, the title should be revised as Intraoperative intraperitoneal chemotherapy using lobaplatin may increases the risk of anastomotic leakage of rectal tumor. Along this line, the authors should provide more information of the relationship between complications and intraoperative intraperitoneal lobaplatin in the other types of cancer patients.

Response: In our revised manuscript, patients were divided into chemotherapy group received intraoperative intraperitoneal chemotherapy and control group. Considering the limited cases of patients using lobaplatin, we didn't explore the respective effects of different chemotherapy types on AL. Patients using different antitumor agents were analyzed together to explore the role of intraoperative intraperitoneal chemotherapy in the occurrence of AL. In this way, we think the title of our article might be suitable. We realized this shortcoming in our research and mentioned it in the discussion section. Moreover, in animal experimental studies, the detrimental effects of intraperitoneal chemotherapy on AL were confirmed for almost all the common antineoplastic drugs in rectal cancer chemotherapy, including mitomycin C, cisplatin, oxaliplatin, 5-fluorouracil, iritotecan, doxorubicin. And we have also discussed these experimental researches in our revised study. As for the relationship between complications and intraoperative intraperitoneal lobaplatin in the other types of cancer patients. The only article that can be found in English literature so far was one paper entitled "Cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy with lobaplatin and docetaxel to treat synchronous peritoneal carcinomatosis from gastric cancer: Results from a Chinese center". One of the 50 patients developed AL after intraperitoneal administration of lobaplatin. But no control group was set in this study. The relationship between AL and use of lobaplatin can not been analyzed.

Comment 5: If authors could provide the examples of images of AL in rectal cancer patients who receive intraoperative intraperitoneal chemotherapy with lobaplatin after anterior resection.

Response: We really appreciate this valuable suggestion. In our revised manuscript, we provided two pictures of AL from patients receiving intraperitoneal administration of lobaplatin. One was from pelvic computed tomography scans, the other was from rectoscopy.

Comment 6: There are few grammar errors in the whole article. For example, "Further investigation were performed to explore the respective impacts of different agents." It should be revised as "Further investigation is performed……"

Response: Thanks for the kind reminding, we have our manuscript checked again by professional language editing company. Now the errors have been modified.

Reviewer #2

Comment 1: Introduction Explanation of abbreviations should be given when first mentioned not only in the abstract, but in the text of the manuscript: AL is a severe and common postoperative issue after anterior resection of rectal neoplasms, with high incidence ranging from 6.1% to 11.9%[1-3].

Response: Thanks for this advice. We have provided the explanation of abbreviations in the text of our revised manuscript.

Comment 2: Result the data presented in the manuscript make it possible to judge the influence of various factors on the frequency of AL. However, the title and purpose of the study is to study the effect of intraperitoneal chemotherapy on the frequency of AL. The main authors' methodological error lies in the fact that they divided patients into groups with and without AL. However, in order to achieve this goal, they need to divide the patients into groups receiving and not receiving intraperitoneal chemotherapy. With this approach, in the group receiving intraperitoneal chemotherapy, AL will develop in 12 of 256 patients (4.7%), and in the group not treated with intraperitoneal chemotherapy-in 8 of 416 patients (2.0%).I think that in this case the differences will be statistically significant. Analysis of other clinical, tumor-related and operation related factors in these group is necessary in order to assess how comparable these groups are. Only in this case it will be possible to assert that the increase in the frequency of AL is associated with the presence of intraperitoneal chemotherapy, and not the inquality of groups in other indicators that also affect this complication. I think that the authors can easily fix it.

Response: We really appreciate this important and valuable advice, which have indicated the study design limitations of our research and provided better way to get it modified. In our revised manuscript, we followed this suggestion. Patients were divided into chemotherapy group received intraoperative intraperatoneal chemotherapy and control group. We compared the incidence of AL in the two groups to explore the association between this newly treatment method and AL. In this way, our research was a retrospective cohort study instead of case control study. Moreover, considering the indication of intraoperative intraperitoneal chemotherapy was patients with locally advanced rectal carcinoma. 195 patients who didn't meet this standard at the date of diagnosis were excluded. Therefore, 477 cases remained and 171 cases received intraperitoneal usage of antitumor agents. AL developed in 13 of 171 patients (7.6%) in the chemotherapy group and 5 of 306 patients (1.6%) in the control group. This difference has been proved to be statistically significant.

Comment 3: However, I think that the first 8 lines of the discussion are related to the results of the study and should be placed in the appropriate section.

Response: Thanks for this suggestion sincerely. In our revised article, we have placed these contents in the result section.

Comment 4: Table 1. There should be no abbreviations in the name of the table. Table 2 - 3. The presented material does not make it possible to understand how the authors calculated the

frequency of the case (percentages). The characteristics should be divided into subgroups, within which the sum of cases will give 100% (for example, as in Table 4). In addition, there is a discrepancy in the number of patients who received chemotherapy. According to the text and in the table, there are 250 of them, but the number of patients who received Lobaplatin and Fluorouracil composes 256 (Table 3). Authors should clarify this discrepancy.

Response: In this revised edition, we have replaced the abbreviations in the name of the table with the full names. As for the calculation method, both the sum of cases of chemotherapy group and control give 100%. And the percentages were calculated by the proportions of cases of respective variables occupied in chemotherapy group and control group, respectively. In regard to the discrepancy, that's because 6 patients received both intraperitoneal administration of lobaplatin and fluorouracil implants. We have clarified this problem in the revised article clearly.

Comment 5: There are grammatical errors, for example: This case control study collected information from 672 consecutive patients who received anterior resection of rectal carcinoma with the double stapling technique in our institution from September 2016 to September 2017. Morphological errors (inconsistent proposal), for example: Peritoneal recurrence is common for patients who have received radical resection of rectal carcinoma, which results from the residual tumor cells after primary tumor removal. Stylistic mistakes, for example: «all locally advanced patients»

Response: We have our manuscript checked again by professional language editing company. Now the errors have been modified.

Reviewer #3
Comment 1: The finding is primitive and limited to one therapeutic agent and not the other one tested in this work. It cannot be generalized. Therefore, it is clear that the title is over and needs to be amended to reflect the limited finding.

Response: We really appreciate this advice from dear reviewers. As we have responded above, in our revised manuscript, patients were divided into chemotherapy group received intraoperative intraperitoneal chemotherapy and control group. Considering the limited cases of patients using lobaplatin, we didn't explore the respective effects of different chemotherapy types on AL. Patients using different antitumor agents were analyzed together to explore the role of intraoperative intraperitoneal chemotherapy in the occurrence of AL. In this way, we think the title of our article might be suitable. We realized this shortcoming in our research and mentioned it in the discussion section. Moreover, in animal experimental studies, the detrimental effects of intraperitoneal chemotherapy on AL were confirmed for almost the common antineoplastic drugs in rectal cancer chemotherapy, including mitomycin C, cisplatin, oxaliplatin, 5-fluorouracil, iritotecan, doxorubicin. And we have also discussed these experimental researches in our revised study.

Comment 2: In their discussion they mention that "However, this correlation was only observed in cases using lobaplatin. Lobaplatin is a type of cytotoxic drug that can inhibit cell proliferation

and induce apoptosis by destroying the structure and function of DNA, especially cells with rapid proliferation. Given that rectal anastomotic stoma healing requires rapid proliferation of regenerative cells, we hypothesize that lobaplatin increased the incidence of AL by inhibiting cell proliferation". First, if this is the case, then the point of the paper should highlight that the finding is limited to a chemotherapy type and possibly the "mode of administration". Second and more important, chemotherapy typically affect cancer cell with much less effect on normal cells; which needs to be mentioned and clarified in this context. Typically, the author should support their argument by experimental data e.g. (in vivo using animal models) if available.

Response: In our revised manuscript, we didn't explore the impacts of different antitumor drugs on bowel anastomoses since the sample size of using lobaplatin was not enough. Indeed, cytotoxic agents have much less effect on normal cells than cancer cells, but the detrimental effects of chemotherapy agents on intestinal anastomoses has been confirmed in numerous previous animal experimental studies, including mitomycin C, cisplatin, oxaliplatin, 5-fluorouracil, iritotecan, doxorubicin. The underlying mechanisms including inhibited fibroblast activity, decreased collagen deposition and reduced neoangiogenesis at the anastomotic site through histological examinations. These outcomes were regarded to be caused by administration of intraperitoneal cytotoxic agents since they can inhibit cell proliferation. These experimental researched has been added in the discussion section in our revised manuscript.

Comment 3: The conclusion does not reflect the contents of the paper.

Response: We have modified our conclusion, and we think now it can reflect the findings of our investigation.

Comment 4: Some statements need to be supported with evidence such as "The reluctance of patients to receive preoperative radiotherapy might be the reason.

Response: We have added more statements to support the evidence. Many of the patients were in poor economic conditions. And some patients declined to receive neoadjuvant therapy for fear about the serious side effects. Therefore, the rate of patients receiving preoperative radiochemotherapy was not very high.

Comment 5: The number of typo and grammar is surprising given that professional company checked the document. The authors are advised to double check and clarify the reason for all of these mistakes such as the following (to mention a few): a. "who didn't received preoperative" b. Lobaplatin is a type of cytotoxic drug that can inhibit cell proliferation and induce apoptosis "by destroying the structure and function of DNA", especially cells with rapid proliferation. Note the inappropriate use of "destroyed" with DNA structure and function c. "a more sufficient impact on anastomotic stoma than fluorouracil implants" sufficient is not clear; do you mean more "efficient"? d. No consistency in the format of "Fluorouracil" /" Fluorouracil" with capital or small "F"?

Response: We have our manuscript checked again by professional language editing company.

Now the errors have been modified.

Finally, we really appreciate your hard and efficient work, every piece of advice is truly precious for us to improve the quality of our work.

With kind regards,

Yours sincerely