

## Peer Review File

Article information: <https://dx.doi.org/10.21037/jgo-23-144>

### Reviewer A

Surgery is the main treatment for colorectal cancer. In this study, Wang et al. retrospectively compared the clinical response and immune feedback of traditional laparoscopic and total laparoscopic radical resection. Based on limited case sample data in a single center, authors roughly concluded that total laparoscopic radical resection is superior to standard laparoscopic-assisted radical resection for the treatment of colorectal cancer because it not only has clinically significant benefits but also has fewer adverse effects on the immune system. Please lower the tone of the words in the conclusion of the research. After all, this retrospective study is relatively rough in design and is a single-center small sample study, which is insufficient to support the author's conclusion. My further specific concerns are outlined below:

**Comment 1:** The full text is insufficient in the summary of existing research, especially in the introduction and discussion parts. It is too simple and the reference citation is inappropriate. Please carefully review the current relevant literature and revise it.

**Reply 1:** We have updated the references as appropriate.

#### Change in text:

1. Devoto L, Celentano V, Cohen R, et al. Colorectal cancer surgery in the very elderly patient: a systematic review of laparoscopic versus open colorectal resection. *Int J Colorectal Dis.* 2017;32(9):1237-1242.
2. Zhou Z, Chen L, Liu J, et al. Laparoscopic Natural Orifice Specimen Extraction Surgery versus Conventional Surgery in Colorectal Cancer: A Meta-Analysis of Randomized Controlled Trials. *Gastroenterol Res Pract.* 2022;2022:6661651.
3. Chin YH, Decruz GM, Ng CH, et al. Colorectal resection via natural orifice specimen extraction versus conventional laparoscopic extraction: a meta-analysis with meta-regression. *Tech Coloproctol.* 2021;25(1):35-48.
4. Biondi A, Grosso G, Mistretta A, et al. Predictors of conversion in laparoscopic-assisted colectomy for colorectal cancer and clinical outcomes. *Surg Laparosc Endosc Percutan Tech* 2014;24:e21-6.
5. Gu C, Wu Q, Zhang X, et al. Single-incision versus conventional multiport laparoscopic surgery for colorectal cancer: a meta-analysis of randomized controlled trials and propensity-score matched studies. *Int J Colorectal Dis* 2021;36:1407-19.
6. Izquierdo KM, Unal E, Marks JH. Natural orifice specimen extraction in colorectal surgery: patient selection and perspectives. *Clin Exp Gastroenterol.* 2018;24;11:265-279.
- 7.

**Comment 2:** Page 2, Line 55-56: “At present, surgery is the main treatment for colorectal cancer, and the laparoscopic surgical approach has become the first choice owing to its advantages in terms of rapid patient recovery”. Is laparoscopic surgery the first choice? Because the full text is not found, from the perspective of the title, the reference is the research related to rectal cancer?

**Reply 2:** We have revised the “has become the first choice” to “has been broadly used” in the sentence in Page2, Line 55-56 and revised the corresponding reference.

**Changes in the text:** At present, surgery is the main treatment for colorectal cancer, **Compared to the open surgery**, the laparoscopic surgical approach has **been broadly used** owing to its advantages in terms of rapid recovery.

**Comment 3:** Methods need to be further checked and improved, such as inclusion criteria, multiple primary tumors, etc?

**Reply 3:** We have revised the exclusion criteria, added the two exclusion criteria which we actually did in the study, such as multiple primary tumors and preoperative steroid use.

**Changes in the text:** Patients were excluded based on the following criteria: (I) serious dysfunction of the heart, kidneys, or other organs; **(II) multiple primary tumors**; (III) imaging evidence of distant cancer cell metastasis; (IV) previous surgical treatment of colorectal cancer; (V) estimated survival time < 6 months; (VI) history of abdominal, pelvic, and anorectal surgery; (VII) patients with autoimmune or infectious diseases and **(VIII) have preoperative steroid use**.

#### **Comment 4,5, 6**

4) The result part is very rough, please provide further detailed description, whether in terms of statistics or language description.

5) Table 1: The TNM staging proportion of the observation group is incorrect, please check.

6) Table 1: Please list more clinicopathological information, such as tumor size and specific TNM stages.

**Reply for 4, 5 and 6:** We added more details in the TNM stages, and tumor size in Table 1.

#### **Changes in the text:**

Clinical characteristics	Control group (n=52)	Observation group (N=60)	t/ $\chi^2$	P
Age (years), mean $\pm$ SD	62.77 $\pm$ 11.38	59.75 $\pm$ 11.82	1.37	0.173
Gender, n (%)			0.12	0.730
Male	32 (61.54)	35 (58.33)		
Female	20 (38.46)	25 (41.67)		
BMI (kg/m <sup>2</sup> ), mean $\pm$ SD	23.05 $\pm$ 2.86	23.35 $\pm$ 2.30	0.63	0.520

Tumor size (mm), mean ± SD	27.90 ± 5.82	28.89 ± 5.43	0.92	0.360
pTNM stages, n (%)				
I	20 (38.46)	24 (40.00)	1.55	0.908
IIA	11 (21.15)	14 (23.33)		
IIB	4 (7.69)	2 (3.33)		
IIIA	3 (5.77)	2 (3.33)		
IIIB	9 (17.31)	12 (20.00)		
IIIC	5 (9.62)	6 (10.00)		

**Comment 7** Table 2: Operation time 30-50 minutes? Are you sure?

**Reply 7:** Sorry for the typing mistake in the operation time, we corrected and revised the operation time in table 2.

**Changes in the text:**

Perioperative outcomes	Control group	Observation group	t/ $\chi^2$	P
Operation time (min)	165.40 ± 49.58	195.72 ± 62.10	2.83	0.006
Intraoperative blood loss (mL)	42.12 ± 19.84	47.67 ± 25.74	1.26	0.209
Duration for the first postoperative exhaust (days)	2.85 ± 1.59	2.33 ± 1.57	1.73	0.086
Duration for the first postoperative defecation (days)	3.83 ± 1.89	3.48 ± 1.84	0.99	0.320
Duration for resumption of a semi-liquid diet (days)	4.86 ± 2.05	4.02 ± 2.07	2.17	0.032
Length of postoperative stay in hospital (days)	9.89 ± 4.48	7.80 ± 3.61	2.74	0.007
Postoperative complications, n (%)				
Incision infection	6 (11.54)	0(0)	7.32	0.009
Anastomotic leakage	2 (3.85)	2 (3.33)	0.02	0.884

**Comment 8** The formulation of the manuscript and the correct use of English needs attention.

**Reply 8:** the manuscript has been proofed carefully.

**Reviewer B**

This study investigated the postoperative clinical outcomes and inflammatory response of total laparoscopy versus conventional laparoscopic-assisted radical resection in the treatment of sigmoid and rectal cancer. Reported results are worthy of publication but require major revision before it can be finally published for the wide scientific readership. Authors are requested to respond to the following queries and modify their manuscript in the light of the following points wherever possible:

**Comment 1:** If the word limit allows, I suggest you extend your introduction section by including a brief comparison of total laparoscopy and conventional laparoscopic-

assisted radical resection.

**Reply 1:** We have revised the first two paragraphs, which mainly described the comparison of total laparoscopy(NOSES) and conventional laparoscopic-assisted radical resection. And we have also described the comparison in the 1<sup>st</sup> paragraph of #Discussion.

**Changes in the text:**

At present, surgery is the main treatment for colorectal cancer. Compared to the open surgery, the laparoscopic surgical approach has been broadly used owing to its advantages in terms of rapid recovery, less intraoperative blood loss and postoperative complications(1). The conventional laparoscopic-assisted radical resection of colorectal cancer is required an auxiliary incision in the abdominal wall for anastomosis and reconstruction of the digestive tract. With the improvement of minimally invasive technology, the emergence of NOSES provides a new method for the treatment of colorectal cancer, including sigmoid and rectal cancer. This operation utilizes a laparoscopic approach for intracorporeal anastomosis without auxiliary incision, by completing the reconstruction of the digestive tract in vivo, which has the advantages of smaller wounds and less invasiveness (2,3).

**Comment 2:** I appreciate your study includes important perioperative outcomes, such as anastomotic leakage, and incision infection. Did you evaluate postoperative bleeding as well? If yes, please include the data.

**Reply 2:** We did not evaluate the postoperative bleeding. But that's a promotive suggestion which we could perform in the further study.

**Comment 3:** This study excluded patients based on the following criteria with estimated survival time <6 months. What were the follow-up protocols of your study?

**Comment 5:** What about evaluations like disease-free survival, progression-free survival, overall survival, and local or metastatic recurrence pattern?

**Comment 7:** What are the future prospects of this study? Please add it to the discussion section.

**Reply 3,5 and 7:** The patients who estimated survival time < 6 months are considered as the exclusion criteria of surgery treatment in our study. Terminal patients are not suggested to receive the surgery treatment. We are going to follow the long-term clinical outcomes for the patients who received the total laparoscopy versus conventional laparoscopic-assisted radical resection. In the progress of this study, we now finished the collection of short-term clinical outcomes, and we will evaluate the disease-free survival, progression-free survival, overall survival, and local or metastatic recurrence pattern in the further following evaluations as reviewer's suggestion. And we added the future prospects in the discussion section as "And the comparison for

long-term clinical outcomes of two surgical techniques need to be evaluated in the further studies.”

**Comment 4:** This study explored several endpoints/outcomes while comparing the two surgical treatments. For any related surgical intervention, primary endpoints like safety, efficacy, and being oncologically clear are very crucial. How did you assess perioperative morbidity?

**Reply 4 :** The evaluation of the perioperative incision infection is specified involving the skin or subcutaneous tissue of the incision site after 30 days of surgery and based on the criteria listed as following: (1) Superficial incisions that have purulent drainage; (2) The presence of organisms in an aseptically obtained culture of tissue or fluid obtained from the superficial incision; (3) The presence of one or more of the following symptoms of infection: pain or tenderness around the incision site, localized swelling, redness, or heat.

**Anastomotic leakage:** The situation which has the infection combined with a leak of luminal contents from a surgical joint, and confirmed by imaging examination, will be classified into anastomotic leakage.

**Comment 6:** Did you perform any molecular analysis to evaluate which of the two surgical techniques is better for reducing tumor cells shedding into the portal/peripheral circulation, and peritoneal cavity? If yes, please share the results. If not, I suggest you include it in your future study.

**Reply 6:** We did not evaluate which of the two surgical techniques is better for reducing tumor cells shedding into the portal/peripheral circulation, and peritoneal cavity. But that’s a promotive suggestion which we could perform in the further study.

### **Reviewer C**

This paper is a well-written original article assessing short term clinical benefit of total laparoscopic surgery with intracorporeal anastomosis compared to conventional laparoscopic-assisted surgery with extracorporeal anastomosis in sigmoid and rectal cancer. Recently, due to advances in the development of surgical instruments and perioperative management, the safety of intracorporeal anastomosis in colon cancer surgery has been reported in many papers. However, its impact on short- and long-term prognosis remains unclear, and judgments regarding the indications for intracorporeal anastomosis should be made with caution. This paper reports that intracorporeal anastomosis and removal of the resected intestinal tract through a natural orifice can improve short-term postoperative outcomes and suppress excessive immune responses. This finding could be very important in the future dissemination of intracorporeal anastomosis.

However, there are several points that remain unclear.

**Comment 1:** Preoperative steroid use may affect postoperative immune indices. Information on preoperative steroid use in patients included in this analysis is needed.

**Reply 1:** All patients included in this study have no steroid use, and we have added it in the exclusion criteria (VIII).

**Changes in the text:** (VIII) have preoperative steroid use.

**Comment 2:** One of the complications of concern in intracorporeal anastomosis is intraperitoneal infection. From this perspective, perioperative antibiotic administration may have a significant impact on the occurrence of postoperative complications. It is advisable to describe what and how antibiotics will be administered.

**Reply 2:** Antibiotics were used prophylactically preoperatively. Cefuroxime was routinely given 30 minutes before the operation and the first day after surgery. Redosing given was required if the operation duration exceeds 3 hours.

**Changes in the text:** We added the antibiotics use description in the 1<sup>st</sup> paragraph of ##Surgical procedure.

**Comment 3:** Figure 1 shows that a drain was placed in both cases. Is there a difference in the detection rate of bacteria in postoperative drain drainage culture tests between intraperitoneal anastomosis cases and extraperitoneal anastomosis cases? Are there any previously published papers that report on this point?

**Reply3:** Cases in two groups routinely have the postoperative drain drainage, but we did not detect the bacteria culture test for all patients. But that's a promotive suggestion which we could perform in the further study.

#### **Reviewer D**

It is very interesting and addresses an important area of research regarding the role of the immune response around surgery for cancer. I think this paper could be improved prior to publication and there are several inconsistencies that need to be addressed.

**Comment 1:** First, I think the authors need to describe clearly and early what is meant by conventional vs. total laparoscopic surgery. It wasn't until the methods that I understand that one was removing the specimens through a natural orifice vs. through the abdominal wall.

**Reply1:** We have revised the first two paragraphs, which mainly described the comparison of total laparoscopy(NOSES) and conventional laparoscopic-assisted radical resection.

Changes in the text: same as the reply1 for comment1 of reviewer B.

**Comment 2:** Second, I think the authors would benefit from reviewing the evidence for total laparoscopic surgery. What is the standard definition or terminology used to describe this? I am not familiar with natural orifice extraction being referred to as total laparoscopic surgery. This nomenclature would benefit from being standardized.

**Reply 2:** As for the standard definition, we use the natural orifice specimen extraction surgery instead of the total laparoscopic surgery.

**Comment 3:** Third, it is unclear why the authors decided to measure the blood levels they choose to use in this study. Can you provide evidence from previous studies that these are the clinically relevant blood tests for the immune system? Are they correlated with the formation of metastases?

**Reply3:** It has been hypothesized that the immunologic response might be related to the surgical outcomes, as the postoperative immune response not only responds to postoperative infection but also to tumor spread and metastases. For example, in the study of Mehigan's, patients with malignancy exhibit significant perioperative immune disturbance with laparoscopically assisted and open surgery. Tang et al. suggested that postoperative immunosuppression provides a window for cancer cell proliferation and awakening dormant cancer cells, leading to rapid recurrences or metastases. The references were added and listed as below:

*Mehigan BJ, Hartley JE, Drew PJ, et al. Changes in T cell subsets, interleukin-6 and C-reactive protein after laparoscopic and open colorectal resection for malignancy. Surg Endosc. 2001;15:1289-93.*

*Tang F, Tie Y, Tu C, Wei X. Surgical trauma-induced immunosuppression in cancer: Recent advances and the potential therapies. Clin Transl Med. 2020;10:199-223.*

**Comment 4:** Finally, the text would benefit from a more thorough discussion of the major studies informing the use of laparoscopy for colorectal cancer in general and the benefits that have been proven. It would also benefit from a discussion of the evidence for enhanced recovery after surgery and the use role of perioperative care in optimizing outcomes described in this study.

**Reply 4:** As the improvement suggestion, we added the discussion about the benefits of enhancement in the recovery in the 1<sup>st</sup> paragraph of discussion.

**Changes in the text:** In summary, total laparoscopic radical resection(NOSES) could effectively decrease the duration for the first postoperative exhaust, first postoperative defecation, resumption to semi-liquid diet, and length of postoperative hospital stay. Our results highlighted the benefits of total laparoscopic radical resection for the treatment of sigmoid and rectal cancer relative to conventional laparoscopic-assisted

radical resection.