


## Original Article

# Preliminary surgical outcomes of laparoscopic right hemicolectomy with transrectal specimen extraction: a propensity score matching study of 120 cases (with video)

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## Abstract

**Background:** Compared with conventional laparoscopic surgery, natural orifice specimen extraction surgery (NOSES) has many advantages. Laparoscopic right colectomy with transvaginal specimen extraction has been reported, but the safety and feasibility of transrectal specimen extraction in male patients with ascending colon cancer remain to be verified. This study aimed to preliminarily evaluate the feasibility and safety of laparoscopic right hemicolectomy with transrectal specimen extraction.

**Methods:** The study was conducted at a single tertiary medical center in China. A total of 494 consecutive patients who underwent laparoscopic right colectomy between September 2018 and September 2020 were included. Transrectal specimen extraction was performed in 40 male patients (the NOSES group). Patients in the NOSES group were matched to the conventional laparoscopic group using propensity score matching at a 1:2 ratio. Short-term and long-term outcomes between the two groups were compared and evaluated.

**Results:** Forty patients in the NOSES group and 80 patients in the conventional laparoscopic group were matched for analysis. Baseline characteristics were balanced after propensity matching. The operative features, including operating time, intraoperative bleeding, and the number of harvested lymph nodes, were statistically comparable in both groups. In terms of post-operative recovery, patients in the NOSES group showed preferable outcomes, as evidenced by less post-operative pain and faster return to flatus, defecation, and discharge. The post-operative complications rate, according to the Clavien–Dindo classification system, was similar in both groups. No differences in overall survival or disease-free survival were observed between the two groups.

**Conclusions:** Laparoscopic right colectomy with transrectal specimen extraction is oncologically safe. Compared with conventional laparoscopic right colectomy, it can reduce post-operative pain, accelerate post-operative recovery, shorten the hospital stay, and achieve better cosmetic effect.

**Keywords:** natural orifice specimen extraction surgery; laparoscopic right colectomy; transrectal specimen extraction

## Introduction

In recent years, the morbidity of colorectal cancer has risen significantly and, according to the latest data of the World Health Organization, both the incidence and the mortality of colorectal cancer rank third among malignant tumors, posing a severe threat to human health [1, 2]. Since Jacob performed the first laparoscopic rectal cancer surgery in 1991 [3], this technique has gained global acceptance. Laparoscopic surgery surpasses conventional laparotomy in efficacy, safety, and reduced trauma when treating colorectal cancer [4]. However, abdominal wall incisions

for specimen extraction are still associated with unexpected outcomes such as increased wound infection, hernia, and post-operative pain [5–8]. Natural orifice specimen extraction surgery (NOSES) is an innovative technique that eliminates abdominal incision. The mesenteric dissection and vascular ligation procedures for NOSES closely resemble those of conventional laparoscopic surgery, with the key difference being the intracorporeal digestive tract reconstruction [9–11] and route of specimen extraction.

Previous studies have demonstrated that NOSES offers advantages in post-operative pain control, bowel recovery, and

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cosmetic effect [12–16]. Laparoscopic right hemicolectomy with transvaginal specimen extraction has been established as safe and feasible for female patients with right colon cancer [17, 18]. However, research on laparoscopic right hemicolectomy with transrectal specimen extraction for male patients is scarce and limited to relatively small case numbers without long-term outcomes. This study aimed to preliminarily assess the feasibility and safety of laparoscopic right hemicolectomy with transrectal specimen extraction.

## Patients and methods

### Patient selection

A total of 494 consecutive patients who underwent laparoscopic right colectomy between September 2018 and September 2020 at Cancer Hospital Chinese Academy of Medical Sciences (Beijing, China) were reviewed. Ten cases of palliative resection and 15 cases of benign disease were excluded. All 40 cases in the NOSES group were male. To maintain homogeneity between the two groups, 227 female patients were excluded and the baseline data including tumor size, age, body mass index (BMI), tumor location, and abdominal surgery history were balanced by using propensity score matching (PSM) at a ratio of 1:2 (Figure 1).

Male patients who met the following criteria were considered suitable candidates for transrectal specimen extraction [19]: (i) imaging diagnosis of T1–3 colon cancer; (ii) the tumor located in the cecum, ascending colon, or colonic hepatic flexure; and (iii) maximal tumor diameter  $\leq 5$  cm. Patients with locally advanced cancer or BMI of  $>30$  kg/m<sup>2</sup> were excluded. The option of the transrectal specimen extraction was presented to the eligible candidates. After patients were fully informed of the advantages and disadvantages of both techniques, those who opted for the transrectal procedure provided informed consent.

### Data collection

After PSM, the study included 40 cases in the NOSES group and 80 cases in the laparoscopic group. The baseline data between the two groups were balanced (Supplementary Table 1). Patient demographics, disease-related features, pathological characteristics, operative characteristics, and short-term and long-term outcomes were analysed. Demographics and disease-related features encompassed age, gender, BMI, tumor location, abdominal surgery history, and preoperative ileus. We examined numerous pathological characteristics, such as pathological type, differential grade, tumor, node, metastasis (TNM) stage (based on the American Joint Committee on Cancer), tumor size, number of

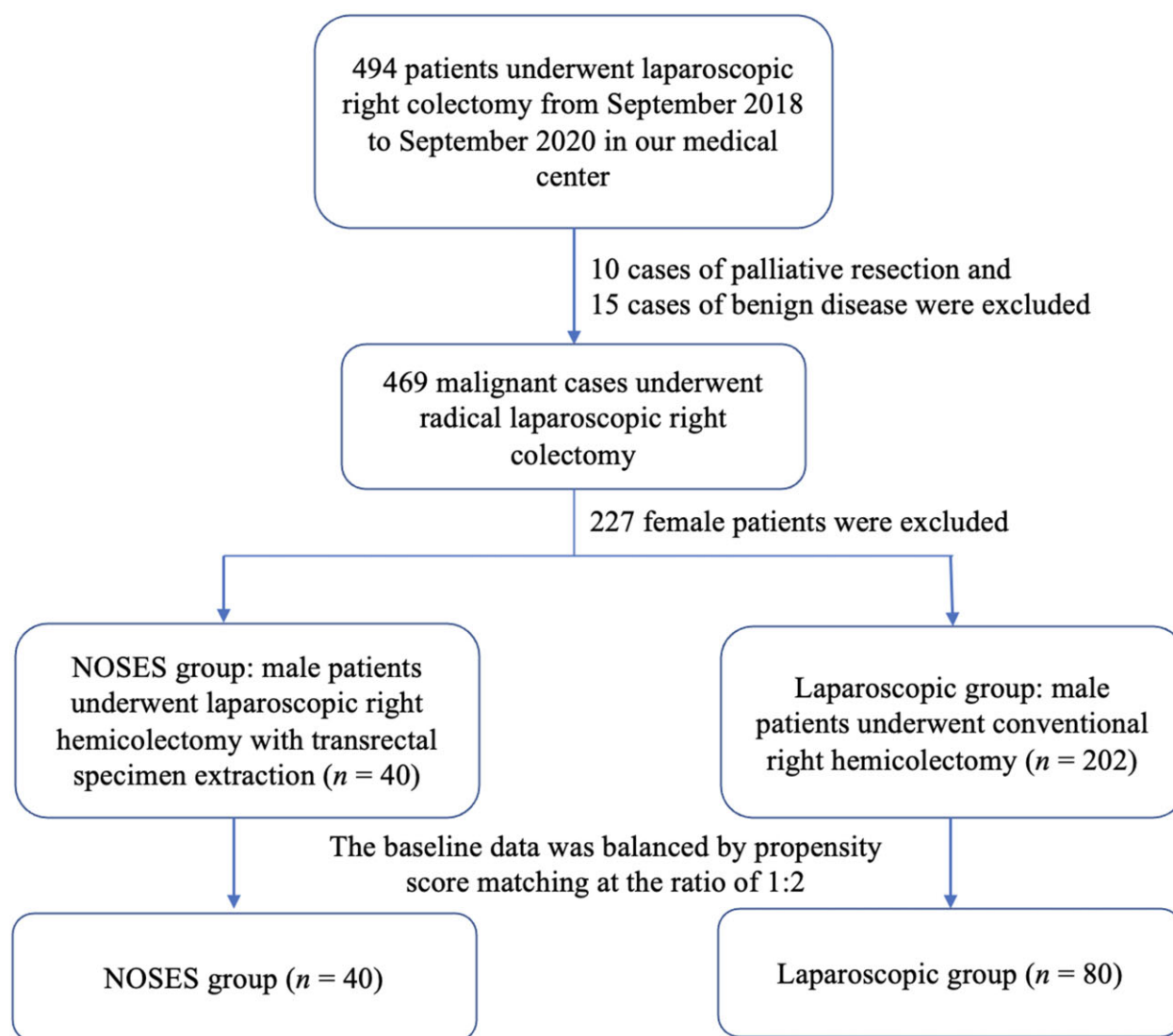


Figure 1. Flowchart of patient selection

harvested lymph nodes, and number of positive lymph nodes. Surgical characteristics encompassed the duration of surgery, estimated blood loss, length of skin incision, and rate of conversion to laparotomy. Short-term (30-day) outcomes included post-operative complications, post-operative pain score, time to first flatus, time to first defecation, post-operative length of stay, rate of reoperation, and rate of readmission. Long-term outcomes comprised disease-free survival (DFS) and overall survival (OS).

## Surgical procedures

Preoperative mechanical bowel preparation was conducted, followed by antibiotic administration. Each patient was positioned in a modified lithotomy fashion, utilizing a five-port technique.

### Laparoscopic group

The mesentery and vessels were dissected and separated according to the principle of complete mesocolic excision. The vertical periumbilical incision or Pfannenstiel incision was made and the mobilized bowel was removed through a protected mini-laparotomy. Subsequently, the terminal ileum and transverse colon were transected under direct vision, followed by a mechanical end-to-side or anti-peristaltic side-to-side anastomosis. Finally, the bowel was reintroduced to the abdominal cavity.

### NORES group

The procedures for dissection and separation prior to digestive reconstruction were identical to those performed in conventional laparoscopic right colectomy.

### Intracorporeal anastomosis

Following transection of the transverse colon and terminal ileum using endoscopic linear staplers, the specimen was placed in an aseptic bag (Figure 2A). The terminal ileum and transverse colon were arranged to overlap by ~5 cm in the same direction. An enterotomy was performed on the antimesenteric side of the ileum at the edge of the staple line (Supplementary Figure 1A). The anvil jaw of the stapler was introduced into the ileum and secured in position. This maneuver was replicated on the

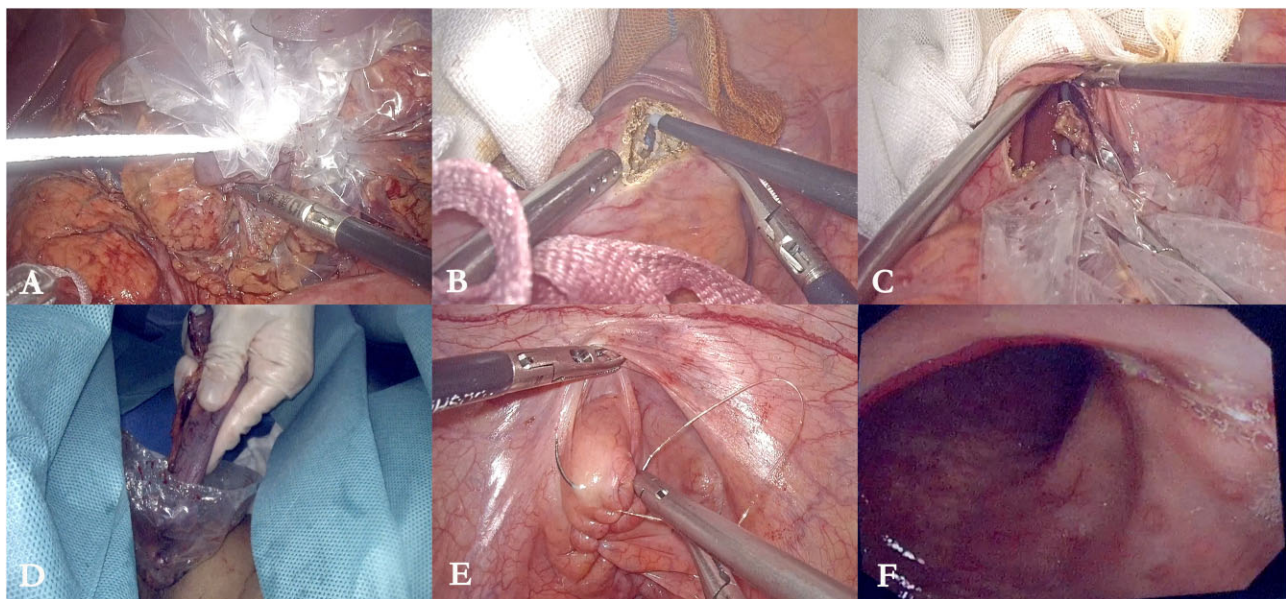
transverse colic side (Supplementary Figure 1B). Subsequently, the cartridge jaw of the stapler was inserted into the transverse colon (Supplementary Figure 1C). The stapler was fired and withdrawn, and the common enterotomy was sealed by using another linear stapler (Supplementary Figure 1D).

### Transrectal specimen extraction (Supplementary Video 1)

The assistant irrigated the rectum by injecting water and a dilute iodine solution through the anus. A longitudinal incision was made on the anterior of wall of the upper rectum (Figure 2B). The assistant employed oval forceps (Figure 2C) to extract the specimen along with the protective sleeve through the incision in the upper rectum (Figure 2D). After the complete extraction of the specimen, a full-layer running suturing was performed to close the incision (Figure 2E). Ultimately, an intraoperative colonoscopy examination was conducted (Figure 2F) to confirm the absence of leakage and stricture at the site of the suturing.

### Perioperative management and follow-up

Perioperative management was consistent across all cases. All patients received patient-controlled analgesia on Days 1 and 2, with non-steroidal anti-inflammatory drugs administered as rescue analgesics. Bowel recovery assessment was based on the passage of flatus and stool. Patients were permitted to eat upon the recovery of bowel motility. Those who were asymptomatic, tolerated three daily meals, and passed stool were deemed suitable for discharge. A follow-up phone call was conducted on the 30th day post-discharge. Adverse events that occurred within 30 days post-surgery were considered complications. All patients in the NORES group underwent colonoscopy examinations 1–3 months after surgery. In accordance with the National Comprehensive Cancer Network guideline, routine follow-up visits were scheduled 1 month post-operation, every 3 months for 2 years, and then every 6 months for 5 years. During the follow-up visits, patients underwent a comprehensive evaluation, including a medical history review, physical examination, carcinoembryonic antigen (CEA) testing, and chest, abdomen, and pelvis CT scans. Colonoscopy was performed at 1 year post-surgery. If the results



**Figure 2.** Procedures of transrectal specimen extraction. (A) The specimen is placed into the sterile protective sleeve. (B) A longitudinal incision is made on the anterior of wall of the upper rectum. (C) and (D) The oval forceps is applied to pull the specimen along with the protective sleeve out through the incision in the upper rectum. (E) The full-layer running suturing is performed to close the incision. (F) Colonoscopy examination is conducted to confirm that there is no leakage and stricture on the site of suturing.



were normal, the colonoscopy was repeated at 3 years and then every 5 years thereafter, provided no advanced adenomas were detected. In addition to routine physical examinations and complementary tests, patients were assessed using the Gastrointestinal Quality of Life Index during follow-up visits.

### Specimen quality

West's classification system was utilized to evaluate the quality of the mesentery [20]. Photographs of the resected fresh specimens were captured in the majority of cases. Based on these photographs, the specimens were categorized into three grades: Grade I, intact mesocolon; Grade II, mesocolon with lacerations; and Grade III, mesocolon with lacerations extending to the bowel. Furthermore, we measured the area of the mesocolon using Camera Measure software developed by E2ESOFT (Shanghai, China).

### Statistical analysis

Continuous variables with normal distributions are presented as mean  $\pm$  standard deviation and were analysed using Student's t-test. Non-normally distributed continuous variables are reported as median and range (minimum–maximum values). Categorical variables are expressed as percentages and were analysed by using Fisher's exact test or a chi-square test, as appropriate. A P-value of  $<0.05$  was considered statistically significant. R software was employed for PSM and SPSS software version 25.0.0 (SPSS Inc., Chicago, IL, USA) was used for all other statistical analyses.

PSM was conducted to select patients at a 1:2 ratio, with a multivariate logistic regression model applied. Covariates incorporated in the propensity score included age, BMI, tumor size, tumor location, TNM stage, and abdominal surgery history.

## Results

### Baseline data and demographic characteristics

Baseline data including BMI, tumor size, and tumor location were not balanced between the NOSES group and the conventional laparoscopic group prior to PSM (all  $P < 0.05$ ; [Supplementary Table 1](#)). After matching, 120 patients were included. The demographic and disease-related characteristics are shown in [Table 1](#). No significant differences were detected in terms of age, BMI, tumor site, TNM stage, or abdominal surgery history (all  $P > 0.05$ ).

### Operative and pathological characteristics

The operative and pathological characteristics are given in [Table 2](#) and [Supplementary Table 2](#). Although the NOSES group exhibited a numerically longer duration of surgery than the laparoscopic group, the difference was not significant ( $P = 0.894$ ). Intraoperative bleeding was similar between the two groups ( $P = 0.327$ ). Patients in the NOSES group had no abdominal incision ( $P < 0.001$ ). One case in the laparoscopic group was converted to open surgery due to intraoperative hemorrhage. No significant differences were found in the resected mesocolon, quality of specimen, TNM stage, differentiated degree, tumor size, number of harvested lymph nodes, or number of positive lymph nodes between the two groups (all  $P > 0.05$ ).

### Post-operative recovery parameters

Post-operative recovery parameters are summarized in [Table 3](#). In the laparoscopic group, two patients underwent reoperation due to anastomotic leakage and bowel obstruction. Patients in the NOSES group experienced less pain on the post-operative day

**Table 1.** Demographics and disease-related characteristics of patients in the NOSES and laparoscopic groups

Characteristic	NOSES (n = 40)	Laparoscopic (n = 80)	P-value
Gender			1.000
Male	40 (100.0%)	100 (100.0%)	
Female	0 (0.0%)	0 (0.0%)	
Age, years	61.5 (40–82)	62 (22–83)	0.762
BMI, kg/m <sup>2</sup>	22.6 $\pm$ 3.6	23.2 $\pm$ 2.8	0.267
Localization of tumor			0.715
Cecum	20 (50.0%)	34 (42.5%)	
Ascending colon	12 (30.0%)	26 (32.5%)	
Hepatic flexure	8 (20.0%)	20 (25.0%)	
Abdominal surgery history	4 (10.0%)	7 (8.8%)	1.000
Preoperative ileus	3 (7.5%)	7 (8.8%)	1.000

Values are presented as median and range (min–max values), or mean  $\pm$  standard deviation or numbers (%).

**Table 2.** Operative and pathological characteristics of patients in the NOSES and laparoscopic groups

Characteristic	NOSES (n = 40)	Laparoscopic (n = 80)	P-value
Operative time, min	170 (110–276)	165 (105–285)	0.894
Estimated blood loss, mL	20 (10–100)	20 (5–400)	0.327
Length of incision, cm	0 (0–0)	6.0 (5.0–20.0)	$<0.001$
Conversion to laparotomy	0 (0.0%)	1 (0.9%)	1.00
Pathological type			0.399
Adenocarcinoma	37 (92.5%)	77 (96.3%)	
Mucinous adenocarcinoma	3 (7.5%)	3 (3.8%)	
Differentiated degree			0.762
High	4 (10.0%)	5 (6.3%)	
Moderate	28 (70.0%)	58 (72.5%)	
Low	8 (20.4%)	17 (21.3%)	
TNM stage			0.817
I	6 (15.0%)	15 (18.8%)	
II	18 (45.0%)	37 (46.3%)	
III	16 (40.0%)	28 (35.0%)	
Tumor size	4.0 (1.5–8.0)	4.5 (1.3–12)	0.241
Harvested lymph nodes	31 (16–76)	35 (12–81)	0.578
Positive lymph nodes	0 (0–11)	0 (0–9)	0.780

Values are presented as median and range (min–max values) or numbers (%).

**Table 3.** Patients in the NOSES group experienced less post-operative pain and demonstrated faster bowel recovery than those in the laparoscopic group during post-operative recovery

Variable	NOSES (n = 40)	Laparoscopic (n = 80)	P-value
Pain score (VAS)			
POD1	2 (0–3)	3 (0–7)	$<0.001$
POD2	1 (0–2)	2 (0–4)	$<0.001$
POD3	0 (0–1)	1 (0–4)	$<0.001$
Reoperation	0 (0.0%)	2 (2.5%)	1.000
Time to first flatus (d)	2 (1–4)	3 (2–5)	$<0.001$
Time to first defecation (d)	3 (2–5)	5 (2–7)	$<0.001$
Length of stay (d)	6 (4–12)	7 (5–27)	$<0.001$
Readmission	0 (0%)	0 (0%)	1.000

Values are presented as median and range (min–max values) or numbers (%). POD, post-operative day.

and demonstrated faster recovery, as evidenced by shorter time to first flatus, defecation, and discharge (all  $P < 0.001$ ).

### Post-operative complications

All post-operative complications according to the Clavien–Dindo classification system are presented in [Table 4](#). A total of 7 (17.5%) adverse events occurred in the NOSES group and 20 (25.0%) in the

**Table 4.** Complications according to Clavien–Dindo classification

Grade	Complication	NOSES (n = 40)	Laparoscopic (n = 80)	P-value
Grade I	Pain	5 (12.5%)	14 (17.5%)	
	Fever	2	4	
	Wound infection	2	3	
	Nausea and vomit	0	2	
	Urinary infection	1	3	
	Chylous ascites	0	1	
Grade II	Blood transfusion	1 (2.5%)	2 (2.5%)	
		1	2	
Grade IIIa	Abdominal infection	1 (2.5%)	2 (2.5%)	
	Anastomotic hemorrhage	0	1	
Grade IIIb	Anastomotic leakage	1	1	
	Ileus	0 (0%)	1 (1.3%)	
		0	0	
Grade IV	ICU management	0 (0%)	1 (1.3%)	
	Mortality	0	1	
Grade V		0 (0%)	0 (0%)	0.505
Grade I–II complications		6 (15.0%)	16 (20.0%)	
Grade III–V complications		1 (2.5%)	4 (5.0%)	
Total number of complications		7 (17.5%)	20 (25.0%)	0.354

Values are presented as numbers of patients with or without a percentage (%). ICU, intensive care unit.

laparoscopic group. In the NOSES group, the incidence rates of Grade I–II and Grade III–IV complications were 15.0% and 2.5%, respectively. In the laparoscopic group, the rates of Grade I–II and Grade III–IV complications were 20.0% and 5.0%, respectively. No fatalities were recorded in either group. No significant differences were observed between the two groups in terms of the total number of complications (17.5% vs 25.0%,  $P = 0.505$ ) or the number of Grade III–V complications (2.5% vs 5.0%,  $P = 0.664$ ). No rectal incision-related complications, such as leakage or stenosis, were reported.

### Long-term outcomes

The median follow-up time for the NOSES group were 37.8 months (range, 23.2–47.4 months) and 35.3 months (range, 22.1–46.5 months) for the laparoscopic group. No difference was found in the quality of life (Supplementary Table 3). In the laparoscopic group, two patients (2.5%) presented with intraperitoneal recurrence and six (7.5%) developed distant metastasis. In the NOSES group, three patients (7.5%) presented with distant metastasis (Supplementary Table 4). Two patients (5.0%) in the NOSES group and four (5.0%) in laparoscopic the group succumbed to recurrent or metastatic disease. During the follow-up period, no significant differences were observed in OS or DFS between the two groups ( $P = 0.552$  or  $P = 0.648$ ) (Figure 3).

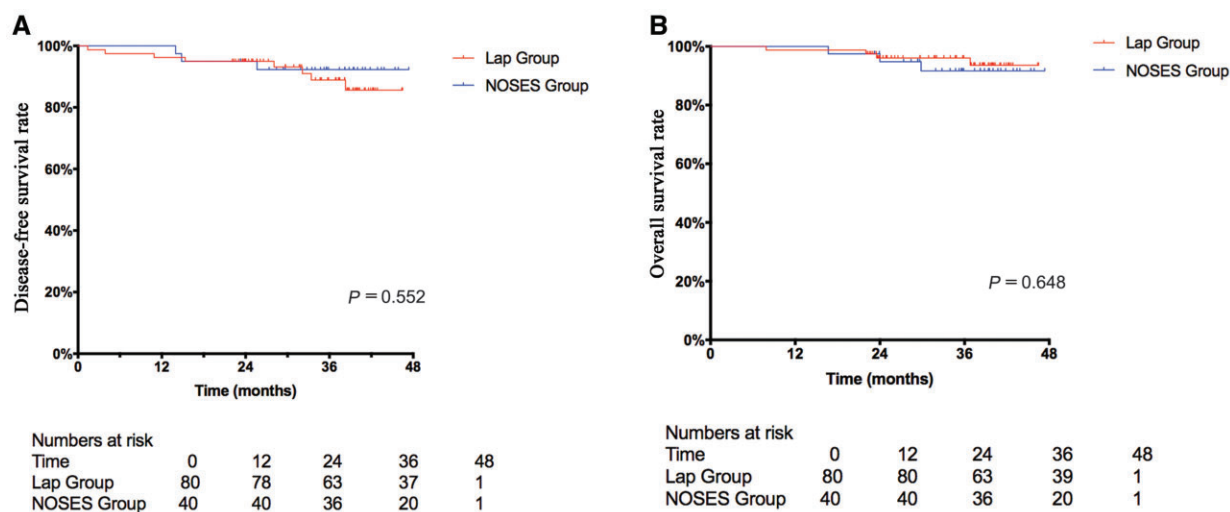
### Discussion

Over the past decade, significant advances in minimally invasive surgery have led to the emergence of various innovative techniques. NOSES, as one representative approach, has gained considerable attention as an alternative to laparoscopic surgery in selected cases. Since Stewert *et al.* [21] first reported colon resection with transvaginal specimen extraction in 1991, the safety and efficacy of NOSES in treating colorectal cancer have been increasingly accepted through 20 years of exploration, practice, and experience. Compared with conventional laparoscopic surgery, NOSES has been shown to reduce post-operative pain, accelerate bowel recovery, shorten hospital stays, and offer improved cosmetic outcomes without compromising long-term oncological outcomes [12–16]. As more evidence has accumulated, the

international NOSES consensus was published in 2019 [19], aiming to standardize and guide the procedure.

The feasibility and safety of transrectal specimen extraction for patients with left-sided colorectal cancer have been demonstrated in previous studies [22–24]. For right-sided colon cancer, some research has reported that specimen can be extracted through a vaginal incision [17, 18]; however, this method is only applicable for women, and its potential impact on sexual and reproductive function remains controversial. Other researchers described extracting the specimen through the left-sided colon and rectum with the aid of colonoscopy [25]. However, this technique is relatively challenging due to the lengthy route the specimen must travel within the colon and the surgeon's need for proficiency in colonoscopy operation. Eshuis *et al.* [26] reported a 20% failure rate for this method. In contrast, transrectal specimen extraction significantly shortened the extraction path and bypassed physiological flexures and stenosis within the colon (such as the splenic flexure and sigmoid flexure), thereby improving its success rate. In our study, all specimens were successfully extracted through a rectal incision and the average maximum tumor diameter was 4.3 cm, which is notably larger than the diameter (2.9 cm) reported in a study of laparoscopic right colectomy with transcolonic specimen extraction [25]. Consequently, transrectal specimen extraction may be more easily achievable for male patients and applicable to a broader range of cases compared with transcolonic specimen extraction.

Regarding feasibility, transrectal specimen extraction has been accepted in NOSES for rectal cancer and sigmoid colon cancer. The unique aspect of right colectomy is the creation of an incision on the rectal wall, which establishes a passageway between the abdominal cavity and the exterior. Previous studies have demonstrated the excellent elasticity and extensibility of the rectum. In this study, all specimens were smoothly extracted without any breakage or rectal rupture. We believe two factors are crucial to this success. First, patient selection is essential. Prior to surgery, careful evaluation of tumor size and stage through imaging examination is necessary. According to the international NOSES consensus [19] and the experience at our center, patients with a maximum tumor diameter of <5 cm and a tumor invasion depth of T1–3 are considered suitable candidates.



**Figure 3.** Kaplan–Meier analysis for comparison of overall survival and disease-free survival of patients in the NOSES group and the laparoscopic group. (A) Comparison of disease-free survival between the NOSES group and the laparoscopic group. (B) Comparison of overall survival between NOSES group and laparoscopic group.

Second, the skill of the specimen extraction is vital. Forceful or rough extraction may cause the specimen to break, potentially leading to tumor dissemination. Typically, we should first pull the terminal ileum out of the anus and then straighten the specimen to prevent intestinal overlapping. The specimen should be gently extracted in an “L” shape to minimize complications.

In terms of safety, the healing of the rectal incision is a significant concern. It is often assumed that an additional incision on the rectal wall might increase the risk of leakage, but this may not necessarily be the case. Theoretically, rectal leakage is influenced by factors such as blood supply [27], tension, location [28, 29], and neoadjuvant radiotherapy [29, 30]. Regarding blood supply, all vessels supplying the rectum are preserved, ensuring sufficient blood supply for the rectal incision. As for tension, the rectum is not resected during the operation and no tension is present. In terms of location, previous studies have shown that low rectal anastomosis is a risk factor for leakage. However, the rectal incision in our study was in the upper segment of the rectum. Additionally, patients with right colon cancer did not receive neoadjuvant radiotherapy. In conclusion, no risk factors related to rectal leakage are present in theory. The results of this study align with the theory, with no leakage occurring in the NOSES group. Another critical problem is achieving aseptic and tumor-free operations. Mechanical bowel preparation, intraoperative peritoneal irrigation, and intraoperative transanal lavage can effectively reduce bacterial load [31]. Placing the specimen in a sterile protective sleeve can prevent the outflow of intestinal content and contamination of the abdominal cavity. The sleeve also isolates the tumor, preventing tumor seeding and spreading during specimen extraction. The oncological safety of this technique is further supported by the long-term outcomes in our study.

In line with previous studies on NOSES, laparoscopic right colectomy with transrectal specimen extraction demonstrated several advantages in post-operative recovery and short-term outcomes, such as reduced post-operative pain, accelerated bowel function recovery, and shorter post-operative length of stay. Additionally, the absence of an abdominal incision resulted in improved cosmetic outcomes.

This study has some limitations. First, this is a retrospective study. Although we employed PSM to eliminate bias and confounding factors as much as possible, PSM itself still has

limitations and some unknown confounding factors cannot be controlled. Second, the relatively small sample size of the study limits its statistical power. Third, our study exclusively included Chinese patients, who generally have a lower prevalence of obesity than Western populations. As a result, the majority of our participants were asthenic, which may have influenced the surgical outcomes and complication rates reported in our study. Fourth, we did not perform water soluble contrast studies, which are considered the gold standard for detecting subclinical anastomotic leaks. As a result, our reported rate of anastomotic leakage may not accurately reflect the true incidence of subclinical leaks in our patient population. Fifth, as our surgical technique involved making an incision in the rectum to extract the specimen and subsequent suturing of the incision, it might potentially affect rectal capacity and compliance. Unfortunately, we did not measure these parameters preoperatively and post-operatively in our study. Sixth, we did not obtain LARS scores and fecal incontinence scores preoperatively and post-operatively, which would have provided a more comprehensive assessment of the impact of this technique on bowel function and patient quality of life.

Laparoscopic right hemicolectomy with transrectal specimen extraction is safe and feasible in selected patients. This technique resulted in less post-operative pain, faster bowel recovery, and improved cosmetic outcomes; meanwhile, it did not increase post-operative complications or compromise oncological outcome.

## Supplementary Data

Supplementary data is available at *Gastroenterology Report* online.

## Authors' Contributions

The author's contributions are as follows: X.S.W. and M.G.Z. designed this study; X.S.W., H.T.Z., Z.L., Q.L., and J.Q.T. provided the administrative support; X.S.W., H.T.Z., J.Q.T., Q.L., and P.S. provided study materials or patients; M.G.Z., P.S., X.Y.H., Z.J., and Z.L. collected and assembled data; M.G.Z. analysed and interpreted data; M.G.Z., Z.L., and X.S.W. wrote and revised the manuscript; M.G.Z. did the figure work. All authors read and approved the final version of the manuscript.

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All the clinical data were collected from the electronic records system of Cancer Hospital Chinese Academy of Medical Sciences. This study involved human participants and was approved by Ethics Committee of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, China). The reference number is NCC201803009. This study complies with the Declaration of Helsinki. All the participated patients signed informed consent. All the participated patients were informed during the follow-up and they agreed to join this research. Written informed consent for publication was obtained from all participants.

## Conflict of Interest

The authors declared that they have no conflicts of interest to this work.

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