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Original Research Article

Is Intraluminal Washout Necessary for Patients with Sigmoid Colon Cancer to Eliminate Exfoliated Cancer Cells as in Patients with Rectal Cancer? A Pilot Study at a Single Institute

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Abstract

Objectives: Rectal stump washout has been widely performed to prevent the implantation of exfoliated cancer cells (ECCs) in patients with rectal cancer. However, it remains unclear whether intraluminal washout before transection is required in patients with sigmoid colon cancer. Therefore, this pilot study was conducted to elucidate the necessity of intraluminal washout for sigmoid colon cancer patients in comparison with rectal cancer patients by cytological assessments.

Methods: A total of 16 patients with sigmoid colon cancer and 24 patients with rectal cancer who underwent sigmoidectomy or anterior resection with anastomosis using double-stapling technique were enrolled. A transanal washout sample was collected before washout and after irrigation with 500 and 1,000 mL of saline. Cytological assessments were conducted according to the Papanicolaou classification, and class IV and V cells were defined as malignant.

Results: Before washout, exfoliated cancer cells were found in 15 of 24 (62.5%) patients with rectal cancer and in 1 of 16 (6.2%) patients with sigmoid colon cancer ($p < 0.001$). Distal-free margin from the tumor was significantly shorter in patients with cancer cells ($p = 0.002$), and the length of the distal-free margin was significantly associated with the tumor location. After irrigation with 500 and 1,000 mL of saline, no cancer cell was found in all patients with sigmoid colon cancer, whereas ECCs were still found in five patients with rectal cancer (20.8%).

Conclusions: Intraluminal washout with 1,000 mL may be sufficient for sigmoid colon cancer patients with longer distal-free margin. A large-scale, randomized controlled study is necessary to confirm these results.

Keywords

distal margin, exfoliated cancer cells, intraluminal washout, sigmoid colon cancer, rectal cancer

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Introduction

Postoperative local recurrence of colorectal cancer causes severe symptoms and leads to poor prognosis. Local recurrence also includes anastomotic recurrence, and previous studies have reported the presence of intraluminal exfoliated cancer cells (ECCs) in patients with colorectal cancer[1,2]. Furthermore, it has been suggested that intraluminal ECCs are viable and that their implantation during surgery in patients with colorectal cancer can cause suture-line recur-

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rence[2-5].

Intraluminal washout is widely performed to prevent the implantation of ECCs in patients with rectal cancer. Although several studies, including prospective clinical studies[6] and meta-analyses[7-11], have evaluated the efficacy of intraluminal washout in preventing local recurrence, it still remains controversial, and the guidelines of the American Society of Colon and Rectal Surgeons indicate that rectal washout for patients with rectal cancer has been weakly recommended based on low-quality evidence. Moreover, although some studies have described an efficient volume of irrigation fluid to eliminate ECCs[11-13], their sample sizes were small, and there was no standard adequate volume of irrigation fluid.

In some institutions, including ours, transanal intraluminal washout is performed even for patients with sigmoid colon cancer who have undergone sigmoidectomy with anastomosis through a double-stapling technique (DST). However, there is limited information on the benefits of this procedure for patients with sigmoid colon cancer. Therefore, we conducted this pilot study to elucidate the necessity of intraluminal washout for patients with sigmoid colon cancer in comparison with patients with rectal cancer by performing cytological assessments.

Methods

Study population

In this prospective observational study, we recruited a total of 40 consecutive patients with sigmoid colon cancer or rectal cancer who underwent sigmoidectomy or anterior resection with anastomosis through DST at the University of Yamanashi (Yamanashi, Japan) between July 2018 and March 2020. Of these 40 patients, 16 and 24 had sigmoid colon cancer and rectal cancer, respectively. The clinicopathological details of these patients were obtained from hospital records and analyzed. The tumor location was diagnosed by enema examination and defined on the basis of the lower edge of the tumor, and the rectum was divided into three sites, viz., recto-sigmoid (RS), upper rectum (above the peritoneal reflection, Ra), and lower rectum (below the peritoneal reflection, Rb), according to the Japanese Classification of Colorectal Carcinoma[14]. In our institute, a standard preoperative bowel preparation includes a combination of magnesium citrate (250 mL) and a sodium picosulfate solution (0.75%, 10 mL). A length of distal-free margin was measured on a resected specimen that was gently stretched and fixed with pins. An undifferentiated histological type comprised a poorly differentiated, mucinous adenocarcinoma and signet-ring cell carcinoma. The protocol for this research project has been approved by a suitably constituted ethics committee of the institution and conforms to the provisions of the Declaration of Helsinki. All informed consent was obtained from the subjects and/or guardians.

Intraluminal washout and sample collection

The length of the distal-free margin was determined according to the location of the tumor as follows: 10 cm at the sigmoid colon, 3 cm at RS and Ra, and 2 cm at Rb[14]. Before dissection, the distal rectum was clamped to occlude the rectal stump to the tumor. An intestinal washout was performed using a transanally inserted nelaton catheter (8.5 mm in diameter) (Izumo Health Co., Ltd., Japan) and normal saline. During the washout, samples were collected at three time points, i.e., before washout and after irrigation with 500 and 1,000 mL of saline.

Cytology

The collected samples were centrifuged at 3,000 rpm for 5 min, and the cell clots were examined after Papanicolaou's staining. Two experienced cytotechnologists analyzed the stained samples to confirm the diagnosis, after which an additional check was conducted by a pathologist. Finally, the samples were classified according to Papanicolaou's classification as follows: samples belonging to classes I, II, and III were categorized as non-malignant and those belonging to classes IV and V as malignant (Figure 1).

Statistical analysis

Fisher's exact test was employed to evaluate the differences in proportions, and Mann-Whitney *U* test was used to evaluate continuous variables. A p-value < 0.05 was considered to be statistically significant. The JMP statistical software package (JMP, version 11, SAS Institute Inc., Cary, NC, USA) was used for data analysis.

Results

Table 1 presents the clinicopathological characteristics of patients with sigmoid colon cancer and those with rectal cancer. Of the 24 patients with rectal cancer, the anal edge of the tumor was located at RS, Ra, and Rb in 10, 8, and 6 patients, respectively. Of the 24 patients, 2 (8.3%) with rectal cancer had received preoperative treatment, whereas none of those with sigmoid colon cancer underwent preoperative treatment. No significant difference was observed in the extent of preoperative bowel preparation, surgical approach, tumor size, histology, and depth of the tumor between the two groups of patients. On the basis of the guidelines described in the Methods section, the distal margin in patients with sigmoid colon cancer was significantly longer than that in patients with rectal cancer ($p < 0.001$).

Figure 2 presents the positive staining rates of ECCs in the irrigation fluid sample collected at the abovementioned three time points. Before washout, the positive staining rate

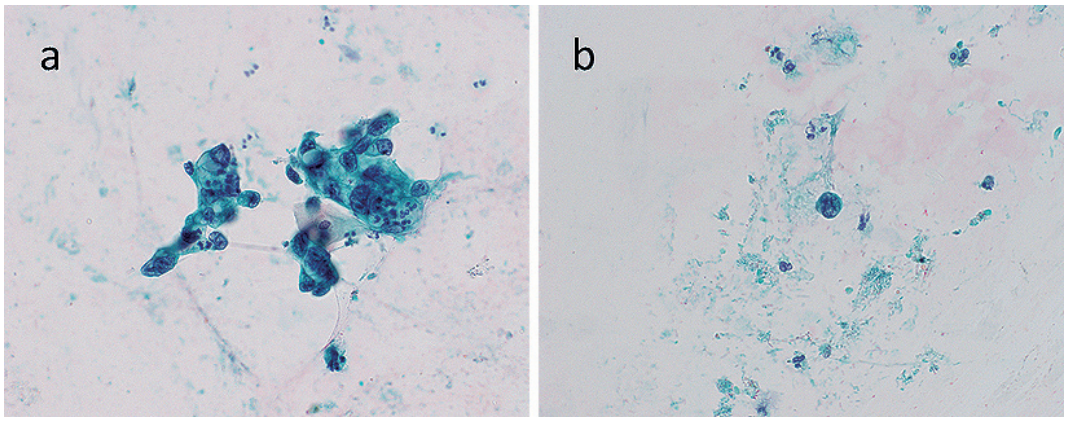

Figure 1. Cytological assessment for ECCs was conducted according to Papanicolaou's classification. a: Class V (malignant), b: Class II (non-malignant).

Table 1. Characteristics of Patients with Sigmoid Colon Cancer and Rectal Cancer.

Variable	Level	Sigmoid colon (n = 16)	Rectum (n = 24)	*p*-value
Sex	Male	9 (56.3)	14 (58.3)	1
	Female	7 (43.8)	10 (41.7)	
Age, years		67.9 ± 8.3	65.0 ± 9.8	0.326
Bowel preparation	Normal	11 (68.8)	19 (79.2)	0.482
	Reduced or none	5 (31.3)	5 (20.8)	
Surgical approach	Open	2 (12.5)	2 (8.3)	0.468
	Laparoscopy	14 (87.5)	20 (83.3)	
	Robot-assisted	0	2 (8.3)	
Distal-free margin, cm		10.7 ± 6.6	3.2 ± 1.3	<0.001
Tumor size, mm		50.5 ± 14.6	42.3 ± 23.3	0.177
Undifferentiated histology	Included	3 (18.8)	9 (37.5)	0.297
	Not included	13 (81.3)	15 (62.5)	
Depth of tumor	T1	0	4 (16.7)	0.287
	T2	2 (12.5)	3 (12.5)	
	T3	10 (62.5)	15 (62.5)	
	T4a	3 (18.8)	2 (8.3)	
	T4b	1 (6.3)	0	

Values are n (%) or mean ± SD unless otherwise indicated.

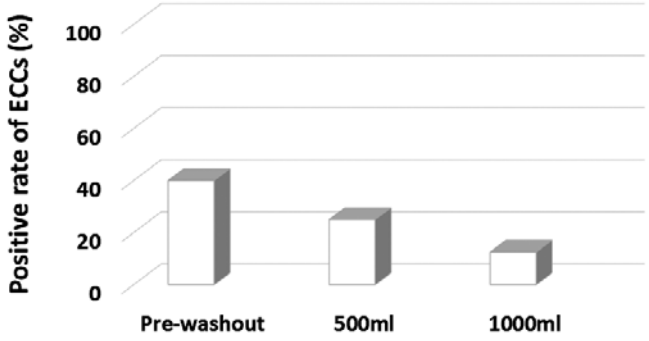

Figure 2. The positive staining rates of ECCs gradually decreased as the amount of irrigation fluid increased in all the 40 study patients.

was 40.0% in all the 40 study patients. However, as the amount of irrigation fluid increased, the positive staining rates gradually decreased (25.0% after irrigation with 500 mL of saline and 12.5% after irrigation with 1,000 mL of saline).

The probable risk factors responsible for the presence of ECCs before washout are presented in Table 2. Although age, sex, tumor size, status of preoperative bowel preparation, histology, and depth of tumor had no significant differences between the two groups, we observed that patients with sigmoid colon cancer ($p < 0.001$) and a longer distal-free margin ($p = 0.002$) exhibited a significantly less positive staining rate of ECCs.

Comparing the positive staining rates of ECCs between patients with sigmoid colon cancer and those with rectal

Table 2. Clinicopathological Risk Factors for the Detection of ECCs before Washout.

Variable	Level	Non-malignant (n = 24)	Malignant (n = 16)	*p*-value
Sex	Male	15 (62.5)	8 (50.0)	0.522
	Female	9 (37.5)	8 (50.0)	
Age, years		67.2 ± 8.4	64.6 ± 10.4	0.422
Tumor location	Sigmoid colon	15 (62.5)	1 (6.3)	<0.001
	Rectum	9 (37.5)	15 (93.8)	
Bowel preparation	Normal	16 (66.7)	14 (87.5)	0.263
	Reduced or none	8 (33.3)	2 (12.5)	
Distal-free margin, cm		8.0 ± 6.5	3.6 ± 2.2	0.002
Tumor size, mm		45.6 ± 20.8	45.5 ± 20.6	0.990
Undifferentiated histology	Included	7 (29.2)	5 (31.3)	1.000
	Not included	17 (70.8)	11 (68.8)	
Depth of tumor	T1-2	4 (16.7)	5 (31.3)	0.441
	T3-4	20 (83.3)	11 (68.8)	

Values are n (%) or mean ± SD unless otherwise indicated.

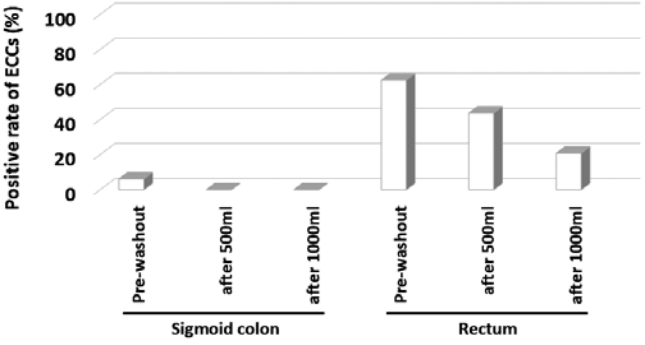

Figure 3. In patients with sigmoid colon cancer, ECCs rarely existed before washout and disappeared after irrigation with 1,000 mL of saline. By contrast, some patients still had ECCs even after irrigation with 1,000 mL of saline in patients with rectal cancer.

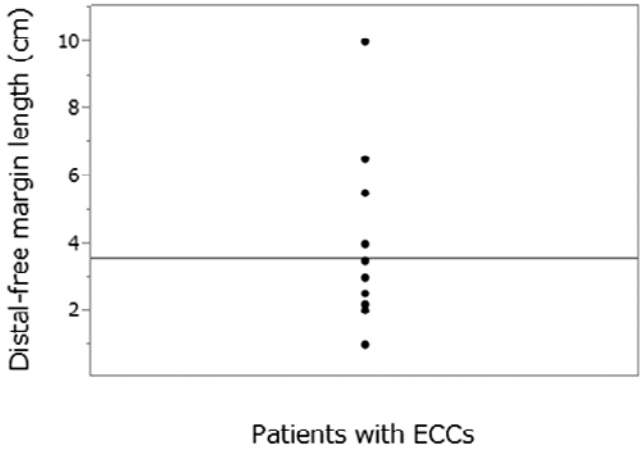

Figure 4. The average length of distal-free margin in patients with ECCs before washout was 3.6 cm with a range of 1.0-10.0 cm.

cancer, we found that the positive staining rate of ECCs before washout was 6.2% (1/16), but there were no malignant findings after irrigation with 500 and 1,000 mL of saline in patients with sigmoid colon cancer (Figure 3). By contrast, 62.5% of patients with rectal cancer had ECCs before washout, and 20.8% still had ECCs after irrigation with 1,000 mL of saline. The lengths of distal-free margin in patients with ECCs before washout were plotted and presented in Figure 4. The distal-free margin of one sigmoid colon cancer patient with ECCs before washout was 10.0 cm.

Discussion

To the best of our knowledge, this is the first study conducted to elucidate the effectiveness of intraluminal washout in eliminating ECCs in patients with sigmoid colon cancer by comparing it with that in patients with rectal cancer. This study demonstrated that patients with rectal cancer required rectal washout with ≥1,000 mL saline irrigation, whereas intraluminal washout with ≥1,000 mL saline irrigation might not be essential for patients with sigmoid colon cancer in whom ECCs rarely existed at the dissection line. This was mainly caused by the longer distal-free margin in patients with sigmoid colon cancer than that in patients with rectal cancer.

ECCs were previously identified at the anastomotic site with high viability in colorectal cancer[1] and detected in the washing fluid of surgical staplers and doughnuts after anterior resection. Gertsch et al. suggested that DST resulted in anastomotic recurrence more often than other anastomotic procedures, such as single stapling and hand suturing[2]. Moreover, studies have reported that a possible mechanism of local recurrence following colorectal cancer surgery was that ECCs existed in the lumen and implanted into the anas-

tomosis[2,5]. Several studies have also demonstrated that human colorectal ECCs could develop into distant metastases in *in vivo* models[3,4]. Taken together, because DST anastomosis during sigmoidectomy or anterior resection has a risk of ECC implantation into the anastomosis, intraluminal washout may be necessary to eliminate ECCs to decrease the risk of local recurrence.

For rectal cancer, the majority of surgeons consider the advantage of performing an intraoperative rectal washout[15], and several studies have also investigated this approach[8-12,16]. Based on short-term results obtained by cytological assessments, Maeda et al. reported that ECCs were detected in 97% of 30 patients in the pre-washout sample[12]. Our study also revealed that 62.5% of 24 patients with rectal cancer had ECCs before rectal washout. Furthermore, in terms of long-term results, Kodera et al. investigated 4,677 patients with rectal cancer and found that those who received rectal washout had significantly lower rates of local recurrence than those who did not (6.0% versus 10.2%, $p < 0.001$), and the absence of rectal washout was the independent risk factor for local recurrence[16]. Several studies have also referred to an efficient volume of irrigation fluid for patients with rectal cancer[11-13]. For instance, Maeda et al. reported that ECCs decreased gradually with increasing irrigation volumes of up to 2,000 mL[12]. Similarly, Zhou et al. recommended 1,500 mL of normal saline to reduce the risk of local recurrence[11]. In our study, we found that ECCs still remained in 20.8% of patients with rectal cancer following rectal washout with 1,000 mL of saline, indicating that we need to reconsider the volume of rectal washout.

By contrast, several studies have demonstrated no clear efficacy of rectal washout in preventing local recurrence[6,17-20]. For instance, Terzi et al. conducted a prospective clinical study on 96 patients with colorectal cancer undergoing anterior resection and concluded that rectal washout did not decrease the rate of local recurrence[6]. One possible reason for this negative result was that the irrigation fluid used for the rectal washout was only 500 mL. In addition, our literature search revealed five previous meta-analyses that were conducted to elucidate whether rectal washout was effective in reducing local recurrence[7-11]. Of these, one meta-analysis reported no significant difference, but the remaining four meta-analyses reported that patients who received rectal washout had significantly lower rates of local recurrence than those who did not. Therefore, whether rectal washout is clinically effective in preventing local recurrence still remains a controversial issue.

A previous study reported that tumor depth had no association with a positive rate of ECCs[13]. Our study also revealed that ECCs were found in 5 of 9 patients with T1 or T2 tumor. These results suggested that intraluminal washout should be considered even for patients with T1 or T2 tumor.

It is also not clear whether the efficacy of intraluminal washout is affected by the tumor location. Maeda et al. concluded that no ECCs were detected after irrigation with 1,500 mL in patients with tumor at Rb, whereas at least 2,000 mL of irrigation fluid was recommended for patients with a tumor at RS or Ra[12]. However, no study has yet elucidated the effectiveness of intraluminal washout in eliminating ECCs in patients with sigmoid colon cancer, and to our knowledge, this is the first study to investigate this aspect in comparison with patients with rectal cancer. Our results demonstrated obvious differences in cytological findings between patients with sigmoid colon cancer and those with rectal cancer, and even before washout, ECCs were rarely detected in patients with sigmoid colon cancer. As the transection line was determined according to the tumor location, the length of the distal-free margin was essentially 10 cm at the sigmoid colon and 2-3 cm at the rectum. Our study also indicated the strong correlation between a longer distal-free margin and less positive cytology in patients with sigmoid colon cancer. From these results, the long distal-free margin should have a strong impact on the positive rate of ECCs.

Some limitations still exist in this study. First, this study was a pilot study and included a small number of patients in each group. Second, only short-term results were examined through a cytological approach. Local recurrence and overall survival rates should be followed up as long-term results to indicate the significance of ECCs. Third, the volume of irrigation fluid used for intraluminal washout was up to 1,000 mL. Although patients with sigmoid colon cancer had no ECCs after irrigation with 1,000 mL of saline, ECCs were found in patients with rectal cancer. Therefore, the efficacy of a larger volume of irrigation fluid must be examined, especially in patients with rectal cancer. A large-scale, randomized controlled study should be conducted in the future to determine short- and long-term results of intraluminal washout for patients with sigmoid colon cancer and those with rectal cancer.

In conclusion, the presence of ECCs correlates with the length of distal-free margin, and rectal washout with ≥1,000 mL of saline irrigation is necessary for patients with rectal cancer, whereas intraluminal washout with 1,000 mL of saline irrigation may be sufficient for those with sigmoid colon cancer.

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Conflicts of Interest

Daisuke Ichikawa received honoraria as a partial support from Company Johnson & Johnson. The remaining authors declare no Conflict of Interests for this article.

Author Contributions

Hiroki Shimizu drafted the manuscript. Makoto Sudo, Shinji Furuya, Koichi Takiguchi, Ryo Saito, Suguru Maruyama, Yoshihiko Kawaguchi, and Hiromichi Kawaida collected samples of intraluminal washout. Tetsuo Kondo performed cytological assessment of washout-fluid samples. Hiroki Shimizu and Daisuke Ichikawa conceived and designed the study and edited the manuscript. All authors read and approved the final version of this manuscript.

Approval by Institutional Review Board (IRB)

Committee of the University of Yamanashi, Approval No. 1931.

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