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

Hiroki Shimizu¹,², Makoto Sudo¹, Shinji Furuya¹, Koichi Takiguchi¹, Ryo Saito¹, Suguru Maruyama¹, Yoshihiko Kawaguchi¹, Hiromichi Kawaida³, Tetsuo Kondo³ and Daisuke Ichikawa¹

¹) First Department of Surgery, Faculty of Medicine, University of Yamanashi, Chuo, Japan
²) Division of Digestive Surgery, Department of Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan
³) Department of Pathology, Faculty of Medicine, University of Yamanashi, Chuo, Japan

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

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-
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 adequate standard 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).

Figure 2. The positive staining rates of ECCs gradually decreased as the amount of irrigation fluid increased 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...
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 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.

**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.

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-
In intraluminal washout for sigmoid colon cancer, 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.

Acknowledgements

The authors thank Dr. Kunio Mochizuki, Kumiko Nakazawa, and Yuki Hanai from Department of Pathology, University of Yamanashi, for the cooperation with cytological assessment of washout-fluid samples performed in this study. The authors thank Dr. Yuki Nakata from First Department of Surgery, University of Yamanashi, for the cooperation with sample collection.
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.

References
1. Umpleby HC, Fermor B, Symes MO, et al. Viability of exfoliated colorectal carcinoma cells. The British journal of surgery. 1984 Sep; 71(9): 659-63.
2. Gertsch P, Baer HU, Kraft R, et al. Malignant cells are collected on circular staplers. Diseases of the colon and rectum. 1992 Mar; 35(3): 238-41.
3. Symes MO, Fermor B, Umpleby HC, et al. Cells exfoliated from colorectal cancers can proliferate in immune deprived mice. British journal of cancer. 1984 Sep; 50(3): 423.
4. Fermor B, Umpleby HC, Lever JV, et al. Proliferative and metastatic potential of exfoliated colorectal cancer cells. Journal of the National Cancer Institute. 1989 Apr; 76(2): 347-9.
5. McGregor JR, Galloway DJ, McCulloch P, et al. Anastomotic suture materials and implantation metastasis: an experimental study. British journal of surgery. 1989 Apr; 76(4): 331-4.
6. Terzi C, Unek T, Sagol O, et al. Is rectal washout necessary in anterior resection for rectal cancer? A prospective clinical study. World journal of surgery. 2006 Feb; 30(2): 233-41.
7. Constantinides VA, Cheetham D, Nicholls RJ, et al. Is rectal washout effective for preventing localized recurrence after anterior resection for rectal cancer? Diseases of the colon and rectum. 2008 Sep; 51(9): 1339-44.
8. Rondelli F, Trastulli S, Cirocchi R, et al. Rectal washout and local recurrence in rectal resection for cancer: a meta-analysis. Colorectal Disease. 2012 Nov; 14(11): 1313-21.
9. Matsuda A, Kishi T, Musso G, et al. The effect of intraoperative rectal washout on local recurrence after rectal cancer surgery: a meta-analysis. Annals of surgical oncology. 2013 Mar; 20(3): 856-63.
10. Zhou C, Ren Y, Li J, et al. Systematic review and meta-analysis of rectal washout on risk of local recurrence for cancer. Journal of surgical research. 2014 Jun; 189(1): 7-16.
11. Zhou C, Ren Y, Li J, et al. Association between irrigation fluids, washout volumes and risk of local recurrence of anterior resection for rectal cancer: a meta-analysis of 427 cases and 492 controls. PloS one. 2014; 9(5): e95699.
12. Maeda K, Maruta M, Hanai T, et al. Irrigation volume determines the efficacy of “rectal washout”. Diseases of the colon and rectum. 2004 Oct; 47(10): 1706-10.
13. Dafnis G, Nordstrom M. Evaluation of the presence of intraluminal cancer cells following rectal washout in rectal cancer surgery. Techniques in coloproctology. 2013 Aug; 17(4): 363-9.
14. PMC JE, API GR. Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma: the 3d English Edition [Secondary Publication].
15. Simillis C, Mistry K, Prabhudesai A. Intraoperative rectal washout in rectal cancer surgery: a survey of current practice in the UK. International Journal of Surgery. 2013 Nov; 11(9): 993-7.
16. Kodera K, Holmberg E, Jorgren F, et al. Rectal washout and local recurrence of cancer after anterior resection. British journal of surgery. 2010 Oct; 97(10): 1589-97.
17. Agaba EA. Does rectal washout during anterior resection prevent local tumor recurrence? Diseases of the colon and rectum. 2004 Mar; 47(3): 291-6.
18. Jorgren F, Johansson R, Armadottir H, et al. The importance of rectal washout for the oncological outcome after Hartmann’s procedure for rectal cancer: analysis of population-based data from the Swedish Colorectal Cancer Registry. Techniques in coloproctology. 2017 May; 21(5): 373-81.
19. Moosvi SR, Manley K, Hernon J. The effect of rectal washout on local recurrence following rectal cancer surgery. The Annals of the Royal College of Surgeons of England. 2018 Feb; 100(2): 146-51.
20. Xingmao Z, Jianjun B, Zheng W, et al. Analysis of outcomes of intra-operative rectal washout in patients with rectal cancer during anterior resection. Medical oncology. 2013 Mar; 30(1): 386.