Introduction

High image quality and image-enhanced endoscopy (IEE) in colonoscopy have improved the detection rate for colorectal adenomas, removal of which can reduce the risk of colorectal cancer (CRC) and associated mortality [1, 2]. Colorectal adenomas and early CRC mainly have been treated with endoscopic mucosal resection (EMR) or hot snare polypectomy (HSP) with electrosurgical generators. Cold snare polypectomy (CSP) without electrcautery is effective for removal of small colorectal adenomas [3–7]. This procedure is characterized by shorter procedure times [3, 4], less postoperative abdominal pain [5], and fewer post-bleeding events in patients on antithrombotic therapy [6, 7] than EMR and HSP. Therefore, CSP has been widely performed in the last decade.

There have been some reports that pathological horizontal margin evaluation cannot be diagnosed in CSP specimens [8–12]. However, after resection of the tumor with CSP, there was
almost no residual tumor [4, 8]. The European Society of Gastrointestinal Endoscopy (ESGE) clinical guidelines for post-polypectomy colonoscopy surveillance recommend early repeat colonoscopy for patients with polyps who have indefinite resection margins at pathology [13, 14]. Inaccurate diagnosis of CSP specimens may be detrimental to patients and improved pathological diagnosis is needed of horizontal margins in CSP specimens.

Specimen fragmentation is the reason for unclear pathological diagnosis of CSP specimens [12]. In addition, we hypothesized that another reason is that CSP specimens are more cramped than EMR specimens. Therefore, we devised a method of pasting the CSP specimens onto filter paper. In previous studies, we successfully improved the pathological diagnosis of horizontal margins using this specimen pasting method [15]. The aim of this study was to prospectively investigate the efficacy of specimen pasting after CSP for pathological evaluation of horizontal margins.

**Patients and methods**

**Study design and protocol**

This single-center, prospective, randomized study was conducted at Osaka Saiseikai Nakatsu Hospital. Colorectal polyps resected by CSP were included in the study between February 1, 2019 and March 31, 2019 after the exclusion of cases fulfilling the following criteria (Fig. 1): colorectal polyp diameter $\geq 10$ mm, polyps other than narrow-band imaging (NBI) international colorectal endoscopic type 2 [16], fragmented specimens, history of colorectal surgery, lost lesions, and failure to follow the study protocol. In this study, the indications for CSP were adenomas $\leq 10$ mm. Enrolled CSP specimens were randomized to the pasting and non-pasting groups using the envelope method. If a patient had multiple polyps resected by CSP, each polyp was randomized on a per-polyp basis. In the pasting group, specimens extended with a toothpick were pasted on filter paper (FILTER PAPER No2; ADVANTEC, Tokyo, Japan) and fixed in 10% buffered formalin. In the non-pasting group, specimens extended with a toothpick were pasted on filter paper (FILTER PAPER No2; ADVANTEC, Tokyo, Japan) and fixed in 10% buffered formalin. In previous studies, we successfully improved the pathological diagnosis of horizontal margins using this specimen pasting method [15]. The aim of this study was to prospectively investigate the efficacy of specimen pasting after CSP for pathological evaluation of horizontal margins.

**Colonoscopy**

For bowel preparation, the patients were administered sennoside the day before colonoscopy and polyethylene glycol electrolyte solution (mobiprep; EA Pharma, Tokyo, Japan) on the day of colonoscopy. All colonoscopy procedures were performed under conscious sedation with midazolam (Sandoz, Tokyo, Japan) and pethidine hydrochloride (Takeda, Tokyo, Japan) by an endoscopist and assisted by a nurse and endoscopy assistant. Oxygen saturation, blood pressure, and pulse rate were monitored during colonoscopy. All procedures were performed by one of 14 gastrointestinal endoscopists (6 specialists and 8 trainees) and assisted by one of eight endoscopy assistants (3 technicians and 5 non-technicians). A colonoscopy (CF-H290I or CF-HQ290I; Olympus Medical Systems, Tokyo, Japan or EC-L590ZW; Fuji Film, Tokyo, Japan) was used with carbon dioxide insufflation. Polyps found on colonoscopy were diagnosed with white light imaging and IEE (NBI or blue laser imaging). The method of polyp resection was selected by the endoscopist. All polyps underwent CSP using the same snare (10-mm, round, stiff Captivator II snare; Boston Scientific, Marlborough, Massachusetts, United States), and was snared to contain normal mucosa around the polyp. Lesion size was estimated based on endoscopic findings and snare opening, and the endoscopic
treatment findings were reported using the NEXUS endoscopy reporting system (Fuji Film).

**Specimen processing after CSP**

CSP specimens were collected in a bottle (Suction polyp trap MH-14; Olympus, Tokyo, Japan) attached to a suction tube via the endoscope working channel. Fragmentation of the collected specimens was evaluated by the endoscopy assistant, and fragmented specimens were excluded from the study. The same endoscopy assistant was responsible for the process from specimen collection to formalin fixation. Specimen processing was performed after all procedures. In the pasting group, the CSP specimen was carefully extended with a toothpick on a plastic plate, and the cut specimen surface was placed on a 20-mm square of filter paper with good water absorbency and pasted. The specimens on filter paper were fixed with 10% buffer formalin (Fig. 2). In the non-pasting group, the specimens were formalin-fixed without pasting. After fixation, the specimens in both groups were serially sectioned at 2-mm intervals, and pathological diagnosis was performed based on evaluation of hematoxylin-eosin-stained samples. Technologists in the pathology laboratory prepared each specimen for pathological diagnosis, which was made by a single pathologist.

**Definitions**

Unclear horizontal margin was defined as involvement of a horizontal margin that could not be pathologically assessed. Fragmented specimens were defined as piecemeal resected specimens, or specimens split during collection. Polyp morphology was defined as protruding for 0-Ip and 0-Is lesions and as superficial for 0-IIa lesions according to the Paris classification [17]. Endoscopy specialists were defined as endoscopists accredited by the Japan Gastroenterological Endoscopy Society; other endoscopists were defined as trainee endoscopists. Gastroenterological endoscopy technicians were defined as assistants accredited by the Japan Gastroenterological Endoscopy Technician Society. Bleeding was defined as postoperative bleeding requiring endoscopic hemostasis. Perforation was defined as confirmed loss of muscle layer at the ulcer floor during colonoscopy or presence of free air confirmed by abdominal radiograph and computed tomography after colonoscopy. Regarding polyp locations, cecum, ascending colon, and transverse colon were defined as right colon and descending colon, sigmoid colon, and rectum were defined as left colon. Lost lesions were defined as specimens that could not be collected after CSP.

**Study outcomes**

The primary endpoint was rate of unclear horizontal margins in lesions resected by CSP. Secondary endpoints were lesion size, location (right colon or left colon), morphology (protruding
type or superficial type), operator (specialist or trainee), endoscopy assistant (technician or non-technician), and adverse events (AEs). If a lethal AE occurred, the study would be halted. The study outcomes were not changed after the trial commencement.

Statistical analysis

The optimal sample size in the present study was estimated based on the rate of unclear horizontal margins in CSP lesions. A preliminary retrospective study at the study institution demonstrated that rates of unclear horizontal margins in the pasting and non-pasting groups were 31% and 50%, respectively (data not published). Therefore, a total of 104 cases were required in each group, considering an \( \alpha \) of 0.05 and power of 0.8. In a previous study, approximately 13% of CSP lesions predicted to be adenomas by endoscopic findings changed to non-adenomas by pathological diagnosis. Therefore, we aimed to include 240 lesions.

Categorical data were presented as proportions, and differences between groups were assessed using Fisher’s exact test. Non-normally distributed continuous variables were presented as medians with interquartile ranges (IQRs) and compared using Wilcoxon’s rank-sum test. Factors associated with unclear horizontal margins were analyzed using univariate and multivariate logistic regression analyses. All P-values were two sided, and \( P<0.05 \) was considered statistically significant. No interim analysis was performed because of the short study period. All data were analyzed with JMP version 11 (SAS Institute, Cary, North Carolina, United States).

Results

Study flow

Between February 1, 2019 and March 31, 2019, 522 lesions in 238 patients were removed by CSP. Recruitment ended when the number of polyps had reached the predetermined number and study participants were followed up to June 30, 2019. Of these, 282 lesions were excluded for the following reasons: polyps other than NICE type 2 (n = 80), fragmented specimens (n = 154), history of colorectal surgery (n = 39), lost lesion (n = 1), Other reasons (n = 8). Therefore, the study included 240 lesions from 142 patients. Recruitment ended when the number of polyps had reached the predetermined number and study participants were followed up to June 30, 2019. After the exclusion of 20 hyperplastic polyps, three sessile serrated lesions (SSls), and one inflammatory polyp based on the pathological diagnosis after CSP, 106 lesions in the pasting group, and 110 lesions in the non-pasting group were included in the final analysis (Fig.1).

Characteristics of patients and lesions

Patient and lesion characteristics in all, pasting, and non-pasting groups are shown in Table 1. The ratio of protruding/superficial lesions was 66 to 40 in the pasting group and 89 to 21 in the non-pasting group, and the ratio of superficial type lesions was significantly higher in the pasting group (\( P=0.003 \)). Sex, age, lesion location, lesion size, pathological diagnosis, AEs, and the skill levels of operators and endoscopy assistants were not significantly different between the two groups.

Clinical outcomes

The number of lesions with unclear horizontal margins was significantly lower in the pasting group (n = 16 [15.1%]) than in the non-pasting group (n = 37 [33.6%]) (Fig.3). Among the CSP specimens with clear horizontal margins, 75 lesions (70.8%) in the pasting group and 67 lesions (60.9%) in the non-pasting group had negative horizontal margins. Sixteen specimens with unclear horizontal margins in the pasting group were improperly sectioned.

Univariate and multivariate analyses of factors associated with unclear horizontal margin involvement

The results of univariate and multivariate analyses to determine factors associated with unclear horizontal margins are shown in Table 2. On univariate analysis, in the non-pasting group, a lesion in the right colon, lesion size of 1 to 4 mm, and low-grade adenoma were significantly associated with unclear horizontal margins. On multivariate analysis, the non-pasting group (odds ratio [OR], 2.69; 95% confidence interval, 1.38 to 5.41; \( P=0.003 \)) and right colon (OR, 1.98; 95% confidence interval, 1.01 to 4.01; \( P=0.047 \)) were independent risk factors for unclear horizontal margin involvement in specimens collected by CSP.

Discussion

In the present prospective RCT, pasting CSP specimens on filter paper improved pathological diagnosis evaluation of horizontal margins by more than 15% compared with non-pasting. CSP was a very useful endoscopic treatment approach for resection of small colorectal adenomas [3–7]. The advantages of CSP include shorter procedure time [3,4] and lower rates of postoperative abdominal symptoms [5] and post-bleeding events in patients on antithrombotic treatment [6,7]. However, because CSP does not burn for treatment, concerns remain regarding postoperative remnants [8–11]. Our results suggest that the pasting method utilized in the present study is inexpensive and can be done easily to improve the pathological evaluation of horizontal margins of specimens resected by CSP.

The horizontal margin was pathologically unknown in 33.6% of formalin-fixed CSP specimens processed without pasting. Previous reports indicate that 18% to 67% of pathologically confirmed horizontal margins could not be evaluated after CSP [8–12,15], thus, our results are never too high. Unclear horizontal margin indicates that the presence or absence of an adenoma at the edge of the specimen cannot be diagnosed. Previous studies suggest that specimen damage caused by CSP and inadequate specimen sectioning are the cause of the unknown breakage [12,18]. In addition, careful sectioning of the specimens is also important. For accurate pathological diagnosis, it is necessary to identify the lesion macroscopically and section it up and down. Ichihara et al. considered that while EMR can confirm the orientation of a specimen by traces of cauterization, CSP can make it difficult to identify the margin of a
specimen when the orientation of the specimen is macroscopically unknown, because it has no cauterizing markers [12]. This inadequate sectioning was reported in 20% of CSP specimens [18]. Pasting the specimen makes up for this shortcoming of CSP. Therefore, it is important to paste and fix a specimen for accurate pathological diagnosis.

Although CSP specimens have a high rate of unknown or positive margins, in most cases, there is no residual tumor. Matsuura et al. reported a true incomplete resection rate of only 3.9% when residual tumor was assessed by EMR performed outside the CSP ulcer [8]. Kawamura et al. reported that only 1.8% of ulcer biopsies from the CSP edge had tumor remnants, a finding that was not inferior to that reported for HSP [4]. These studies showed that little tumor remains after CSP. Matsuura et al. recommend the resect-and-discard approach because the pathological evaluation of horizontal margins in CSP specimens is not useful due to the discrepancy with true tumor remnants, but we disagree. In ESGE clinical guidelines, the follow-up period after polypectomy is considered based on the pathological diagnosis, and early re-colonoscopy is recommended for lesions with indistinct margins. Therefore, unclear pathological diagnosis after CSP forces patients to undergo unnecessary colonoscopies. We believe that we need to improve this unclear horizontal margin rate of diagnosis.

In this study, the median lesion diameter of the polyps was 4 mm, which was too small to paste using pins. While in some reports, pins have been used to fix CSP specimens [6, 19], other investigators have reported that CSP specimens were not pin-

| Table 1 Patient and lesion characteristics in all, pasting, and non-pasting groups. |
|------------------|------------------|------------------|------------------|------------------|
|                  | All              | Pasting group    | Non-pasting group | P               |
| Lesion, n        | 216              | 106              | 110              | 0.626           |
| Sex, n (%)       |                  |                  |                  | 0.626           |
| ▪ Male           | 81 (61.4)        | 47 (62.7)        | 48 (57.8)        |                  |
| ▪ Female         | 51 (38.6)        | 28 (37.3)        | 35 (42.2)        |                  |
| Age, median (IQR), years | 69 (60–75) | 69 (62–75)     | 69 (60–75)       | 0.772           |
| Lesions per patient, median (IQR) | 2.5 (1–4)       | 2.5 (1–4)       | 2.5 (1–4)       |                  |
| Lesion location, n (%) |                  |                  |                  | 0.783           |
| ▪ Right colon    | 125 (57.9)       | 60 (56.6)        | 65 (59.1)        |                  |
| ▪ Left colon     | 91 (42.1)        | 46 (43.4)        | 45 (40.9)        |                  |
| Morphology, n (%)|                  |                  |                  | 0.003           |
| ▪ Protruding type| 155 (71.8)       | 66 (62.3)        | 89 (80.9)        |                  |
| ▪ Superficial type| 61 (28.2)       | 40 (37.7)        | 21 (19.1)        |                  |
| Lesion size, n (%)|                  |                  |                  | 0.167           |
| ▪ 1–4 mm         | 160 (74.1)       | 74 (69.8)        | 86 (78.2)        |                  |
| ▪ 5–9 mm         | 56 (25.9)        | 32 (30.2)        | 24 (21.8)        |                  |
| Pathological diagnosis, n (%) |                  |                  |                  | 0.212           |
| ▪ High-grade adenoma | 26 (12.0)    | 16 (15.1)        | 10 (9.1)         |                  |
| ▪ Low-grade adenoma | 190 (88.0)  | 90 (84.9)        | 100 (90.9)       |                  |
| Adverse events, n (%) |                  |                  |                  |                  |
| ▪ Perforation    | 0 (0.4)          | 0 (1.3)          | 1 (1.2)          | 0.425           |
| ▪ Bleeding       |                  |                  |                  | 0.272           |
| Operators, n (%) |                  |                  |                  |                  |
| ▪ Specialist     | 50 (23.1)        | 22 (20.8)        | 28 (25.5)        |                  |
| ▪ Trainees       | 166 (76.9)       | 84 (79.2)        | 82 (74.5)        |                  |
| Endoscopy assistants, n (%) |                  |                  |                  |                  |
| ▪ Technicians    | 90 (41.7)        | 40 (37.7)        | 50 (45.5)        |                  |
| ▪ Non-technicians| 126 (58.3)       | 66 (62.3)        | 60 (54.5)        |                  |
| IQR, interquartile range. |                  |                  |                  |                  |
ned because they were small and fragile [10, 20, 21]. In particular, Hirose et al. did not pin even CSP specimens > 10 mm in diameter [20]. For the same reason, it is difficult to extend a specimen after formalin fixation. In addition, Japan Gastroenterological Endoscopy Society Guidelines recommend that pasting be performed before formalin fixation for accurate pathological diagnosis [22]. Therefore, we have devised a method to fix the extended specimens on filter paper. With this method, it is important that the edge of a curled CSP specimen first be extended with a toothpick on a plastic plate, because extending specimens is difficult on filter paper that easily absorbs water. After extending a specimen, the cut specimen surface is pasted on filter paper. Specimen processing time after CSP is less than 1 minute (no data) and we believe that there are few disadvantages to this method. By using this method, even small and fragile CSP specimens can be stretched and fixed without damage during pasting.

The risk factors for unclear horizontal margins were non-pasting group and lesions located in the right colon. Other studies have reported that risk factors for incomplete resection by CSP are female sex, descending colon, sigmoid colon, and trainee endoscopists [8, 9, 15]. We added these factors as secondary endpoints in our analysis, but the results were different
in our study. Therefore, larger studies will be needed to clarify the true risk factors.

There were 16 specimens (15.1 %) with unclear horizontal margin in the pasting group. All of the cases with unclear margins in the pasting group had been obliquely sectioned. These specimens were very similar to the inadequately sectioned specimens shown by Ito et al [18]. The reason for this is considered to be the small margin of normal mucosa at the time of CSP, which made it difficult to see the top and bottom of the specimen even if it was pasted. When Kim et al. resected polyps with CSP, they ensnared 1 to 2 mm of the normal mucosa surrounding the polyp for a complete resection [23]. In addition, this normal mucosa can also be used as a marker of up and down when sectioning a specimen. Therefore, in CSP, the polyp should be resected including some normal mucosa surrounding it.

Local recurrence caused by positive vertical margins is also a very important concern. Ito et al. reported that VMX/+ was positive in 6 % of CSP cases. However, their report did not reveal the respective proportions of VMX and VM+. Improvement in the accurate pathological diagnosis for vertical as well as horizontal margins is needed. No method has been proposed so far to overcome this challenge. Therefore, we believe that further research is needed.

Fragmented lesions, SSLs, and hyperplastic polyps were excluded to accurately evaluate the efficacy of filter paper paste. A fragmented lesion is one of the causes of unclear horizontal margins and an important issue to be resolved. In our study, 29.5 % of CSP specimens were fragmented before pasting. Ichihara et al. reported that 12 % of specimens were fragmented at the time of collection after CSP [12]. A specimen is damaged by collection through the suction channel. Therefore, Kishida et al. significantly reduced the splitting rate by removing the suction button (button-attached 36.6 % vs. button-removed 22.4 %, P<0.001) [24]. These specimens were excluded because pathological evaluation of horizontal margins is difficult, even if the specimens were pasted on filter paper. The endoscopic characteristics of SSL are flat morphology, color similar to normal mucosa, mucus adhesion, and unclear borders, making it difficult to get an overall picture of the tumor [25–27]. Previous studies have reported that SSLs have a higher rate of incomplete CSP resection compared to adenomas (31.0 % vs. 7.2 %) [18,28]. We mainly used non-magnified endoscopy, which is easy to use to identify errors in endoscopic diagnosis; therefore, these lesions were excluded and only adenomas were included in the study. Patients who had previously undergone colorectal surgery were excluded, because it would affect the difficulty of treatment and make it difficult to determine the location of a lesion.

The present study has several limitations. First, we used a snare compatible with both CSP and EMR. The use of dedicated CSP snares, which are sharper than conventional snares, might have resulted in a lower rate of unclear horizontal margins. Secondly, there may have been insufficient blinding of pathologists. In this study, the allocation was also blinded to pathologists. However, they may have been able to infer the group assignments from the microscopic image. In addition, all lesions were diagnosed by a single pathologist, which means that subjective factors may have affected these results. Third, fragmentation of the collected specimens was assessed by the endoscopy assistant, which may be a source of selection bias. However, we believe that selection bias was minimized because randomization was performed after fragmented specimens were excluded. Fourth, in this study, a single pathologist diagnosed all lesions.

Conclusions
The observed reduction in the rate of unclear horizontal margins in extended specimens pasted on filter paper in the present study indicates the utility of this approach for accurate pathological examination after CSP.

Competing interests
The authors declare that they have no conflict of interest.

Clinical trial

UMIN-CTR
UMIN00035456
TRIAL REGISTRATION: Single-Center, Randomized, prospective trial at www.umin.ac.jp

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