Impact of preoperative biopsy on severe submucosal fibrosis on endoscopic submucosal dissection for colorectal laterally spreading tumors: a propensity score analysis

Shusei Fukunaga, Yasuaki Nagami, Masatsugu Shiba, Taishi Sakai, Hirotugu Maruyama, Masaki Ominami, Koji Otani, Shuhei Hosomi, Fumio Tanaka, Koichi Taira, Tetsuya Tanigawa, Hirokazu Yamagami, Toshio Watanabe, Yasuhiro Fujiwara

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DOI	10.1016/j.gie.2018.08.051
Highlights	<ul style="list-style-type: none">・表面型大腸腫瘍患者が診断目的の術前生検を受けると、粘膜下層に内視鏡的粘膜切除術（EMR）を困難にする高度線維化が生じやすくなることが初めて証明されました。・生検を受けた病変の治療には、内視鏡的粘膜下層剥離術（ESD）が有効で、ESD は生検の影響を受けないことがわかりました。

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Description	<p>研究グループは、表面型大腸腫瘍に診断目的の術前生検を行うことによって粘膜下層に高度の線維化が起きやすくなることを初めて証明しました。</p> <p>表面型大腸腫瘍の内視鏡治療には、内視鏡的粘膜切除術（EMR）、内視鏡的粘膜下層剥離術（ESD）の2種類がありますが、生検後は EMR が困難になるため、診断目的の術前生検を避けて治療方針を決定することが望まれます。</p> <p>一方で、生検が行われても ESD による治療には悪影響がないことも明らかになりました。</p> <p>“検査の有無で治療法の選択肢が変わる？表面型大腸腫瘍の術前生検は内視鏡治療に悪影響” 大阪市立大学. https://www.osaka-cu.ac.jp/ja/news/2018/181031-2. (参照 2018-10-31)</p>
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Department of Gastroenterology, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan

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Corresponding Author: Yasuaki Nagami, Department of Gastroenterology, Osaka City University Graduate School of Medicine, 1-4-3, Asahimachi, Abeno-ku, Osaka, 545-8585, Japan

Tel: +81-6-6645-3811

Fax: +81-6-6645-3813

E-mail: yasuaki-75@med.osaka-cu.ac.jp

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ABSTRACT

Background and Aims: It is thought that preoperative biopsy for superficial-type colorectal tumors should be avoided because submucosal fibrosis caused by biopsy makes endoscopic mucosal resection impossible. However, few studies have reported the influence of biopsy on colorectal endoscopic submucosal dissection (ESD). This study aimed to examine the effect of biopsy on submucosal fibrosis and treatment outcomes of ESD for laterally spreading tumors (LSTs).

Methods: Between April 2005 and September 2015, 441 consecutive patients underwent colorectal ESD in Osaka City University Hospital. Using propensity score matching and inverse probability of treatment weighting (IPTW), we retrospectively evaluated risk factors for severe submucosal fibrosis and treatment outcomes for patients with LST, with or without preoperative biopsy.

Results: A total of 428 LSTs resected using ESD were enrolled. After matching, there were 136 matched pairs of lesions that did or did not receive biopsies. Preoperative biopsy increased severe fibrosis compared to that in the non-biopsy group (20.6% versus 11.0 %; $p = 0.03$) and was significantly associated with severe fibrosis after matching (odds ratio [OR], 2.09; 95% confidence interval [CI], 1.07–4.10; $p = 0.03$). After adjustment with IPTW, biopsy also increased the risk of severe fibrosis (OR, 2.33; 95% CI, 1.17–4.63; $p = 0.02$). However, no significant differences were observed between the two groups in treatment outcomes.

Conclusions: Preoperative biopsy for colorectal LSTs might cause severe submucosal

fibrosis, but has no adverse influence on clinical outcomes of ESD.

INTRODUCTION

Colorectal endoscopic submucosal dissection (ESD) enables en bloc resection of laterally spreading tumors (LSTs), regardless of tumor size, and allows precise assessment of histological curability.¹⁻⁴ We sometimes encounter fibrosis in the submucosal layer during ESD for colorectal LSTs. Severe submucosal fibrosis makes it difficult to separate the submucosal layer from the muscle layer in ESD and can lead to higher incidence of perforation and incomplete resection, as well as longer procedure time.⁵⁻¹⁰ Preoperative biopsy is sometimes performed in LSTs to diagnose definite histology and/or invasion depth.^{11,12} However, preoperative biopsy may cause fibrosis in the submucosal layer, leading to a positive non-lifting sign on endoscopic mucosal resection (EMR), and subsequently making EMR impossible.¹³⁻¹⁵ Therefore, avoidance of a biopsy has been recommended for definite histological diagnosis by the Japan Gastroenterological Endoscopy Society (JGES) guidelines for colorectal ESD/EMR.¹⁶ Few studies have reported the effect of preoperative biopsy on submucosal fibrosis and treatment outcomes in colorectal ESD.

We therefore conducted a comparative study to examine the effect of preoperative biopsy on submucosal fibrosis and treatment outcomes to support our hypothesis that preoperative biopsy causes severe submucosal fibrosis and makes ESD difficult.

METHODS

Patients

This was a retrospective, matched cohort study conducted at a single referral hospital. Between April 2005 and September 2015, 441 consecutive patients with 478 colorectal tumors underwent ESD in our department. Patients with lesions that received any local injection were excluded because injection into the submucosa could cause tissue damage, followed by submucosal fibrosis.¹⁵⁻¹⁷ Patients who failed to complete the ESD

procedure were also excluded because evaluation of submucosal fibrosis could be insufficient during ESD. Preoperative biopsy was sometimes performed based on the endoscopist's judgement for definite histological diagnosis, especially at the referring hospital. Clinical information on preoperative biopsy was collected retrospectively from electronic medical records. We compared the outcomes of 2 groups of patients: those who underwent biopsy for colorectal LST before ESD and those who did not undergo biopsy. Propensity score analysis was performed to reduce the effects of selection bias for preoperative biopsy and other possible confounding factors.¹⁸

The protocol of this study was approved by the ethics committee of the Osaka City University Graduate School of Medicine (number 3368). Written informed consent was obtained from each patient who underwent colorectal ESD. We disclosed the information about this study on our website's home page, and subjects had the opportunity to decline participation.

Indications for ESD

Initial preoperative endoscopic examination using magnifying narrow-band imaging and chromoendoscopy was performed in all cases to evaluate the depth of invasion of LST for the purpose of determining the treatment method.^{19,20} When deep submucosal invasion was strongly suspected, surgical resection was recommended. ESD was essentially indicated for lesions that required en bloc resection and when resection was likely to be difficult using conventional EMR technique. The indications for ESD of LSTs followed the JGES guidelines that were based on a draft proposal by the Colorectal ESD Standardization Implementation Working Group.^{16,21,22}

ESD procedure

All patients received a low-fiber diet for lunch and dinner on the day before ESD. All procedures began in the afternoon after achieving successful bowel preparation with oral administration of polyethylene glycol starting early in the morning.

Experienced endoscopists performed all procedures using intravenous sedation. A single-channel endoscope with a water-jet system (PCF-Q260JI; Olympus Medical

Systems Co., Tokyo, Japan) and a transparent hood (D-201-11804; Olympus) attached to the tip of the endoscope, a carbon dioxide insufflator (UCR; Olympus), and a standard electrosurgical generator (ICC 200 or VIO300D; ERBE Elektromedizin GmbH, Tübingen, Germany) were used. We mainly used a bipolar needle knife (B knife; XEMEX Co, Tokyo, Japan) or a monopolar needle knife (Flush knife, DK2618JN; Fujifilm Medical, Tokyo, Japan). A Coagrasper (FD-410LR; Olympus) or HemoStat-Y (H-S2518; Pentax Co, Tokyo, Japan) was also used to stop bleeding or prevent hemorrhage before cutting vessels.

After 5% glucose injection to confirm appropriate submucosal elevation, 0.4% sodium hyaluronate solution (Mucoup; Boston Scientific Co, Tokyo, Japan) was injected into the submucosal layer. Then, a partial mucosal incision was made from the anal side, and a certain degree of submucosal dissection was performed. After the submucosal layer became sufficiently visible, the circumferential mucosal incision was completed. Finally, submucosal dissection was achieved.

Pathological examination

Fixed ESD specimens were sectioned perpendicularly at 2-mm intervals, stained with hematoxylin and eosin, and examined microscopically. Histological types, depth of invasion, lateral and vertical margins, and lymphovascular invasion were evaluated in each slice according to the Japanese classification of colorectal carcinoma.²³

Endoscopic evaluation of submucosal fibrosis

Submucosal fibrosis was evaluated on the basis of the findings obtained at the time of injection of sodium hyaluronate with indigo carmine during ESD. Based on a previous report, we classified the degree of submucosal fibrosis into the following 3 categories: F0, no fibrosis, which is demonstrated as a blue transparent layer; F1, mild fibrosis, which appeared as a white web-like structure; and F2, severe fibrosis, which appeared as white muscle-like structure without a blue transparent layer⁵. Patients were divided into F0/1 and F2 groups.

Outcome assessment

The main outcome was the incidence of F2. Outcomes were evaluated based on the risk of F2. Secondary clinical outcomes of ESD included procedure time, procedure speed, en bloc resection, en bloc resection with free margin, perforation, and delayed bleeding.

Definition

LST

LSTs were defined as lesions ≥ 10 mm in diameter with a low vertical axis extending laterally along the interior luminal wall.²⁴ LSTs were classified into granular type (LST-G) and non-granular type (LST-NG) on the basis of images obtained using indigo carmine dye spray according to the Japanese Classification of Colorectal Carcinoma.²³

Procedure time and speed

Procedure time was defined as the period from the start of the circumferential mucosal incision to the removal of the tumor. Procedure speed was calculated by determining the procedure time for the oval area (mm^2) of the resected specimen as follows: $3.14 \times 0.25 \times \text{long axis} \times \text{minor axis}$.²⁵

Adverse events

Perforation was defined as the condition in which the abdominal cavity is visible from the colorectal lumen or free air is seen on abdominal radiograph or computed tomography.¹⁶ Delayed bleeding was defined as a decrease in hemoglobin by >2 g/dL or confirmation of marked hemorrhage at a certain period of time after ESD.²⁶

Statistical analysis

Data are presented as the mean $\pm$ standard deviation for continuous variables and as numbers for categorical variables. The continuous variables were evaluated using the Student *t*-test. The categorical variables were evaluated with the chi-squared test or the Fisher exact test because of small sample sizes. Propensity score matching analysis was performed for preoperative biopsies to reduce the effects of selection bias.¹⁸ This

method was proposed to evaluate statistically causal effects free from confounding effects by mathematically refashioning an observational study into a randomized study.²⁷⁻²⁹

We selected 9 confounding variables, namely, age, sex, location, tumor diameter, LST type, visibility of the lesion, location with respect to the fold, relationship with colonic flexure, and fold conversion, that could influence the outcomes, based on our clinical knowledge and experience (Table 1). Of these, large tumor size, LST-NG type, and fold conversion were associated with submucosal fibrosis according to previous reports.³⁰⁻³² In addition, we speculated that location and endoscopic visibility might be associated with the submucosal depth and width of tissue injury by biopsy, which could influence the submucosal fibrosis. All 9 factors could be obtained even before treatment. Then, we generated a model for calculating propensity scores using logistic regression. A propensity score-matched cohort was identified by attempting to match each patient receiving biopsy with a patient not receiving biopsy (a 1:1 match), using a greedy matching technique.

The validity and reliability of the model were evaluated by estimating the area under the receiver operating characteristics curve using c-statistics and Hosmer-Lemeshow goodness-of-fit tests, respectively. We used the standardized difference to measure covariate balance, whereby an absolute standardized difference (ASD) above 10% represents a meaningful imbalance. All treated patients were matched to the closest control within 0.10 standard deviations of the logit of the estimated propensity score. After matching, crude comparisons were performed using the Mantel-Haenszel chi-squared test or McNemar's test for binary data and paired *t*-tests. The risk of F2 was estimated by calculating the odds ratio (OR) and 95% confidence interval (CI) using logistic regression analysis with generalized estimating equations (GEE) to analyze repeated measures data.³³ The GEE model focused on patients with any multiple LSTs as repeated measures. The propensity score analysis focused on LSTs.

In addition, the inverse probability of treatment weighting (IPTW) method was applied to assess the sensitivity of the results.³⁴⁻³⁷ This method can adjust for confounding factors and can evaluate causal effects without reducing sample size using the estimated propensity scores to assign weights to the data.

Statistical analyses were performed using IBM SPSS software, version 23.0 for Windows (IBM Corp, Armonk, NY, USA). All statistical tests were 2-sided, and a value of $p < 0.05$ was considered statistically significant.

RESULTS

Baseline patient characteristics

A total of 392 patients with 428 LSTs who underwent ESD as their first treatment were enrolled after exclusion of 50 non-LSTs. Of these, 25 lesions that received any local injection were excluded. Furthermore, 1, 1, and 6 lesions that were not completely treated because of perforation, deep submucosal invasion, or poor endoscope maneuverability, respectively, were excluded. Consequently, 369 patients with 395 LSTs were assessed in the present study. Of these, 18 had 2 synchronous lesions and 1 had 3 synchronous lesions; 4 patients had metachronous lesions that were treated another day. One patient had 2 synchronous and 1 metachronous lesions. Preoperative biopsies of 136 lesions were taken in 131 patients (Fig. 1). The clinicopathological characteristics of the study subjects are shown in Table 1. Rectal lesions underwent biopsy more frequently and had a higher incidence of LST-G. The 2 groups were similar with respect to age, sex, tumor diameter, visibility of the lesion, location with respect to the fold, and relationship with the colonic flexure.

After propensity score matching, 136 matched pairs of lesions did or did not undergo biopsy. There were 2 situations where a matched pair involved two lesions from the same patient with one lesion on a preoperative biopsy and one without biopsy. The baseline characteristics of the 2 groups were comparable.

Effect of preoperative biopsy on F2 in the submucosal layer

Of the 395 lesions, F2 in the submucosal layer was observed in 64 lesions. Before

propensity score matching, 28 of the 136 lesions (20.6 %) that were biopsied had F2, whereas 36 of the 259 lesions (13.9 %) had F2 in the control group ($p = 0.09$). On the contrary, after propensity score matching, the preoperative biopsy group (28/136, 20.6%) showed more cases with F2 than the non-biopsy group (15/136, 11.0%; $p = 0.03$) (Table 2). A representative case is shown in Fig. 2.

Risk factors for F2

Preoperative biopsy was significantly associated with F2 after matching (OR, 2.09; 95% CI, 1.07–4.10; $p = 0.03$), whereas no significant association was observed before matching (OR, 1.61; 95% CI, 0.93–2.77; $p = 0.09$) (Table 3). In addition, fold conversion was also significantly associated with F2 after matching (OR, 2.49; 95% CI, 1.29–4.82; $p = 0.007$).

The influence of biopsy on F2 remained robust following adjustments for the propensity score (OR, 2.12), location (OR, 2.15), LST (OR, 2.09), fold conversion (OR, 2.12), LST and fold conversion (OR, 2.12), and all 9 covariates (OR, 2.21) (Table 4).

In addition, after adjustment for confounding factors using IPTW, biopsy also increased the risk of F2 (OR, 2.33; 95% CI, 1.17–4.63; $p = 0.02$) (Table 5).

Evaluation of propensity score matching

The propensity score model showed good calibration (Hosmer-Lemeshow test, $p = 0.09$) and discrimination between biopsy and non-biopsy groups (c statistic = 0.65). The c -statistic was calculated by measuring the receiver operating characteristic curve to assess the validity of the model. All LSTs were matched to the closest control within 0.09 ASD of the logit of the estimated propensity score.

Clinical outcomes after propensity score matching

After matching, no significant differences were observed between the groups in terms of procedure time, procedure speed, en bloc resection, en bloc resection with free margin, perforation, and delayed bleeding (Table 6).

In each group, only one patient with delayed perforation underwent emergency surgical treatment. All patients with delayed bleeding recovered with endoscopic hemostasis, without surgical intervention, although 2 patients required blood transfusion in the non-biopsy group.

The rate of deep submucosal invasion after ESD due to underestimation of the depth of invasion was only 5.5% (15/272) because we excluded the lesions which had confidently been diagnosed as deep submucosal invasive cancer. However, there was no significant difference in the rate of deep submucosal invasion between the biopsy and non-biopsy groups (8.1% vs 2.9%, respectively, $p = 0.12$). Moreover, all deep submucosal invasive cancers did not have F2 detected endoscopically in the present study. F2 in deep submucosal invasive cancers was demonstrated only in 3 and 2 lesions of biopsy and non-biopsy group, respectively.

DISCUSSION

We demonstrated that the incidence of severe fibrosis in the preoperative biopsy group was significantly higher than that in the non-biopsy group using propensity score analysis. To the best of our knowledge, this is the first report to demonstrate the relationship between preoperative biopsy and fibrosis during ESD. On the contrary, our study demonstrated no significant differences between the groups in terms of procedure time, en bloc resection rate, and adverse events.

Preoperative biopsy for LSTs tended to cause severe fibrosis in the submucosal layer in the present study. This may be because mechanical stimulation from the biopsy procedure led to submucosal fibrosis.^{13,14} In terms of histology, myofibroblasts stimulated by tissue damage can induce collagen fiber production leading to submucosal fibrosis.^{38,39}

Previous studies have shown that severe submucosal fibrosis causes technical difficulty in endoscopic resection.^{5,7,8,13-15,40} Jeong et al⁴⁰ reported that advanced endoscopic submucosal fibrosis led to a longer procedure time, lower en bloc resection rate, and higher frequency of adverse events in gastric ESD. Matsumoto et al⁵ demonstrated that

ESD for colorectal lesions with severe fibrosis resulted in a lower complete en bloc resection rate and a higher perforation rate, even with an experienced operator. Moreover, several reports on colorectal ESD also reported that submucosal fibrosis was a significant risk factor associated with perforation using multivariate analysis.^{7,8} However, the above reports did not investigate the effect of preoperative biopsy on submucosal fibrosis.

In contrast, 2 reports demonstrated the relationship between preoperative biopsy and colorectal endoscopic resection.^{14,15} Han et al¹⁴ showed that a history of biopsy affected the non-lifting sign on EMR for submucosal invasive colorectal carcinoma. However, that study focused only on EMR, and no outcome for intramucosal lesions was described. Kim et al¹⁵ reported that prior biopsy sampling significantly reduced complete resection and increased recurrence in colorectal endoscopic resection. Although this was the first report that demonstrated the influence of biopsy for colorectal tumors on the treatment outcomes of various endoscopic resection techniques, very few ESD procedures were included.

Unlike previous reports, previous biopsy had no significant effects on treatment outcomes in our study. Although there was no clear reason for this result, we assumed possible mechanisms. First, it was relatively easy to recognize the fibrotic area requiring dissection within a visual field because submucosal fibrosis caused by biopsy might be less extensive than that due to cancer invasion or peristaltic motion of a large protruded lesion, although it is sometimes difficult to distinguish. We examined the extent of histological fibrosis among 25 post-biopsy lesions which had F2 fibrosis detected endoscopically and 5 deep submucosal invasive cancers by reviewing the slides of the specimens. Interestingly, the median maximum width of histological submucosal fibrosis with dense fibrotic change reaching the vertical margin of the specimen in the post-biopsy lesions was significantly narrower than that in submucosal invasive cancers (2672.4 μm vs 6660.1 μm , $p = 0.01$). Second, it was possible to dissect the localized fibrotic area by injecting solution such as hyaluronic acid into both sides of the fibrotic

area and creating countertraction (Fig. 3).

This study has 4 important strengths. First, we found that preoperative biopsy significantly influenced severe fibrotic change in the submucosal layer during ESD. No prior reports examined the association between ESD and preoperative biopsy by focusing on the degree of submucosal fibrosis. Second, we adjusted for confounding factors that were present before treatment and could influence the relationship between severe fibrosis and preoperative biopsy, such as age, sex, location, tumor characteristics, and endoscopic visibility and used propensity matched analysis to adjust for selection bias. The propensity score matching method was proposed to reduce potential confounding effects by mathematically refashioning a retrospective study.^{27-29,34} Moreover, a randomized controlled trial (RCT) comparing biopsy and non-biopsy groups is ethically not feasible because preoperative biopsy to obtain histologic verification of endoscopic diagnosis was not recommended in the JGES guidelines.¹⁶ Under such circumstances, the present study might be more appropriate than an RCT by applying a propensity score-based statistical method.⁴¹ Third, IPTW based on propensity scoring, which can be analyzed without reducing the sample size, was also performed to assess the sensitivity of the results.^{35,35-37} IPTW indicated that the results were qualitatively similar to those of the matching analysis and that preoperative biopsy was an independent risk factor for severe fibrosis.⁴² Fourth, our study demonstrated that preoperative biopsy might not have a negative influence on actual clinical outcomes of ESD for LSTs, even though biopsy caused severe submucosal fibrosis.

Our study had some limitations. First, the statistical power of the present study might be low because this was a small-sample, retrospective study in a single center. However, there have been no reports similar to our study design referring to sample size. Selection bias can be reduced by propensity score analysis that can provide unbiased estimations, unless the true confounders related to the outcome are not included in the propensity model.^{18,27-39} Second, there was no assessment of differences in biopsy methods. Most biopsy specimens were obtained by the referring physician in smaller hospitals without

magnified endoscopy as a modality of preoperative diagnosis. Therefore, no clear indication of the biopsy method was defined between hospitals and no detailed information could be obtained. Third, the outcomes of this study were based on the fibrosis in the endoscopic findings during procedure. Histological assessment is often more objective than clinical assessment. Lee et al³⁰ reported objective classification of fibrosis using histological evaluation with Masson's trichrome stain. However, many studies reported clinical evaluation of the degree of fibrosis by the endoscopist during the procedure.^{5,6,10} Histological assessment of resected specimens is sometimes influenced by the dissection procedure, dissection depth, and thermal injury due to electrocoagulation. Therefore, histological severity of fibrosis does not necessarily reflect endoscopic severity. Fourth, deep submucosal invasion could cause severe submucosal fibrosis and could affect the results. As mentioned above, however, we think that the influence of deep submucosal invasion on the outcome was small because of the number of severe fibrosis in biopsy and non-biopsy groups.

In conclusion, our results indicate that preoperative biopsy for colorectal LSTs may cause severe submucosal fibrosis. However, preoperative biopsy may have no negative influence on actual clinical outcomes of ESD. A large-scale study may be needed to investigate the actual effect of fibrosis caused by biopsy.

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Table 1. Baseline characteristics before and after propensity score matching.

		Before matching (n = 395)				After matching (n = 272)			
		Non-biopsy (n = 259)	Biopsy (n = 136)	P value	ASD	Non-biopsy (n = 136)	Biopsy (n = 136)	P value	ASD
Age (y), mean $\pm$ SD		70.5 $\pm$ 8.8	70.9 $\pm$ 9.8	0.75	0.04	71.4 $\pm$ 8.9	70.9 $\pm$ 9.8	0.67	0.05
Sex	Female	114 (44.0)	61 (44.9)	0.87	0.02	62 (45.6)	61 (44.9)	1.00	0.01
	Male	145 (56.0)	75 (55.1)			74 (22.8)	75 (55.1)		
Location	Right	163 (62.9)	71 (52.2)	0.03	0.10	77 (56.6)	71 (52.2)	0.52	0.02
	Left	52 (20.1)	22 (16.2)			21 (15.4)	22 (16.2)		
	Rectum	44 (17.0)	43 (31.6)			38 (27.9)	43 (31.6)		
Tumor diameter (mm), mean $\pm$ SD		34.3 $\pm$ 14.8	36.7 $\pm$ 16.0	0.14	0.16	35.9 $\pm$ 15.7	36.7 $\pm$ 16.0	0.68	0.05
LST type	G	104 (40.2)	81 (59.6)	<0.01	0.40	83 (61.0)	81 (59.6)	0.83	0.03
	NG	155 (59.8)	55 (40.4)			53 (39.0)	55 (40.4)		
Visibility of the lesion	Horizontally	185 (71.4)	106 (77.9)	0.19	0.15	104 (76.5)	106 (77.9)	0.87	0.03
	Vertically	74 (28.6)	30 (22.1)			32 (23.5)	30 (22.1)		
Location with respect to the fold	Between	43 (16.6)	32 (23.5)	0.11	0.17	27 (19.9)	32 (23.5)	0.53	0.09
	Across	216 (83.4)	104 (76.5)			109 (80.1)	104 (76.5)		
Relationship with colonic flexure	Non-colonic flexure	174 (67.2)	104 (76.5)	0.06	0.21	103 (75.7)	104 (76.5)	1.00	0.02
	Colonic flexure	85 (32.8)	32 (23.5)			33 (24.3)	32 (23.5)		
Fold conversion	Negative	170 (65.6)	104 (62.5)	0.58	0.06	86 (63.2)	85 (62.5)	1.00	0.02
	Positive	89 (34.4)	32 (37.5)			50 (36.8)	51 (37.5)		

Values are number (%) unless otherwise indicated.

ASD, absolute standardized difference; G, granular type; LST, laterally spreading tumor; NG, non-granular type; SD, standard deviation.

Table 2. Incidence of F2 before and after propensity score matching.

	Before matching (n = 395)			After matching (n = 272)		
	Non-biopsy (n = 259)	Biopsy (n = 136)	<i>P</i> value	Non-biopsy (n = 136)	Biopsy (n = 136)	<i>P</i> value
F2	36 (13.9%)	28 (20.6%)	0.09	15 (11.0%)	28 (20.6%)	0.03

Values are number (%).

F2, severe fibrosis

Table 2. The incidence of F2 before and after propensity score matching.

	Before matching (n = 395)			After matching (n = 272)		
	Non-biopsy (n = 259)	Biopsy (n = 136)	<i>P</i> value	Non-biopsy (n = 136)	Biopsy (n = 136)	<i>P</i> value
F2	36 (13.9%)	28 (20.6%)	0.09	15 (11.0%)	28 (20.6%)	0.03

Values are number (%).

F2, severe
fibrosis

Table 3. Risk factors for F2 by logistic regression analysis before and after propensity score matching.

		Before matching (n = 395)				After matching (n = 272)			
		n	case (%)	Crude OR (95% CI)	P value	n	case (%)	Crude OR (95% CI)	P value
Age (continuous, per 10 years old)		395	64 (16.2)	1.02 (0.99-1.05)	0.19	272	43 (15.8)	1.02 (0.98-1.05)	0.42
Sex	Female	175	32 (18.3)	1.00		123	20 (16.3)	1.00	
	Male	220	32 (14.5)	0.76 (0.45-1.30)	0.32	149	23 (15.4)	0.94 (0.49-1.80)	0.85
Location	Right	234	37 (15.8)	1.00		148	25 (16.9)	1.00	
	Left	74	19 (25.7)	1.84 (0.98-3.45)	0.06	43	10 (23.3)	1.49 (0.66-3.37)	0.34
	Rectum	87	8 (9.2)	0.54 (0.24-1.21)	0.13	81	8 (9.9)	0.54 (0.23-1.26)	0.15
Tumor diameter (mm)		395	64 (16.2)	1.00 (0.99-1.02)	0.72	272	43 (15.8)	1.00 (0.98-1.02)	0.87
LST type	G	185	25 (13.5)	1.00		164	21 (12.8)	1.00	
	NG	210	39 (18.6)	1.46 (0.85-2.52)	0.18	108	22 (20.4)	1.74 (0.90-3.38)	0.10
Visibility of the lesion	Horizontally	291	44 (15.1)	1.00		210	31 (14.8)	1.00	
	Vertically	104	20 (19.2)	1.34 (0.75-2.40)	0.33	62	12 (19.4)	1.39 (0.67-2.87)	0.38
Location with respect to the fold	Between	75	6 (8.0)	1.00		59	5 (8.5)	1.00	
	Across	320	16 (18.1)	2.55 (1.06-6.15)	0.04	213	38 (17.8)	2.35 (0.88-6.26)	0.09
Relationship with colonic flexure	Non-colonic flexure	278	39 (14.0)	1.00		207	29 (14.0)	1.00	
	Colonic flexure	117	25 (21.4)	1.67 (0.95-2.91)	0.07	65	14 (21.5)	1.69 (0.84-3.39)	0.14
Fold conversion	Negative	255	31 (12.2)	1.00		171	19 (11.1)	1.00	
	Positive	140	33 (23.6)	2.23 (1.30-3.83)	0.004	101	24 (23.8)	2.49 (1.29-4.82)	0.007
Biopsy	Negative	259	36 (13.9)	1.00		136	15 (11.0)	1.00	
	Positive	136	28 (20.6)	1.61 (0.93-2.77)	0.09	136	28 (20.6)	2.09 (1.07-4.10)	0.03

CI, confidence interval; F2, severe fibrosis; LST, laterally spreading tumor; G, granular type; NG, non-granular type; OR, Odds ratio; SD, standard deviation.

Table 4. Univariate and multivariate odds ratios of F2 for biopsy compared with non-biopsy after propensity score matching.

	After matching	
	OR (95% CI)	<i>P</i> value
Unadjusted	2.09 (1.07-4.10)	0.03
Adjusted for propensity score	2.12 (1.06-4.26)	0.03
Adjusted for location	2.15 (1.10-4.23)	0.03
Adjusted for LST	2.09 (1.06-4.11)	0.03
Adjusted for fold conversion	2.12 (1.08-4.19)	0.03
Adjusted for LST and fold conversion	2.12 (1.07-4.17)	0.03
Adjusted for all covariates	2.21 (1.12-4.35)	0.02

CI, confidence interval; *F2*, severe fibrosis; *OR*, Odds ratio.

Table 5. Propensity score matched and weighted Odds ratios of F2 for biopsy compared with non-biopsy.

	OR (95% CI)	<i>P</i> value
Before PS matching	1.61 (0.93-2.77)	0.09
After PS matching	2.09 (1.07-4.10)	0.03
IPTW	2.33 (1.17-4.63)	0.02

F2, severe fibrosis; *IPTW*, Inverse probability of treatment weighting; *PS*, Propensity score; *OR*, Odds ratio; *CI*, confidence interval.

Table 6. Clinical outcomes after propensity score matching.

	After matching (n = 272)		<i>P</i> value
	Non-biopsy (n = 136)	Biopsy (n = 136)	
Procedure time (min.), mean $\pm$ SD	113.5 $\pm$ 76.3	124.3 $\pm$ 98.1	0.33
Procedure speed (mm ² /min.), mean $\pm$ SD	14.7 $\pm$ 9.2	14.0 $\pm$ 8.0	0.44
En-bloc resection	132 (97.1)	134 (98.5)	0.68
En-bloc resection with free margin	119 (87.5)	115 (84.6)	0.61
Perforation	9 (6.6)	3 (2.2)	0.15
Delayed bleeding	9 (6.6)	11 (8.1)	0.82

Values are number (%) unless otherwise indicated.

SD, standard deviation

Procedure speed, $(3.14 \times 0.25 \times \text{long axis} \times \text{minor axis}) \div \text{procedure time}$.

Figure legends

Figure 1. Diagram of the study design

ESD, endoscopic submucosal dissection; LST, laterally spreading tumor

Fig. 2. ESD procedures for LST in which preoperative biopsy was performed. A,

Preoperative biopsy was taken at the anal side in LST. B, After 5% glucose injection,

0.4% sodium hyaluronate solution with indigo carmine was injected into the submucosa.

C, A ball-tipped flush knife was used for mucosal incision. D, F2 was revealed just

under the biopsy scar. E, The fibrotic area was localized within one visual field. F, The

fibrotic area was successfully dissected.

ESD, endoscopic submucosal dissection; LST, laterally spreading tumor; F2, severe

fibrosis

Author Contribution

Shusei Fukunaga: Endoscopy, Clinical management of patients, Conception, Buildup of date base, and Manuscript production

Yasuaki Nagami: Endoscopy, Clinical management of patients, Study design, Statistical analysis, and Manuscript production

Masatsugu Shiba: Clinical management of patients and Statistical analysis

Taishi Sakai: Endoscopy and Clinical management of patients

Hirotsugu Maruyama: Endoscopy and Clinical management of patients

Masaki Ominami: Endoscopy and Clinical management of patients

Koji Otani: Clinical management of patients

Shuhei Hosomi: Clinical management of patients and Adviser for manuscript production

Fumio Tanaka: Clinical management of patients and Adviser for manuscript production

Koichi Taira: Clinical management of patients and Adviser for manuscript production

Tetsuya Tanigawa: Clinical management of patients and Adviser for manuscript production

Hirokazu Yamagami: Clinical management of patients and Adviser for manuscript production

Toshio Watanabe: Clinical management of patients and Adviser for manuscript production

Yasuhiro Fujiwara: Clinical management of patients and Supervisor for overall study

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Abbreviations:

ESD - endoscopic submucosal dissection

LST - laterally spreading tumor

GEE - generalized estimating equations

IPTW - inverse probability of treatment weighting

OR - odds ratio

CI - confidence interval;

EMR - endoscopic mucosal resection

JGES - Japan Gastroenterological Endoscopy Society

F0 - no fibrosis

F1 - mild fibrosis

F2 - severe fibrosis

LST-G - LST granular type

LST-NG - LST non-granular type

GEE - generalized estimating equations

ASD - absolute standardized differences

RCT - randomized controlled trial