lesions with a biopsy diagnosis of group III may have been followed without ER depending on the patient's background or the preference of the primary physician. However, as this study had a large sample size and high statistical power, we think the results of our study will be useful.

In conclusion, a biopsy diagnosis of borderline lesion or undifferentiated type cancer is more likely than other histologic diagnoses to show a discrepancy with the histologic diagnosis obtained from the ER specimen. Endoscopic characteristics should be considered together with the biopsy diagnosis to determine the treatment strategy for these lesions.

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ORIGINAL ARTICLE

FACTORS RELATED TO LATERAL MARGIN POSITIVITY FOR CANCER IN GASTRIC SPECIMENS OF ENDOSCOPIC SUBMUCOSAL DISSECTION

NAOMI KAKUSHIMA, HIROYUKI ONO, MASAKI TANAKA, KOHEI TAKIZAWA, YUICHIRO YAMAGUCHI AND HIROYUKI MATSUBAYASHI

Division of Endoscopy, Shizuoka Cancer Center, Shizuoka, Japan

Background: With the widespread use of endoscopic submucosal dissection (ESD), more large early gastric cancers (EGC) have become candidates for endoscopic resection. A precise diagnosis of the extent of cancer is indispensable to obtain R0 resection. The aim of the present study was to clarify the factors related to lateral margin positivity for cancer in specimens resected by ESD for EGC.

Methods: Among 1549 EGC treated by ESD during September 2002 to December 2008, lesions that were resected in an en-bloc fashion and resulted in a pathological diagnosis of lateral margin positive (LM+) for cancer, were extracted. The reason for LM+ and pathological characteristics of the lesions were studied and compared to lesions successfully resected with margins negative for cancer.

Results: There were three types of lesion that resulted in LM+ resection: lesions with a flat spreading area, lesions with an unexpected nearby lesion, and lesions with lateral extension beneath a non-cancerous mucosa. Compared to lesions resected with margins negative for cancer, diameter of the tumor, recurrent-type cancer, submucosal cancer, and undifferentiated-type cancer were factors significantly related to LM+ resection.

Conclusion: Other than misdiagnosing a small portion of cancer extension, lateral margin positivity for cancer by ESD could result from a neighboring lesion and an unexpected lateral submucosal cancer extension. To avoid LM+ resection of EGC by ESD, one should be careful of unexpected lateral extension and simultaneous multi-lesions.

Key words: diagnosis, early gastric cancer (EGC), endoscopic submucosal dissection (ESD), lateral margin.

INTRODUCTION

Early gastric cancer (EGC) is defined to a mucosal or submucosal invasive cancer (T1 cancer) irrespective of the presence of lymph node metastasis. Lesions indicated for endoscopic resection (ER) should be EGC with no risk of nodal metastasis and that can be resected in a single fragment. Using a large Japanese database of more than 5000 EGC patients who underwent gastrectomy with D2 lymph node dissection, particular conditions of node-negative cancer have been defined. At present, lesions with preoperative endoscopic diagnosis of differentiated-type intramucosal cancer without ulcer findings, differentiated-type intramucosal cancer no larger than 3 cm in diameter with ulcer findings, differentiated-type minute invasive submucosal (<500 μm below muscularis mucosa) cancer no larger than 3 cm in diameter are considered indications for ER.[1]

Endoscopic submucosal dissection (ESD) is the latest ER method that is characterized by circumferential mucosal incision and submucosal dissection. In Japan, ESD is now gaining acceptance as the standard endoscopic resection technique for EGC, especially for large or ulcerative lesions.[2,3] The merit of ESD is that the incision line can be made freely, so that a mucosal cancer of any size and location can be resected in one specimen. An en bloc resection will provide accurate pathological information of tumor depth, size, lymphovascular infiltration and whether the specimen margin is free of cancer. However, to minimize the procedure time and to prevent complications, it is better to remove the lesion with a minimum margin around the lesion. Therefore, a precise diagnosis of the extent of the cancer is indispensable before carrying out an ESD.

Unlike colorectal or esophageal lesions, the borders of which are rather easy to recognize by chromoendoscopy with indigocarmine or lugol staining, the borders of EGC are sometimes difficult to diagnose because the background mucosa is affected by acute or chronic inflammation. Chromoendoscopy with indigocarmine is useful in the detection and diagnosis of EGC and has been practically used for more than 40 years.[4,5] Indigocarmine accumulates in the grooves and emphasizes the structural changes of the gastric mucosa. However, with the prevalence of endoscopic screening, more EGC with little difference of elevation or completely flat lesions have been able to be detected. These EGC lesions with slight mucosal change are often candidates for ESD. However, if the border of the lesion is not identified, the lateral margin may be positive for cancer, and endoscopic treatment will be unsuccessful. The aim of the present study was to clarify the factors related to lateral margin positivity for cancer in specimens resected by ESD for EGC.

Correspondence: Naomi Kakushima, Division of Endoscopy, Shizuoka Cancer Center, 1007 Shimonagakubo, Nagaizumi, Suntougun, Shizuoka 411-8777, Japan. Email: kakushin-tky@umin.ac.jp

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METHODS

From September 2002 to December 2008, 1575 EGC were treated by ESD at Shizuoka Cancer Center Hospital. Lesions with margins that were not suitable for histological assessment due to burning effect or piecemeal resection, and lesions that were resected for the purpose of jumbo biopsy, were excluded. A total of 1549 lesions were included for this study. Clinicopathological data were extracted by charts, endoscopy reports and pathology reports. Lesions with a pathological diagnosis of lateral margin positivity (LM+) of cancer were extracted. The reason for LM+ and clinicopathological characteristics of the lesions were studied and compared to lesions successfully resected with margins negative for cancer.

Method of preoperative diagnosis

Lesions were thoroughly rinsed with water containing pronase and dimethicone (Kissei Pharmaceutical Co., Nagano, Japan). Diagnosis of the extent of cancer was made by normal endoscopy (GIF-H260; Olympus, Tokyo, Japan) and chromoendoscopy with 0.2% indigocarmine. Slight changes of mucosal height, texture and color were carefully observed to assess the extent of cancer. Then, several biopsies around the estimated border of the lesion were taken to confirm areas with non-neoplastic mucosa (negative biopsies). For lesions with a diameter ≤20 mm, three or four biopsies were taken around the lesion at even intervals. For larger lesions, more biopsies were taken around the lesion so that they became a reference for marking before the ESD procedure. All endoscopic images of each negative biopsy site were recorded. Preoperative screening endoscopy was carried out at least once before performing ESD. The duration of screening endoscopy and ESD was 4 weeks (range 1 to 6 weeks). The indication for ESD was the finding of an early gastric cancer with no apparent submucosal deep invasion, diagnosed by endoscopy and chromoendoscopy.

Method of ESD

After rinsing the lesion with water containing pronase and dimethicone, the lesion extent was once again confirmed by normal observation and chromoendoscopy with indigocarmine. Marking was made around the lesion referring to the biopsy scars previously taken. When the biopsy scars were not recognized, the previous endoscopic images of biopsies were taken into account. Then, the mucosal incision line was made around the marking dots. The method of gastric ESD was carried out as described previously.[2,6] The electrosurgical endoscopic knife mainly used was the insulated tip diathermic knife (IT-knife 1 or 2).

Pathological assessment

After removing the lesion, the specimen was fixed in a formalin solution and was serially cut at 2-mm intervals parallel to a line that included the closest resection margin of the specimen. The margins of the tumor, depth, histological type, size, macroscopic appearance, presence of ulceration and lymphovascular infiltration were assessed pathologically. Endoscopic characteristics of the lesions were classified according to the Paris endoscopic classification.[7] Histological classification was microscopically carried out according to the revised Vienna classification of gastrointestinal epithelial neoplasia.[8] The location was described by UML classification: three portions are defined by subdividing both lesser and greater curvatures into three equal lengths: U, upper; M, middle; L, lower.[9]

Written informed consent was collected from all patients undergoing ESD. This retrospective study was approved by the institutional review board of our hospital (no. 22-J72-22-1–3). Statistical analysis was done using Student's *t*-test, chi-squared test or Fisher's exact test for univariate analysis, and logistic regression was applied for multivariate analysis. All analysis were done by StatView, version 5.0 (SAS Institute Inc., Cary, NC, USA), and a P value of ≤0.05 was considered significant.

RESULTS

Pathological characters of lesions with a lateral margin positive for cancer

Among 1549 lesions, 18 lesions were diagnosed as LM+ for cancer. Table 1 shows a comparison between the pathological characteristics of LM+ lesions and lesions with a cancer-free lateral margin (LM–). By univariate analysis, diameter of the tumor, macroscopic type (protruded type, recurrent type), location (upper gastric area), depth (submucosal cancer),

Table 1. Comparison between lesions LM+ for cancer and those LM– for cancer in specimens resected by ESD for EGC

	LM+ ($n = 18$)	LM– ($n = 1531$)	P value
Lesion diameter median (mm)	41 (19–91)	18 (1.3–110)	<0.001
Macroscopic type (Protruded/Depressed/Mixed/Recurrence)	9/4/3/2	469/851/164/47	0.01
Location† (U/M/L)	9/7/2	309/676/546	0.008
Circumference‡ (LC/GC/A/P)	9/1/4/4	603/214/331/383	NS
Ulceration (no/yes)	10/8	1221/310	0.059
Depth§ (M/SM–)	6/12	1201/330	0.0001
Main histology (differentiated/undifferentiated)	14/4	1462/69	0.002
Mixed histology (no/yes)	8/10	1166/365	0.004

EGC, early gastric cancer; ESD, endoscopic submucosal dissection; LM+, lateral margin positive; LM–, lateral margin negative; NS, not significant.
†L, lower; M, middle; U, upper.
‡A, anterior; GC, greater curvature; LC, lesser curvature; P, posterior wall.
§M, mucosal; SM–, submucosal or deeper.

undifferentiated type and a mixed histology were factors significantly related to LM+ lesions. By multivariate analysis, diameter of the tumor, recurrent type, submucosal cancer, and undifferentiated type were significant variables related to LM+ lesions (Table 2).

Reasons for LM+ after ESD

There were three reasons for LM+. First, 13 lesions (72%) were considered as having a misdiagnosis of the tumor extent. These lesions had a small portion of cancer that extended between negative biopsies (Fig. 1). Preoperative negative biopsies were carried out in all cases, but were positive in three cases. The cutting line during ESD was made outside sufficient of the negative biopsy scar, but was inefficient. The endoscopic and pathological characteristics of these 13 lesions are shown in Table 3. The type of lesion was protruding or slightly elevated type in nine cases, mixed histology was revealed in eight cases and the depth was submucosal invading cancer in nine cases. Second, three lesions had another unexpected lesion existing beside the lesion. (Fig. 2) Negative biopsies were carried out in all cases, but they were not successful in detecting the neighboring lesion. Third, there were two cases that had lateral invasion of cancer in the submucosa under a non-cancerous mucosa (Fig. 3).

Table 2. Multivariate analysis for factors related to lateral margin positive resection

Variable	Odds ratio (95% CI)	*P* value
Diameter < 30 mm	0.2 (0.07–0.65)	0.0067
Recurrence type	12.5 (1.6–96.4)	0.0149
Submucosal invasion	4.5 (1.5–13.9)	0.0073
Undifferentiated histology	10.4 (1.5–71.1)	0.016

CI, confidence interval.

DISCUSSION

The present study showed factors other than technical reasons related to LM+ after ESD for EGC. The most common reason for LM+ was misdiagnosis of the extent of cancer. To achieve a margin free of cancer by ESD, negative biopsies were obtained before the ESD procedure and the circumferential cutting line was made outside those negative biopsies. However, in some cases, three or four negative biopsies were not able to detect a small portion of extending cancer. Endoscopic pictures of the lesions with LM+ were retrospectively examined, but it was difficult to delineate the correct margin of the cancer. From our study, large lesions, undifferentiated-type cancer, and lesions with submucosal invasion were factors that were significantly related to LM+ lesions and, therefore, the borders of these lesions are considered to be rather difficult to delineate. This result was

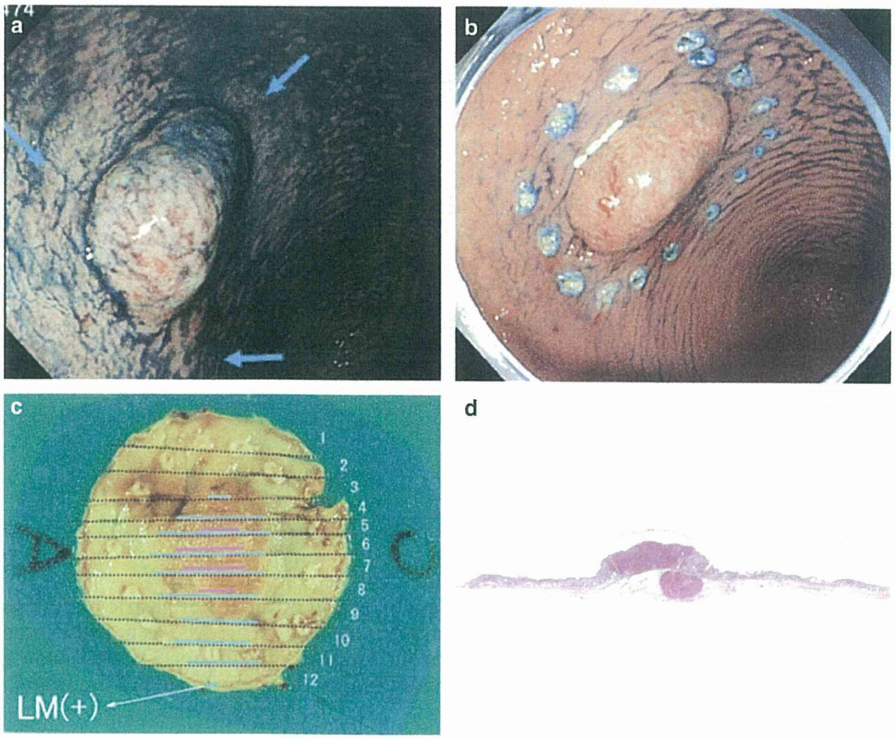

Fig. 1. (a) 0-I type lesion, 15 mm in size, was observed on the anterior wall of the gastric antrum. Preoperative diagnosis was mucosal cancer. Three biopsies around the estimated tumor margin (blue arrows) were taken as negative biopsies. (b) Markings were made around the lesion with reference to previous biopsy scars. (c) Endoscopic submucosal dissection (ESD) of the specimen revealed lateral margin positivity for cancer at the lesser curvature side of the lesion. (d) Loupe view of slide no. 8. Lesion was 0-I+IIb, 36 mm in size, with a depth of SM2. Histology of the protruded component (0-I) was moderately differentiated adenocarcinoma, and for the flat component (0-IIb) it was well-differentiated adenocarcinoma.

Table 3. Endoscopic and pathological characteristics of 13 lesions with a mucosal cancer extension misdiagnosed by endoscopy

Case	Type	Color	Diameter (mm)	Histology	Depth	Ulceration	No. LM+ slides
1	0-IIa	Normal	65	Well	M	Yes	2
2	Recurrence	Reddish	39	Well	SM2	Yes	1
3	0-IIa+IIc	Pale	76	Well > mod	SM2	Yes	1
4	0-I	Reddish	25	Pap > por > well	SM2	No	1
5	0-IIa	Reddish	30	Mod > por	SM–	No	3
6	0-IIa	Normal	48	Well > mod	SM1	No	1
7	Recurrence	Reddish	50	Por > sig	SM–	Yes	4
8	0-I	Normal	36	Mod > por	SM2	No	1
9	0-IIa	Pale	87	Mod > well	M	No	1
10	0-IIc	Pale	91	Sig	M	Yes	2
11	0-IIa	Pale	42	Mod > well > por	SM2	No	1
12	0-I+IIc	Red & pale	64	Sig	SM–	Yes	2
13	0-IIc	Reddish	24	Well	M	No	1

LM+, lateral margin positive; M, mucosal; mod, moderately differentiated adenocarcinoma; pap, papillary adenocarcinoma; por, poorly differentiated adenocarcinoma; sig, signet ring cell carcinoma; SM–, submucosal or deeper; SM1, submucosal <500 μm; SM2, submucosal ≥500 μm; well, well-differentiated adenocarcinoma.

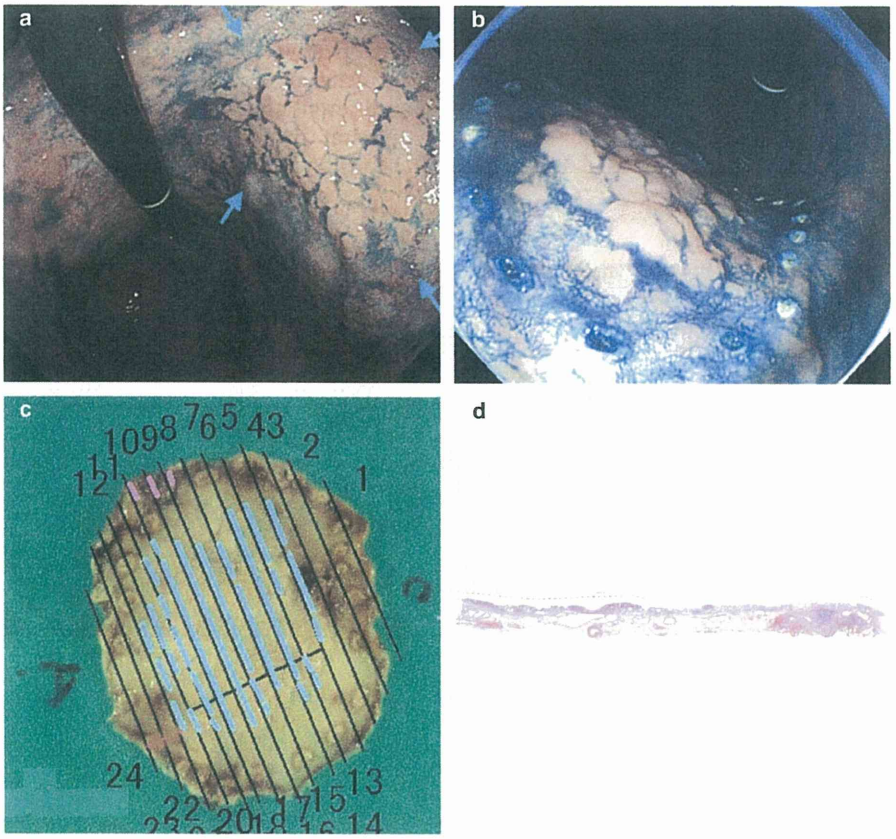

Fig. 2. (a) 0-IIa type lesion, 30 mm in size, was observed on the anterior wall of the lower gastric body. Preoperative diagnosis was mucosal cancer. Four biopsies were taken around the estimated tumor margin (blue arrows) as negative biopsies. (b) Markings were made around the lesion with reference to previous biopsy scars. (c) Endoscopic submucosal dissection (ESD) of the specimen revealed lateral margin positivity for cancer by a nearby lesion (pink lines) at the anterior side of the lesion. (d) Loupe view of slide no. 10 shows a nearby lesion at the edge of the specimen.

compatible to previous studies.[10,11] Before the technique of ESD was developed, these lesions were not considered appropriate for endoscopic resection because en bloc resection was difficult by conventional EMR methods.

The technique of ESD enables widespread resection, and the depth of submucosal dissection can be controlled by direct vision, so that large lesions and cancer with minute submucosal invasion have become candidates for endoscopic

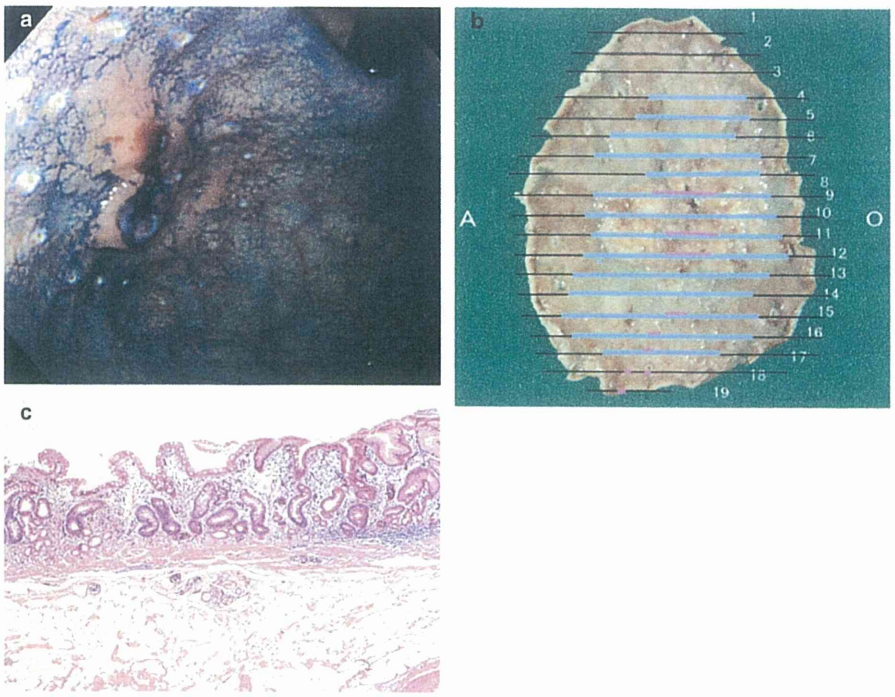

Fig. 3. (a) Marking was made around a 0-IIa+IIc lesion, 30 mm in size, on the posterior wall of the upper gastric body. The estimated depth of the lesion was SM2; however, ESD was carried out because of the patient's age and concomitant disease. (b) Endoscopic submucosal dissection (ESD) of the specimen revealed lateral margin positivity for cancer at the posterior side of the tumor in the submucosa. (c) Loupe view of slide no. 19 shows a laterally spreading cancer in the submucosa under a non-cancerous mucosa.

resection. Sufficient margin around the lesion is secured by cutting outside the marking dots, but, unexpectedly, in our study, another lesion existing nearby was another factor that led to LM+. These lesions should have been detected at screening endoscopy. However, due to them being the same color as the surrounding mucosa and having a flat morphology (0-IIb), they were overlooked. If the area of the neighboring lesion could be identified and considered to be a mucosal lesion, another ESD could be the treatment option.

Lateral extension in the submucosa beneath a non-cancerous mucosa was observed in two cases. This rare phenomenon is also reported in cancer of the esophagogastric junction, where a Barrett's cancer sometimes extends orally beneath a non-cancerous squamous epithelium.[12] As the lateral extending part is covered by a normal mucosa, it seems to be impossible to diagnose by normal endoscopy and chromoendoscopy. Using endoscopic ultrasonography, it may be difficult to detect a small portion of submucosal cancer at the edge of a lesion. These two cases had massive submucosal invasion at the center of the lesion; therefore, a rather larger resection for lesions with a possibility of submucosal invasion would be reasonable.

In the present study, the preoperative diagnosis of the lesion extent was made by normal endoscopy and chromoendoscopy. Recently, chromoendoscopy with an acetic acid-indigocarmine mixture (AIM) has been reported to improve the diagnostic yield in terms of recognizing the lesion extent.[13–15] The diagnostic performance of AIM in delineating the tumor border was reported to be more than 90% in differentiated-type EGC and 70% in undifferentiated EGC.[14] However, there are lesions that become less clear after AIM, and the usefulness of AIM is also reduced in lesions with ulceration. In our study, the result of LM– resection by using normal chromoendoscopy with negative biopsy was 99% (1531/1549). Whether AIM can provide a sufficiently accurate diagnosis to avoid preoperative negative biopsies should be studied in the future.

CONCLUSION

Other than misdiagnosing a small portion of cancer extension, lateral margin positivity for cancer by ESD could result from a neighboring lesion and an unexpected lateral submucosal cancer extension. To avoid LM+ resection of EGC by ESD, one should take care of unexpected lateral extension and simultaneous multi-lesions.

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Risk factors for recurrence of artificial gastric ulcers after endoscopic submucosal dissection

Authors

Y. Huang[1], N. Kakushima[1], K. Takizawa[1], M. Tanaka[1], H. Ikehara[1], Y. Yamaguchi[1], H. Matsubayashi[1], H. Ono[1], T. Oishi[2], T. Nakajima[2]

Institutions

[1] Endoscopy Division, Shizuoka Cancer Center, Shizuoka, Japan
[2] Pathology Division, Shizuoka Cancer Center, Shizuoka, Japan

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Corresponding author
N. Kakushima, MD, PhD
Endoscopy Division
Shizuoka Cancer Center
1007 Shimonagakubo
Nagaizumi
Suntogun Shizuoka
411-8777
Japan
Fax: +81-55-9895783
kakushin-tky@umin.ac.jp

It has been reported previously that artificial gastric ulcers caused by endoscopic submucosal dissection (ESD) would heal within 8 weeks, irrespective of their size and location. The aim of this retrospective study was to describe long-term outcomes of gastric ESD ulcers. Check-up of ulcers was performed by periodic endoscopy. The rate of ESD ulcer recurrence and clinicopathological factors that may relate to recurrence were assessed. During the median observation period of 33 months, a benign ulcer recurrence occurred in 10 lesions in 10 patients (2.1 %). Univariate analysis showed that *Helicobacter pylori* infection and presence of pathological ulcer findings within the ESD specimen were significantly related to the risk of ESD ulcer recurrence. Although the frequency is low, there is a possibility of ESD ulcer recurrence in patients with *H. pylori* infection and in patients who undergo ESD for a lesion with ulceration.

Introduction

Endoscopic submucosal dissection (ESD) has been performed around the world as a treatment for early gastric cancer (EGC) when the risk of lymph node metastasis is diagnosed as being very low or negligible [1]. ESD is characterized by circumferential mucosal incision and submucosal dissection of the lesion using specially developed endoscopic knives [2]. The artificial gastric ulcers tend to become larger, because gastric lesions treated by ESD include larger lesions than those treated by conventional endoscopic mucosal resection [3].

It has been reported previously that artificial gastric ulcers caused by ESD (ESD ulcer) would heal within 8 weeks, irrespective of their size and location [4]. As these ulcers are created artificially by ESD, different from peptic ulcers, they are considered to have a good outcome with little risk of recurrence [5]. However, to our knowledge, no previous study has reported on the long-term clinical outcomes of gastric ESD ulcers. The objective of this study was to describe possibilities of gastric ESD ulcer recurrence and assess the clinicopathological factors that may relate to recurrence.

Patients and methods

A total of 1590 EGCs in 1371 consecutive patients were treated by ESD at Shizuoka Cancer Center Hospital, Shizuoka, between September 2002 and August 2008. Written informed consent was obtained from all patients before their ESD procedures. Patients who were followed for less than 12 months after ESD, and those who underwent additional treatment according to the pathological result of ESD specimens, such as gastrectomy, argon plasma coagulation, and photodynamic therapy, were excluded from the study. Finally, a total of 487 lesions in 395 patients (320 men, 75 women; median age 70 years, range 34–91 years) were included. Clinicopathological data were retrieved from medical records, endoscopic reports, and histopathological reports.

ESD was performed using an insulated-tip knife 1 or 2 as described by Ono et al. [6]. As a rule, after ESD all patients received omeprazole (40 mg) intravenously for 2 days followed by oral 10 mg rabeprazole daily for 2 months.

Follow-up endoscopy was scheduled 2 months after ESD and annually thereafter regardless of symptoms in order to evaluate healing of the ESD ulcer and metachronous neoplastic lesions. Ulcer recurrence at the scar of ESD was recorded, and biopsy specimens were taken to determine whether the ulcer was benign. ESD ulcer recur-

Table 1 Clinical characteristics of patients and endoscopic submucosal dissection (ESD) ulcer recurrence.

Factors	ESD ulcer recurrence		*P* value
	Yes (n = 10)	No (n = 385)	
Age, mean ± SD, years	69.3 ± 12.1	69.3 ± 9.0	0.99
Sex, male/female	9/1	311/74	0.70
History of peptic ulcer, yes/no	4/6	57/328	0.053
Anticoagulant/antiplatelet treatment			0.99
Used	1	50	
Not used	9	335	

Table 2 Clinical and pathological characteristics of lesions (n = 487).

Factors	ESD ulcer recurrence		P value
	Yes (n = 10)	No (n = 477)	
Location, n			0.92
Upper third	2	107	
Middle third	5	207	
Lower third	3	163	
Circumference, n			0.40
Anterior wall	4	106	
Posterior wall	2	134	
Greater curvature	0	63	
Lesser curvature	4	174	
Gross type, n			0.49
Elevated type	2	156	
Depressed type	7	243	
Mixed type	1	78	
H. pylori, n			0.049
Positive	9	269	
Negative	1	208	
Tumor size, mean ± SD, mm	60.0 ± 27.2	50.0 ± 19.8	0.11
Ulcer findings, n			0.00001
Present	9	111	
Absent	1	366	
Tumor depth, n			0.64
Mucosa	8	410	
Submucosa	2	67	

rence was defined as a benign ulcer occurring at the scar of ESD confirmed by follow-up endoscopy.

Patient characteristics and histological findings of ESD specimens were collected, and factors related to ESD ulcer recurrence were analyzed statistically by using Student t test, Fisher's exact test, and the chi-squared test. A *P* value of less than 0.05 was considered statistically significant. All analyses were performed with Statview version 5.0 (SAS Institute Inc., Cary, North Carolina, USA). For detection of *Helicobacter pylori*, paraffin wax embedded gastric biopsy specimens were obtained and underwent staining with hematoxylin-eosin and immunohistochemistry (anti-*H. pylori* antibody: Dakocytomation, Glostrup, Denmark) [7]. This study was approved by the hospital's institutional review board.

Results

The clinical characteristics of 395 patients, divided into two groups according to ESD ulcer recurrence, are shown in **Table 1**. There were no statistical differences in age, sex, past history of peptic ulcer, and daily usage of anticoagulant/antiplatelet agents, between the two groups.

The clinical and pathological characteristics of 487 lesions are shown in **Table 2**. ESD ulcer recurrence occurred in 10 of 487 lesions (2.1 %). A representative case of artificial gastric ulcer recurrence after ESD is shown in **Fig. 1**. Univariate analysis showed that *H. pylori* infection and presence of pathological ulcer

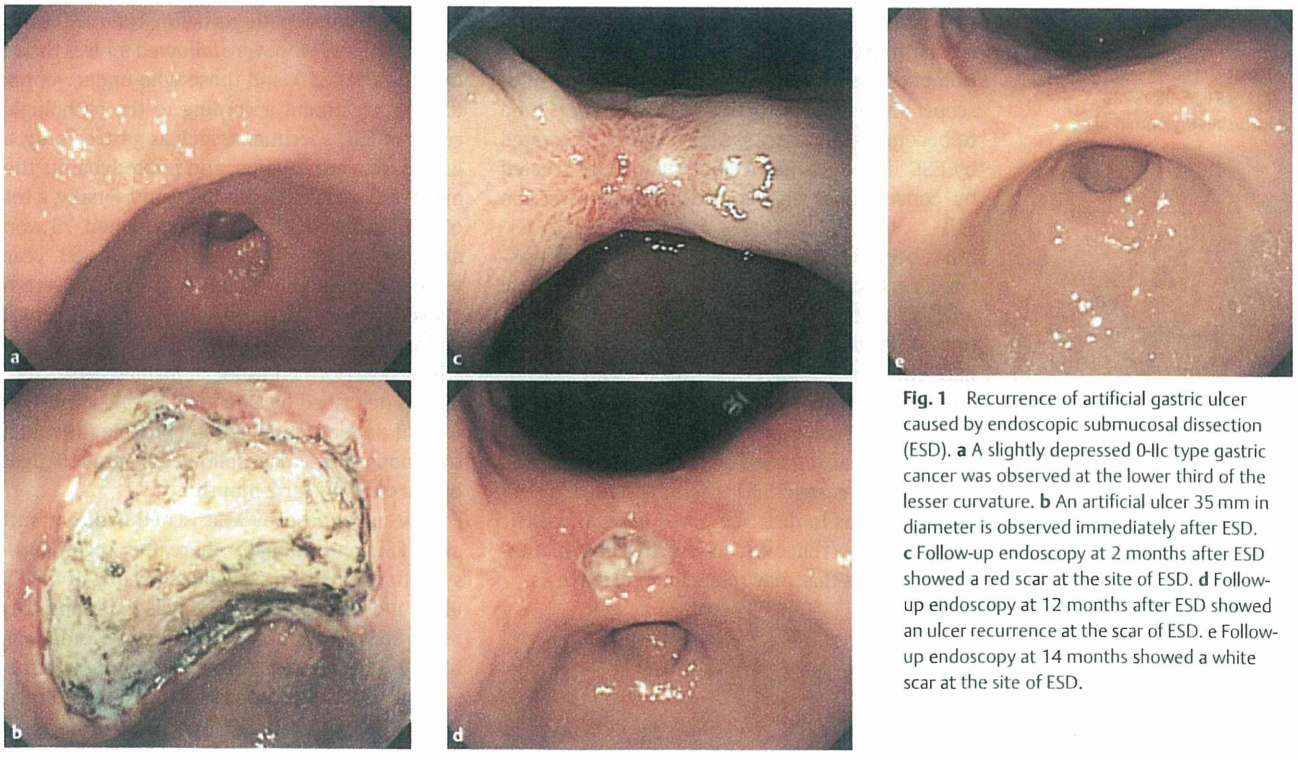

Fig. 1 Recurrence of artificial gastric ulcer caused by endoscopic submucosal dissection (ESD). **a** A slightly depressed 0-IIc type gastric cancer was observed at the lower third of the lesser curvature. **b** An artificial ulcer 35 mm in diameter is observed immediately after ESD. **c** Follow-up endoscopy at 2 months after ESD showed a red scar at the site of ESD. **d** Follow-up endoscopy at 12 months after ESD showed an ulcer recurrence at the scar of ESD. e Follow-up endoscopy at 14 months showed a white scar at the site of ESD.

findings within the lesion were significantly related to the risk of ESD ulcer recurrence ($P < 0.05$). Multivariate analysis was not performed because there were only two factors demonstrated by univariate analysis.

The clinical data and pathological findings of 10 patients with ESD ulcer recurrence are summarized in **Table 3**. The median observation period was 33 months (range 12–76 months). The median period to ulcer recurrence after ESD was 18 months (range 6–76 months). Among the 10 patients, two patients were found by endoscopy earlier than scheduled because of clinical symptoms of epigastralgia; the remaining patients were asymptomatic and ulcer recurrence were found during the periodic endoscopy. Repeated ESD ulcer recurrence was found in three patients during the follow-up endoscopy without any symptoms. Nine of the 10 patients with ESD ulcer recurrence were histologically *H. pylori*-positive, and eradication was performed in the three patients with ESD ulcer recurrence.

Table 3 The Clinical and pathological characteristics of 10 patients with ESD ulcer recurrence.

Case No.	Age, years/sex	Agents*	History of gastric/duodenal ulcer	*H. pylori*	Location	Type of tumor	Size, mm	Invasion†	Ulcer findings	Symptoms	Recurrence period, months	Recurrence times
1	53/M	–	–	+	Upper	IIa	128	SM2	+	–	6	2
2	84/M	+	–	+	Middle	IIc	42	M	+	–	7	1
3	82/M	–	–	+	Upper	IIc	63	SM2	+	–	8	3
4	71/M	–	–	+	Middle	IIc	45	M	+	–	12	1
5	57/M	–	+	+	Lower	IIc	51	M	+	–	12	1
6	84/F	–	–	+	Lower	IIc	50	M	+	–	24	2
7	75/M	–	+	+	Lower	IIc	45	M	+	Epigastralgia	32	1
8	71/M	–	+	+	Middle	IIa	35	M	+	–	36	1
9	60/M	–	+	–	Middle	IIc	49	M	+	Epigastralgia	48	1
10	56/M	–	–	+	Middle	IIa+IIc	80	M	–	–	76	1

*Anticoagulant/antiplatelet agents. †M/SM, mucosa/submucosa.

Discussion

In the present study, the rate of ESD ulcer recurrence was 2.1% during 3 years of follow-up. There was no tendency for recurrence related to location, size, and depth of tumor resected by ESD. *H. pylori* infection is considered to be a risk factor for peptic ulcer recurrence and several cohort studies have estimated that the lifetime risk of ulcer disease in *H. pylori*-positive individuals is 3–10 times that in *H. pylori*-negative individuals [8]. It is also reported that *H. pylori* status does not influence artificial gastric ulcer healing after ESD [9]. However, in the long follow-up period after ESD, whether *H. pylori* status is related to ESD ulcer recurrence remains unclear. In the present study the rate of *H. pylori* infection was significantly higher in ESD ulcer recurrence patients than in patients with no ulcer recurrence, indicating that *H. pylori* infection may relate to ESD ulcer recurrence after ESD.

From the analysis of pathological factors, the presence of pathological ulcer findings within the lesion was significantly higher in patients with ESD ulcer recurrence. Ulcer findings within the lesion may be caused by tumor invasion, concomitant peptic ulcer or ulcer scar, and from biopsy injuries. It is reported that pathological ulcer findings within the lesion would adversely affect the contractility of the gastric muscle layer and delay the ulcer size reduction after ESD [10]. We assume that ulcer scar covered with thin regenerative mucosa and less mucosal blood flow due to thickness of fibrosis would decrease the defense mechanism of gastric mucosa. Therefore it is conceivable that when there is any inflammation (e.g. caused by *H. pylori* infection) that exceeds the defense mechanism at the scar of ESD, it may easily cause a recurrence of ESD ulcer.

The present study has several limitations. First, the analysis is based on retrospective data. Second, recurrence cases are relatively small and the follow-up period is still limited. However, this is the first study to describe the rate and related factors of recurrence of benign ESD ulcers.

In conclusion, although the frequency is low, there is a possibility of gastric ESD ulcer recurrence. *H. pylori* infection and presence of pathological ulcer findings within the lesion would be risk factors for recurrence.

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ORIGINAL ARTICLE

INFLUENTIAL FACTORS IN PROCEDURE TIME OF ENDOSCOPIC SUBMUCOSAL DISSECTION FOR GASTRIC CANCER WITH FIBROTIC CHANGE

SHIGENORI NAGATA,[1] YU-FEN JIN,[1] MIKI TOMOEDA,[1] MASANORI KITAMURA,[1] MICHIKO YUKI,[1] HIDENORI YOSHIZAWA,[1] CHIAKI KUBO,[1] YURI ITO,[2] NORIYA UEDO,[3] RYU ISHIHARA,[3] HIROYASU IISHI[3] AND YASUHIKO TOMITA[1]

Departments of [1]Pathology, [2]Cancer Control and Statistics and [3]Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

Background: Factors correlating with the technical difficulty of endoscopic submucosal dissection (ESD) for early gastric cancer (EGC) are still unclear. EGC coexisting with fibrosis inside lesions has been a common therapeutic indication for ESD. The aim of this study was to clarify the most important factor related to difficult ESD for EGC.

Patients and Methods: Fifty-six patients (49 male and seven female, median age 66 years) who received ESD at a single institute for EGC with fibrosis in the resected lesion were selected. Various clinicopathological factors, including the histological findings of fibrotic changes within the cancer area in the resected specimen, were evaluated statistically for correlation with ESD procedure time.

Results: Univariate linear regression analysis with logarithmic ESD procedure time revealed the upper-third portion of lesion in the stomach ($P = 0.02$), histological classification of dense fibrosis (ulcer/ulcer scar-III/IV) within EGC ($P < 0.001$), and presence of peptic ulcer other than EGC ($P = 0.04$). Areas of the resected specimen ($P < 0.001$) and fibrosis ($P < 0.001$) were significant factors related to prolonged operation times. Multivariate analysis demonstrated that the upper-third portion of lesion ($P = 0.007$), ulcer/ulcer scar-III/IV findings ($P = 0.006$), and area of resected specimen ($P = 0.006$) were significant independent factors influencing ESD procedure time.

Conclusion: Histological findings of fibrotic changes coexisting with EGC are closely related to technical difficulty in ESD as well as the location of tumors. Preoperative precise evaluation of fibrotic changes within EGC may be helpful to predict a technical difficulty in ESD.

Key words: early gastric cancer, endoscopic submucosal dissection, fibrosis, peptic ulcer, procedure time.

INTRODUCTION

Since the late 1990s, endoscopic submucosal dissection (ESD) has been accepted in Western countries and Japan as an innovative procedure to remove gastrointestinal neoplasms using newly developed endoscopic knives.[1–5] Despite its disadvantages, such as the need for greater endoscopic skills and a higher incidence of complications in comparison with those of conventional endoscopic mucosal resection (EMR),[6–10] ESD for early gastric cancer (EGC) has spread throughout Japan within a short period, and has made it possible to remove larger EGC lesions in one fragment (en bloc resection).[3,5,11] Preliminary studies have demonstrated the advantage of ESD over conventional EMR for removing larger or ulcerated EGC lesions in an en bloc manner.[12–14] Thus, ESD allows precise histological assessment of the resected specimens, and it may prevent residual disease.[15–17]

Correspondence: Yasuhiko Tomita, Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari, Osaka 537-8511, Japan. Email: yasuhiko-tomita@umin.ac.jp

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Although ESD for EGC has increased rates of en bloc and histologically complete resection, and may reduce local recurrence, increased procedure time and complication risks with ESD compared with EMR remain problematic,[10,12] but factors correlating with the technical difficulty in ESD for EGC are still unclear. One significant characteristic of EGC is its frequent coexistence with peptic ulcer,[18,19] and peptic ulcers can be histologically classified according to their shape and size, activity (open ulcers or ulcer scars), depth of penetration (submucosa, muscularis externa, or beyond), or a combination of criteria.[20] The presence of ulcer or scar in EGC has been a common therapeutic indication for ESD, and is responsible for the technical difficulty of the procedure.[1]

As the main reasons for difficult gastric ESD are still unknown, the present study aimed to clarify the factors related to technical difficulty in ESD for EGC with ulcer changes. Fifty-six lesions of gastric cancer removed from the same number of patients with ESD, all of which were sole lesions limited to the mucosa or shallow submucosa accompanied by fibrotic change inside, were examined by histological assessment. Herein, we evaluated the impact on ESD procedure time of various clinicopathological factors, including size variables observed in resected specimens.

METHODS

Patients

During the period from May 2007 to September 2008, 435 consecutive patients received ESD for EGC at the Department of Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan. After review of histological specimens, 56 cases of EGC with fibrosis were selected for the present study. The cases of recurrent tumors with fibrosis that was created by previous endoscopic treatment were excluded. There were 49 male and seven female patients, with a median age of 66 years (mean, 67.0 years; range, 51–84 years). All the patients were confirmed as harboring EGC by pre-therapeutic biopsy. Clinical evaluation confirmed that they met the guideline criteria or fulfilled the expanded indications for ESD according to the Gastric Cancer Treatment Guidelines of the Japanese Gastric Cancer Association.[21] The patients were sedated by intravenous injection of 2.5 mg midazolam (Dormicum; Astellas Pharma Inc., Tokyo, Japan) and 7.5 mg pentazocine (Pentagin; Daiichi-Sankyo Pharmaceutical Co., Ltd, Tokyo, Japan), and an additional 1.25–2.5 mg midazolam was given for continuous sedation as needed throughout the procedure. Surgeons consisted of 10 gastroenterologists who had experienced more than 100 gastric ESD during the period of 4–16 years (four surgeons with less than 5 years' experience, three with 5–10 years, and three with more than 10 years).

ESD procedure

All procedures were performed with a videoendoscope (GIFQ240; Olympus Medical Systems Co., Ltd, Tokyo, Japan) that was fitted with a disposable attachment (D-201–11804; Olympus) on its tip. A needle knife (KD-1L-1; Olympus), an insulated-tip (IT) knife (KD-610L; Olympus), and hemostatic forceps (Coagrasper, FD-410LR; Olympus) were used during the procedure.[22] The Intelligent Cut and Coagulation 200 (ICC-200; Erbe Elektromedizin GmbH, Tübingen, Germany) or Power Supply Diathermy 60 (PSD-60; Olympus) was used as an electrical surgical unit. A solution of 2% epinephrine (Bosmin; Daiichi-Sankyo) with 20% concentrated glycerin–fructose (10% Glycerol solution; Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) was used for submucosal injection.

Histopathological examination

The length and width of the resected specimens were measured on gross examination. EGC is confined to the mucosa or submucosa (T1 cancer) regardless of regional lymph node metastasis. The location of EGC was defined as the upper, middle and lower thirds of the stomach, together with four equal parts of the gastric circumference: lesser curvature, greater curvature, anterior wall, and posterior wall (Post). The macroscopic type of EGC was divided into superficial elevated (0-IIa), flat (0-IIb), and superficial depressed type (0-IIc). The resected specimens were fixed in 15% formalin, cut at a 2 mm interval, and processed for paraffin embedding (Fig. 1). Histological classification was assessed, such as: differentiated adenocarcinoma (well or moderately differentiated adenocarcinoma or papillary adenocarcinoma) or undifferentiated adenocarcinoma (poorly differentiated adenocarcinoma or signet-ring-cell carcinoma); size and depth of tumor; lymphatic and vascular involvement; and tumor involvement to the lateral and vertical margins. Open ulcers and ulcer scars in EGC were identified, respectively, by histological ulceration and microscopic fibrosis at least in the submucosal layer. The depth of ulcerative changes was defined histologically as UL (ulcer/ulcer scar)-II if fibrotic change was limited to the submucosal layer with interruption of the muscularis mucosa, or as UL-III/IV if the proper muscle was contained in ESD specimens and if we could confirm fusion of the proper muscle and muscularis mucosae by routine hematoxylin–eosin-staining and/or desmin (Dako A/S, Glostrup, Denmark) immunostaining. The areas of resected specimen, tumor, and fibrosis were calculated as the product of length and width in cm^2 or mm^2.

Statistical analysis

To identify variables influencing ESD procedure time, univariate and multivariate linear regression analysis was performed with logarithmic time (minutes) of operation as a dependent variable using StataCorp 2009 (StataCorp LP, College Station, TX, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

Clinical outcomes

Of all the 56 patients treated with ESD for EGC, complete en bloc resection was achieved in 55 (98.2%) patients, while piecemeal resection was performed in one (1.8%). Five patients underwent additional gastrectomy with lymph node dissection to avoid risk of metastasis. All the patients enrolled in this study received endoscopic follow up for 15–32 months (median, 23 months). Metachronous gastric cancer developed in nine patients in another area of the stomach (previous to the present cancer in four and after in five). No patients had metastases to the lymph nodes or distant organs, such as the liver and lungs, during the study period. None of the patients died of gastric cancer in the present study. Procedure-related bleeding was seen in four (7.1%) of the 56 patients, who were treated successfully with endoscopic clipping or coagulation. Perforations related to ESD occurred in one (1.8%) patient and were managed conservatively after endoscopic closure with clipping. There were no treatment-related deaths (procedure-related mortality rate, 0%).

Histological examination

The median area of resected specimens was 13.9 cm^2 (range, 5.9–46.8 cm^2). Eleven cases were superficially elevated, and the other 45 were flat or superficially depressed. Fifty-one tumors were classified as differentiated adenocarcinoma (well or moderately differentiated adenocarcinoma or papillary adenocarcinoma), and five as undifferentiated adenocarcinoma (poorly differentiated adenocarcinoma or signet-ring-cell carcinoma). Forty-nine cases were intramucosal cancer without lymphatic or vascular involvement, and seven had minute submucosal invasion (pSM1, <0.5 mm).

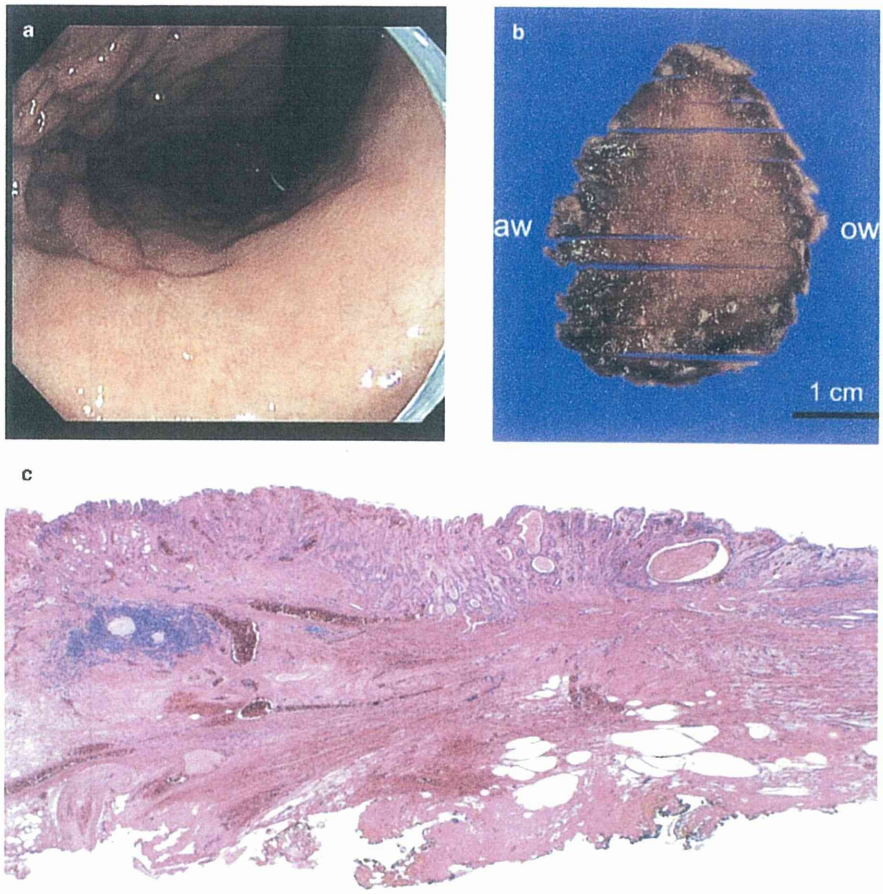

Fig. 1. Early gastric cancer with peptic ulcer. (a) Superficial carcinoma lesion located in the angle of the stomach, with fold convergence. (b) Resected specimen fixed in 15% formalin and cut at 2 mm intervals. (c) Intramucosal carcinoma coexisting with dense fibrosis of peptic ulcer/ulcer scar-III/IV (hematoxylin–eosin, ×20). ow, oral wedge (margin); aw, anal wedge.

Lymphatic invasion of carcinoma at the submucosal layer was detected in one case of invasive cancer. Open ulceration in EGC was found in two patients, and the median area of fibrosis was 93 mm^2 (range, 6–1444 mm^2). Ulcerative changes were defined as fibrosis that involved the submucosal layer (UL-II) and muscularis propria or beyond (UL-III/IV) in 42 and 14 cases, respectively.

Difference in ESD procedure time according to clinicopathological factors

ESD procedure time was 141 ± 72 (mean ± SD) minutes. Univariate linear regression analysis with logarithmic time of ESD procedure as a dependent variable demonstrated that the following factors were significant: location of the lesion in a one-third portion of the stomach ($P = 0.02$), histological classification of ulcerative changes within EGC ($P < 0.001$), presence or absence of peptic ulcer other than EGC ($P = 0.04$), and area of the specimen ($P < 0.001$) and fibrosis ($P < 0.001$; Table 1). Multivariate linear regression model with factors proven to be significant in univariate analysis showed location of the lesion ($P = 0.007$), histological classification of ulcerative changes within EGC ($P = 0.006$), and area of the specimen ($P = 0.006$) as independent factors related to longer ESD procedure time (Table 2). Adjusted R-square value of the factors was 0.557.

DISCUSSION

ESD is a new method for curative treatment of early gastrointestinal neoplasms, which was developed in order to increase the en bloc resection rate, especially for lesions >20 mm in diameter. Drawbacks of ESD include the fact that it is a technically difficult procedure that is associated with a high perforation rate.[8,10,23,24] Previously, upper location and larger tumor size, and the presence of ulceration or scarring have been reported to affect the difficulty of ESD, thus tending to prolong the procedure time.[24,25] The present study clearly demonstrated that actual size of the resected specimen and fibrotic area were factors influencing ESD procedure time.

In the present study, upper location of EGC in the stomach and UL-III/IV fibrotic change coexisting within the tumor were both significant factors related to prolonged ESD procedure time. Since lesions in the upper third of the stomach tend to be more tangential to endoscopes, it may be difficult to recognize and access the lesions during treatment.[25] In the present study, UL-III/IV was defined as ulcerative change accompanied by fusion of muscularis mucosae and proper muscle, which was confirmed in the resected specimens.

The kind of knives and devices used is also considered to be an important factor related to ESD procedure time. In the present study, all the procedures were performed with the same videoendoscope. An IT knife 2 was mainly used during

Table 1. Univariate linear regression analysis of clinicopathological factors influencing ESD procedure time in 56 patients with EGC with ulcerative change

Factors	Category	No. of lesions	Coefficient of logarithmic ESD time	95% confidence interval		*P*
Age (years)	1: ≤60	14				
	2: >60	42	−0.02	−0.33	0.28	0.88
Sex	1: Male	49	0.36	−0.02	0.75	0.06
	2: Female	7				
Location of the lesion						
Portion of the stomach	1: U or M	14				
	2: L	42	−0.35	−0.64	−0.07	0.02
Part of the gastric circumference	1: Less	24	0.08	−0.32	0.49	0.68
	2: Gre	10	−0.05	−0.52	0.42	0.84
	3: Ant	8				
	4: Post	14	0.15	−0.29	0.59	0.51
Tumor size in largest diameter (mm)	1: ≤20	36				
	2: 20–30	11				
	3: >30	9				
Macroscopic type of EGC occupying the largest area	1: 0-IIa or IIb	12				
	2: 0-IIc	44	0.12	−0.20	0.44	0.46
Histological type of EGC based on the predominant pattern	1: Well-differentiated type	51				
	2: Undifferentiated type	5	−0.36	−0.81	0.09	0.12
Depth of tumor invasion	1: pM	49				
	2: pSM (<0.5 mm)	7	0.01	−0.38	0.41	0.95
Lymphatic invasion	1: Absent	55				
	2: Present	1				†
Venous invasion	1: Absent	56				
	2: Present	0				†
Activity of peptic ulcer within EGC	1: Open ulcer	2				†
	2: Ulcer scar	54				
Histological classification of ulcerative changes within EGC	1: UL-II	42				
	2: UL-III/IV	14	0.63	0.38	0.88	<0.001
Resectability of ESD	1: En bloc resection	55				
	2: Piecemeal resection	1				†
Peptic ulcer other than EGC	1: Absent	43				
	2: Present	13	0.38	0.02	0.74	0.04
Detection of peptic ulcer within EGC on pre-therapeutic endoscopy	1: Undetected	37				
	2: Detected	19	−0.05	−0.33	0.23	0.72
Surgeons' experience of operation (years)	1: ≤5	15				
	2: ≤10	23	0.09	−0.24	0.42	0.6
	3: >10	18	0.03	−0.31	0.38	0.85
Size variables (continuous scale)						
Area of specimen (cm^2)			0.03	0.01	0.04	<0.001
Area of tumor (cm^2)			0.04	−0.002	0.09	0.06
Area of fibrosis (cm^2)			0.12	0.08	0.16	<0.001

†Variables excluded because the category included fewer than three cases, which meant a regression model could not be carried out.

Ant, anterior wall; CI, confidence interval; EGC, early gastric cancer; ESD, endoscopic submucosal dissection; Gre, greater curvature; L, lower third; Less, lesser curvature; M, middle third; pM, pathological mucosal invasion; Post, posterior wall; pSM, pathological submucosal invasion; U, upper third; UL, ulcer/ulcer scar.

Table 2. Multivariate linear regression analysis of dependent factors possibly influencing endoscopic submucosal dissection procedure time

Variables	Category	95% confidence interval		*P*-value
Location of the lesion	1: U or M			
	2: L	−0.599	−0.197	0.007
Histological classification of ulcerative changes within EGC	1: UL-II			
	2: UL-III/IV	0.362	0.774	0.006
Peptic ulcer other than EGC	1: Absent			
	2: Present	−0.117	0.452	0.24
Size variables				
Area of specimen (cm^2)		0.005	0.027	0.006
Area of fibrosis (cm^2)		−0.022	0.089	0.23

CI, confidence interval; EGC, early gastric cancer; L, lower third; M, middle third; U, upper third; UL, ulcer/ulcer scar.

the procedure, and a needle knife was temporarily used only when fibrous submucosal tissue was difficult to dissect with the IT knife 2; thus, the affect of difference in the devices used cannot be evaluated in the present analysis. In general, technical aspects, such as surgeons' experience, are also considered as a factor of time of operation. In the present study, surgeons' experience of operations (years) was not a statistically significant factor. In our institute, all ESD procedures are performed under supervision of expert endoscopists, so surgeons with less experience can easily obtain technical advice when necessary.

We conclude that the factors that directly affect the procedure time of ESD for EGC with ulcerative changes include the portion of the lesion in the stomach, coexisting UL-III/IV histological changes, and the actual size of the resected specimen. This result suggests that the density of fibrotic change rather than the location of fibrosis in the lesion affects ESD procedure time. Preoperative evaluation of fibrotic changes by ultrasonographic or radiographic examination may be helpful to predict the technical difficulty in ESD, and surgeons should perform dissection on an appropriate line to avoid prolonged ESD procedure time.

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Lymphatic Tumor Emboli Detected by D2-40 Immunostaining Can More Accurately Predict Lymph-node Metastasis

Hiromitsu Moriya · Makoto Ohbu · Nobuyuki Kobayashi · Satoshi Tanabe · Natsuya Katada · Nobue Futawatari · Shinichi Sakuramoto · Shiro Kikuchi · Isao Okayasu · Masahiko Watanabe

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Abstract

Background Resected specimens of superficial squamous cell carcinoma of the esophagus (SSCCE) underwent D2-40 immunostaining to accurately assess lymphatic tumor emboli (LY) and to analyze correlations between LY and lymph node metastasis (N). This present study was designed to determine the accuracy of LY grade for predicting the risk of N.

Materials and methods We studied 75 patients with SSCCE who underwent surgical resection of their tumors. Resected specimens were sliced into continuous sections at 5 mm intervals. Intramucosal cancers are classified into three groups (m1, m2, m3), and submucosal cancers are also divided into three groups (sm1, sm2, sm3). The numbers of LY present in lymphatic ducts on D2-40 immunostaining, venous tumor emboli (V) on CD34 immunostaining, and lymphatic tumor emboli (ly) and V on hematoxylin-eosin staining (HE) and elastica van Gieson staining (EVG) were counted for each case. The presence of lymphatic tumor emboli was graded according to the total number of LY per case as follows: 0, LY0; 1 to 2, LY1; 3 to 9, LY2; and 10 or more, LY3.

Results All m1 and m2 cases were LY− and N− Lymphatic tumor emboli were present in 54% of m3 cases, 70% of sm1 cases, 54% of sm2 cases, and 75% of sm3 cases. Determination of N was positive in 18% of m3 cases, 47% of sm1 cases, 36% of sm2 cases, and 62% of sm3 cases. The frequency of LY significantly correlated with the number of N ($p < 0.0001$). Multiple regression analysis showed that only LY and V significantly correlated with N. When the detection rate of N was compared between LY and ly, LY was superior to ly in terms of specificity, accuracy, positive predictive value, and false positive rate. As for LY grade, N was positive in 39.1% of LY1 cases, 81.8% of LY2 cases, and 100% of LY3 cases. Even in LY-, N was positive in one sm1 case and in two sm2 cases. In the sm1 case, the depth of invasion was 350 μm from the lower margin of the muscularis mucosae.

Conclusions Evaluation of lymphatic invasion on the basis of LY is more accurate for the prediction of N than conventional techniques, and LY grade strongly correlates with N. In patients with SSCCE, mucosal cancers (m1, m2, and m3) and submucosal cancers with a depth of invasion of ≤200 μm from the lower margin of the muscularis mucosae on endoscopic mucosal resection have a low risk of N if the number of LY is 0. Endoscopic mucosal resection alone can provide good treatment outcomes in such patients.

H. Moriya (✉) · N. Kobayashi · N. Katada · N. Futawatari · S. Sakuramoto · S. Kikuchi · M. Watanabe
Department of Surgery, School of Medicine, Kitasato University, Tokyo, Japan
e-mail: hmoriya@med.kitasato-u.ac.jp

M. Ohbu
Department of Pathology, School of Allied Health Sciences, Kitasato University, Tokyo, Japan

S. Tanabe
Department of Internal Medicine, School of Medicine, Kitasato University, Tokyo, Japan

I. Okayasu
Department of Pathology, School of Medicine, Kitasato University, Tokyo, Japan

Introduction

Esophageal squamous cell carcinoma is associated with a high risk of lymph node metastasis (N) and a poor

prognosis [1]. However, superficial squamous cell carcinoma of the esophagus (SSCCE) without N has a favorable prognosis after endoscopic resection [2]. Even SSCCE with N often responds to additional treatment, such as surgery and chemoradiotherapy [3]. Therefore, accurate prediction of N plays an important role in the determination of treatment policy.

The Japan Esophagus Society classifies SSCCE into six categories according to the depth of invasion to determine treatment policy and assess prognosis. Intramucosal cancers are classified into three groups: m1, cancer confined to the mucosal epithelium; m2, cancer confined to the lamina propria mucosae; and m3, cancer reaching the muscularis mucosae. Submucosal cancers are also divided into three groups: sm1, cancer confined to the upper third of the submucosa; sm2, cancer confined to the middle third of the submucosa; and sm3, cancer confined to the lower third of the submucosa [4, 5] (Fig. 1). Endoscopically resected specimens are classified as described above for intramucosal cancers. Submucosal cancers are divided into two groups according to whether the depth of invasion is 200 μm or less (pSM1) or greater than 200 μm (pSM2) from the lower margin of the muscularis mucosae. The treatment guidelines for SSCCE are based on this classification [6]. These guidelines consider tumors with an estimated depth of invasion of m1 or m2 that involve two thirds or less of the circumference of the esophagus to have an absolute indication for endoscopic resection. Tumors with an estimated depth of invasion of m3 or sm1 without N on diagnostic imaging studies are considered to have a relative indication for endoscopic resection. Tumors with an estimated depth of invasion of sm2 or sm3 have an indication for open surgery, in principle. Endoscopic resection can be performed for m1 and m2 tumors because such lesions are usually free of N. In contrast, m3 and sm1 tumors have an increased risk of N; diagnosis of N before and after endoscopic treatment is thus very important. Examinations such as endoscopic ultrasonography, (EUS) computed tomography (CT), and magnetic resonance imaging (MRI) are used for further assessment of N.

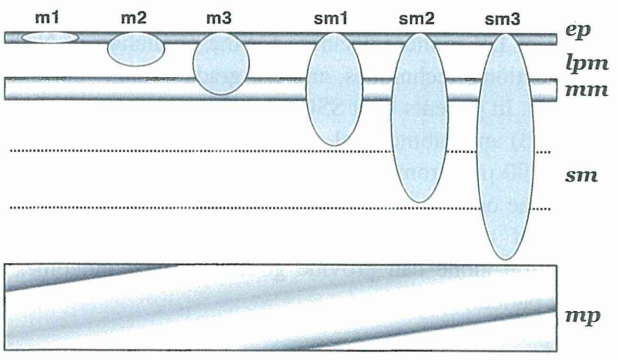

Fig. 1 Histological classification of superficial carcinoma of the esophagus. Intramucosal carcinoma is divided into m1, m2, and m3, and submucosal carcinoma is divided into sm1, sm2 and sm3. *ep* surface squamous epithelium; *lpm* lamina propria mucosae; *mm* muscularis mucosae; *sm* submucosa; *mp* muscularis propria

During growth and development of malignant tumors, cancer cells are thought to enter lymphatic vessels and take root in lymph nodes, leading to N. Lymphatic invasion by cancer cells is considered a preliminary step in the development of N. Analysis of lymphatic invasion may therefore allow accurate prediction of N. Lymphatic invasion is reported to be the most reliable histopathological finding linked to the risk of N on examination of resected specimens [3, 7, 8]. Lymphatic invasion can usually be identified on hematoxylin-eosin staining (HE) or elastica van Gieson staining (EVG). Tumor emboli in the lumen of vessels covered by a single layer of endothelial cells not including erythrocytes are regarded to represent lymphatic invasion. However, complete occlusion of lymphatic vessels by cancer cells is not considered lymphatic invasion. Moreover, when tumors are fixed in formalin, a gap due to tissue shrinkage appears between tumor cells and the surrounding stroma. Such retraction artifacts are sometimes misinterpreted to be lymphatic tumor emboli [9, 10]. Recent studies have described several lymphatic endothelial markers [11–13]. One of these markers, D2-40, is a novel monoclonal antibody against Mr 40000 O-linked sialoglycoprotein that reacts with a fixation-resistant epitope in lymphatic endothelium [9, 10]. Many studies have reported that D2-40 is useful for the identification of lymphatic tumor emboli in the stroma of neoplasms and tumors because D2-40 does not react with the vascular endothelium of arteries, veins, or capillaries [9–18]. Therefore, we examined the frequencies of lymphatic tumor emboli identified by D2-40 immunostaining (LY) and of lymphatic tumor emboli identified by HE and EVG (ly) in specimens of esophageal superficial cancer. We then examined correlations of LY and ly with N to determine whether LY and ly can be used to predict N.

Materials and methods

Patients

The study group consisted of 75 patients who underwent surgery for SSCCE at Kitasato University Hospital and Kitasato University East Hospital from 1990 through 2005. The depth of tumor invasion was m1 in 2 patients (2%), m2 in 7 patients (9%), m3 in 11 patients (14%), sm1 in 17 patients (22%), sm2 in 22 patients (29%), and sm3 in 16 patients (21%) (Table 1). All patients underwent periesophageal and perigastric lymph node dissection. None of the patients received preoperative radiotherapy or

Table 1 Relation between depth of invasion and lymphatic tumor emboli, venous tumor emboli, and lymph node metastasis

Depth	Cases	LY	ly	V	N
m1	2	0	0	0	0
m2	7	0	0	0	0
m3	11	6 (54.5%)	5 (45.5%)	1 (9.1%)	2 (18.2%)
sm1	17	12 (70.6%)	14 (82.4%)	4 (23.5%)	8 (47.1%)
sm2	22	12 (54.5%)	17 (72.3%)	13 (54.5%)	8 (36.4%)
sm3	16	12 (75.0%)	15 (93.8%)	16 (100.0%)	10 (62.5%)
Total	75	40 (53.3%)	51 (68.0%)	34 (45.3%)	28 (37.3%)

LY lymphatic tumor emboli detected by D2-40 immunostain; *ly* lymphatic tumor emboli detected by hematoxylin-eosin and elastica van Gieson stains; *V* venous tumor emboli detected by elastic van Gieson stain and CD34 immunostain; *n* metastasis of dissected lymph nodes; *n'* lymph node metastasis detected by image analysis after operation; *N* means patients with n or n'

chemotherapy. Lymph node metastasis was assessed on the basis of pathological features of the dissected lymph nodes at the time of operation. If N was confirmed, the case was evaluated to be N+. If N was not detected, the evaluation was N−.

Immunohistoenzyme chemistry

The surgically resected esophagus was fixed in 10% formalin and was then sliced into continuous sections at intervals of about 5 mm, from the proximal end of the esophagus to the esophagogastric junction. Thirty tissue blocks were prepared per case on average. All blocks were embedded in paraffin and cut into 3-μm-thick sections, which were processed for staining with HE and with EVG.

To retrieve antigens, tissue specimens were soaked in 0.01 M citrate buffer (pH 6) and heated in a microwave oven three times for 5 min. The tissue specimens of whole cancer lesions were allowed to react with D2-40 monoclonal antibody (1:40 dilution; Clone D2-40, Signet Laboratories, Dedham, MA) and anti-CD34 monoclonal antibody (1:100 dilution; Clone NU-4A1, Nichirei, Tokyo) for 1 h at room temperature. Next, the specimens were allowed to react with peroxidase-conjugated anti-mouse IgG antibodies (Nichirei) for 30 min, and peroxidase activity was detected with H_2O_2/diaminobenzidine substrate solution (Wako, Tokyo). The sections were counterstained with hematoxylin (Fig. 2).

Assessment of tumor emboli

A tumor embolus detected on visual inspection of lymphatic vessels labeled with D2-40 was counted as 1. The total number of LY were counted for each case. Lymphatic tumor emboli were evaluated by two pathologists who were blinded to N. Venous tumor emboli (V) were similarly evaluated with the use of CD34, and the total number was recorded. As part of routine pathological evaluation, tissue specimens were also stained with HE and with EVG; ly and v were then assessed by two pathologists.

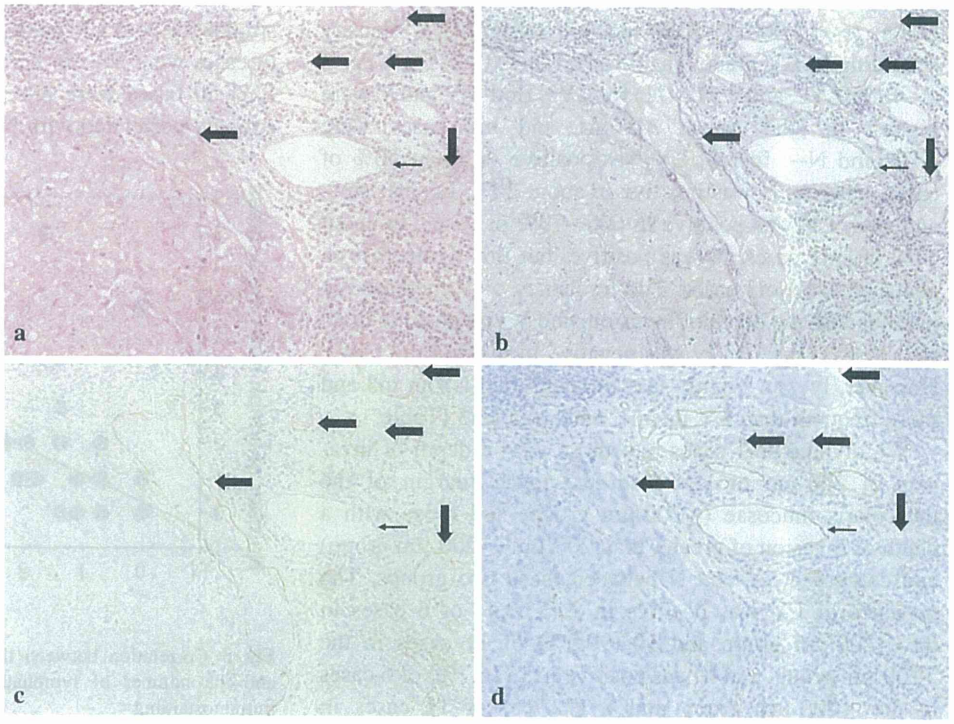

Fig. 2 Histologic appearance of tumor emboli. **a** Hematoxylin-eosin stain; (**b**) elastica van Gieson stain; (**c**) D2-40 immunostain; (**d**) CD34 immunostain. *Thick arrows*, lymphatic tumor emboli; *thin arrows*, veins