An extra 1-cm margin during endoscopic submucosal dissection can control subsquamous tumor extension of esophagogastric junction adenocarcinoma

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Introduction

The incidence of esophagogastric junction (EGJ) adenocarcinoma has increased during recent decades, especially in Western countries. This is mainly related to the increasing prevalence of gastroesophageal reflux disease and obesity, and the decreasing prevalence of *Helicobacter pylori* infection. The incidence of EGJ adenocarcinoma is also increasing in Eastern countries, including Japan, and is expected to continue doing so for similar reasons. Endoscopic submucosal dissection (ESD) is a minimally invasive and curative treatment for superficial gastrointestinal cancer, as it provides a high rate of en bloc resection, regardless of lesion size, and facilitates accurate histopathological diagnosis [1]. Several retrospective studies have recently indicated that ESD is safe and effective for EGJ adenocarcinomas [2–6].

EGJ adenocarcinomas can extend laterally and invade the neighboring normal esophageal squamous epithelium; this is known as subsquamous tumor extension (STE) [7]. Because STE may not be observable at the esophageal epithelium, it is difficult to accurately define the proximal tumor margin during ESD for EGJ adenocarcinomas; this can lead to postoperative positive lateral margins [3, 5, 6]. In a previous study, we retrospectively showed that an extra 1-cm safety margin achieved 100% negative lateral margins during ESD for EGJ adenocarcinomas, even when the STE was < 7 mm [4].

In response to the lack of prospectively collected data, the present prospective feasibility study aimed to evaluate whether considering an extra 1-cm safety margin was effective in preventing positive lateral margins during ESD for EGJ adenocarcinomas.

Patients and methods

Patients

The protocol for this single-center prospective feasibility study was approved by the ethics committee of the Osaka City University Hospital (approval: 3064) and was registered in the University Hospital Medical Network Clinical Trial Registry (UMIN000017120). All patients provided written informed consent before enrollment. The inclusion criteria comprised: (1) an EGJ tumor diagnosed as Siewert's type II classification; (2) pathological diagnosis of definite or suspected adenocarcinoma; (3) cT1a-M (invasion limited to the muscularis mucosae); (4) no signs of lymph node or distant metastasis; (5) age > 20 years; and (6) provided written informed consent. The exclusion criteria comprised: (1) no informed consent; (2) possibility of pregnancy or lactation; (3) severe mental illness; (4) continuous systemic steroid use; (5) active bacterial or fungal infection; (6) cardiac infarction or unstable angina pectoris within the last 3 months; (7) uncontrollable hypertension or diabetes mellitus; (8) pulmonary disease requiring oxygen; or (9) judged

to be ineligible at the discretion of a study investigator. The sample size was, based on our previous experience, calculated as per the number of patients that we could feasibly enroll during the study period.

Outcome measures

The primary outcome of interest was the rate of complete resection (R0 resection rate), i. e., an en bloc resection with histologically cancer-free margins. The secondary outcomes of interest were the treatment outcomes (curative resection and adverse events [AEs]) and the incidence, extension length, and preoperative diagnostic rate of the STE. Preoperative diagnostic rate was defined as accuracy rate. We determined that STE could be diagnosed if the presence and length of STE were consistent with preoperative endoscopic diagnosis.

Defining STE and the 1-cm safety margin

Preoperative endoscopy was performed using an Evis Lucera Elite System (Olympus, Tokyo, Japan) with a magnifying upper gastrointestinal endoscope (GIF-H260Z; Olympus). We delineated the lesion's area via magnifying endoscopy with narrow-band imaging (NBI) (▶Fig. 1a). In all cases, the presence of STE was determined on the basis of the following modalities: 1) white light endoscopy (WLE) for visualizing a slight elevation (▶Fig. 1b) [4]; 2) non-magnifying endoscopy with NBI for visualizing a pale brownish area (▶Fig. 1c) [5]; 3) magnifying endoscopy with NBI for visualizing an irregular microvascular pattern under the normal squamous epithelium (▶Fig. 1d) [7]; and 4) magnifying endoscopy with acetic acid application for visualizing a small white sign (▶Fig. 1e) [8].

The marking dots were generally positioned at least 2 mm outside the tumor margin. However, these dots were placed 1 cm away from the oral side of any suspected STE or 1 cm away from the squamous-columnar junction in cases without endoscopic signs of STE. The 1-cm safety margin was measured using a 1-cm marking on the tip of the device's sheath (▶Fig. 1f, ▶Fig. 1g, ▶Fig. 1h). ESD was performed as previously reported [4]. A flush knife (Flush knife, DK2620JN; Fujifilm Medical, Tokyo, Japan) was used as the main electrosurgical knife.

Pathological examination

The fresh and post-formalin fixation specimens were photographed with a scale. The resected specimens were cut into 2-mm slices from the oral side to the anal side, and each slice was evaluated to determine the histological type, size, depth of invasion, lateral and vertical margins, and lymphovascular invasion status of the EGJ tumor. In cases with STE, the length of the extension was determined with a scale using microscopy, and the lesion was then mapped on the photographs of the fresh specimen. The length of the STE was based on the photograph of the fresh specimen. In the same case as described in ▶Fig. 1, an EGJ adenocarcinoma arising from the Barrett's esophagus was defined as a Barrett's adenocarcinoma and was identified from the presence of esophageal glands at the anal side of the carcinoma (▶Fig. 2).

Gastroesophageal reflux disease or proton pump inhibitor (PPI) use sometimes makes it difficult to assume that double
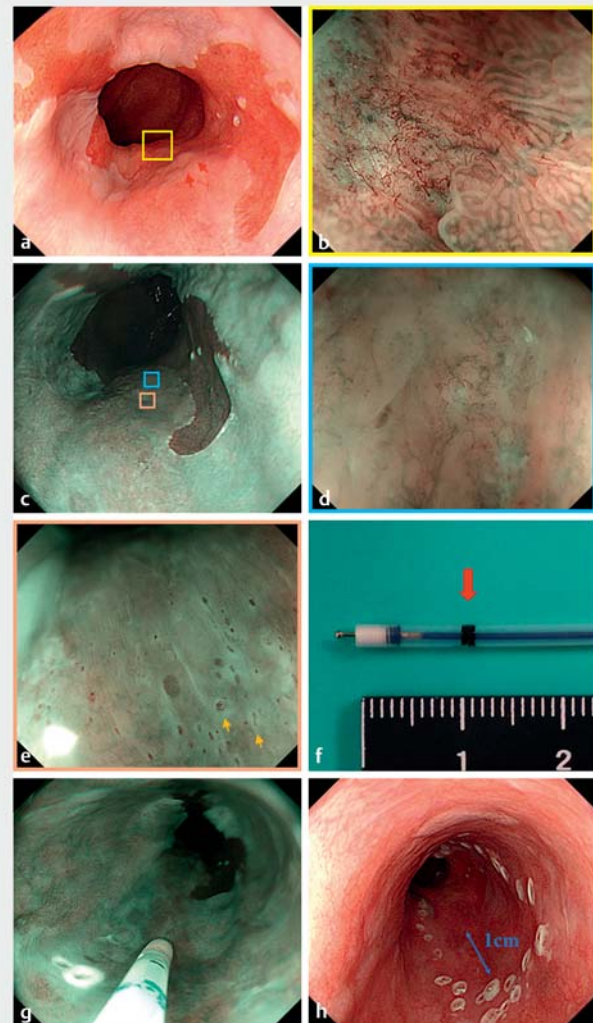
▶**Fig. 1** Endoscopic sign of EGJ adenocarcinomas and placement of the 1-cm safety margin. **a** Demarcation line with an irregular microvascular or microsurface pattern during magnifying endoscopy with NBI. **b** Slight elevation (red arrow) during white light endoscopy. **c** Pale brownish area during non-magnifying endoscopy with NBI. **d** Irregular microvascular pattern under the normal squamous epithelium during magnifying endoscopy with NBI. **e** Small white sign (orange arrow) during magnifying endoscopy with acetic acid application. **f** One-cm marking on the tip of the device's sheath. **g** Measurement of the 1-cm safety margin using the device. **h** Marking dots around the lesion. EGJ, esophagogastric junction; NBI, narrow-band imaging

muscularis mucosa and columnar epithelial island. Thus, in this study, esophageal glands on the anal side of the lesion were considered as an accurate definition of Barrett's adenocarcinoma. All adenocarcinomas that were not considered as Barrett's adenocarcinomas were classified as non-Barrett's adenocarcinomas.
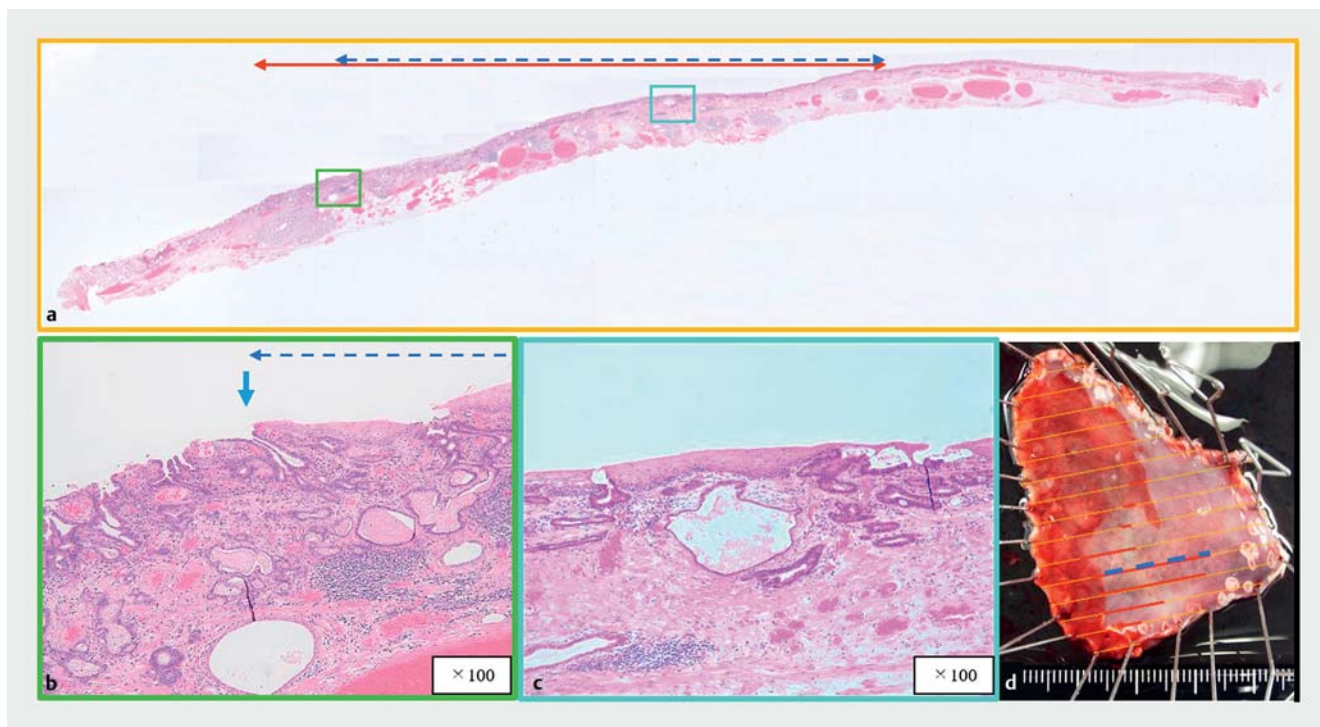
▶ **Fig. 2** Calculation of STE length. **a** Length of the STE as calculated using microscopy with a scale. The red arrow depicts the range of the lesion, and the blue arrow depicts the range of STE. **b,c** STE. **d** Length calculated by mapping the lesion on the photographs of the fresh specimen using the aforementioned reference length. STE, subsquamous tumor extension

Definitions

PPI intake was defined as taking PPI before endoscopy. It was defined as *Helicobacter pylori* infection, if any following test was positive, the urea breath test, serum anti-*Helicobacter pylori* body titer, and stool antigen test. The presence of circular Barrett mucosa extending longitudinally for 3 cm or more was defined as long-segment Barrett's esophagus (LSBE), and the others were defined as short-segment Barrett's esophagus (SSBE) [9]. Gastric atrophy was definition based on Kimura-Takemoto Classification. Mild atrophy was defined as C-1 and C-2, moderate was as C-3 and O-1, severe was O-2 and O-3, and no was as C0.

An en bloc resection was defined as the resection of a single specimen that included all the marking dots. Complete resection was defined as an en bloc resection with histologically cancer-free margins. Curative resection of a Barrett's adenocarcinoma was defined as the complete resection of a differentiated adenocarcinoma with an invasion depth of T1a-DMM (deep muscularis mucosa) and no lymphovascular invasion, as these patients have a very low risk of metastasis [10]. Curative resection of a non-Barrett's adenocarcinoma was defined as the complete resection of an adenocarcinoma with an invasion depth of < 500 µm from the muscularis mucosa, no lymphovascular invasion, and a poorly differentiated component [11].

Perforation was defined as an endoscopically visible hole in the esophageal or gastric wall that exposed the mediastinal or peritoneal cavity. Delayed bleeding was defined as bleeding with hematemesis or melena that required endoscopic reinter-

vention or transfusion after the ESD procedure. Esophageal stricture was defined as diameter reduction that prevented the passage of a standard 9.2-mm endoscope at the follow-up endoscopic examination (1 to 2 months after ESD).

Statistical analyses

Continuous variables were reported as median (range) and analyzed using the Mann-Whitney U test, categorical variables were reported as number (percentage) and analyzed using Fisher's exact test. Differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using EZR software (version 1.35; Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R software (The R Foundation for Statistical Computing, Vienna, Austria).

Results
Clinical characteristics

Fourteen patients presented with a suspected EGJ adenocarcinoma between September 2015 and December 2018. One patient with histologically no cancer and another in whom an evaluation for STE was impossible, because the specimen was not cut from the oral side to the anal side, were excluded. Thus, 12 patients (▶ **Table 1**), five of whom were histologically diagnosed with a Barrett's adenocarcinoma, were enrolled in this study.

▶ **Table 1** Patient characteristics

	Total	Barrett's adenocarcinoma	Non-Barrett's adenocarcinoma
	n = 12	n = 5	n = 7
Age, years, median (range)	64 (50 – 80)	63 (50 – 76)	64 (60 – 80)
Sex, male/ female, n	9/3	5/0	4/3
ASA PS, 1/2/3, n	2/8/2	5/0/0	2/3/2
Medication, PPI, n	5	1	4
Helicobacter pylori infection[1], n	3	0	3
SSBE/LSBE, n	8/0	5/0	3/0
Gastric atrophy, no/mild/moderate, n	6/2/4	5/0/0	1/2/4
Tumor size, mm, median (range)	28 (5.0 – 53)	28 (13.0 – 37)	28(5.0 – 53)
Circumference, %, median (range)	29.2 (8.3 – 91.7)	16.7 (8.3 – 58.3)	33.3 (8.3 – 91.7)
Macroscopic appearance, elevated/flat/depressed, n	4/3/5	1/1/3	3/2/2
Depth of cancer, mucosa/submucosa, n	8/4	4/1	4/3

ASA PS, American Society of Anesthesiologists Physical Status; PPI, proton pump inhibitor; SSBE, short-segment Barrett's epithelium; LSBE, long-segment Barrett's epithelium.
[1] Status of *Helicobacter pylori* in two cases was not investigated.

▶ **Table 2** Primary and secondary outcomes.

|	Total	Barrett's adenocarcinoma	Non-Barrett's adenocarcinoma
	n = 12	n = 5	n = 7
Result of resection, n (%)			
▪ En bloc resection	12 (100)	5 (100)	7 (100)
▪ Complete resection (R0)	11 (91.7)	5 (100)	6 (85.7)
▪ Negative lateral margin	12 (100)	5 (100)	7 (100)
▪ Negative vertical margin	11 (91.7)	5 (100)	6 (85.7)
▪ Curative resection	8 (66.7)	4 (80.0)	4 (57.2)
Adverse events of ESD procedure, n (%)			
▪ Stricture	2 (16.7)	0 (0)	2 (28.6)
▪ Perforation	0 (0)	0 (0)	0 (0)
▪ Delayed bleeding	0 (0)	0 (0)	0 (0)
Subsquamous tumor extension (STE)			
▪ Incidence of STE, n (%)	9 (75.0)	4 (80.0)	5 (71.4)
▪ Preoperative diagnosis of STE, n (%)	8 (66.7)	5 (100)	3 (42.9)
▪ Length of STE, mm, median (range)	5.7 (1.0–24.8)	6.2 (5.7–21)	3.6 (1.0–24.8)

ESD, endoscopic submucosal dissection; STE, subsquamous tumor extension.

Primary endpoints

The complete resection rate was 91.7% (95% confidence interval [CI]: 62.5–100.6) (11/12) (▶ **Table 2**). The remaining patient with a positive vertical margin had an EGJ adenocarcinoma with massive submucosal invasion. The rate of negative lateral margins was 100% (95% CI: 71.8–103.9), and all lesions were laterally included within the 1-cm safety margin.
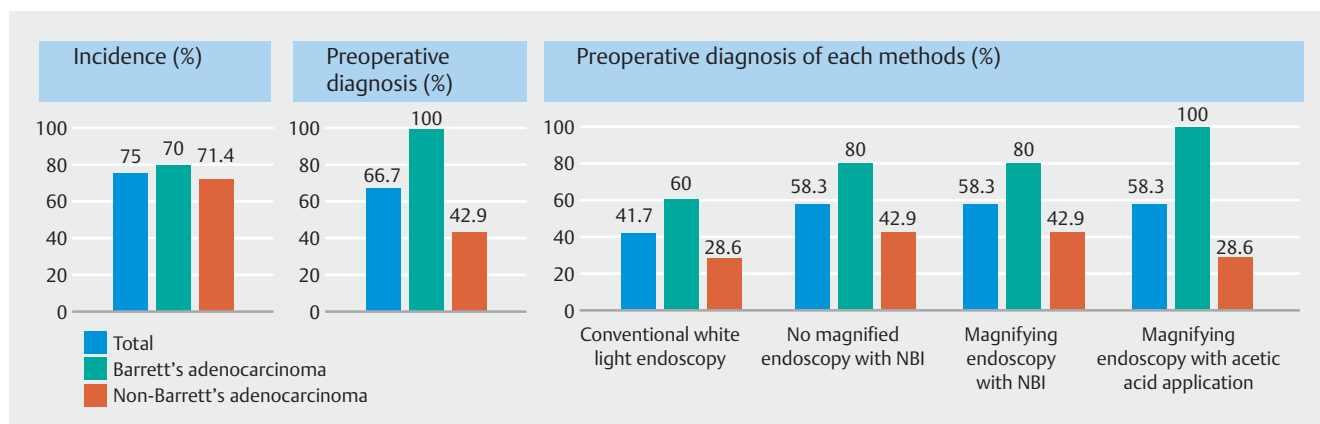
▶ **Fig. 3** Preoperative diagnosis rates for STE using various modalities. Preoperative diagnosis was defined as accuracy. EGJ, esophagogastric junction; NBI, narrow-band imaging; STE, subsquamous tumor extension
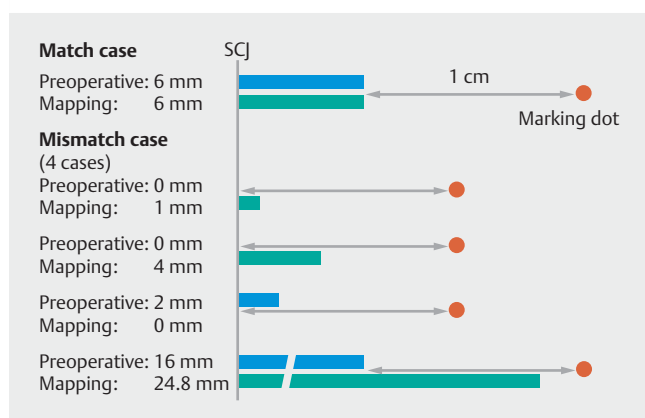
▶ **Fig. 4** Four misdiagnosed cases of STE. The white bar shows preoperative diagnosis, while the gray bar shows pathological result. The black dots show oral side marking. No findings were detected in two cases during preoperative endoscopy. The final case was expected to have 16-mm submucosal tumor extension; however, the pathological extension was 24.8 mm. SCJ, squamous-columnar junction; STE, subsquamous tumor extension

Presence of STE

STE was observed in 75 % of patients (9/12; ▶ **Table 2**). The median length of the STE was 5.7 mm (range: 1–24.8 mm; ▶ **Table 2**). The preoperative diagnostic rates for the four modalities are shown in ▶ **Fig. 3**. Magnifying endoscopy with acetic acid application identified STE in 100 % (5/5) of patients with a Barrett's adenocarcinoma but in only 28.6 % (2/7) of patients with a non-Barrett's adenocarcinoma. The preoperative diagnostic rates for STE of EGJ adenocarcinomas were 66.7 % using all four modalities, 41.7 % using WLE, 58.3 % using magnifying endoscopy with NBI, and 58.3 % using magnifying endoscopy with acetic acid application. Four patients were misdiagnosed during preoperative endoscopy (▶ **Fig. 4**), all with non-Barret's adenocarcinoma. Sensitivity and specificity rates for STE of EGJ adenocarcinomas were both 66.7 % using all four modalities (▶ **Table 3**). Histological type was well-differentiated adenocarcinoma in

three patients and moderately in one patient. In all cases, invasion depth of STE was the lamina propria mucosa.

Clinical outcomes

The curative resection rate was 66.7 % (8/12). Two patients had an esophageal stricture. There was no bleeding or perforation in any of the patients (▶ **Table 2**).

Discussion

This prospective feasibility study revealed that an extra 1-cm safety margin is safe and helpful for achieving negative lateral margins during an ESD for EGJ adenocarcinomas. Despite the difficulty in accurately diagnosing STE preoperatively, all lesions were included within the 1-cm margin. To the best of our knowledge, this is the first prospective study in this setting.

Our findings agree with the previously reported R0 resection rates of 79 % to 89.8 % and AE rates of 3.9 % to 27.3 % after an ESD for EGJ adenocarcinomas [2, 3, 5, 6]. However, these studies included cases with positive lateral margins due to STE (7.5 %–18.0 % of cases) [2, 3, 5, 6]. A multicenter retrospective study also revealed a positive lateral margin rate of 7.5 % (24/321 patients) [6]. These patients may require additional surgery and may have an increased risk of local recurrence [5, 8]. Because positive lateral margin has often been reported only on the oral side, we considered it important to address such an incomplete resection. Therefore, we evaluated the STE of the oral side, and we did not identify any patients with positive lateral margins after we included an extra 1-cm safety margin during ESD for EGJ adenocarcinomas, and all lesions were included within this 1-cm margin, regardless of the STE status.

Magnifying endoscopy with NBI and acetic acid application is reportedly useful for diagnosing STE [7, 8, 10]. A questionnaire survey revealed that the rates of STE diagnosis were 55 % (97/175 patients) with WLE and 60 % (42/70 cases) with magnifying endoscopy with NBI [8]. In another study, review of endoscopic images after histological examination of ESD specimens revealed STE diagnostic rates of 50 % (5/10 patients) with WLE, 43 % (3/7 patients) with magnifying endoscopy and NBI, and

▶ **Table 3** Diagnostic performance (accuracy [preoperative diagnosis], sensitivity, specificity, PPV and NPV) of STE.

	Accuracy (%) (95 % CI)	Sensitivity (%) (95 % CI)	Specificity (%) (95 % CI)	PPV (%) (95 % CI)	NPV (%) (95 % CI)
Diagnostic performance using all four modalities					
▪ Total	66.7 (45.1–80.0)	66.7 (52.3–75.6)	66.7 (23.6–93.4)	85.7 (67.2–97.2)	40.0 (14.1–56.0)
▪ Barrett's adenocarcinoma	100 (70.2–100)	100 (81.4–100)	100 (25.5–100)	100 (81.4–100)	100 (25.5–100)
▪ Non-Barrett's adenocarcinoma	42.9 (20.5–65.4)	40.0 (24.3–55.8)	50.0 (10.8–89.5)	66.7 (40.6–93.0)	25.0 (5.4–44.8)
Diagnostic performance using each modality of total					
▪ White light endoscopy	41.7 (20.9–55.1)	33.3 (19.5–42.3)	66.7 (23.6–93.4)	85.7 (67.2–97.2)	40.0 (14.1–56.0)
▪ Non-magnifying endoscopy with NBI	58.3 (36.8–71.7)	55.6 (41.2–64.5)	66.7 (23.6–93.5)	83.3 (61.8–96.7)	33.3 (11.8–46.7)
▪ Magnifying endoscopy with NBI	58.3 (36.8–71.7)	55.6 (41.2–64.5)	66.7 (23.6–93.5)	83.3 (61.8–96.7)	33.3 (11.8–46.7)
▪ Magnifying endoscopy with acetic acid application	58.3 (36.8–71.7)	55.6 (41.2–64.5)	66.7 (23.6–93.5)	83.3 (61.8–96.7)	33.3 (11.8–46.7)

CI, confidence interval; EGJ, esophagogastric junction; NBI, narrow-band imaging; NPV, negative predictive value; PPV, positive predictive value; STE, subsquamous tumor extension

100 % (6/6 patients) with magnifying endoscopy and acetic acid application [8].

The diagnostic rates in the present study were similar to those reported for WLE and magnifying endoscopy with NBI previously; however, the diagnostic rate for acetic acid application was lower than that in a previous report. This might be caused by the difference in the method of diagnosis. In a previous report, the endoscopic images of STE were reviewed retrospectively only in cases that yielded pathological results after ESD [8]. This study revealed that magnifying endoscopy with acetic acid application had higher accuracy in diagnosing STE of Barrett's adenocarcinomas than that of non-Barrett's adenocarcinomas, with the shorter length of STE of non-Barrett's adenocarcinoma being a possible explanation for the reduced accuracy. The median lengths of STE of Barrett's adenocarcinoma and non-Barrett's adenocarcinoma were 6.2 (5.7–21) mm and 3.6 (1.0–24.8) mm, and in two patients with non-Barrett's adenocarcinoma, the lengths of STEs were not detected endoscopically. In addition, because PPI use and preceding biopsy are known to occasionally result in non-cancerous squamous epithelium covering the existing tumor [12], they might contribute to a difference in diagnosis.

Nonetheless, our study is unique in that we prospectively applied the four imaging modalities in all patients, which revealed that magnifying endoscopy with acetic acid application was more accurate for diagnosing STE of Barrett's adenocarcinomas than for diagnosing the STE of non-Barrett's adenocarcinomas.

This study has several strengths, including its prospective design and the use of all four imaging modalities for all patients, which allowed us to calculate each modality's diagnostic ability for STE. Another strength includes the accurate measurement of the 1-cm margin using a mark that was placed 1 cm from the tip of the device's sheath. Finally, it is notable that the length of the STE was effectively evaluated using a photograph of the fresh specimen, based on the positional relationship between the STE and the 1-cm safety margin.

The present study also has several limitations, the first being its small single-center design; hence, there is a need for larger multicenter studies that will validate our findings. Secondary, we did not evaluate long-term outcomes. Further well-designed studies are needed to address these issues.

Conclusions

Our findings suggest that an extra 1-cm safety margin is useful for achieving complete resection of EGJ adenocarcinomas during ESD, warranting validation in a large cohort study.

Competing interests

The authors declare that they have no conflict of interest.

Clinical trial

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