Endoscopic Ultrasonography Miniature Probe Performance for Depth Diagnosis of Early Gastric Cancer with Suspected Submucosal Invasion

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Background/Aims: The accurate assessment of the depth of invasion of early gastric cancer (EGC) is critical to determine the most appropriate treatment option. However, it is difficult to distinguish shallow submucosal (SM1) invasion from deeper submucosal (SM2) invasion. We investigated the diagnostic performance of endoscopic ultrasonography (EUS) using a miniature probe for EGC with suspected SM invasion.

Methods: From April 2008 to June 2018, EGCs with suspected SM invasion were analyzed retrospectively. The EGCs examined by a 20 MHz high-frequency miniature probe was included in our study. Esophago-gastric junction cancers and patients treated by chemotherapy before resection were excluded. The sensitivity and specificity for the detection of SM2 invasion by EUS were compared with those of white light imaging (WLI). Additionally, factors related to depth underestimation or overestimation were investigated using multivariate analysis.

Results: A total of 278 EGCs in 259 patients were included in the final analysis. The sensitivity and specificity for SM2 or deeper by EUS were 73.7% (87/118) and 74.4% (119/160), respectively. The sensitivity and specificity by WLI were 47.5% (56/118) and 68.1% (109/160), respectively. The sensitivity of EUS was significantly superior to that of conventional endoscopy (p<0.01). Multivariate analysis revealed that an anterior location of the EGC was an independent risk factor for underestimation by EUS (odds ratio, 3.3; 95% confidence interval, 1.1 to 9.8; p=0.03). Conclusions: The depth diagnostic performance for EGCs with suspected SM invasion using EUS was satisfactory and superior to that of conventional endoscopy. Additionally, it is important to recognize factors that may lead to misdiagnosis in those lesions. (Gut Liver 2020;14:581-588)

Key Words: Endosonography; Stomach neoplasms; Invasion depth
MATERIALS AND METHODS

1. Patients

From April 2008 to June 2018, 1,209 consecutive patients with 1,999 EGCs suspicious of SM invasion diagnosed by endoscopic examination at our institution were analyzed retrospectively. This study was approved by National Cancer Center Hospital Ethics Committee (approval number: 2016-447). Among those, the EGCs examined by high-frequency miniature probe were included our study. The following cases were excluded: lesions located at the esophago-gastric junction (Siewert type 2) and lesions in patients who had received chemotherapy between examinations and resection (Fig. 1). The technique has been previously described in detail. Briefly, we performed EUS using 20 MHz high-frequency ultrasound probes (UM-3R; Olympus Medical Systems, Tokyo, Japan). After washing the lesion, we filled the stomach with deaerated water through an endoscope channel. After the lesion was submerged, we performed ultrasound with the miniature probe. EUS was performed by three endoscopists (H. Takamaru, H. Takisawa, and S.Y.) on a separate day from the index WLI examination. All EUS findings were supervised by one expert endosonographer (S.Y.). The final depth diagnosis via EUS was agreed upon after discussion between the three endosonographers in this study. In addition, WLI examination for depth diagnosis and EUS examination for depth diagnosis was performed by a different endoscopist.

2. Methods

The endoscopic criteria for deep obvious SM2 EGCs by WLI were as follows: an uneven, irregular, or nodular surface, a marked margin elevation or submucosal tumor-like protrusion without flexibility, a deep ulceration, an irregular protrusion (in lesions with a 0-I component), a remarkable redness, and an enlarged, clubbing, or fused folds (Fig. 2). If the endoscopic findings listed above were identified, we decided to refer the patient

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**Fig. 1.** Patient flowchart.
SM, submucosal; EUS, endoscopic ultrasonography; EG, esophago-gastric.

**Fig. 2.** Representative endoscopic findings of gastric cancer with deep submucosal invasion. The lesion showed an uneven, irregular, or nodular surface (A-D), an irregular protrusion (A), a remarkable redness (A, B), and stiffness (B).
When the depth diagnosis based on the above endoscopic criteria was of lower confidence, (i.e., patients were judged as SM1>SM2 or SM2>SM1) the endoscopists decided to perform EUS for a more detailed examination.

We reviewed endoscopic reports, electronic medical records, and histological results of EGCs. Endoscopic and histological findings evaluated were as follows: patient’s age, sex, histologically defined size of the lesion, endoscopic morphology, direction of the wall upon which the lesion was located (anterior wall, posterior wall, lesser curvature, or greater curvature), location of the lesion in the stomach (upper-third, U; middle-third, M; or lower-third, L), histological differentiation, endoscopically or histologically diagnosed ulceration, estimated depth by conventional endoscopy or by EUS, and histological depth of invasion. The EUS criteria of M-SM1 EGCs was an intact 3rd layer (submucosal layer) or 3rd layer with irregularity. SM2 EGCs were defined as narrowing or an interruption of the 3rd layer (Fig. 3A). To differentiate submucosal fibrosis with submucosal invasion, we focused on the shape of the hypo-echoic image of the 2nd layer that narrowed the 3rd layer. If the hypo-echoic component showed a fan-like appearance we defined the narrowed 3rd layer to be due to submucosal fibrosis (Fig. 3B). The histological depth of invasion was considered the gold standard of depth diagnosis.

We calculated the sensitivity and specificity of WLI and EUS for SM2 or deeper for diagnostic performance to distinguish M-SM1 and SM2 or deeper. The sensitivity and specificity by EUS was compared to those by WLI. We investigated the factors related to the underestimation (i.e., histologically SM2 or deeper EGC but diagnosed M-SM1 by EUS) or overestimation (i.e., histologically M-SM1 EGC but diagnosed SM2 or deeper by EUS) using multivariate analysis. Co-variate factors were selected according to previous studies.

3. Statistical analysis

Results were expressed as mean±standard deviation (SD) or median and range. We used the McNemar test to compare the sensitivity and specificity of WLI and EUS diagnosis. The odds ratio and 95% confidence interval were computed using binomial logistic regression analysis. A two-tailed p-value <0.05 was considered to be statistically significant. Statistical analysis was performed using the JMP SAS version 13.0.0 (SAS Institute, Cary, NC, USA).

RESULTS

A total of 278 EGCs in 259 patients were included for final analysis. Demographics and EGC data are listed in Table 1. The mean age of patients was 69.8 years old (SD, ±9.1). There were 197 males (76.1%) and 62 females (23.9%). Among all of the EGCs, the median size of tumors resected was 20.0 mm (range, 3.0 to 110.0 mm). EGCs were located most commonly along the lesser curve 89 (32%) and posterior wall of the stomach 75 (27%). The prevalence of endoscopic and histological ulceration was 18.3% and 16.9%, respectively. About half of the EGCs (55.4%) were treated endoscopically. More than half of the EGCs showed histology of differentiated type (66.2%), followed by mixed type of differentiated predominant (21.2%). Only a small number of EGCs with mixed type of undifferentiated predominant or undifferentiated type were also seen. Regarding the ratio of histological depth of invasion, M, SM1, SM2, and muscularis propria was 42.8%, 15.1%, 37.8%, and 4.3%, respectively.

Table 2 shows the diagnostic performance of WLI and EUS. The sensitivity of EUS was significantly superior to that of conventional endoscopy (73.7% vs 47.5%, p<0.01). In this study, diagnostic sensitivity, specificity and overall accuracy for SM2 or deeper by EUS was 73.7% (87/118), 74.4% (119/160), and 74.1% (206/278), respectively. Alternatively, sensitivity, specificity, and overall accuracy by WLI was 47.9% (56/118), 68.1% (109/160), and 59.4% (165/278), respectively.

Table 3 shows the ratio of EGCs misdiagnosed by EUS. Thirty-one EGCs were underestimated as SM1 invasion by EUS, while 41 EGCs overestimated as SM2 invasion. The mean size of EGCs showed no differences in each group. Cases of EUS underesti-
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Table 1. Demographic Data of the Patients and Lesions

| Variable                        | Value (%) |
|---------------------------------|-----------|
| Sex                             | 197       |
| Male                            | 62        |
| Female                          | 69.8±9.1  |
| Size of lesion, median (IQR), mm| 20.0 (3.0–110.0) |
| Macroscopic feature             |           |
| 0-I                             | 23 (8.2)  |
| 0-IIa                           | 99 (35.6) |
| 0-IIb                           | 4 (1.4)   |
| 0-IIc                           | 152 (54.8)|
| Location of the lesion          |           |
| Upper                           | 103 (37.1)|
| Middle                          | 111 (40.0)|
| Lower                           | 64 (23.0) |
| Position in the gastric wall     |           |
| Anterior wall                   | 46 (16.5) |
| Posterior wall                  | 75 (27.0) |
| Greater curvature               | 68 (24.5) |
| Lesser curvature                | 89 (32.0) |
| Ulceration (endoscopic)         |           |
| Positive                        | 51 (18.3) |
| Negative                        | 227 (81.7)|
| Ulceration (histological)       |           |
| Positive                        | 47 (16.9) |
| Negative                        | 231 (83.1)|
| Primary treatment               |           |
| ESD                             | 154 (55.4)|
| Surgery                         | 124 (44.6)|
| Histology                       |           |
| Differentiate type              | 184 (66.2)|
| Mixed/differentiated predominant| 59 (21.2) |
| Mixed/undifferentiated predominant| 17 (6.1) |
| Undifferentiated type           | 18 (6.5)  |
| Histological depth of invasion  |           |
| Mucosal cancer (M)              | 119 (42.8)|
| Shallow submucosal invasion (SM1)| 42 (15.1) |
| Deep submucosal invasion (SM2)  | 105 (37.8)|
| Invasion to muscularis mucosa (MP)| 12 (4.3) |

We found 278 early gastric cancer lesions with 259 patients available for analysis.

IQR, interquartile range; ESD, endoscopic submucosal dissection.

mation tended to be located in the anterior wall of the stomach (22.6% vs 14.6%) and have 0-I type macroscopic feature (19.4% vs 7.7%), while a small number of lesions were located in lower-third of the stomach (9.7% vs 24.7%). In comparison, EGCs overestimated by EUS tended to be located also in the anterior wall of the stomach (33.3% vs 15.2%), 0-IIc type macroscopic feature (70.7% vs 51.9%) and were associated with the presence of endoscopic ulceration (29.3% vs 14.8%) (Fig. 4). After adjustment by multivariate analysis, lesion location on the anterior wall was the independent risk factor for underestimation by EUS (odds ratio, 3.3; 95% confidence interval, 1.1 to 9.8; p=0.03). We found no significant factors for overestimation by EUS (Table 4).

DISCUSSION

Our study results indicate that EUS has superior sensitivity for SM2 or deeper compared to WLI for EGC suspicious for SM invasion. Treatment options for EGC are determined by clinicopathological factors including the depth of invasion. Higher sensitivity for SM2 or deeper enable adequate decision making and thereby avoiding redundant ER.

The patients included in our study were all examined using 20 MHz miniature probe during EUS examinations. This high-frequency miniature probe has higher resolution and less penetration compared to conventional EUS. This is most suitable for depth diagnosis for EGC.

Multivariate analysis revealed that anterior lesion location was an independent risk factor of underestimation by EUS. We speculated that anterior lesion location was different from other locations in terms of EUS probe approach. The anterior wall might be a difficult location to approach the probe correctly, as the miniature probe should approach the EGC lesions horizontally (Fig. 5).

In several previous reports, the diagnostic performance of EUS has been evaluated by accuracy. However, accuracy depends on the prevalence of SM2 or deeper EGC in the study subjects. On the other hand, sensitivity and specificity could evaluate the diagnostic performance, irrespective of SM2 or deeper EGC prevalence. Therefore, we believe diagnostic performance by EUS was firmly evaluated in our study.

Several studies have mentioned the sensitivity for SM2 or deeper EGC by EUS ranging from 66.3% to 84.0%, similar to the findings in our study.

Several previous reports have identified risk factors associated with diagnostic accuracy such as size of the lesion, protruded (0-I) type EGCs, location of the upper-third of the stomach (U), and ulceration for misdiagnosis by EUS. One of the reasons why these findings were not significant in our study was partially due to the differences in our study subjects and EUS method. With regards to study subjects, we only included EGCs suspicious for SM invasion, while other studies have included a high number of muscularis propria gastric cancers. EUS examination is not necessary in practice for lesions in which the depth diagnosis is evident based on endoscopic evaluation because of time and procedure related costs. Our study analysis only focused on EGC suspicious of SM invasion. Therefore, we
analyzed only EGCs suspected to have SM invasion to simulate actual clinical practice. Because EGCs are generally smaller than advanced gastric cancer, there were no differences in lesion size in the underestimated, overestimated, and the adequately diagnosed groups (Table 3). In some previous reports, EUS examinations were performed by conventional EUS or miniature probe.20,21,23 This is one of the reasons that the upper-third stomach location was not a risk factor of misdiagnosis in our study, because it is often difficult to scan lesions located in the upper-third of the stomach using conventional EUS, while we have used only the miniature probe for EUS examination. The “0-I” macroscopic subtype has also been reported to be a factor for misdiagnosis,13,15 however, macroscopic features had no relationship to misdiagnosis in our study. A few number of EGCs with 0-IIb were included our study cohort, which was rarely seen in other studies, which might also affect the results. Several previous studies have reported ulceration as one of the risk factors for misdiagnosis,4,13,14,19,20 while both endoscopic and histological ulceration did not reach statistical significance in our study. Because histological findings were not suitable for

| Table 2. Diagnostic Performance of WLI and EUS |
|-----------------------------------------------|
|                                 | WLI     | EUS     | p-value* |
| Histology                        |         |         |         |
| M/SM1 (n=160)                    | 109 (68.1) | 119 (74.4) | 0.20     |
| SM2/deeper (n=118)               | 62 (52.5)  | 31 (26.3)  | <0.01    |
| Overall accuracy                 | 59.4    | 74.1    |
| Sensitivity for SM2 or deeper    | 47.5    | 73.7    |
| Specificity for SM2 or deeper    | 68.1    | 74.4    |
| PPV for SM2 or deeper            | 54.8    | 79.3    |
| NPV for SM2 or deeper            | 63.7    | 68.0    |

Data are presented as number (%) or percent. WLI, white light image; EUS, endoscopic ultrasound; M, mucosal cancer; SM1, shallow submucosal; SM2, deep submucosal; PPV, positive predictive value; NPV, negative predictive value.

*McNemar test.

| Table 3. Rate of Endoscopic Findings Resulting in the Overestimation and Underestimation of Depth Diagnosis by Endoscopic Ultrasound |
|---------------------------------------------------------------------------------------------------------------------------------|
| Risk factor for under/overestimation | Underestimation (n=31) | Adequate or overestimation (n=247) | Overestimation (n=41) | Adequate or underestimation (n=237) |
| Size of the lesion, mm                | 21.3±12.5               | 23.8±15.0              | 22.2±13.0              | 23.8±15.1              |
| Position in the gastric wall          |                         |                         |                         |                         |
| Lesser curvature                      | 10 (33.3)               | 82 (33.2)              | 16 (39.0)              | 73 (30.8)              |
| Greater curvature                     | 7 (22.6)                | 61 (24.7)              | 6 (14.6)               | 62 (26.2)              |
| Anterior wall                         | 7 (22.6)                | 36 (14.6)              | 10 (33.3)              | 36 (15.2)              |
| Posterior wall                        | 7 (22.6)                | 68 (27.5)              | 9 (22.0)               | 66 (27.9)              |
| Location of the lesions               |                         |                         |                         |                         |
| Upper                                | 15 (48.4)               | 88 (35.6)              | 15 (36.6)              | 88 (37.1)              |
| Middle                               | 13 (41.9)               | 98 (39.7)              | 18 (43.9)              | 93 (39.2)              |
| Lower                                | 3 (9.7)                 | 61 (24.7)              | 8 (19.5)               | 56 (23.6)              |
| Macroscopic feature (predominant)    |                         |                         |                         |                         |
| 0-I                                  | 6 (19.4)                | 17 (7.7)               | 1 (2.4)                | 22 (9.3)               |
| 0-IIa                                | 9 (29.3)                | 90 (36.4)              | 11 (26.8)              | 88 (37.1)              |
| 0-IIb                                | 1 (3.2)                 | 3 (1.2)                | 0                      | 4 (1.7)                |
| 0-IIc                                | 15 (48.4)               | 137 (55.5)             | 29 (70.7)              | 123 (51.9)             |
| Ulceration status (endoscopic)       |                         |                         |                         |                         |
| Ulcer (-)                            | 27 (87.1)               | 204 (82.6)             | 29 (70.7)              | 202 (85.2)             |
| Ulcer (+)                            | 4 (12.9)                | 43 (17.4)              | 12 (29.3)              | 35 (14.8)              |

Data are presented as mean±SD or number (%).
predictive factors prior to treatment, we used endoscopic ulceration in logistic regression analysis. To further assess the impact of ulceration, we also evaluated histological ulceration during post hoc analysis and found no correlation with misdiagnosis (Supplementary Table 1). One of the reasons for this discrepancy from previous reports is the number of lesions analyzed, as well as the differences of selected ECGs in our study.

We speculate that the 0-IIc macroscopic feature might work as a co-effective factor in logistic regression analysis, because both endoscopic and histological ulceration showed the highest ratio of 0-IIc (70.6% and 74.5% among all of lesions with ulceration, detail data not shown).

Table 4. Associated Endoscopic Findings of the Overestimation and Underestimation of Depth Diagnosis by Endoscopic Ultrasound

| Variable                          | Underestimation | Overestimation |
|-----------------------------------|-----------------|---------------|
|                                   | Multivariate OR (95% CI) | p-value | Multivariate OR (95% CI) | p-value |
| Position in the gastric wall       |                 |             |                           |         |
| Lesser curvature                  | 1 (reference)   |             | 1 (reference)             |         |
| Greater curvature                 | 1.5 (0.5–4.8)   | 0.45        | 0.4 (0.2–1.2)             | 0.12    |
| Anterior wall                     | 3.3 (1.1–9.8)   | 0.03*       | 1.3 (0.5–3.3)             | 0.56    |
| Posterior wall                    | 1.1 (0.4–3.4)   | 0.87        | 0.7 (0.3–1.6)             | 0.37    |
| Location of the lesions           |                 |             |                           |         |
| Upper                             | 1 (reference)   |             | 1 (reference)             |         |
| Middle                            | 0.8 (0.3–1.9)   | 0.60        | 0.9 (0.4–2.0)             | 0.83    |
| Lower                             | 0.3 (0.1–1.0)   | 0.06        | 0.8 (0.3–2.0)             | 0.57    |
| Macroscopic feature (predominant) |                 |             |                           |         |
| 0-I                               | 1 (reference)   |             | 1 (reference)             |         |
| 0-IIa                             | 0.4 (0.1–1.2)   | 0.11        | 3.0 (0.4–25.3)            | 0.31    |
| 0-IIb                             | 0.7 (0.1–9.1)   | 0.78        | 0.0 (0.0–0.0)             | 0.99    |
| 0-IIc                             | 0.4 (0.1–1.3)   | 0.11        | 5.0 (0.6–39.9)            | 0.13    |
| Ulceration status (endoscopic)    |                 |             |                           |         |
| Ulcer (-)                         | 1 (reference)   |             | 1 (reference)             |         |
| Ulcer (+)                         | 0.8 (0.3–2.4)   | 0.69        | 1.8 (0.8–4.2)             | 0.12    |

OR, odds ratio; CI, confidence interval.  
*p<0.05.
On the viewpoint of practice, our study demonstrates the diagnostic performance of additional EUS examination followed by WLI diagnosis, because the WLI diagnosis was not blinded to each endosonographer. The additional EUS showed 28.2% of overestimation of all overestimated patients while it was 42.3% by WLI alone (Supplementary Fig. 1). Similarly, EUS added after WLI showed only 5.7% of underestimation while WLI underestimated 53.7% (Supplementary Fig. 2). Taking into account these results, additional EUS examination adds value when the depth diagnosis is difficult by WLI only. Additionally, we have to be careful of overestimation even if it was diagnosed by EUS examination.

There are various limitations to the present study which warrant discussion. First, our study was a single center, retrospective study. Second, the diagnosis by WLI could not be blinded as practice, some kind of carry over effect is suspected and affects the result. On the other hand, in our institution, EUS diagnosis was supervised by one expert endosonographer. Therefore, uniform quality of diagnosis is ensured. Thirdly, only EGCs suspicious for SM invasion were analyzed, potentially leading to selection bias. The primary aim of EUS examination for EGCs is to determine the depth of tumor invasion and therefore determine the appropriate treatment option. Mocellin and Pasquali has reported that a study within a practical setting is important. In this point, our study was considered to be close to “real clinical practice.” Another limitation to our study is histological evaluation after resection. In the current study, histological depth diagnosis was defined as the gold standard. For histological evaluation, sections were made in each 2 mm for ER and 5 mm in each for surgery. Different section widths could be considered as potential bias.

In conclusion, this is the first report to identify the anterior wall as an independent risk factor to underestimate the depth diagnosis of EGC by EUS examination using high-frequency miniature probe. EUS examination with 20 MHz miniature probe had higher sensitivity for EGC suspicious of SM invasion. As a result, EUS could reduce the incidence of redundant ER for SM2 invasive EGC with careful examination especially for EGCs located in anterior wall of the stomach.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Study concept and design: H.Takamaru, S.Y. Data acquisition: H.Takamaru, S.Y., H.Takissawa, I.O., H.K., S.S., K.T., Y.S. Data analysis and interpretation: H.Takamaru, S.Y., S.S., K.T. Drafting of the manuscript: H.Takamaru, S.Y. Critical revision of the manuscript for important intellectual content: S.Y., K.T., Y.S. Statistical analysis:
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