Endoscopic Ultrasound Elastography as a Novel Diagnostic Method for the Assessment of Hardness and Depth of Invasion in Colorectal Neoplasms

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Keywords

Colorectal neoplasm · Endoscopic ultrasound · Endoscopic ultrasound elastography · Magnifying chromoendoscopy

Abstract

Introduction: We aimed to compare the efficacy of endoscopic ultrasound elastography (EUS-EG) with that of magnifying chromoendoscopy (MCE) and endoscopic ultrasonography (EUS) for the diagnosis of the depth of invasion in colorectal neoplasms. This is an important clinical issue as the depth of invasion is associated with the risk of metastasis. **Methods:** Consecutive patients with suspected superficial colorectal neoplasms, evaluated by MCE, EUS, and EUS-EG, for whom endoscopic submucosal dissection was considered, were enrolled in 2018 (derivation study) and in 2019–2020 (validation study). The primary clinical endpoint was the diagnostic yield differentiating intramucosal and shallow submucosal neoplasms from deep submucosal (dSM) and advanced colorectal cancers. In addition, inter- and intra-observer agreements of the elastic score of colorec-

tal neoplasm (ES-CRN) were evaluated by 2 expert and 2 non-expert endoscopists. **Results:** Thirty-one (33 lesions) and 50 (55 lesions) patients were enrolled in the derivation and validation studies, respectively. Sensitivity, specificity, positive, and negative predictive values, and accuracy of assessment of the depth of submucosal or deeper invasion in the derivation and validation groups were as follows: EUS-EG, 100/88.2/86.7/100/93.3% and 77.8/86.1/73.7/88.6/83.3%; MCE, 66.7/94.4/90.9/77.3/81.8% and 84.2/91.4/84.2/91.4/88.9%; and EUS, 93.3/77.8/77.8/93.3/84.8% and 89.5/65.7/58.6/92.0/74.1%, respectively. For the 2 expert endoscopists, interobserver agreement for the ES-CRN (first and second assessments) in the derivation group was 0.84 and 0.78, respectively; these values were 0.73 and 0.49, respectively, for the 2 non-expert endoscopists. **Discussion/Conclusion:** All 3 modalities presented similar diagnostic yield. Inter- and intra-observer agreements of the ES-CRN were substantial, even for non-expert endoscopists. Therefore, EUS-EG may be a useful modality in determining the depth of invasion in colorectal neoplasms.

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Introduction

Endoscopic resection, including endoscopic *mucosal* resection and endoscopic submucosal dissection (ESD), is widely used to treat colorectal neoplasms with invasion limited to the mucosa or shallow submucosa [1–3]. Appropriate diagnosis of intramucosal colorectal neoplasms is important, as these lesions have negligible risk for lymph node metastasis [4, 5]. However, submucosal invasive colorectal carcinoma has a risk for lymph node metastasis of 10–15% [6–8]. The rate of lymph node metastasis for submucosal invasive colorectal carcinomas is non-uniform and being dependent on the depth of submucosal invasion and other factors. When the submucosal invasion depth is limited to <1,000 μm , without lymphatic or venous invasion, sprouting, or poorly differentiated components, the rate of lymph node metastasis is approximately 0% [5]; thus, endoscopic resection would be indicated.

Currently, the depth of invasion of colorectal neoplasms is estimated using magnifying endoscopy (ME) with image enhancement, including the optical digital method [9–11], dye-spraying method [12–14], and endoscopic ultrasonography (EUS) [15–17]. However, predicting the depth of invasion remains difficult in some cases, particularly for protruded-type lesions [18, 19]. In these cases, another imaging modality might be necessary, such as EUS elastography (EUS-EG) which quantifies the relative hardness (or elasticity) of different tissues [20]. EG has been effectively used to identify neoplastic lesions in the breast, pancreas, prostate, lymph nodes, and rectum [20–27]. To date, a few studies have reported on the use of elastography to evaluate rectal neoplasms [27, 28]. However, the use of elastography with echoendoscopic assessment of whole colorectal neoplasms and, in particular, for early colorectal cancer, has not been previously clarified. Moreover, elastic scores for the colorectum have not yet been determined. As such, the applicability of elastography to diagnose the depth of invasion colorectal neoplasms has not been evaluated. Accordingly, our aim in this study was to compare the efficacy of EUS-EG with that of EUS and magnifying chromoendoscopy (MCE) for the diagnosis of the depth of invasion of colorectal neoplasms.

Materials and Methods

Selection of Patients

Consecutive patients who underwent colonoscopy and echoendoscopy for suspected superficial colorectal neoplasms for whom ESD was considered, at Nagoya University Hospital, Japan,

between March and December 2018, were eligible for the derivation study. Consecutive patients meeting these criteria, between January 2019 and January 2020, were eligible for the validation study. Exclusion criteria were as follows: age >90 years (as super-old patients are more likely to have unexpected events which would increase the procedure time); a lesion size for the target lesions for endoscopic assessment of <20 mm, scarring of lesions (subjective assessment at the time of the examination); inflammatory bowel diseases; and pregnancy. All patients had undergone examination using conventional white light imaging (WLI) endoscopy and MCE (MCE; CF-HQ290ZI; Olympus Medical Systems Corp., Tokyo, Japan or EC-600ZP7; Fujifilm Co., Tokyo, Japan) before EUS-EG. EUS-EG was performed using a forward-viewing radial-array echoendoscope (EG-580UR; Fujifilm Co., Ltd; see online suppl. Table; for all online suppl. material, see www.karger.com/doi/10.1159/000511589). Endoluminal carbon dioxide (CO_2) insufflation was used for all patients during the endoscopic procedure (CO_2 gas supply system GW-100; Fujifilm Co., Tokyo, Japan). All lesions were treated using endoscopic or surgical resection within 1 month of imaging, with the final diagnosis confirmed histopathologically.

Principles of EUS-EG

In elastography, tissue strain, which is negatively correlated with tissue elasticity, is generated by applying slight manual compression and comparing the data obtained before and after compression. The data are compared using a cross-correlation technique to determine the amount of displacement each small portion of tissue undergoes in response to the compression applied by the ultrasound transducer [29, 30]. The elasticity modulus can be calculated from the strain and stress on the structures examined. EUS-EG provides a relative value of strain in a specific region of interest (ROI). Tissue strain is expressed with color maps on a B-mode image, with soft tissue expressed in red and hard tissue in blue. Based on the fact that a tumor becomes fibrotic and harder as it invades deeper tissues, in this study, we examined whether the invasion depth could be diagnosed by comparing the hardness of the tumor relative to the surrounding tissue.

EUS and EUS-EG Settings

All EUS procedures were performed by experienced echoendoscopists (M.E. and T.K.), and each having conducted >100 EUS and >3,000 ME procedures of the colon. The echoendoscope was inserted through the anus, following standard colonoscopy, with patient positioning adjusted to place the lesion under gravity. EUS was performed with the patient under sedation (intravenous administration of 0.05 mg/kg midazolam for induction and additional 1 mg doses, as needed, for restlessness and body movement during the procedure), with heart rate, blood pressure, and hemoglobin oxygen saturation (SpO_2) monitored. An antispasmodic agent (butylscopolamine, 20 mg or glucagon, and 0.5 or 1 mg) was administered immediately before inserting the endoscope, unless contraindicated based on the patient's medical history. An additional dose of 10 mg of butylscopolamine or 0.5 mg of glucagon was administered in the presence of strong bowel peristalsis. Overall, antispasmodic agents were not used in 10 patients; glucagon was used in 8 patients; and butylscopolamine in the remaining 63.

Insufflated CO_2 was removed to decompress the bowel lumen, and deaerated water was infused using an irrigation pump (UWS-1; Olympus Optical Co., Ltd., Tokyo, Japan). Observation was per-

Fig. 1. Setting of the ROI for EUS-EG of colorectal neoplasms. ROI, region of interest; EUS-EG, endoscopic ultrasound elastography.

formed while replenishing water, as appropriate. The EG-580UR echoendoscope (Fujifilm Co., Ltd.), equipped with a Sonart SU-1 ultrasound processor (Fujifilm Co., Ltd.), was used in all cases. EUS-EG was performed in the radial scanning mode, with lesions identified under B-mode observation, at 12-MHz for both signal transmission and reception.

The ROI was selected to be as close as possible to the target lesion size, with the area from the surface of the lesion to the serous membrane included for evaluating tissue elasticity. The location was set to the deepest suspected depth of invasion of the lesion (Fig. 1). The echoendoscope was fixed on the lesion for a few seconds to obtain a stable image which is required for elastic pattern definition. From a cine-loop of the EUS-EG imaging obtained, all lesion images were stored as still images (bitmap format) for offline analysis. The elastic score of the pancreas was used as the reference tissue hardness for calibration.

Analysis of EUS-EG Images

EUS-EG images were classified into 3 patterns using the elastic score of colorectal neoplasm (ES-CRN; Fig. 2) as follows: ES-CRN 1, the tumor region is softer (green, yellow, and red coloring) than the surrounding area (blue); ES-CRN 2, the tumor region is hard-

er (blue) or as hard (blue) as the surrounding area; and ES-CRN 3, a honeycomb-like pattern is observed within the lesion. ES-CRN 1 lesions were defined as having less than slight submucosal (SSM) invasion and ES-CRN 2 and 3 lesions as having deeper submucosal invasion. The EUS-EG-derived measure of the hardness of the tumor was compared to that of the muscle layer within the ROI; if the tumor had a hardness equal to or higher than that of the muscle layer, the tumor mainly turns blue. The tumors were classified based on a review of the color-coded images obtained by experienced echoendoscopists in the derivation study.

Reliability of the ES-CRN Classification

Reliability of the ES-CRN classification was evaluated in addition to the derivation study between 2 expert (T.Y. and H.S.) and 2 non-expert (I.H. and K.Y.) endoscopists who were blinded to information regarding WLI, MCE, EUS, and the final histological diagnosis. Expert endoscopists had each performed >3,000 ME and >100 EUS procedures of the colon and had observed EUS-EG procedures. Non-experts had each performed >3,000 ME procedures but had neither observed nor performed EUS-EG procedures and had limited experience performing EUS of the colon. EUS-EG images were reviewed after a short lecture on the ES-CRN

Fig. 2. Elastic score of colorectal neoplasm (ES-CRN). **a** ES-CRN 1: the lesion is mostly in the soft tissue range (green, yellow, and red), with the hard tissue range (blue) being almost exclusive to the muscularis propria (MP; fourth layer of B mode). **b** ES-CRN 2: the lesion is mostly in the hard tissue range (blue). **c** ES-CRN 3: honeycomb-like pattern within the lesion. ES-CRN, elastic score of colorectal neoplasm; MP, muscularis propria.

classification, with reassessment 2 weeks after, using a random order of the same images.

Inter- and intra-observer agreements were calculated using the Kappa coefficient, interpreted as follows: poor, 0–0.20; fair, 0.21–0.40; moderate, 0.41–0.60; substantial, 0.61–0.80; and excellent, 0.81–1.00 [31]. Notably, experienced echoendoscopists who per-

formed EUS-EG were excluded from the process of reliability examination.

Analysis of MCE and EUS Images

MCE images were diagnosed using the pit pattern [12]. The index for dSM or deeper invasion on MCE was defined by VI high-

Fig. 3. Study flow chart: **a** derivation study, **b** validation study. EUS-EG, endoscopic ultrasound elastography, ES-CRN, elastic score of colorectal neoplasm.

grade or VN pit pattern. In comparison with the ES-CRN classification, the depth of invasion recorded during MCE and EUS examination was used, with no further verification of images.

Assessment of Diagnosis Using Each Modality

In both of the derivation and the validation studies, lesions difficult to evaluate by each modality were excluded. The lesions difficult to evaluate were judged at the time of diagnosis for each modality. All remaining lesions were assessed in real time by either 1 of the 2 expert endoscopists (T.Y., H.S.) for MCE and by either 1 of the experienced echoendoscopists (M.E., T.K.) for EUS. All analyses were performed without knowledge of the MCE findings.

In the derivation study, for EUS-EG, all lesions were diagnosed solely from still images by either one of the experienced echoendoscopists. These lesions were then assessed by 4 doctors (2 experts and 2 non-experts) to determine the reliability of assessment. In the validation study, all lesions were assessed in real time for EUS-EG by either one of the experienced echoendoscopists, without knowledge of the MCE findings (Fig. 3). The diagnostic ability was calculated with exclusion of cases difficult to assess for all modalities. The diagnosis of EUS-EG was assigned a level of confidence (high or low) based on predictive accuracy, with a >90% accuracy classified as a diagnosis with “high confidence” [32].

Table 1. Patients' characteristics and indications for EUS-EG

Patients' characteristics	Derivation study, <i>n</i> = 33	Validation study, <i>n</i> = 55
<i>N</i> (male/female)	33 (20/13)	55 (29/26)
Age, median (range), years	70 (50–87)	70 (34–85)
<i>Tumor location</i> R/S/D/T/A/C, <i>n</i>	12/10/0/5/5/1	22/10/5/4/9/5
Tumor size, median (range), mm	36 (15–100)	25 (10–82)
<i>Morphology type, n</i> Is/Ip/Ila/Is + Ila/Is + Iic/Ila + Is/Ila + Iic	9/5/3/4/1/7/4	16/1/17/7/0/6/8
Endoscopic/surgical resection, <i>n</i>	20/13	35/20
Depth of final diagnosis of histopathological assessment, <i>n</i> m/sSM/dSM/MP/SS	16/4/5/7/1	33/3/12/5/2
<i>Insertion time, median (range), s</i> Total	60 (5–900)	60 (1–1,800)
Lesion site		
Rectum	5 (5–30)	5 (1–60)
Sigmoid colon	75 (10–300)	42 (15–120)
Descending colon	na	180 (62–780)
Transverse colon	180 (120–600)	390 (120–570)
Ascending colon	420 (180–900)	270 (120–380)
Cecum	600	420 (178–1,800)

R, rectum; S, sigmoid colon; D, descending colon; T, transverse colon; A, ascending colon; C, cecum; m, intramucosal cancer; sSM, slight submucosal; dSM, deep submucosal; EUS-EG, endoscopic ultrasound elastography; MP, muscularis propria; SS, subserosa; na, not applicable; *N*, number.

Histopathological Assessment

Resected specimens were immediately placed in formalin and embedded in paraffin wax for histological examination. Pathological diagnoses were established by experienced pathologists, on the basis of hematoxylin-eosin and immunopathological staining. The vertical depth of submucosal invasion was defined as <1,000 and ≥1,000 μm as sSM and dSM invasion, respectively [5].

Outcome Measures

Primary Endpoint: Diagnostic Ability

The primary endpoint of this study was the diagnostic ability. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the ES-CRN classification and MCE/EUS diagnosis yield for a dSM or deeper lesion were compared.

Secondary Outcomes: Clinical Efficacy

Diagnostic performance for each ES-CRN classification was evaluated. Inter- and intra-observer agreements between the 2 expert and 2 non-expert endoscopists were evaluated. Technical feasibility was defined as the time from insertion of the echoendoscope into the anus to arrival at the target lesion. Adverse events were defined as any deviation from the clinical course after EUS-EG, as observed by the 2 expert endoscopists. All patients were contacted within 1 month of the procedure to determine the presence of any late-onset adverse events.

Sample Size Calculation

For dSM or deeper lesions, pooled estimates from a meta-analysis of MCE and reported a sensitivity and specificity of 81 and 95%, respectively [33]. Assuming that EUS-EG has the equivalent diagnostic ability as MCE, we set an accuracy >80% as necessary criterion. A power analysis indicated that 23 and 51 lesions were needed to achieve this criterion in the derivation and the validation studies, based on a 20 and 5% significance level for the derivation and validation study, respectively, a statistical power of 80%, and an effect size of 0.5, using a 1-sided equivalence. Therefore, 33 and 55 lesions were estimated to be needed, accounting for a reasonable anticipated dropout rate, in the derivation and the validation study, respectively.

Statistical Analysis

Continuous variables, including patients' age and lesion size, were presented as median and range. Sensitivity, specificity, PPV, and NPV were calculated for the EUS-EG-, MCE-, and EUS-based classification of lesions, using the histopathological diagnosis as the reference. Inter- and intra-observer agreements for the EUS-EG classification were calculated using the Kappa coefficient. Insertion time was reported as median and interquartile range. All analyses were performed using SPSS software (version 24; SPSS Japan, Tokyo).

Fig. 4. A 30-mm, morphology type Is, lesion at the site of rectum with the final diagnosis of MP invasion confirmed by histopathological examination. **a** Findings on WLI showing a protruded lesion that is slightly reddish and bleeds easily. **b** Findings on MCE showing a VI high-grade pit pattern. **c** EUS findings showing invasion of the tumor into the third layer; of note, the demarcation with

the fourth layer is partially obscured. **d** EUS-EG findings showing that the tumor region is almost blue, indicative of an ES-CRN 2. MP, muscularis propria; WLI, white light imaging; MCE, magnifying chromoendoscopy; EUS, endoscopic ultrasonography; EUS-EG, endoscopic ultrasound elastography; ES-CRN, elastic score of colorectal neoplasm.

Results

Derivation Study and Validation Study

Thirty-one (33 lesions) and 50 (55 lesions) patients were enrolled in the derivation and validation studies, respectively (Fig. 3; Table 1). EUS-EG was performed on the

same day as MCE for 27 and 48 lesions for the derivation and the validation study, respectively, and within 2 weeks after MCE for the remaining 6 and 7, respectively. The echoendoscope was able to reach the target lesion in all cases, with no adverse events noted in both studies. Of the 33 and 55 lesions in the derivation and the validation

Table 2. Diagnostic performance of each modality for dSM invasions or deeper lesions

Derivation study, <i>n</i> = 33	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)
EUS-EG*	100 (84.1–100)	88.2 (76.1–88.2)	86.7 (72.9–86.7)	100 (86.2–100)	93.3 (79.5–93.3)
MCE	66.7 (50.1–72.1)	94.4 (80.6–99.0)	90.9 (68.3–98.3)	77.3 (66.0–81.0)	81.8 (66.8–86.8)
EUS	93.3 (76.6–98.8)	77.8 (63.9–82.3)	77.8 (63.9–82.3)	93.3 (76.6–98.8)	84.8 (69.7–89.8)
Validation study, <i>n</i> = 55	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)
EUS-EG**	77.8 (60.4–89.0)	86.1 (77.4–91.7)	73.7 (57.2–84.3)	88.6 (79.6–94.4)	83.3 (71.7–90.8)
MCE**	84.2 (68.8–92.6)	91.4 (83.1–96.0)	84.2 (68.8–92.6)	91.4 (83.1–96.0)	88.9 (78.0–94.8)
EUS**	89.5 (72.8–96.9)	65.7 (56.7–69.8)	58.6 (47.7–63.5)	92.0 (79.3–97.7)	74.1 (62.3–79.3)

N, number; EUS-EG, endoscopic ultrasound elastography; MCE, magnifying chromoendoscopy; EUS, endoscopic ultrasonography; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval. * Excluding 3 cases that are difficult to evaluate for EUS-EG. ** Excluding 1 case that is difficult to evaluate for respective modality.

study, respectively, 13 and 18 lesions were surgically resected due to suspected dSM invasion based on WLI, MCE, and EUS findings. When the diagnosis for each modality was different, the MCE, which has the most evidence at present, was given priority. All lesions were treated by endoscopic or surgical resection within 1 month of the endoscopic procedures, with the final diagnosis confirmed by histopathological examination. Figure 4 shows a case presentation including MCE, EUS, and EUS-EG findings.

For each imaging modality, sensitivity, specificity, PPV, NPV, and accuracy of differentiating dSM or deeper lesions in the derivation, and validation studies were as follows: EUS-EG, 100/88.2/86.7/100/93.3% and 77.8/86.1/73.7/88.6/83.3%; MCE, 66.7/94.4/90.9/77.3/81.8% and 84.2/91.4/84.2/91.4/88.9%; and EUS, 93.3/77.8/77.8/93.3/84.8% and 89.5/65.7/58.6/92.0/74.1%, respectively (Table 2). In the derivation study, EUS-EG had the highest accuracy, with MCE having the highest accuracy in the validation study. There was no statistical difference between the 3 modalities in either study. For EUS-EG diagnoses, a high confidence was obtained in 23/30 (76.7%) and 51/54 (94.4%) of cases in the derivation and the validation study, respectively. The distribution of the depth of invasion, confirmed by histopathological examination, for each ES-CRN classification (1 through 3), for MCE and EUS, is shown in Table 3.

Of the 33 and 55 lesions examined, 21 and 30 lesions included a type Is or Ip morphology, which are protruded lesions, in the derivation and the validation study, respectively. For these 21 and 30 protruded lesions, as well as for lesions with other types of morphology, diagnostic abili-

Table 3. Distribution of the depth of invasion, as confirmed by histopathological assessment, for each ES-CRN pattern (EUS-EG), MCE, and EUS

Prediction of the depth of lesions for each modality	Derivation study/validation study Histopathological assessment, <i>n</i>				
	m	sSM	dSM	MP	SS
<i>EUS-EG</i>					
≤sSM					
ES-CRN 1	14/28	1/3	0/2	0/2	0/0
≥dSM					
ES-CRN 2	1/5	1/0	4/9	4/3	1/2
ES-CRN 3	0/0	0/0	1/0	3/0	0/0
<i>MCE</i>					
≤sSM	15/30	2/2	2/2	2/1	1/0
≥dSM	1/3	0/0	5/10	5/4	0/2
<i>EUS</i>					
≤sSM	13/22	1/2	1/1	0/1	0/0
≥dSM	3/10	1/1	6/11	7/4	1/2

N, number of cases; ES-CRN, elastic score of colorectal neoplasm; EUS-EG, endoscopic ultrasound elastography; MCE, magnifying chromoendoscopy; EUS, endoscopic ultrasonography; m, intramucosal cancer; sSM, slight submucosal; dSM, deep submucosal; MP, muscularis propria; SS, subserosa.

ties for dSM or deeper lesions are reported for each imaging modality in the derivation and the validation study in Table 4. No significant difference in the diagnostic yield was observed between the 3 imaging modalities or morphology types.

Table 4. Diagnostic performance of each diagnostic modality for dSM invasions or deeper lesions, including morphology type Is or Ip (I)/not including morphology type Is or Ip (II)

Derivation study I: N = 21/II: N = 9	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)
EUS-EG					
I	100 (78.7–100)	83.3 (67.3–83.3)	81.8 (64.4–81.8)	100 (80.8–100)	90.5 (72.2–90.5)
II	100 (66.5–100)	100 (73.2–100)	100 (66.5–100)	100 (73.2–100)	100 (70.2–100)
MCE					
I	55.6 (35.1–55.6)	100 (84.6–100)	100 (63.1–100)	75.0 (63.5–75.0)	81.0 (63.4–81.0)
II	75.0 (39.0–92.9)	80.0 (51.2–94.3)	75.0 (39.0–92.9)	80.0 (51.2–94.3)	77.8 (45.8–93.7)
EUS					
I	100 (78.7–100)	83.3 (67.3–83.3)	81.8 (64.4–81.8)	100 (80.8–100)	90.5 (72.2–90.5)
II	75.0 (39.0–92.9)	80.0 (51.2–94.3)	75.0 (39.0–92.9)	80.0 (51.2–94.3)	77.8 (45.8–93.7)
Validation study I: N = 30/II: n = 24	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)
EUS-EG					
I	88.9 (62.5–97.9)	76.2 (64.9–80.1)	61.5 (43.3–67.8)	94.1 (80.2–98.9)	80.0 (64.2–85.4)
II	66.7 (45.4–66.7)	100 (87.2–100)	100 (68.1–100)	83.3 (72.7–83.3)	87.5 (71.5–87.5)
MCE					
I	77.8 (52.4–91.1)	90.5 (79.6–96.2)	77.8 (52.4–91.1)	90.5 (79.6–96.2)	86.7 (71.5–94.7)
II	90.0 (69.2–97.2)	92.9 (78.0–98.0)	90.0 (69.2–97.2)	92.9 (78.0–98.0)	91.7 (74.3–97.7)
EUS					
I	88.9 (62.2–98.0)	57.1 (45.7–61.0)	47.1 (32.9–51.9)	92.3 (73.8–98.6)	66.7 (50.6–72.1)
II	90.0 (68.1–98.0)	85.7 (70.1–91.4)	81.8 (61.9–89.1)	92.3 (75.5–98.5)	87.5 (69.2–94.2)

N, number; EUS-EG, endoscopic ultrasound elastography; MCE, magnifying chromoendoscopy; EUS, endoscopic ultrasonography; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

In the derivation study, while MCE and EUS provided a useful diagnostic image for all lesions, the EUS-EG image was difficult to assess for 3 lesions as the ROI could not be adequately set, preventing the evaluation of the area immediately surrounding the lesion. Two of these lesions were located in the rectum and the other in the sigmoid colon, with a lesion size of 15, 30, and 36 mm, respectively, and morphology type Is, Ip, and Ila + IIC, respectively. The depth of invasion on final histopathological diagnosis was sSM for 2 lesions and dSM in the third. In the validation study, each modality had one lesion that was difficult to assess: a lesion located on a fold for EUS and EUS-EG and a lesion with mucous adhesion for MCE.

Reliability Examination in the Derivation Study

In the reliability examination, inter-observer agreement was substantial to excellent between the 2 experts (κ -coefficient: 0.84 and 0.78 for the first and second assessments, respectively) and moderate to substantial for the 2 non-experts (κ -coefficient: 0.73 and 0.49 for the first

and second assessments, respectively). Intra-observer agreement was substantial for the 2 experts (κ -coefficients of 0.78 and 0.79 for the first and second assessments, respectively) and moderate to substantial for the 2 non-experts (κ -coefficient of 0.68 and 0.52 for the first and second assessments, respectively). Sensitivity, specificity, PPV, NPV, and accuracy of diagnosis for dSM or deeper lesions and the number of EUS-EG diagnosis of high confidence for each observer (first assessment) are shown in Table 5.

Discussion/Conclusion

To our knowledge, this is the first study to evaluate the diagnostic ability and clinical efficacy of EUS-EG using a forward-viewing radial-array echoendoscope to determine the depth of invasion in colorectal neoplasms. Real-time tissue elastography is used to visualize tissue hardness [34, 35], producing a color overlay on the B-mode image with 256 levels of color gradation of tissue hard-

Table 5. Reliability examination in the derivation study and the diagnostic performance of EUS-EG for dSM invasions or deeper lesions, as assessed by each observer

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Accuracy, % (95% CI)
Expert A (T.Y.) HC 19/30	92.3 (73.9–98.5)	82.4 (68.3–87.1)	80 (64.0–85.4)	93.3 (77.4–98.7)	86.7 (70.7–92.1)
Expert B (H.S.) HC 24/30	100 (84.1–100)	88.2 (76.1–88.2)	86.7 (72.9–86.7)	100 (86.2–100)	93.3 (79.5–93.3)
Non-expert A (I.H.) HC 16/30	100 (82.9–100)	58.8 (45.8–58.8)	65.0 (53.9–65.0)	100 (77.8–100)	76.7 (61.9–76.7)
Non-expert B (K.Y.) HC 18/30	92.3 (73.3–98.6)	58.8 (44.3–63.6)	63.2 (50.2–67.5)	90.9 (68.5–98.3)	73.3 (56.9–78.8)

EUS-EG, endoscopic ultrasound elastography; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; HC, high confidence.

ness, from soft (red) to moderate (green) and hard (blue) [36]. Real-time tissue elastography was initially used to assess breast tissue, thyroid gland, prostate, and liver, using extracorporeal ultrasound. Similarly, EUS-based elastography is used to evaluate biliary and pancreatic diseases and digestive tract submucosal tumors [37]. For pancreatic lesions (classified into 5 elastic scores), EUS-EG differentiates benign from malignant lesions, with a sensitivity and specificity ranging between 92.3–100% and 67.0–80.0%, respectively [21, 38]. There have been reports of endorectal elastography providing valuable additional information for grayscale ultrasound imaging [20, 39]. Although there are a few reports using elastography for colorectal tumors, there have been no reports of elastography using echoendoscopy for the tumors throughout the whole colorectum.

The diagnostic performance of MCE and EUS has previously been reported. For dSM or deeper lesions, pooled estimates from a meta-analysis of MCE, using the pit-pattern classification, reported a sensitivity and specificity of 81 and 95%, respectively [33]. For EUS, when clear images were obtained, the accuracy for differentiating Tis and T1a cancers from T1b cancer was high, at approximately 90% [16, 17, 40]. In our study, the diagnostic performance of EUS-EG was comparable to that of MCE and EUS. However, determining the depth of protruded-type colorectal neoplasms could be difficult using MCE and EUS, with these lesions often classified as “non-invasive” using MCE, even if they deeply invade the submucosal layer [18]. Protruded-type lesions are similarly difficult to image using EUS due to high rate of deep echo attenua-

tion and more significant difficulty in vertically scanning semi-pedunculated and other types of protruded lesions [19]. Therefore, with MCE, there is a risk of considering the lesion to be shallower than the real depth, while with EUS, the depth may be estimated to be deeper than the real depth of the lesion. For these reasons, we included a range of lesion morphologies in our study, including Is or Ip which are protruded lesions. In our study, the decrease in diagnostic ability of each modality was not significant, even for the protruded lesions. For cases of misdiagnosis, compared to histopathological findings, the depth of invasion was lesser for 6 lesions using MCE and greater for 11 lesions using EUS and for 2 lesions using EUS-EG. Out of these, 5 lesions misdiagnosed by MCE were correctly diagnosed by EUS-EG. Six lesions misdiagnosed by EUS were correctly diagnosed by EUS-EG. This suggests that using EUS-EG may lead to a correct diagnosis of cases that have been misdiagnosed by conventional modalities. However, this study has small numbers, and our findings warrant confirmation by larger cohorts.

In the derivation study, 3 protruded lesions that were difficult to assess using EUS-EG were adequately visualized for diagnosis by MCE and EUS; however, in the validation study, all protruded lesions were adequately visualized by EUS-EG. It is possible that the reason for the noted difficulty in evaluation might reflect the learning curve rather than the morphology of the lesion. It has been shown that elastography results can vary widely depending on the skill level of the examiner in obtaining a stable image and in the selection and settings of the ROI for measurement [41]. In this study, some difficulties

were experienced in appropriately setting the ROI for EUS-EG, indicating the continued need to advance technical aspects of EUS-EG. Similarly, EUS-EG can be cumbersome in other gastrointestinal regions due to effects of breathing and other movements. In our study, patient positioning and adjustments of the scope were used to control these influences during imaging in the colorectum.

Image-enhanced endoscopy of MCE is used with the Japan NBI Expert Team classification of the depth of invasion in colorectal neoplasms, with moderate inter-observer (κ -coefficient, 0.52) and excellent intra-observer (κ -coefficient, 0.88) agreements [42], based on the review of 199 lesions by 2 experienced colonoscopists. By comparison, a moderate to substantial intra- and inter-observer agreement was achieved by non-expert endoscopists using the ES-CRN classification. Based on these results, we consider that the ES-CRN classification was relatively simple to use and easy to understand, even for endoscopists unfamiliar with EUS-EG.

EUS is not usually performed for colorectal lesions due to difficulty with inserting a regular echoendoscope. However, the safety of advancing a forward-oblique-viewing upper echoendoscope to the sigmoid/left-sided colon for EUS imaging of benign and malignant colonic lesions has previously been reported [43]. Another report confirmed these results, with colorectal lesions being reached using a forward-viewing radial-array echoendoscope without adverse events, with the time to reach the lesion was similar to that of standard colonoscopy techniques [44]. Therefore, a forward-viewing radial-array echoendoscope can be safely used as the staging tool of choice for colon cancer. In our study, all lesions were similarly reached without adverse events.

The limitations of our study should be noted. First, this was a single-center case series; as such, selection bias cannot be denied. Second, all lesions were eligible for ESD or surgical resection, and there were no small lesions, which would have been better treated by EMR. Third, the lesions with scarring were excluded to reduce an impact of fibrosis. In fact, most intramucosal cancers were treated endoscopically in this study; there were few lesions with fibrosis in the result. Finally, the diagnostic ability of each modality was calculated excluding the cases that were difficult to assess. However, in the validation study, only one case was difficult to assess in each modality, respectively. Based on our findings, we propose that the ES-CRN classification (based on EUS-EG images) offer a potential adjunct for MCE and EUS to diagnose the depth of invasion in colorectal neoplasms. As well, with an accumulation of

evidence, it might be possible to substitute EUS-EG, combined with the ES-CRN, for MCE or EUS in the future. We note cases which were difficult to assess or were misdiagnosed using MCE or EUS, with the diagnosis corrected using EUS-EG and EUS-EG and this with substantial interobserver agreement, even for non-experts. More accurate determination of submucosal invasion is valuable to inform appropriate treatment strategies. Further prospective studies should accumulate evidence of clinical usefulness of the ES-CRN classification for diagnosing the depth of colorectal neoplasms invasion.

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Statement of Ethics

This study was approved by the Research Ethics Board at Nagoya University Hospital (IRB No. 2015-0381) and registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; study number UMIN000035902). Written informed consent was obtained from all patients. This study conforms to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

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