Medicine®

OBSERVATIONAL STUDY

OPEN

Risk Factors of Submucosal or Lymphovascular Invasion in Early Gastric Cancer <2 cm

Yu Yi Choi, MD, Su Jin Kim, MD, Cheol Woong Choi, MD, PhD, Dae Hwan Kang, MD, PhD, Hyung Wook Kim, MD, PhD, Su Bum Park, MD, PhD, and Hyeong Seok Nam, MD

Abstract: Although prediction of submucosal (SM) or lymphovascular (LV) invasion is important before endoscopic resection of early gastric cancer (EGC), it can only be confirmed following endoscopic resection. After endoscopic resection, patients with SM or LV invasion may require additional surgery due to high risk of lymph node metastasis.

We conducted a retrospective study to identify risk factors for SM or LV invasion before endoscopic submucosal dissection (ESD) of EGC. Between January 2009 and May 2014, we reviewed the data of patients with EGC who met the absolute indications for ESD before procedure: well and/or moderately differentiated adenocarcinomas, tumors ≤ 2 cm in length and absence of ulcer or ulcer-scar.

During study period, a total of 308 lesions in 297 patients were included. SM or LV invasion was detected in 34 lesions (34/308, 11.0%). Multivariate analysis revealed that a moderately differentiated adenocarcinoma (odds ratio [OR] 4.157, $P = 0.000$) and location of the stomach (the upper and middle third; OR 3.100, $P = 0.008$) were significant risk factors for SM or LV invasion.

Careful consideration of endoscopic treatment decision might be necessary for the patients with a moderately differentiated adenocarcinoma and EGC located on the upper and middle third of the stomach.

(*Medicine* 95(22):e3822)

Abbreviations: CI = confidence interval, EGCe = arly gastric cancer, ESD = endoscopic submucosal dissection, EUS = endoscopic ultrasonography, LVl = ymphovascular, OR = odds ratio, SMs = ubmucosal.

INTRODUCTION

Early gastric cancer (EGC) is a gastric cancer limited to the mucosa or submucosa, irrespective of lymph node metastasis. From the past, surgical gastrectomy with lymph node dissection is a golden standard of treatment for EGC because all gastric cancer may have a possibility of lymph node metastasis. Recently, endoscopic submucosal dissection (ESD) has become widely used as a curative modality for EGC with low risk of lymph node metastasis. Recent reports examining the long-term outcomes of endoscopic resection for EGC (differentiated-type adenocarcinoma; no surface ulceration; and a diameter of ≤2 cm) showed comparable overall survival with surgery.[1–3] For the endoscopic treatment of EGC, Japanese[4] and Korean[5] gastric cancer treatment guidelines are almost the same. According to the guidelines, ESD is indicated as a standard treatment for lesions meeting the following criteria (absolute indications): well to moderately differentiated adenocarcinoma limited to the mucosa, within 2 cm in length, absence of ulcer or ulcer scar, and without lymphovascular (LV) invasion.[4,5]

Submucosal (SM) and LV invasions are independent risk factors for lymph node metastases and a critical prognostic factor in patients with EGC.[6–10] SM invasion has been reported as an independent risk factor for LV invasion in endoscopically resected EGC, and the incidence of lymph node metastasis is significantly high in SM invasive EGC.[8,11,12] This can be explained by the rare distribution of lymph capillaries in the mucosal layer. Although lymph capillaries are found in the deep lamina propria adjacent to and within the muscularis mucosa, most large lymph vessels are located in the submucosa.[13,14] However, there is no way to precisely evaluate SM and LV invasion before endoscopic resection. Thus, when SM or LV invasion are found in endoscopically resected specimens, additional surgical treatment is sometimes required.

Therefore, we retrospectively analyzed the clinicopathologic associated factors with SM or LV invasion after endoscopic resection of EGC which met absolute indication before ESD.

PATIENTS AND METHODS

Patients

From January 2009 and May 2014, the medical records of the patients who underwent ESD at Pusan National University Yangsan Hospital in Korea due to EGC were reviewed, retrospectively. During study period, a total of 408 EGC lesions were resected based on an endoscopic forceps biopsy. Before endoscopic resection, lesions with ulceration, a diameter of >2 cm, an undifferentiated or mixed adenocarcinoma histology were excluded from this study. Included lesions were lesions meeting the absolute indication before ESD (well and/or moderately differentiated adenocarcinomas, tumors ≤2 cm in length and absence of ulcer or ulcer-scar tissue before endoscopic resection).[4,5] In the present study, the risk factors were analyzed based on individual tumors because some patients had multiple lesions resected. After exclusion, a total 308 EGC lesions (in 297 patients) were included in this study (Figure 1). Written informed consent was obtained from all patients prior to the

Editor: Xiao-Dong Chen.
Received: January 3, 2016; revised: April 30, 2016; accepted: May 10, 2016.
From the Department of Internal Medicine, Pusan National University School of Medicine and Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, Korea.
Correspondence: Cheol Woong Choi and Dae Hwan Kang, Department of Internal Medicine, Medical Research Institute, Pusan National University School of Medicine and Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, 20, Geumo-ro, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do 50612, Korea (e-mail: luckyace@hanmail.net [CWC] and sulsulpul@naver.com [DHK]).
YYC and SJK contributed equally to this work and share first authorship. CWC and DHK contributed equally to this work and are co-correspondences for this article.
The authors have no funding and conflicts of interest to disclose.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000003822

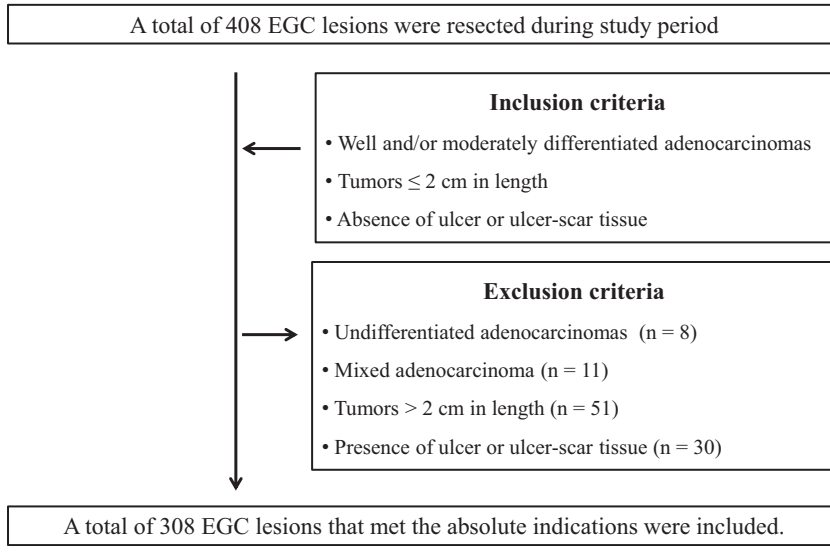

FIGURE 1. Flow chart illustrating the process of selecting lesions of early gastric cancer resected by endoscopic submucosal dissection. EGC = early gastric cancer, ESD = endoscopic submucosal dissection.

procedure. The study was approved by the ethics committee of the Institutional Review Board.

Endoscopic and Clinicopathologic Factors

ESD was performed by a method previously reported.[15] All of the endoscopic reports and data were reviewed and analyzed by 2 endoscopists (YYC and SJK). The maximum diameter was measured from the ESD specimen. The macroscopic appearances of lesions were determined after reviewing endoscopic photograph. The gross type of the superficial lesions was defined by the Paris classification.[16] The surface appearance of the lesions (such as surface redness, nodularity, presence of ulceration or ulcer scar), SM fibrosis, and location of the lesions were also evaluated. The location of lesions was described according to the Japanese Classification of Gastric Cancer as upper, middle, and lower third of stomach.[17] We defined the surface redness as reddish tint compared to the surrounding mucosa color and surface nodularity as the irregularly raised or nodular mucosa.

Two pathologists reviewed all of the endoscopic resected tissue slides blindly and reevaluated each discordant case under multiheaded microscope until agreement. The resected specimen was stretched and pinned. After the specimen was fixed with formalin, it was sectioned at 2 mm intervals. If piecemeal-resected specimens, those were reconstructed as much as possible.

Statistical Analysis

Univariate analysis was performed using chi-squared test or Fisher exact test for categorical variables and Student *t* test for continuous variables. A forward stepwise multiple logistic regression model was used for statistically significant variables ($P < 0.05$) in the univariate analysis to identify risk factors for SM or LV invasion of EGC. $P < 0.05$ is considered as statistically significant. SPSS program version 18.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analysis.

RESULTS

During study period, a total of 308 EGC lesions met the inclusion criteria was included in this study. The patients' mean age was 65.78 ± 9.49 years. The patient population was predominantly male (246/308, 79.9%). The most common gross type of lesion was the depressed type (197/308, 64.0%) and the most predominant location was the lower third (230/308, 74.7%). The mean tumor size of all lesions was 11.05 ± 4.73 mm. Most lesions were solitary (280/308, 90.9%) and well-differentiated adenocarcinomas (208/308, 67.5%). Among 308 lesions, 34 showed SM invasion (11.0%) and 5 showed LV invasion (1.6%). All of the LV invasive cancer was SM cancers. The baseline characteristics were shown in Table 1.

TABLE 1. Baseline Characteristics of Patients Included in the Study

	Total (n = 308)
Mean age, y (±SD)	65.78 ± 9.49
Male, n (%)	246 (79.9)
Gross type, n (%)	
Elevated	54 (17.5)
Flat	57 (18.5)
Depressed	197 (64.0)
Surface redness, n (%)	210 (68.2)
Tumor location, n (%)	
Upper	15 (4.9)
Middle	63 (20.4)
Lower	230 (74.7)
Tumor number, n (%)	
Solitary	280 (90.9)
Multiple	28 (9.1)
Tumor size, n (%)	
≤10 mm	151 (49.0)
>10 mm	157 (51.0)
Histological type, n (%)	
Well differentiated	208 (67.5)
Moderately differentiated	100 (32.5)
SM invasive cancer, n (%)	34 (11.0)
SM and LV invasive cancer, n (%)	5 (1.6)

LV = lymphovascular, SD = standard deviation, SM = submucosa.

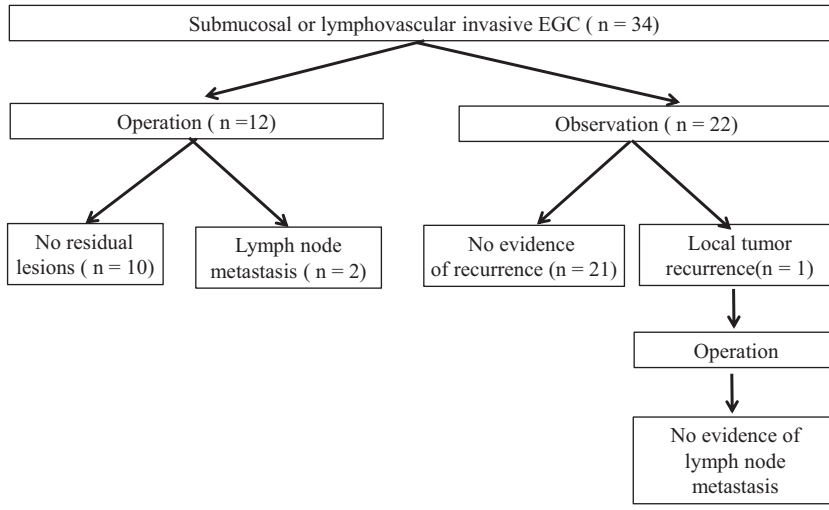

FIGURE 2. Flow chart of the results of 34 submucosal or lymphovascular invasive early gastric cancers. EGC = early gastric cancer.

Twelve of 34 patients with SM or LV invasive EGC underwent additional radical surgical treatments within 2 months after ESD. Lymph node metastasis was found in 2 patients (2/12). A local recurrence was found in 1 patient after 6 months after ESD. The patient underwent additional surgical treatment, and there was no lymph node metastasis. Twenty-one patients did not undergo additional surgical treatment, and there was no evidence of recurrence during follow-up period (range, 17–63 months). A flow diagram of the results of 34 patients with SM or LV invasive EGC was shown in Figure 2.

The clinicopathological data of the lesions, with and/or without SM or LV invasion, are summarized in Table 2.

TABLE 2. Characteristics and Associated Risk Factors for Submucosa or Lymphovascular Invasive Early Gastric Cancer: Univariate Analysis

	SM/LV Invasion (−) (n = 274)	SM/LV Invasion (+) (n = 34)	*P* Value
Mean age, y (±SD)	65.83 ± 9.28	65.35 ± 11.21	0.782
Male gender, n (%)	221 (80.7)	25 (73.5)	0.328
Gross type, n (%)			0.532
Elevated	46 (16.8)	8 (23.5)	
Flat	50 (18.2)	7 (20.6)	
Depressed	178 (65.0)	19 (55.9)	
Surface redness, n (%)	183 (66.8)	27 (79.4)	
Tumor location, n (%)			0.004
Upper	10 (3.6)	5 (14.7)	
Middle	53 (19.3)	10 (29.4)	
Lower	211 (77.0)	19 (55.9)	
Tumor number, n (%)			0.531
Solitary	250 (91.2)	30 (88.2)	
Multiple	24 (8.8)	4 (11.8)	
Mean tumor size, mm (±SD)	10.92 ± 4.81	12.15 ± 3.96	0.153
Tumor size, n (%)			0.544
≤10 mm	136 (49.6)	15 (44.1)	
>10 mm	138 (60.4)	19 (55.9)	
Invasion depth, n (%)			
m	274 (100)	1 (2.9)	
SM1	0 (0)	16 (47.1)	
SM2	0 (0)	8 (23.5)	
SM3	0 (0)	9 (26.5)	
Mean SM depth, μm (±SD)		748.97 ± 629.61	
Histological type, n (%)			<0.001
Well differentiated	195 (71.2)	13 (38.2)	
Moderately differentiated	79 (28.2)	21 (61.8)	

LV = lymphovascular, SD = standard deviation, SM = submucosa.

TABLE 3. Associated Risk Factors for Submucosa or Lymphovascular Invasive Early Gastric Cancer: Multivariate Analysis

	Odds Ratio	95% CI	*P* Value
Depressed gross type	0.631	0.289–1.374	0.246
Upper and middle location	3.100	1.421–6.766	0.004
Tumor size: >10 mm	1.055	0.492–2.265	0.890
Tumor number: multiple	1.848	0.548–6.223	0.322
Moderately differentiated histology	4.157	1.920–9.002	<0.001
Surface redness	1.945	0.770–4.908	0.159

CI = confidence interval.

Univariate analysis revealed significant differences in tumor location ($P = 0.004$) and histological type ($P < 0.001$) between EGCs with and without SM or LV invasion. Among the EGCs with SM or LV invasion, 44.1% (15/34) were located in the middle and upper thirds of the stomach, and 61.8% (21/34) were moderately differentiated adenocarcinomas.

Multivariate analysis revealed that upper and middle location of the lesion ($P = 0.004$, odds ratio [OR] 3.100, 95% confidence interval [CI] 1.421–6.766) and moderately differentiated histology ($P < 0.001$, OR 4.157, 95% CI 0.770–4.908) were significant risk factors (Table 3).

DISCUSSION

The overall 5-year survival rate for treated EGC is >90%, and the most important prognostic factor is lymph node metastasis.[18] Ideal indication for ESD is EGC without risk of lymph node metastasis. However, it is impossible to know lymph node metastasis accurately before surgical resection. Therefore, the indications for ESD were determined based on the data of surgical resections to date. Among the absolute indications for endoscopic treatment of EGC, histologic type (well and/or moderately differentiated adenocarcinomas), tumors size (≤2 cm in length), and absence of ulcer can be predicted or diagnosed before endoscopic resection from endoscopic diagnostic approach. However, it is difficult to predict the depth of invasion (lesions limited to the mucosal layer) precisely. In the recent years, accurate prediction of tumor invasion depth in EGC is crucial because we could provide the patients the opportunity of an endoscopic treatment. To date, several studies about the endoscopic prediction of tumor invasion depth in EGC showed 72% to 78% accurate prediction rate by conventional endoscopy.[19–22] Although, endoscopic ultrasonography is a widely used for detecting the depth of EGC in many institutions, the reported accurate prediction rate of depth of invasion in EGC was 67.4% to 85%.[19,22] In addition, among absolute indication, it is impossible to determine LV involvement before resection.

For the prediction of submucosal invasive cancer, various endoscopic morphologic factors were reported; large tumor size (≥30 mm in diameter), irregular surface (including nodules in the depressed area), SM tumor-like lesions, fold thickness, and fusion of convergent folds in the case of a lesion with fold convergence.[20,22] In this study, we included only EGCs met the absolute indication (lesion size <2 cm and no ulceration) before resection. Therefore, the most common reported significant morphologic endoscopic findings were not significant predictable factors for SM or LV invasion.

In the present study, a moderately differentiated adenocarcinoma histology (OR 4.157; 95% CI 1.920–9.002; $P < 0.001$) (compared with well-differentiated adenocarcinoma) was found to be a significant risk factor for SM or LV invasion. Although well to moderately differentiated EGCs are accepted as an absolute indication for ESD, a moderately differentiated adenocarcinoma was a significant risk factor for SM or LV invasion compared with well-differentiated adenocarcinoma.[23,24] Furthermore, a moderately differentiated EGC is often associated with a mixed histological type of EGC which is a risk factor for lymph node metastasis.[24,25] Mixed histological type EGCs are comprised histologically of nonhomogenous mixtures of intestinal type and diffuse type carcinomas. Although the clinical outcomes and significance of mixed histological type EGCs treated with ESD are poorly understood, complete resection rate was lower and local recurrence was more frequent than other types of EGC.[26] In the present study, 11 lesions were mixed histology during data collection, but these lesions were excluded during data analysis because we limited the study EGC lesions with purely well- to moderate-differentiated carcinoma. More precautions are required for moderately differentiated EGC was found from endoscopic forceps biopsy compared with well-differentiated adenocarcinoma before ESD.

Unexpectedly, tumors located in the upper and middle third of the stomach had a significantly higher risk for SM or LV invasion in the present study. Tumors located in the upper and middle third of the stomach were larger (11.63 ± 4.66 mm vs 10.86 ± 4.75 mm, $P = 0.214$), although the difference was not significant. We could not know why the location of the EGC was the significant risk factor. One study revealed differences in thickness of according to the location of the stomach. The entire wall thickness is thicker in the antrum than the body and cardia. And, the thickness of the SM layer decreased from the antrum to the cardia and the body.[14] Thus, EGC located in the upper portion of the stomach might be even deep invasive cancer though same size. The other possible explanation is technical factor associated with endoscopic experience. EGC located lower third of the stomach, especially antrum, might be easily detected by unexperienced hand or unclosed observed endoscopy. However, to detect EGCs in the upper and middle third of the stomach, more experienced handed or closed observed endoscopic procedure might be required. Therefore, EGC lesions located in the upper and middle third of the stomach might be delayed diagnosed. Further studies might be required to elucidate the reasons of this finding.

Gotoda et al[8] reported the well or moderately differentiated adenocarcinoma with minute SM invasion (≤500 μm, SM1). However, other studies reported that 6.3% to 15% of superficial SM invasive (SM1, <500 μm) associated with lymph node metastasis.[23,27] In the present study, 13 of 34 patients with SM or LV invasive EGC underwent additional radical surgical treatments. Principally, after diagnosis of SM or LV invasive caner, we explained the risk of lymph node metastasis and

recommended operation. However, only 12 patients accepted additional operation, just after ESD. Lymph node metastasis was found in 2 patients with SM invasion. One patient presented with a local recurrence during follow-up after ESD. After additional surgery, lymph node metastasis was not found. The remainder of patients did not want additional surgical operation (all lesions were no evidence of LV invasion). During follow-up period, no recurrence of gastric cancer was found during follow-up (range, 17–63 months).

There are several limitations in the present study. First, it was retrospectively conducted in a single center. The number of enrolled cases was lack for supporting for these risk factors definitely. Accumulation of data from multicenter and prospective studies may more accurately predict the SM or LV invasive EGC in small size ($\leq$2 cm). However, the identified risk factors, including other studies so far, may be helpful for further studies and evidences to cautious endoscopic treatment of EGC. Second, among endoscopic findings, we used only lesion diameter and macroscopic appearances of lesion for the analyses. If recent diagnostic technologies such as narrow band image, magnifying endoscopy, endoscopic ultrasound, or endomicroscopy might be used, more accurate predictable data might be collected.

In summary, this study identified 11% of EGC lesions showed SM or LV invasions, although those were included in the absolute indications before ESD. Associated risk factors with SM or LV invasions were histologic type (moderately differentiated adenocarcinoma) and location (in the upper and middle third of the stomach) of the lesion. Therefore, precautions are required in the management of patients with these 2 risk factors. Before ESD, for lesions with the 2 risk factors, endoscopists should explain patients for the possibility of SM or LV invasion after ESD.

REFERENCES

1. Chiu PW, Teoh AY, To KF, et al. Endoscopic submucosal dissection (ESD) compared with gastrectomy for treatment of early gastric neoplasia: a retrospective cohort study. *Surg Endosc.* 2012;26:3584–3591.
2. Choi IJ, Lee JH, Kim YI, et al. Long-term outcome comparison of endoscopic resection and surgery in early gastric cancer meeting the absolute indication for endoscopic resection. *Gastrointest Endosc.* 2015;81:333–341e1.
3. Choi KS, Jung HY, Choi KD, et al. EMR versus gastrectomy for intramucosal gastric cancer: comparison of long-term outcomes. *Gastrointest Endosc.* 2011;73:942–948.
4. Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2010 (ver. 3). *Gastric Cancer.* 2011;14:113–123.
5. Lee JH, Kim JG, Jung HK, et al. Clinical practice guidelines for gastric cancer in Korea: an evidence-based approach. *J Gastric Cancer.* 2014;14:87–104.
6. An JY, Baik YH, Choi MG, et al. Predictive factors for lymph node metastasis in early gastric cancer with submucosal invasion: analysis of a single institutional experience. *Ann Surg.* 2007;246:749–753.
7. Fujimoto A, Ishikawa Y, Akishima-Fukasawa Y, et al. Significance of lymphatic invasion on regional lymph node metastasis in early gastric cancer using LYVE-1 immunohistochemical analysis. *Am J Clin Pathol.* 2007;127:82–88.
8. Gotoda T, Yanagisawa A, Sasako M, et al. Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers. *Gastric Cancer.* 2000;3:219–225.
9. Kim H, Kim JH, Park JC, et al. Lymphovascular invasion is an important predictor of lymph node metastasis in endoscopically resected early gastric cancers. *Oncol Rep.* 2011;25:1589–1595.
10. Sekiguchi M, Sekine S, Oda I, et al. Risk factors for lymphatic and venous involvement in endoscopically resected gastric cancer. *J Gastroenterol.* 2013;48:706–712.
11. Holscher AH, Drebber U, Monig SP, et al. Early gastric cancer: lymph node metastasis starts with deep mucosal infiltration. *Ann Surg.* 2009;250:791–797.
12. Kosuke N, Oguma H, Yamamoto M. Early gastric cancer with lymph node metastasis. *Ann Surg.* 2011;253:840–841author reply 1.
13. Lehnert T, Erlandson RA, Decosse JJ. Lymph and blood capillaries of the human gastric mucosa. A morphologic basis for metastasis in early gastric carcinoma. *Gastroenterology.* 1985;89:939–950.
14. Sako A, Kitayama J, Ishikawa M, et al. Impact of immunohistochemically identified lymphatic invasion on nodal metastasis in early gastric cancer. *Gastric Cancer.* 2006;9:295–302.
15. Choi CW, Kim HW, Shin DH, et al. The risk factors for discrepancy after endoscopic submucosal dissection of gastric category 3 lesion (low grade dysplasia). *Dig Dis Sci.* 2014;59:421–427.
16. Participants in the Paris Workshop. The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon: November 30 to December 1, 2002. *Gastrointest Endosc.* 2003;58(Suppl):S3–S43.
17. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer.* 2011;14:101–112.
18. Okada K, Fujisaki J, Yoshida T, et al. Long-term outcomes of endoscopic submucosal dissection for undifferentiated-type early gastric cancer. *Endoscopy.* 2012;44:122–127.
19. Choi J, Kim SG, Im JP, et al. Comparison of endoscopic ultrasonography and conventional endoscopy for prediction of depth of tumor invasion in early gastric cancer. *Endoscopy.* 2010;42:705–713.
20. Choi J, Kim SG, Im JP, et al. Endoscopic prediction of tumor invasion depth in early gastric cancer. *Gastrointest Endosc.* 2011;73:917–927.
21. Sano T, Okuyama Y, Kobori O, et al. Early gastric cancer. Endoscopic diagnosis of depth of invasion. *Dig Dis Sci.* 1990;35:1340–1344.
22. Tsujii Y, Kato M, Inoue T, et al. Integrated diagnostic strategy for the invasion depth of early gastric cancer by conventional endoscopy and EUS. *Gastrointest Endosc.* 2015;82:452–459.
23. Kim JH, Lee YC, Kim H, et al. Additive lymph node dissection may be necessary in minute submucosal cancer of the stomach after endoscopic resection. *Ann Surg Oncol.* 2012;19:779–785.
24. Yamada T, Sugiyama H, Ochi D, et al. Risk factors for submucosal and lymphovascular invasion in gastric cancer looking indicative for endoscopic submucosal dissection. *Gastric Cancer.* 2014;17:692–696.
25. Iwamoto J, Mizokami Y, Ito M, et al. Clinicopathological features of undifferentiated mixed type early gastric cancer treated with endoscopic submucosal dissection. *Hepatogastroenterology.* 2010;57:185–190.
26. Han JP, Hong SJ, Kim HK. Long-term outcomes of early gastric cancer diagnosed as mixed adenocarcinoma after endoscopic submucosal dissection. *J Gastroenterol Hepatol.* 2015;30:316–320.
27. Kang HJ, Kim DH, Jeon TY, et al. Lymph node metastasis from intestinal-type early gastric cancer: experience in a single institution and reassessment of the extended criteria for endoscopic submucosal dissection. *Gastrointest Endosc.* 2010;72:508–515.