Digestive Endoscopy (2008) **20**, 17–21 doi:10.1111/j.1443-1661.2007.00770.x

ORIGINAL ARTICLE

MIXED INTESTINAL- AND DIFFUSE-TYPE HISTOLOGY IS A RISK FACTOR FOR LYMPH NODE METASTASIS OF SUBMUCOSAL INVASIVE GASTRIC CANCER

Naoko Tsuji,[1,2] Shingo Ishiguro,[1] Yasuko Umehara[2] and Masatoshi Kudo[3]

[1]*Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka,* [2]*Department of Gastroenterology, Sakai Hospital, Kinki University School of Medicine, Sakai and* [3]*Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-sayama, Japan*

Background: Endoscopic submucosal dissection is expected to increase the number of node-negative submucosal invasive gastric cancers, particularly differentiated-type adenocarcinomas that can be treated conservatively.
Methods: Two hundred and seven consecutive surgically treated cases of differentiated-type early gastric cancer with submucosal invasion were analyzed clinicopathologically. Comparison was made between patients with node-positive ($n = 33$) and node-negative cancer ($n = 174$). Whole sections of surgical specimens were reviewed and reclassified as pure intestinal type or mixed type. The intramucosal and submucosal components were also described histologically, and the depth of invasion from the muscularis mucosae as well as the width of submucosal invasion was measured.
Results: Twenty-four of 33 (73%) node-positive cases were of the mixed type, whereas 71 of 174 (41%) node-negative cases were of the mixed type ($P < 0.01$). As for the intramucosal histology, the ratio of mixed-type was also higher in the node-positive group (58% *vs* 34%; $P < 0.05$). Other factors associated with lymph node metastasis were larger tumor size ($P = 0.003$), deeper submucosal invasion ($P < 0.001$) and wider submucosal extension ($P = 0.004$), and lymphatic permeation ($P < 0.001$). Multivariate analysis demonstrated that lymphatic permeation ($P = 0.001$, OR 4.76), and mixed-type histology (OR 2.56) were independent risk factors.
Conclusions: Histological heterogeneity is a risk factor for metastasis of submucosal invasive gastric cancer to lymph nodes. Heterogeneity of mucosal components is also a significant risk factor and thus a good predictor of lymph node metastasis, potentially useful in distinguishing patients ineligible for conservative therapy.

Key words: endoscopic submucosal dissection, lymph node metastasis, mixed-type histology, submucosal invasive gastric cancer.

INTRODUCTION

Endoscopic mucosal resection (EMR) allows conservative treatment of early gastric cancer.[1] However, these are technical limitations to EMR. Stomach-conserving therapy was used to treat a subset of intramucosal gastric cancers. The Japanese Gastric Cancer Association recommended EMR for treatment of ulcer-free intramucosal differentiated adenocarcinoma smaller than 2 cm.[2] Endoscopic submucosal dissection (ESD) allows en bloc resection of larger tumors and has extended the indications for conservative therapy.[3]

We previously investigated 740 consecutive cases of surgically treated intramucosal gastric cancer and reported that mixed histology is a risk factor for lymph node metastasis from intramucosal gastric cancer.[4] In the present study, we investigated surgically resected submucosal invasive gastric cancer, especially differentiated-type adenocarcinoma, to clarify the risk factors for lymph node metastasis and to evaluated which tumor characteristics could be used to expand the indication for ESD to treat gastric cancer.

Correspondence: Naoko Tsuji, Department of Pathology, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari, Osaka 537-8511, Japan. Email: tsuji@sakai.med.kindai.ac.jp

Received 24 April 2006; accepted 6 August 2006.

METHODS

The study population comprised 207 consecutive patients with submucosal invasive gastric cancer treated surgically at Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan. All tumors were single primary adenocarcinomas, and no double or multiple tumors were found at the time of surgery. All specimens were fixed in 10% formalin and cut serially into 5 mm slices parallel with the lesser curvature. All tissue sections were stained with hematoxylin–eosin. Comparison was made between patients with node-positive ($n = 33$) and node-negative cancer ($n = 174$). Tumors were characterized by macroscopic appearance, histological type, depth of invasion, and lymphatic permeations. The major histological type of each tumor was determined according to the Japanese Classification of Gastric Carcinoma.[5] In addition to major histological type, all other minor histological types were also examined and reclassified as pure intestinal type, mixed type, or pure diffuse type. Papillary adenocarcinoma, and well- and moderately differentiated

tubular adenocarcinoma were classified as pure intestinal-type tumor, and poorly differentiated adenocarcinoma and signet ring cell carcinoma were classified as pure diffuse-type tumors. Tumors containing both types were classified as mixed type. If there was an element of diffuse-type cancer cells even in one part, it was classified as mixed type. The intramucosal and submucosal components were also examined, and the depth of invasion from the muscularis mucosae and the width of submucosal extension were measured.

Frequencies of various characteristics were compared between node-positive and node-negative groups. Statistical analysis was performed using the χ^2 test for categorical data and Mann–Whitney *U*-test for continuous data. A multivariate analysis of the risk factor for lymph node metastasis was performed using a logistic regression method. SPSS software (SPSS, Chicago, IL, USA) was used and statistical significance was accepted at a *P*-value of less than 0.05.

RESULTS

Univariate analysis of risk factors predicting lymph node metastasis

The clinicopathological characteristics of the 207 patients are shown in Table 1. There were 33 (16%) node-positive patients and 174 (84%) node-negative patients. The average age of node-positive patients was 58 years, and that of node-negative patients was 61 years. Female sex (39% *vs* 20%, $P < 0.05$), main histology of moderately differentiated tubular adenocarcinoma (tub2) (67% *vs* 48%, $P < 0.05$), and positive lymphatic permeation (79% *vs* 36%; $P < 0.001$) were associated with lymph node metastasis. There was no statistical difference in tumor location or macroscopic appearance between the groups. Twenty-four of 33 (73%) node-positive cases were of the mixed type, whereas 71 of 174 (41%) node-negative cases were of the mixed type. The percentage of cases with mixed-type histology was significantly higher in

Table 1. Results of univariate analysis between node-positive and node-negative groups

	n(+) ($n = 33$)	n(−) ($n = 174$)	*P*-value
Age, mean ± SD, years	57.85 ± 11.79	61.36 ± 10.30	0.078
Sex (%)			
Male	20 (61)	139 (80)	
Female	13 (39)	35 (20)	0.016
Size, mean ± SD, mm	49.57 ± 25.00	36.48 ± 21.43	0.003
Location (%)			
Upper third	4 (12)	35 (20)	
Middle third	7 (21)	55 (32)	
Lower third	22 (67)	84 (48)	0.151
Anterior wall	7 (21)	33 (19)	
Posterior wall	9 (27)	52 (30)	
Lesser curvature	12 (36)	66 (38)	
Greater curvature	5 (15)	23 (13)	0.972
Macroscopic appearance (%)			
Elevated	12 (36)	51 (29)	
Depressed-ulcer(−)	5 (15)	56 (32)	
Depressed-ulcer(+)	16 (48)	67 (39)	0.144
Main histology (%)			
Pap	6 (18)	24 (14)	
Tub1	5 (15)	66 (38)	
Tub2	22 (67)	84 (48)	0.040
Histology (%)			
Pure intestinal	9 (27)	103 (59)	
Mixed	24 (73)	71 (41)	<0.001
Intramucosal histology (%)			
Pure intestinal	14 (42)	115 (66)	
Mixed	19 (58)	59 (34)	0.010
Submucosal histology (%)			
Pure intestinal	20 (61)	129 (74)	
Mixed	13 (39)	35 (20)	
Pure diffuse	0 (0)	10 (6)	0.029
Depth of submucosal invasion, mean ± SD, mm	3.388 ± 2.575	1.886 ± 1.611	<0.001
Width of submuosal extension, mean ± SD, mm	22.221 ± 19.083	12.353 ± 12.452	0.004
Lymphatic permeation			
ly(−)	7 (21)	111 (74)	
ly(+)	26 (79)	63 (36)	<0.001

n(+), node-positive group; n(−), node-negative group; pap, papillary adenocarcinoma; tub1, well-differentiated tubular adenocarcinoma; tub2, moderately differentiated tubular adenocarcinoma.

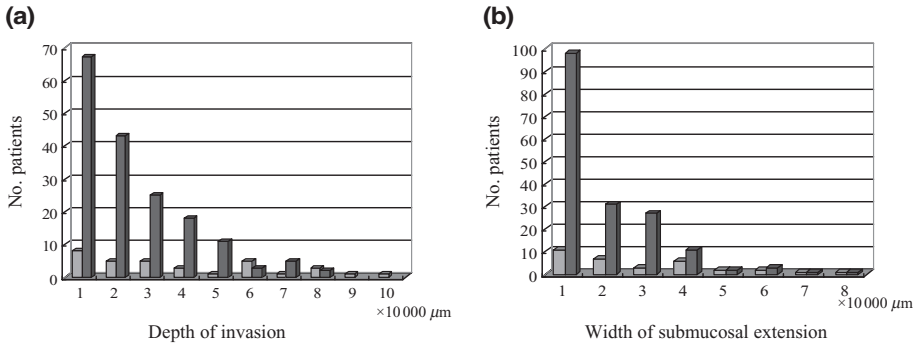

Fig. 1. Distributions of the (a) depth of invasion from the muscularis mucosae and (b) width of submucosal extension. Node-positive group showed deeper submucosal invasion (mean ± SD: 3388 ± 2575 μm *vs* 1886 ± 1611 μm, $P < 0.001$) and wider extension (mean ± SD: 22 221 ± 19 083 μm *vs* 12 353 ± 12 452 μm, $P < 0.01$). □, n(+); ■, n(−).

the node-positive group ($P < 0.01$). Intramucosal components were also described and reclassified, and the ratio of mixed-type histology was also higher in the node-positive group (58% *vs* 34%; $P < 0.05$). In submucosal histology, pure diffuse type was seen only in node-negative group. The distributions of the depth of invasion from the muscularis mucosae and width of submucosal invasion are shown in Fig. 1. The node-positive group showed deeper submucosal invasion (mean ± SD: 3.4 ± 2.6 mm *vs* 1.9 ± 1.6 mm, $P < 0.001$) and wider extension (mean ± SD: 22.2 ± 19.0 mm *vs* 12.4 ± 12.4 mm, $P < 0.01$). The minimal depth of invasion in the node-positive group was 425 μm. This patient was a 47-year-old woman with mixed-type tumor (Fig. 2a–e). The predominant histology was moderately differentiated adenocarcinoma, the minor components of the mucosa were signet ring cell carcinoma and well-differentiated adenocarcinoma, and the submucosal component was well-differentiated adenocarcinoma. Histological examination of the metastatic lymph node revealed poorly differentiated adenocarcinoma. The depth of invasion in all other node-positive patients was greater than 500 μm.

Multivariate analysis

The independent risk factor for lymph node metastasis of mixed-type histology was evaluated in a multivariate analysis that included all of the variables associated with lymph node metastasis in univariate analysis (Table 2). Besides variables such as lymphatic permeation and deeper submucosal invasion, this analysis showed that mixed histology was also an independent predictor of lymph node metastasis. Overall, patients with mixed histology had a 2.5-fold increase in the risk of lymph node metastasis compared with patients with pure intestinal type ($P < 0.05$).

DISCUSSION

Endoscopic mucosal resection was stomach-conserving therapy without lymph node dissection. EMR was first used to treat intramucosal adenocarcinoma of the stomach in the 1990s.[1] The criteria for tumors suited to EMR were developed from data derived from surgically resected early gastric cancers. The principles of the criteria were exception of node-positive cases and en bloc resection. According to the guidelines for the treatment of gastric cancer of the Japanese Gastric Cancer Association, EMR is indicated for the treatment of ulcer-free intramucosal differentiated-type adenocarcinoma smaller than 2 cm.[2] The advent of a new method, ESD, has allowed simple en bloc resection of larger tumors.[3] This technique allows more patients to undergo treatment that preserves the stomach. Gotoda *et al*. proposed expanding the criteria for local treatment of gastric cancers to node-negative cases of differentiated-type adenocarcinoma with submucosal invasion, smaller than 3 cm, no lymphatic or vascular permeation, and less than 500 μm depth of invasion.[6] None of our node-positive patients fulfilled these criteria. The recommendation of Gotoda *et al*. to expand the indications for local treatment was based on a large data set from surgical specimens and not on endoscopically resected specimens.[6] These criteria are not thought to guarantee the safety of conservative therapy. The resected stomachs we reviewed were cut serially every 5 mm, but we now cut EMR and ESD specimens every 2 mm. The information obtained from the EMR and ESD specimens is more precise than that from surgical specimens. Measurements of the depth of submucosal invasion and lymphatic permeation from EMR and ESD specimens are more accurate than those from surgical specimens. The probability that a case is node negative is thought to be very high if the endoscopically resected material meets the criteria proposed by Gotoda *et al*.[6]

In Japan, the histological type of gastric cancer is determined according to the Japanese Classification of Gastric Carcinoma,[5] and the major histological type is described. However, some gastric cancers show histological heterogeneity and, in the present study, mixed histology was an independent predictor of lymph node metastasis. We recommend describing both major histological type and any minor components in the pathological descriptions of biopsy specimens before ESD and resected specimens after ESD. We may sometimes waver between endoscopic therapy and surgery when the size of the tumor is approximately 3 cm or depth of invasion is approximately 500 μm. In such circumstances, histological type will give us good information.

There are other problems with node-negative criteria made by surgical specimens. Lymph node metastasis is typically evaluated on only one or two slices of the resected lymph nodes. Nodal micrometastasis of routinely diagnosed node-negative cases has been reported in many studies. Endo *et al*. reported that nodal micrometastasis was present in 19% of patients with node-negative submucosal gastric cancer, and they concluded that EMR is not suitable for patients with submucosal gastric cancer.[7] In Japan, the clinical outcome of early gastric cancers is excellent, and many Japanese surgeons emphasize the importance of lymph node dissection.[8] However, lymph node dissection has been shown to be irrelevant to the prognosis of breast cancer.[9]

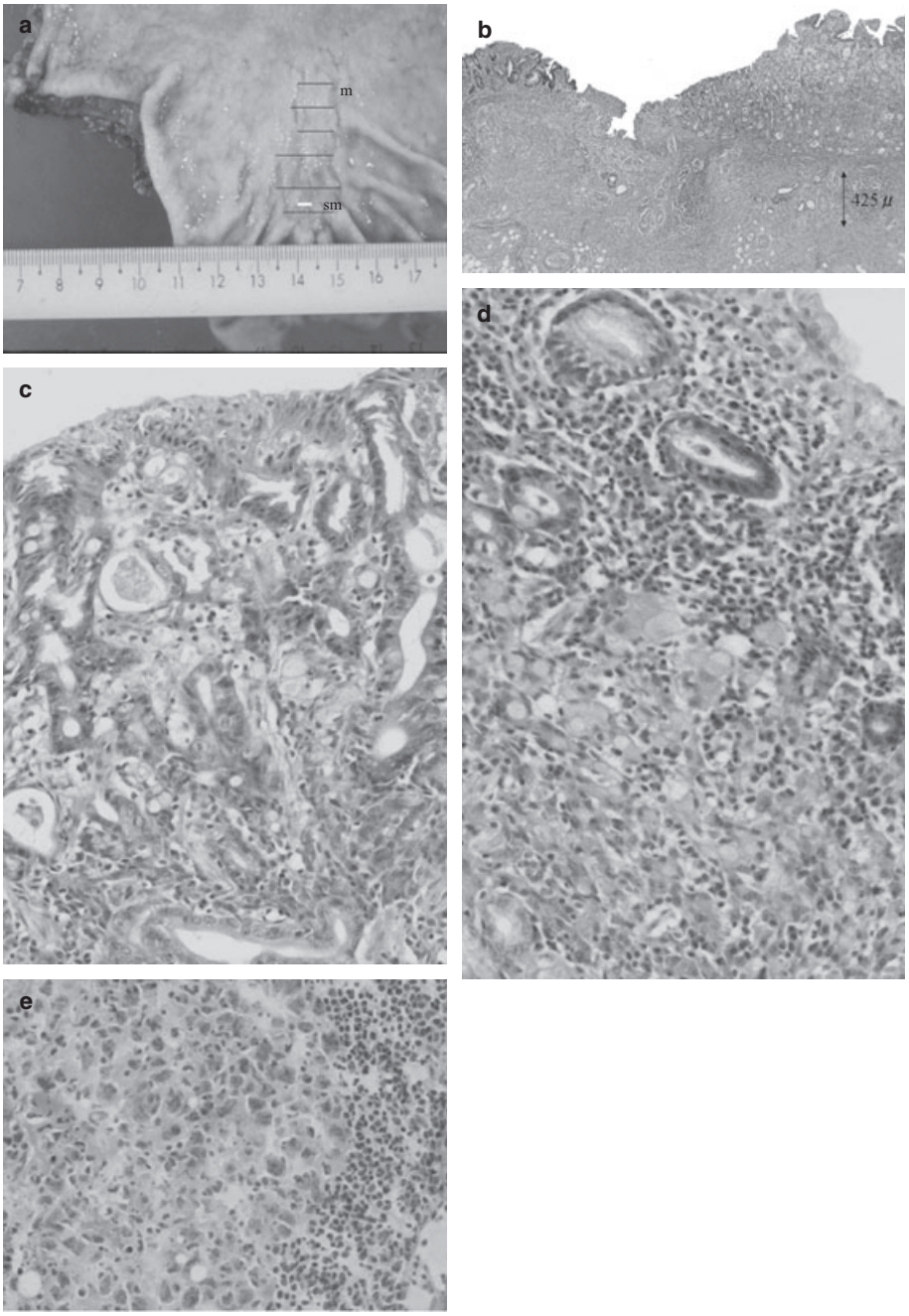

Fig. 2. (a) A 47-year-old node-positive woman with mixed intestinal- and diffuse-type histology. The gross specimen shows depressed-type submucosal gastric cancer. (b) Hematoxylin–eosin stain of the same case. Depth of submucosal invasion was 425 μm. (c) Predominant histology is differentiated-type adenocarcinoma, but (d) a signet ring cell component is also visible. (e) Poorly differentiated adenocarcinoma is seen in a metastatic lymph node.

Table 2. Result of multivariate analysis between node-positive and node-negative groups

	n(+) ($n = 33$)	n(−) ($n = 174$)	*P*-value	OR	95%CI
Size, more than 30 mm	23	91	0.522	1.382	0.514–3.717
Histology, mixed	24	71	0.038	2.562	1.052–6.238
Depth of submucosal invasion, more than 2 mm	19	61	0.565	1.310	0.522–3.291
Width of submucosal extension, more than 20 mm	15	29	0.331	1.685	0.589–4.822
Lymphatic permeation, positive	26	63	0.001	4.764	1.848–12.282

n(+), node-positive group; n(−), node-negative group; OR, odds ratio; 95%CI, 95% confidence interval.

Breast-conserving therapy is one of the early organ-conserving therapies, and long-term follow-up data led to this disturbing conclusion. Regarding the prognosis of intramucosal gastric cancer treated by EMR, Ono *et al*. concluded that EMR can provide a favorable long-term survival rate.[10] In the present study, the average disease-free survival of recurrent patients was approximately 5 years, which means that long-term follow up is necessary after conservative therapy. EMR and ESD provide good quality of life for patients with early gastric cancer. Most of our elderly patients who had undergone gastrectomy with lymph node dissection died of other causes.

In the present study, only one node-positive patient showed less than 500 μm submucosal invasion, but tumor size was larger than 3 cm, and none of the node-positive patients fulfilled Gotoda's criteria. Therefore, we agree with the opinion of Gotoda *et al*. But, in a very delicate case such as tumor size approximately 3 cm with suspected submucosal invasion and the biopsy specimen showing any amount of diffuse-type cancer cells, we should be careful with ESD. We believe that the indication for conservative therapy can be extended to pure-intestinal type submucosal invasive cancer patients and elderly patients.

Whether ESD for node-negative sm-invasive carcinoma is sufficient or not for the treatment of choice is a difficult question to answer. In our study, the recurrent patients of the node-negative group had tendency of male sex, relatively elderly age, and pure intestinal-type histology. Those patients had recurrence in spite of lymph node dissection; therefore, we believe ESD is also indicated for such patients. But we should also inform patients about the risk of recurrences, and the risk factors of recurrences in node-negative patients should be studied. At present, we cannot know the true safety of ESD for submucosal invasive gastric cancer, and only follow-up data will reveal the actual safety of ESD. Detailed examination of the ESD specimens and systematic long-time follow up is recommended for the estimation of the safety of stomach conservative therapy.

CONCLUSIONS

Mixed histology is an independent risk factor for lymph node metastasis of submucosal invasive gastric cancer. Heterogeneity of mucosal components is also a significant risk factor, and may be useful for identifying cases not suited for conservative therapy.

REFERENCES

1. Tada M, Murakami A, Karita M, Yanai H, Okita K. Endoscopic resection of early gastric cancer. *Endoscopy* 1993; **25**: 445–50.
2. Japanese Gastric Cancer Association. *Guidelines for the Treatment of Gastric Cancer.* Tokyo: Kanehara, 2001.
3. Gotoda T, Kondo H, Ono H. A new endoscopic mucosal resection procedure using an insulation-tipped electrosurgical knife for rectal flat lesions: Report of two cases. *Gastrointest. Endosc.* 1999; **50**: 560–3.
4. Tsuji N, Ishiguro S, Suzuki N. Risk factors for lymph node metastases of intramucosal gastric cancer, a case-control study. *Gastroenterol. Endosc.* 1999; **41**: 1059–65.
5. Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinoma. 2nd English Edition. *Gastric Cancer* 1998; **1**: 10–24.
6. 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**: 219–25.
7. Endo K, Kohnoe S, Okamura T *et al.* Evaluation of endoscopic mucosal resection and nodal micrometastasis in pN0 submucosal gastric cancer. *Oncol. Rep.* 2005; **13**: 1059–63.
8. Okamura T, Tsujitani S, Korenaga D *et al.* Lymphadenectomy for cure in patients with early gastric cancer and lymph node metastasis. *Am. J. Surg.* 1988; **155**: 476–80.
9. Fisher B, Anderson S, Bryant J *et al.* Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. *N. Engl. J. Med.* 2002; **347**: 567–75.
10. Ono H, Kondo H, Gotoda T *et al.* Endoscopic mucosal resection for treatment of early gastric cancer. *Gut* 2001; **48**: 225–9.