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Autofluorescence imaging for predicting development of metachronous gastric cancer after *Helicobacter pylori* eradication

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Key words
autofluorescence imaging, early gastric cancer, endoscopic submucosal dissection, *Helicobacter pylori*, metachronous gastric cancer.

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Abstract

Background and Aims: Although *Helicobacter pylori* eradication decreases the incidence of metachronous gastric cancer after endoscopic treatment for early gastric cancer (EGC), metachronous cancer still develops after successful eradication, particularly in patients with severe corpus gastritis. We investigated whether the extent of atrophic fundic gastritis diagnosed by autofluorescence imaging (AFI) videoendoscopy is predictive of development of metachronous gastric cancer after *H. pylori* eradication in patients treated with endoscopic submucosal dissection (ESD) for EGC.

Patients and Methods: A total of 82 patients who underwent ESD for EGC from 2003 to 2006, who received eradication therapy participated in this study. The extent of chronic atrophic fundic gastritis was evaluated by AFI and categorized into closed and open type. The main outcome was the incidence of metachronous gastric cancer detected by annual surveillance endoscopy.

Results: During a median observation period of 55 months, metachronous gastric cancer developed in 12 of 82 patients (14.6%). Multivariate Cox's proportional hazard analysis revealed that open-type, atrophic fundic gastritis diagnosed by AFI was significantly associated with development of metachronous gastric cancer (hazard ratio: 4.88, 95% confidence interval [CI]: 1.32–18.2, $P = 0.018$) after adjustment for age, sex, histological intestinal metaplasia, serum pepsinogen level, and *H. pylori* status.

Conclusions: Metachronous EGC developed after successful *H. pylori* eradication, and extensive atrophic fundic gastritis diagnosed by AFI was a significant predictor, thus it could identify patients undergoing ESD for EGC who still required intensive surveillance after eradication.

Introduction

Endoscopic submucosal dissection (ESD) is now widely performed for treatment of early gastric cancer (EGC) in Japan.[1–4] Multiple gastric cancers have been found in 9.0–11.5% of gastric cancer patients[3,5–7] and are more frequent in EGC than in advanced cancer patients. Moreover, metachronous multiple gastric cancer developed in 2.7–14.0% of the patients who underwent endoscopic mucosal resection within 3–5 years.[3,6,8,9] Recently, it has been indicated that *Helicobacter pylori* eradication therapy decreases the incidence of metachronous EGC after endoscopic resection.[10] In our experience, however, metachronous EGC still developed even after achieving successful eradication (11.2% in 33 months), and it was particularly more frequent in patients with severe corpus gastritis.[11]

Autofluorescence imaging (AFI) videoendoscopy produces real-time pseudocolor images based on natural tissue autofluorescence emitted by light excitation from endogenous fluorophores such as collagen, nicotinamide, adenine dinucleotide, flavin and porphyrins. In the AFI images, the mucosa that has more inflammation, atrophy or intestinal metaplasia induced by *H. pylori* infection looks bright green, whereas, normal fundic mucosa looks purple or deep green. In per-patient analysis, the accuracy of green mucosa with atrophy and intestinal metaplasia was 88% and 81%, and in per biopsy analysis, 76% and 76%, respectively.[12] Therefore, green mucosa in the gastric body represents the extent of chronic atrophic fundic gastritis. The aims of the present study were to investigate the extent of chronic atrophic fundic gastritis diagnosed by AFI, and whether it could be a predictor for the development of metachronous gastric

cancer after *H. pylori* eradication in patients who have undergone ESD for EGC.

Methods

Study participants

This was a prospective cohort study performed at the Department of Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan. A total of 100 patients who underwent ESD for EGC from November 2003 to May 2006 and who gave written informed consent to participate in this study were enrolled. Patients were excluded if they had a history of *H. pylori* eradication, nonsteroidal anti-inflammatory drugs (NSAIDs) or anticoagulants, hemorrhagic diseases, major organ failure or drug allergy. The study was approved by the ethical committee of our institution.

All patients were interviewed on their past medical and family histories. A structural questionnaire elicited information on demographic data, drinking and smoking habits. Drinking and smoking were defined as regular when consumption was > 35 g for ethanol or five cigarettes per day.

Serum sample

Serum samples were obtained and were analyzed for IgG *H. pylori* antibodies with an enzyme linked immunosorbent assay (ELISA) kit using the E plate test (Eiken Kagaku, Inc., Tokyo, Japan). Serum level of pepsinogen I and pepsinogen II was also assessed by ELISA (Eiken Kagaku).

Endoscopic procedure

Esophago-gastro-duodenoscopy (EGD) was performed with videoendoscopes that worked in high-resolution, white light mode and AFI mode (EVIS-FQ260Z; Olympus Medical Systems Co. Ltd, Tokyo, Japan). Before ESD, the extent of atrophic fundic gastritis in AFI images was assessed and categorized into six types that were based on the Kimura–Takemoto classification.[13] If the cardia was surrounded by purple mucosa (AF-C-1, AF-C-2 and AF-C-3), it was defined as closed type (Fig. 1), and if there was a green mucosa on the cardia (AF-O-1, AF-O-2 and AF-O-3), it was defined as open type (Fig. 2). Two biopsy specimens were taken at each site from the greater curvature of the antrum, and the greater and lesser curvature of the corpus.

Histological evaluation

Biopsy specimens were fixed in formalin, embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin. Severity of neutrophil (activity) and lymphocytic infiltration (inflammation), glandular atrophy (atrophy) and intestinal metaplasia was graded according to the updated Sydney system[14] (none, 0; mild, 1; moderate, 2; and severe, 3). Presence or absence of *H. pylori* was assessed histologically by Giemsa staining.

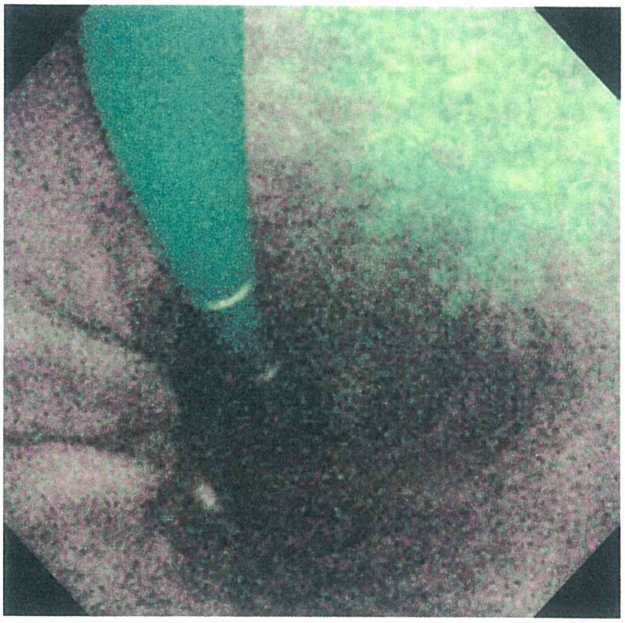

Figure 1 Representative case of closed type atrophic fundic gastritis in autofluorescence imaging (AFI) images. Cardia is surrounded by purple mucosa, and a color border between green and purple is observed at a lesser curvature of gastric body.

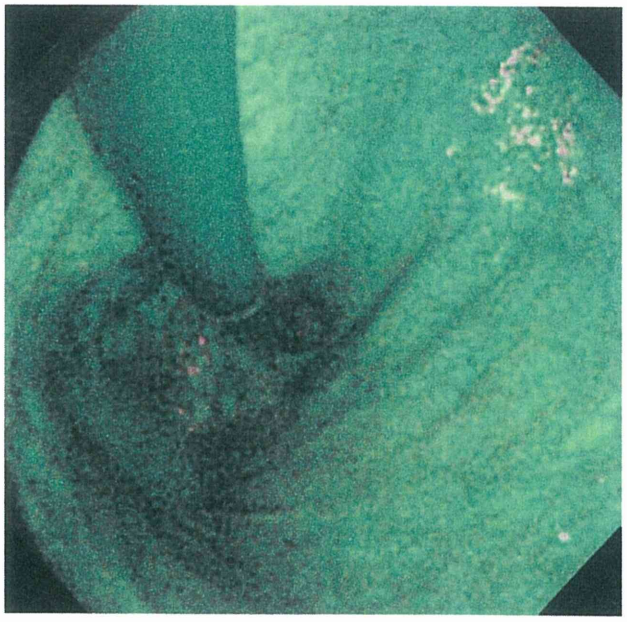

Figure 2 Representative case of open type atrophic fundic gastritis in autofluorescence imaging (AFI) images. The entire gastric mucosa including cardia looks bright green.

Helicobacter pylori status and eradication therapy

Patients were considered to be infected with *H. pylori* if any of the serum tests or histology was positive. Infected patients

were treated with 1 week of anti-*H. pylori* therapy that consisted of amoxicillin 1500 mg, clarithromycin 800 mg and rabeprazole 20 mg, 3 months after ESD. Successful eradication was diagnosed by urea breath test (UBiT-IR 300; Otsuka Electronics Co. Ltd, Osaka, Japan). The patients who failed the first regimen were retreated with second-line therapy of amoxicillin 1500 mg, metronidazole 500 mg and rabeprazole 20 mg. Patients in whom *H. pylori* was not eradicated after second-line therapy were followed up as those with persistent *H. pylori* infection.

Follow-up endoscopy

Two months after ESD, EGD was performed before eradication therapy to exclude the presence of synchronous multiple neoplasia. After that, surveillance endoscopy was scheduled annually after eradication therapy to diagnose metachronous EGC, using AFI videoendoscopy. The detected lesions were biopsied and removed by ESD if the histological findings of the biopsy specimens indicated that they were category 3–5 according to the revised Vienna classification.[15] Metachronous EGC was defined as lesions diagnosed as category 4 or 5 that were detected > 1 year after eradication therapy. Incidence of metachronous EGC was thoroughly studied by the end of June 2010.

Statistical analysis

Statistical analysis was performed with SPSS version 11.0 (SPSS, Chicago, IL, USA). The scores for neutrophil and lymphatic infiltration, glandular atrophy and intestinal metaplasia according to the Updated Sydney System and the serum level of pepsinogen were compared by Mann–Whitney *U*-test. Other clinical characteristics (sex, type of extension of atrophy, alcohol and smoking habits) were compared by the χ^2 test or Fisher's exact test when it was appropriate. The cumulative incidence of metachronous gastric cancer after *H. pylori* eradication was analyzed using the Kaplan–Meier method, and the difference between the curves of open- and closed-type was tested by Log-rank test. A Cox's proportional hazards regression model was used to analyze independence of the association between the extent of green mucosa in AFI images and development of metachronous EGC. Age, sex, intestinal metaplasia in the lesser curvature of the corpus, serum pepsinogen status, and *H. pylori* status were selected as candidate covariates for multivariate analysis. $P < 0.05$ was considered to indicate statistical significance.

Results

Eighteen patients in whom AFI endoscopy was not available and who did not undergo AFI observation were excluded, which left a total of 82 patients who were followed up and analyzed. The patients' demographic and clinical characteristics are shown in Table 1. In the AFI images, 31 patients had open-type, chronic atrophic fundic gastritis, and 51 had closed type. Among 82 patients who were analyzed, 73 were *H. pylori*-positive and received eradication therapy, while the remaining nine patients were negative and were not prescribed anti-H. *pylori* treatment. In 58 of 73 *H. pylori*-positive patients, the first eradication therapy was successful, and second-line therapy was successful in five patients. Thus, a total of 72 patients were followed up as an *H. pylori* negative group. Ten patients who failed first- and second-line eradication therapy were followed up as a persistent *H. pylori* infection group (Fig. 3).

Table 1 Clinicopathological features of 82 patients

Mean age (years old [SD])	65.2(8.5)
Male/female	68/14
Current smokers	36
Regular alcohol intake	40
Cancer lesions	105
Single/multiple	64/18
Location	
Upper third	32
Middle third	35
Lower third	38
Macroscopic type	
Elevated	57
Depressed	48
Mean tumor size (mm (SD)) mean(SD)	14.0(7.5)
Histological type	
Differentiated	103
Undifferentiated	2

All participants received follow-up endoscopy (median duration of follow-up period, 55 months; range, 14–72 months). Metachronous EGC developed in nine (12.5%) of 72 patients without *H. pylori* infection, and in three (30.0%) of 10 patients who had persistent *H. pylori* infection (Fig. 3). All metachronous EGC detected had a small size (mean tumor size, 6.0 ± 3.6 mm), was confined to the mucosa, and could be treated by ESD. Pathologically, all EGC was of the differentiated type.

The most suitable cut-off points for pepsinogen for metachronous EGC, obtained by receiver operating characteristic curve, were pepsinogen I ≤ 22 ng/mL or pepsinogen I/II ratio ≤ 1.8. Using the most suitable cut-off point for pepsinogen I/II ratio, the sensitivity and specificity for metachronous EGC was 63.6% and 41.0%, respectively.

Investigating predictive factors by univariate analysis, age ($P = 0.028$), intestinal metaplasia in the lesser curvature of the corpus ($P = 0.012$), and open-type atrophic fundic gastritis diagnosed by AFI ($P < 0.001$) were significantly associated with the development of metachronous EGC (Table 2). The cumulative 4-year incidence of metachronous EGC was 27.8% in patients with open-type atrophic fundic gastritis diagnosed by AFI and 4.1% in those with closed type, respectively ($P < 0.001$, Fig. 4). Cox's proportional hazard regression model revealed that only open-type, atrophic fundic gastritis, demonstrated by AFI, was an independent predictor for development of metachronous EGC after adjustment for age, sex, intestinal metaplasia in the lesser curvature of the corpus, serum pepsinogen status and *H. pylori* status (adjusted hazard ratio 4.88, 95% confidence interval [CI] 1.32–18.2, $P = 0.018$, Table 3). When we reduced the covariates to age and intestinal metaplasia in the lesser curvature of the corpus, which were significant predictors by univariate analysis, the independent significance of open-type, atrophic fundic gastritis, demonstrated by AFI, remained.

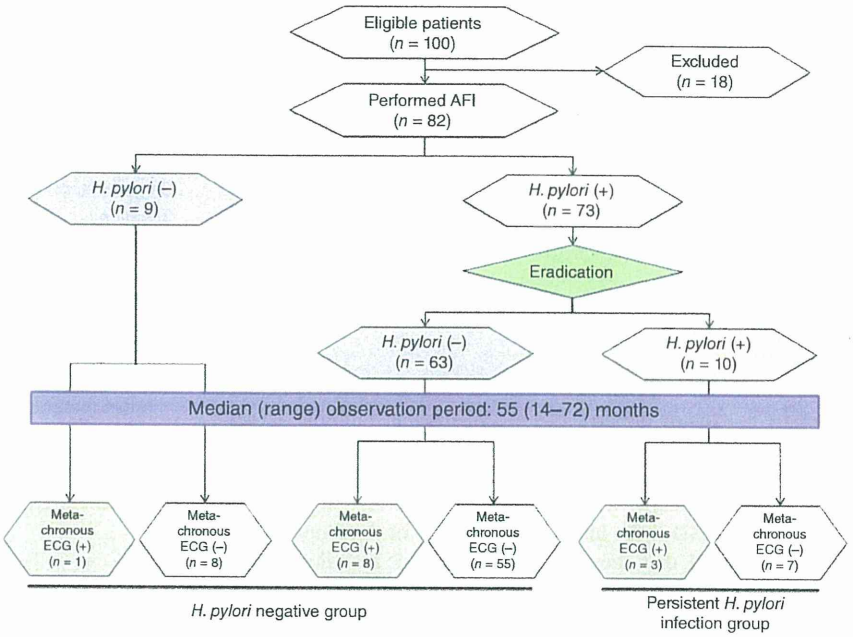

Figure 3 Flow diagram of the study.

Discussion

In the present study, we found that a considerable number of metachronous EGCs developed in patients who received ESD for EGC after successful eradication of *H. pylori*. This shows that early recognition and management of multiple EGCs is one of the keys to obtaining good prognosis after treatment of EGC with ESD. Moreover, a wide area of atrophic fundic gastritis diagnosed by AFI was an independent predictor for development of metachronous EGC.

Our previous study has shown that atrophy in biopsy specimens of the lesser curvature of the corpus is associated strongly with gastric cancer risk.[11] In the current study, intestinal metaplasia at the lesser curvature of the corpus was associated with development of metachronous EGC, by univariate analysis; however, it was not statistically significant in multivariate analysis after adjusting for the extent of atrophic fundic gastritis diagnosed by AFI. Biopsy evaluates only a narrow area in the whole gastric mucosa, therefore it cannot evaluate the actual extent or distribution of the gastric mucosal changes related to gastric carcinogenesis, such as mucosal atrophy or intestinal metaplasia, and it might cause sampling error. This suggests that the evaluation of gastritis by endoscopy is more suitable for the assessment of risk of development of metachronous EGC compared with point evaluation by biopsy.

To diagnose the extent of chronic atrophic fundic gastritis by endoscopy, we have developed the endoscopic Congo red test and have demonstrated that the method can identify patients at high risk for development of gastric cancer, in a long-term cohort study.[16,17] However, the endoscopic Congo red test requires gastrin injection to stimulate acid secretion, dye spraying over the entire gastric mucosa, and observation of the color change in the Congo red dye for several minutes. Therefore, it has not been used widely in clinical practice because of its complicated and time-consuming procedure, and potential adverse effects of drug or dye administration. AFI is a new endoscopic imaging technology that uses illumination of different wavelength light through a filter in a light source, and it can be performed subsequently to white light endoscopy by simply pressing a small button on the videoendoscope. The method demands neither drug injection nor dye spraying. AFI is more convenient and less invasive than chromoendoscopy, which might facilitate its implementation in routine examination.[18]

There is another noninvasive method to evaluate the risk of development of gastric cancer. The serum pepsinogen level is correlated with the extent of chronic, atrophic fundic gastritis and is used for screening of EGC that arises from atrophic gastric mucosa.[19–21] It detects the presence of atrophic gastritis and is therefore more applicable to intestinal-type gastric cancer that develops predominantly in post-ESD patients. A recent study has indicated that combination of *H. pylori* serology and pepsinogen level is good for predicting gastric cancer development.[22] It has been demonstrated that those who had a pepsinogen level that indicated atrophic gastritis had a significantly higher risk (6–8 times increase) of developing gastric cancer, during a mean observation period of 4.7 years, than those with a normal pepsinogen level who were negative for *H. pylori* antibody. However, because the serum pepsinogen level is altered by *H. pylori* eradication,[23] it cannot be used for patients who already have been treated by eradication therapy. Moreover, different cut-offs used in different studies might affect the sensitivity and specificity of the results.

Recently, the prophylactic effect of *H. pylori* eradication on the incidence of metachronous gastric cancer after endoscopic resection of EGC has been demonstrated in a randomized controlled trial.[10] It has been shown that the odds ratio (OR) for developing metachronous cancer was 0.353 in favor of *H. pylori* eradication. In the present study, 12 (14.6%) metachronous EGCs developed

Table 2 Clinicopathological factors in accordance with development of metachronous early gastric cancer (EGC)

	Metachronous EGC		*P*-value
	Developed ($n = 12$)	Not developed ($n = 70$)	
Sex			
Male	12	56	0.115
Female	0	14	
Mean age (years old [SD])	70.3 (5.1)	64.4 (8.7)	0.028
Alcohol drinking			
Yes	6	34	1.00
No	6	36	
Smoking			
Yes	8	28	1.00
No	4	42	
Extent of chronic atrophic fundic gastritis by AFI			
Open type	9	22	< 0.001
Closed type	3	48	
Pepsinogen I (ng/mL)			
≤ 22	4	26	0.849
> 22	7	40	
Not evaluated	1	4	
Pepsinogen I/II ratio			
≤ 1.8	5	30	0.940
> 1.8	6	36	
Not evaluated	1	4	
Helicobacter pylori status			
Negative or eradicated	9	63	0.159
Persistent infection	3	7	
Median (range) gastritis score			
Corpus lesser curvature			
Inflammation	2 (1–2)	2 (0–3)	0.238
Atrophy	3 (1–3)	3 (0–3)	0.294
Intestinal metaplasia	3 (1–3)	2 (0–3)	0.012
Corpus greater curvature			
Inflammation	2 (0–3)	2 (0–3)	0.221
Atrophy	1 (0–2)	1 (0–3)	0.464
Intestinal metaplasia	1 (0–2)	0 (0–2)	0.667
Antrum greater curvature			
Inflammation	2 (1–3)	2 (0–3)	0.860
Atrophy	2 (1–3)	2 (0–3)	0.242
Intestinal metaplasia	1 (0–3)	1 (0–3)	0.149

AFI, autofluorescence imaging.

during a mean follow up period of 55 months in patients who had undergone ESD for EGC after *H. pylori* eradication therapy. Although our study was not designed to compare incidence of metachronous EGC in patients who received eradication therapy with those who did not, we could not find any association between *H. pylori* status and development of metachronous EGC. We speculated that when severe atrophy or intestinal metaplasia has already developed widely, the effect of *H. pylori* eradication for reducing development of EGC is limited. This is in line with the results of a large randomized study[24] and other non-randomized studies.[11,25,26] They have suggested that *H. pylori* eradication is not beneficial in preventing cancer development in patients with pre-

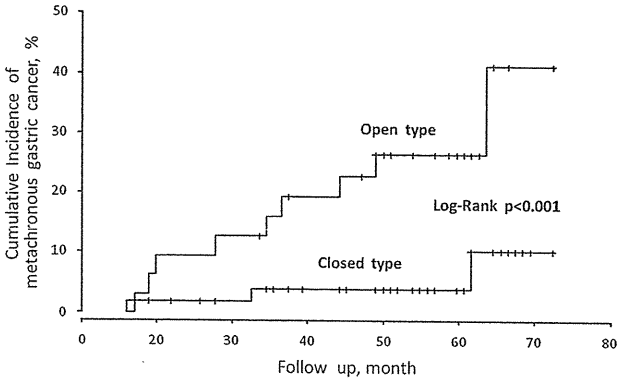

Figure 4 Kaplan–Meier analysis of metachronous gastric cancer development after endoscopic submucosal dissection (ESD) with respect to extent of atrophy diagnosed by autofluorescence imaging (AFI).

Table 3 Multivariate analysis for development of metachronous early gastric cancer (EGC)

Extent of green mucosa by AFI	Adjusted hazard ratio	95% CI	*P*-value
Closed type	1		
Open type	4.88	1.32–18.2	0.018

Adjusted for age, sex, intestinal metaplasia in corpus lesser curvature, serum pepsinogen level and *Helicobacter pylori* status. AFI, autofluorescence imaging; CI, confidence interval.

cancerous lesions such as atrophy, intestinal metaplasia or dysplasia. Taking this into consideration, we would like to emphasize that surveillance endoscopy for early detection of metachronous EGC is essential for management of ESD patients, even if they received eradication therapy for *H. pylori* because a considerable number of metachronous EGCs would still develop.

There are several limitations to consider in this study. First, the median observation period of this study was 55 months. Gastric cancer usually grows slowly, and it has been reported that the doubling time of EGC ranges from 2 to 10 years.[27] In this aspect, a longer observation period is needed to draw conclusions about the effect of *H. pylori* eradication on the development of new neoplasms. Second, we classified the extent of atrophic fundic gastritis into open and closed type in this study. Further studies are needed to determine cut-off levels that could identify patients at high risk for developing metachronous EGC, by AFI. Third, all metachronous EGCs were small intramucosal carcinomas that were classified as Category 4: mucosal high grade neoplasia in the revised Vienna classification.[14] There is a question as to whether EGC is a pseudo-cancer and not a truly lethal disease.[28] Therefore, whether detection of metachronous neoplasm would affect the prognosis of EGC patients who are treated by ESD warrants further investigation.

In conclusion, patients with extensive atrophic fundic gastritis diagnosed by AFI are at high risk for developing metachronous gastric cancer after ESD for EGC, even though they have achieved successful eradication of *H. pylori*. Scheduled surveillance endoscopy is strongly endorsed in such patients.

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Endoscopic Doppler US for the prevention of ulcer bleeding after endoscopic submucosal dissection for early gastric cancer: a preliminary study (with video)

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Background: After endoscopic submucosal dissection (ESD) for early gastric cancer (EGC), delayed bleeding occurs in 1.7% to 38% of cases. Routine coagulation of all nonbleeding visible vessels (NBVVs) in post-ESD ulcers is currently performed as standard practice, but it cannot eliminate bleeding. An endoscopic Doppler US (DOP-US) probe system has possible benefits for the prediction of recurrent bleeding in peptic ulcer hemorrhage.

Objective: To establish optimum use and evaluate feasibility of DOP-US for post-ESD ulcers.

Design: Case series study.

Setting: Cancer referral center.

Patients: Eight patients with mucosal EGC larger than 2 cm without ulceration or scarring and 2 patients with EGC less than 3 cm with scarring.

Interventions: We searched for a positive DOP-US signal (DOP-US+), which was defined as pulsatile sound at a depth of 1.5 mm, and NBVVs or areas with DOP-US+ were coagulated with hemostatic forceps. A multibending, double-channel videoendoscope that was fitted with a transparent hood was used.

Main Outcome Measurements: Detectability of DOP-US signals in post-ESD ulcers.

Results: One of 13 oozing bleeding sites, 24 (18%) of 136 NBVVs, and 7 areas without any bleeding stigmata had DOP-US+ and were coagulated until the signal became silent. One hundred twelve NBVVs (82%) and 8 adherent clots without DOP-US signals were left untreated. No delayed bleeding was experienced at 30 days. Median time required for Doppler examination was 34 minutes, but it improved to 18 and 19 minutes in patients 9 and 10, respectively.

Conclusions: DOP-US might be helpful in the endoscopic management of post-ESD ulcers in EGC. Our setting and maneuver warrant further investigation to clarify whether DOP-US can reduce delayed bleeding and avoid unnecessary coagulation for NBVVs in post-ESD ulcers.

Abbreviations: ESD, endoscopic mucosal dissection; EGC, early gastric cancer; NBVV, nonbleeding visible vessel; DOP-US, Doppler US; DOP-US−, negative Doppler US signal; DOP-US+, positive Doppler US signal.

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Endoscopic submucosal dissection (ESD) is indicated for early gastric cancer (EGC) in Japan. Delayed bleeding is one of the major complications of gastric ESD and occurs in 1.7% to 38% of patients.[1] Routine coagulation of all nonbleeding visible vessels (NBVVs) at the ulcer base decreases the rate of delayed hemorrhage[2] and is performed as standard practice. However, it cannot eliminate the bleeding, and there remain a few patients with severe bleeding who return to the emergency department after discharge.[3] Accordingly, we suspected that we might miss a nonvisible vessel that had the potential risk of hemorrhage; moreover, any preventive effect of drug therapy for delayed hemorrhage from arterial bleeding might be limited.

Endoscopic Doppler US (DOP-US) is a technique that provides information on the presence or absence of blood flow at the probe as sound from a transceiver placed at a preselected scanning depth beneath the mucosa.[4] Possible benefits of this system for the prediction of recurrent bleeding from NBVVs[5] of bleeding ulcers and for improvement of outcome in patients with peptic ulcer disease by intervention with endoscopic treatment of NBVVs[6,7] have been reported.

The aims of this preliminary evaluation were to establish optimum use of DOP-US for post-ESD ulcers and to evaluate whether it is feasible to investigate further its efficacy for the prevention of delayed bleeding after ESD for EGC.

Take-home Message

- Routine coagulation of all nonbleeding visible vessels (NBVVs) in ulcers after endoscopic submucosal dissection (ESD) for early gastric cancer (EGC) decreases the rate of delayed hemorrhage, but it does not eliminate the bleeding.
- An endoscopic Doppler US (DOP-US) probe system efficiently detected a positive signal in 1 of 13 oozing, bleeding ulcers, 24 of 136 NBVVs, and 7 areas with no bleeding stigmata. DOP-US–guided coagulation may reduce delayed bleeding and avoid unnecessary coagulation of NBVVs in post-ESD ulcers.

PATIENTS AND METHODS

Study sample

This was a case series study that was performed in the Endoscopy Unit of the Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan. We enrolled 8 patients with mucosal EGC that was larger than 2 cm without ulceration or scarring and 2 with EGC less than 3 cm with scarring who gave written informed consent and underwent ESD. Patients who were on anticoagulation therapy, with a history of gastrectomy or major organ failure, were excluded.

The study protocol was approved by the ethics committee at our center and registered at the University Hospital Medical Network Clinical Trials Registry (UMIN000002745).

DOP-US probe system

A 20-MHz pulsed-wave Doppler US unit (VTI Endoscopic Doppler System, Vascular Technology Inc, Nashua, NH) was used. It had 3 preset scanning depths: from the surface to 1.5, 4, and 7 mm and an audible Doppler signal output. The unit was portable, battery powered (8 AA-type batteries), measured 10.8 cm (depth) × 9.5 cm (width) × 13.3 cm (height), and weighed 0.68 kg. The single-use Doppler probe for EGD was 2.5 mm in diameter and 165 cm long (Fig. 1).[4]

Endoscopic procedure and treatment protocol

Just after completing the ESD procedure, the ulcer was examined carefully and treated by using the following protocol. All procedures were performed by the same endoscopist (N.U.). Before starting the Doppler examination, blood or mucous clots were washed by using a water jet from the videoendoscope. A DOP-US probe was inserted through an accessory channel and placed in contact with the ulcer base to search for a positive Doppler signal (DOP-US+). The maximum Doppler shift signal could be achieved at an angle of 45 degrees to the blood flow. The submucosal vessels run parallel, and the intramuscular artery penetrates perpendicular to the muscularis propria. Therefore, we attempted to apply the Doppler probe as near as possible to 45 degrees to the ulcer base. Once we detected a DOP-US+, the probe angle was varied to hear the maximum sound. A DOP-US+ was defined as a pulsatile sound at a shallow (1.5 mm) depth. For a large ulcer, the area was divided into left and right sides, and each side was examined at serial points in a zigzag line. The endoscopic findings of the ulcer were diagnosed and classified according to the stigmata of recent bleeding from peptic ulcers, ie, the Forrest classification,[8] and the presence or absence of a Doppler signal was documented in a dedicated schematic map. Electrical coagulation was performed for active bleeding sites and for areas with a DOP-US+ by using hemostatic forceps (FD-410LR; Olympus Medical Systems, Tokyo, Japan). An electrosurgical unit (Intelligent Cut and Coagulation 200; ERBE, Tübingen, Germany) was used in a soft coagulation mode of 80 W. Areas with bleeding stigmata that were negative for Doppler signaling were left untreated. The coagulated points were reinspected for a persistent signal, and coagulation was repeated until the DOP-US+ disappeared. Water jet function–equipped videoendoscopes (EVIS-Q260J and EVIS-H260Z; Olympus Medical Systems) were subsequently used for the Doppler examination in some cases. However, a multibending, double-channel videoendoscope (EVIS-2TQ260M; Olympus Medical Systems) that was fitted with a transparent hood (D-201-13404; Olympus Medical Systems) was used most often for the procedure.

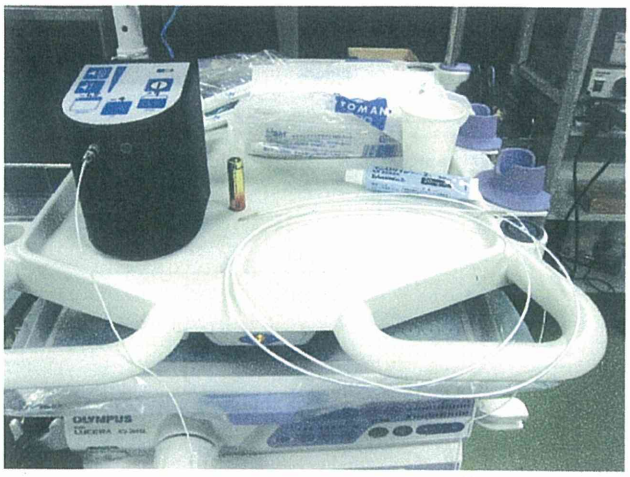

Figure 1. The DOP-US system consisted of a battery-powered portable transceiver and a disposable probe. It converted blood flow signals at the tip of the probe into sound output from a speaker.

Rabeprazole 20 mg (Eisai Co Ltd, Tokyo, Japan) was administered to all patients for 8 weeks.[3] Second-look endoscopy was not performed in any of the patients.

Outcome measures

The detectability of DOP-US signals in post-ESD ulcers was evaluated as the primary endpoint. Incidence of delayed bleeding, procedure time, and adverse events were assessed as secondary endpoints. Delayed bleeding was defined as hematemesis or melena that required endoscopic hemostasis and decreased hemoglobin count by more than 2 g/dL from day 2 until day 30. The procedure time was measured from the start of the Doppler examination until the end of the procedure and it included the time required to perform coagulation for areas with a DOP-US+ or active bleeding. Adverse events were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

RESULTS

Demographics of the patients with the lesions, blood vessel appearance, Doppler findings, and procedure data are provided in Table 1. A total of 13 oozing bleeding sites were observed in 10 patients and 1 had a DOP-US+. Conspicuous bleeding had already stopped during the ESD procedure; therefore, oozing bleeding just after ESD was minor. All sites were coagulated, bleeding was stopped, and the DOP-US+ became silent. Of 136 NBVVs, 24 (18%) were DOP-US+ and were coagulated. After coagulation (once at 19 sites, twice at 3 sites, and 3 times at 2 sites), all signals became silent. The remainder of the 112 NBVVs (82%) and 8 adherent clots were DOP-US− (Fig. 2); thus, they were left untreated. A DOP-US+ was heard at 7 areas that had no bleeding stigmata, and the areas were coagulated until the signals became silent (Fig. 3, Video 1). No delayed bleeding occurred in the 10 patients within 30 days. The median time required for performing the Doppler procedure was 34 minutes, but it improved with experience so that only 18 and 19 minutes were required in patients 9 and 10, respectively. There were no procedure-related adverse events that were more severe than grade 3 in National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

DISCUSSION

In this study, the use of DOP-US changed endoscopic management of post-ESD ulcers in 10 patients. A total of 32 DOP-US+ areas in the 10 patients with post-ESD ulcers were treated under DOP-US guidance, and none of the 112 DOP-US negative (DOP-US−) NBVVs rebled without endoscopic treatment.

Among the 32 areas with DOP-US+, 7 had no endoscopic sign of bleeding stigmata. Such areas were located mostly at the periphery of the ulcers. In 2 of 7 areas that had a DOP-US+ but no endoscopic finding of bleeding stigmata, vessels were identified in the submucosa after washing out and watching the region carefully. In post-ESD ulcers, the submucosa in the central area was almost removed by ESD, whereas the submucosa in the peripheral area remained partially intact. We speculate that the vessels in the central area were already cut and coagulated during ESD, but the vessels in the peripheral area remained viable beneath the submucosa and were missed by conventional observation. Such nonvisible vessels could become a potential cause of delayed bleeding.

We found a DOP-US+ in 24 of 139 NBVVs (17%), but more than 80% of the NBVVs were DOP-US−, and there was no delayed bleeding, although they were left untreated. Post-ESD ulcers were fresh and were not coated with fibrinopurulent exudates, as are peptic ulcers; therefore, even small NBVVs could be observed on the ulcer base. However, routine coagulation of all NBVVs is currently recommended at the end of ESD, even if there is no evidence of bleeding. Taking all of this into consideration, we might be overtreating many NBVVs in post-ESD ulcers that have no risk of delayed bleeding. In patient 10, an 8-cm specimen was removed for a 6-cm tumor. Although none of the 23 NBVVs in large ulcers was treated because they did not have a DOP-US+, no delayed bleeding occurred. Wong[9] suggested that if active blood flow in bleeding lesions has ceased, for example, as a result of spontaneous intravascular thrombosis, the risk of recurrent bleeding is reduced. We observed a few NBVVs that appeared as thick pulsating vessel stumps, which were not treated because there was no Doppler signal; however, delayed bleeding did not occur from these vessels. We suspect that such vessels would be spontaneously organized and disappear after a while. Repeated electrical coagulation was required for persistent signals in 3 DOP-US+ NBVVs. Confirmation of the disappearance of the

TABLE 1. Summary of results in this study

	Tumor				Vessel appearance and Doppler signal						
Patient	Location	Type	UI	Size (mm)	Oozing bleeding	Nonbleeding visible vessel	Adherent clot	Area without stigmata of recent bleeding	Endoscope	Transparent hood	Procedure time (min)
1	M, LC	0IIa	–	25	○	●○○○○○○		●	GIF-Q260J	+	37
2	L, PW	0IIc	+	10		●○○○○○○	○○○○		GIF-Q260J	+	33
3	M, LC	0IIc	–	35	○○○○	●●●●○○○○○○ ○○○○	○○		GIF-2TQ260M	+	31
4	L, LC	0IIc	–	20		●●●○○○○○○ ○○○	○		GIF-H260Z	+	42
5	M, LC	0IIc	+	25	●○○○	○○○○○○○○○ ○○○○	○	●	GIF-Q260J	+	49
6	M, LC	0IIa	–	40	○	●●●●●●●●○ ○○○○○○○○○ ○○○			GIF-2TQ260M	+	39
7	L, GC	0IIa	–	40	○○	●○○○○○○○○ ○○○○○○○○		●	GIF-2TQ260M	+	26
8	L, PW	0IIc	–	22	○	●●●●●○○○○		●●	GIF-2TQ260M	+	34
9	U, PW	0IIa	–	35		●○○○○○○○○ ○○○○○		●●	GIF-2TQ260M	+	18
10	M, LC	0IIa	–	57		○○○○○○○○○ ○○○○○○○○○ ○○○○○			GIF-2TQ260M	+	19

AW, Anterior wall; *GC*, greater curvature; *L*, lower third; *LC*, lesser curvature; *M*, middle third; *PW*, posterior wall; *U*, upper third; *UI*, ulcer scar; ●, positive Doppler US signal; ○, negative Doppler US signal.

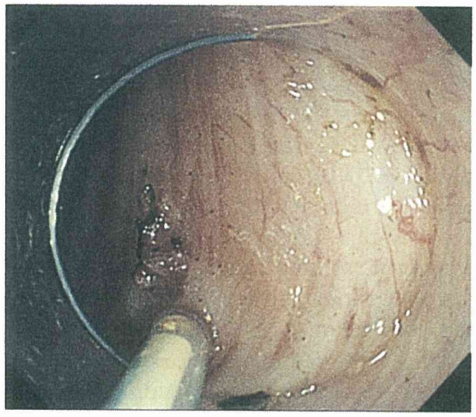

Figure 2. The NBVV was observed in the center of the post-ESD ulcer, and because there was no Doppler signal, the vessel was not treated.

blood flow signal by electrical coagulation with DOP-US might also contribute to risk reduction for delayed bleeding. We also expect that the use of DOP-US at the time of the first ESD procedure significantly reduces the incidence of delayed bleeding; therefore, routine second-look endoscopy might not be necessary as standard practice.

Post-ESD ulcers were larger than ordinary peptic ulcers (in particular, we included lesions >2 cm); therefore, it required more than 30 minutes for Doppler examination at the beginning of the study. The transparent hood that is used commonly in ESD was a good accessory for the Doppler procedure because it could be fixed to the proximal mucosa to ensure that the probe was in stable contact with the ulcer base. The multibending, double-channel videoendoscope (EVIS-2TQ260M; Olympus Medical Systems) was also beneficial for the procedure. We searched for DOP-US signals with a probe that was inserted through 1 channel of the videoendoscope, and we coagulated the DOP-US+ area by a hemostatic forceps through another channel. When using an ordinary single-channel videoendoscope, we sometimes missed the DOP-US+ area during replacement of the Doppler probe with the hemostatic forceps. Moreover, the videoendoscope was equipped with a dedicated water-jet channel that was connected to an electrical pump, so that the blood or mucous clots on the ulcer could be flushed out without withdrawing the devices that occupied the working channels. Although the Doppler examination can be performed with a conventional videoendoscope, the multibending function of the videoscope facilitated approaching the ulcer that was located in an area that was difficult to reach by conventional endoscopy.[10]

In conclusion, we detected Doppler US signals efficiently in post-ESD ulcers. Our instrumental setting and maneuver warrant further investigation to clarify whether DOP-US can reduce delayed bleeding and avoid unnec-

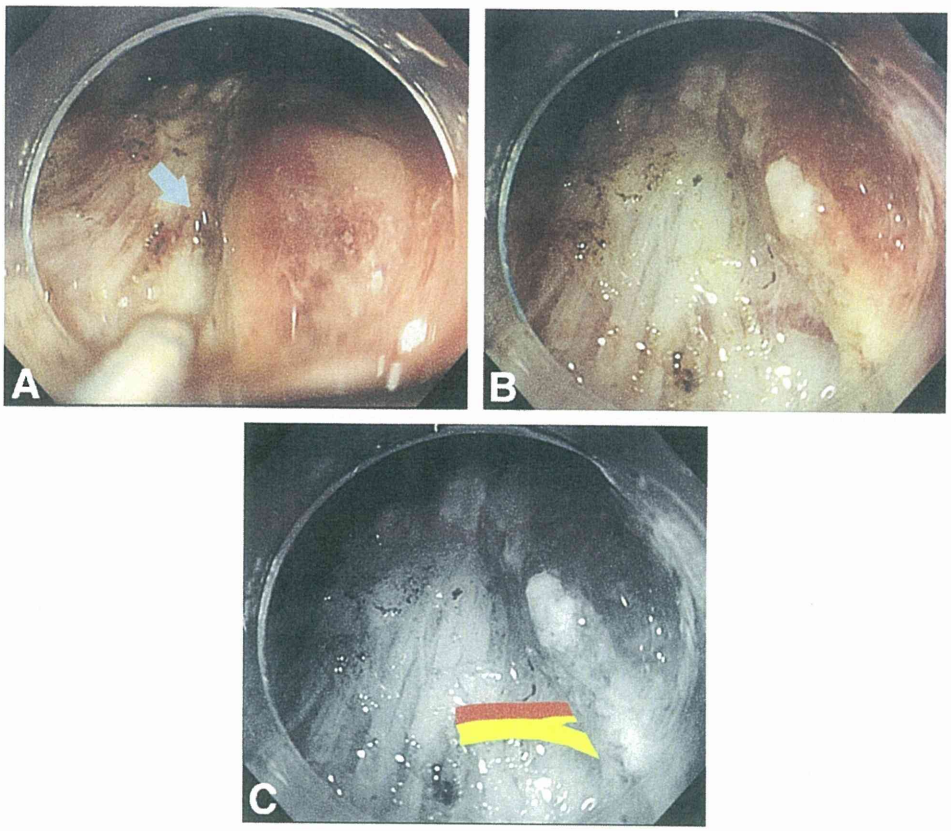

Figure 3. **A**, A DOP-US+ was detected at the periphery of the post-ESD ulcer, although the area had no obvious endoscopic appearance of bleeding stigmata (*arrow*). **B**, After washing the area with a water jet, the vessel beneath the submucosa became visible. **C**, The reddish vessel was more perceptible, but there was a strong pulsatile signal in an area adjacent to the reddish vein (yellow), which indicated the presence of an accompanying artery (red).

essary coagulation of NBVVs in post-ESD ulcers in patients treated for EGC.

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Helicobacter pylori Eradication Prevents Extension of Intestinalization Even in the High-Risk Group for Gastric Cancer

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Key Words
Helicobacter pylori eradication · Gastric cancer · Atrophic gastritis · CDX2

Abstract
Background/Aims: CDX2 is associated with the intestinal phenotype in the gastrointestinal tracts and is expressed in the intestinal type of gastric cancer. *Helicobacter pylori*-associated atrophic gastritis is characterized by aberrant expression of CDX2. The aim was to investigate the effects of eradication to the expression of genes related to the gastric and intestinal phenotype including *CDX2*. We compared the effect of eradication between the patients at high risk for gastric cancer and controls. ***Methods:*** 20 patients with endoscopic resection for early gastric cancer and 12 sex- and age-matched controls were studied. *CDX2* and mucin mRNA expressions were examined using whole biopsy specimens and microdissected gastric glands taken from corpus lesser and greater curves before and 1 year after eradication. ***Results:*** *CDX2* and *MUC2* expressions in the cancer group were significantly higher than in the controls and were significantly decreased after eradication. *MUC5AC* ($p = 0.01$) and *MUC6* ($p = 0.02$) expression significantly increased in the control group; the difference between the two groups became significant after eradication. *CDX2* expression in the glands without goblet cells was detectable and disappeared after eradication. ***Conclusion:*** *H. pylori* eradication can reverse gastric phenotype and diminish aberrant *CDX2* expression in the early stage of intestinalization.

Introduction

The extent and severity of *Helicobacter pylori*-induced atrophic gastritis, particularly corpus atrophy, is the most important risk factor for the development of gastric cancer [1–3]. Although recent papers have reported a prophylactic effect of *H. pylori* eradication on the development of gastric cancer, the molecular pathogenesis of prevention remains unclear.

Mucins are heavily glycosylated proteins. Twelve core proteins for human mucins (MUC1, 2, 3, 4, 5AC, 5B, 6, 7, 8, 9, 11, 12) have been identified [4–7]. The normal gastric mucosa shows cell type-specific expression of MUC5AC

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and MUC6 and does not express MUC2. Both cancer and intestinal metaplasia (IM) are associated with alterations in the pattern of mucin expression; loss of expression of MUC5AC and increased mucin heterogeneity have been reported in gastric cancer and underexpression of MUC5AC and MUC6 along with de novo expression of MUC2 have been described in IM.

CDX proteins function as intestine-specific transcription factors and act as master regulators of intestinal development and differentiation [8–10]. In Cdx2-transgenic mice, aberrant Cdx2 expression induces IM and plays a significant role in the genesis and progression of gastric carcinoma [11]. In particular, loss of CDX2 expression leads to focal gastric differentiation in the colon [12], while aberrant expression of CDX2 in the upper gastrointestinal tract is a key event in the pathogenesis of Barrett's mucosa in the esophagus and of IM in the stomach [13, 14]. CDX2 is expressed in adenocarcinomas from various organs such as the stomach, colon and pancreas, and may be clinically useful in predicting the outcome of patients with advanced cancer [15–19]. Here we investigated *CDX2* mRNA expression in metaplastic and non-metaplastic gastric glands among patients with early intestinal-type gastric cancer compared with control subjects using fixed-point biopsy samples. We also assessed the effects of *H. pylori* eradication on gastric and intestinal phenotypes and *CDX2* expression.

Methods

This was a case-control study of patients before and after endoscopic submucosal dissection (ESD) for early gastric cancer and non-cancer controls. Patients were enrolled for the study between October 2006 and April 2008.

Patients

Patients with ESD for early stage, non-cardia intestinal-type gastric cancer without lymph node metastasis were enrolled as the high-risk group for gastric cancer. The control group consisted of subjects who had been previously diagnosed as *H. pylori*-positive gastric ulcer or atrophic gastritis. Exclusion criteria included prior *H. pylori* eradication, use of anti-secretory or non-steroidal anti-inflammatory drugs (NSAIDs), hemorrhagic diseases, insulin-dependent diabetes mellitus, cirrhosis, or renal failure. Demographic data collected at study entry included age, gender, smoking habit, alcohol consumption and drug treatments. Drinking and smoking were defined as 'regular' when consumption was >35 g for ethanol or 5 cigarettes per day, respectively. The study was approved by the Osaka Medical Center for Cancer and Cardiovascular Diseases Ethical Committee and Kawasaki Medical School Ethical Committee, and written informed consent was obtained from each patient.

Biopsy Samples

Endoscopies were performed by experienced endoscopists after patients had fasted for 12 h. Two specimens from each sample site, the greater and lesser curves of the corpus, were taken using the endoscopic forceps (FB231K(A); Olympus, Tokyo, Japan) under the endoscopic examinations before and 1 year after eradication; one was used as a whole sample and the other for laser-captured microdissection. The biopsy samples were immediately frozen with liquid nitrogen and stored at −80°C until use.

Laser-Captured Microdissection

The frozen samples obtained at endoscopy were embedded in optimal cutting temperature compound (Sakura Finetek USA, Inc., Torrance, Calif., USA) and cut into serial 8-μm sections. Before microdissection, up to 8 sections from each block were mounted on slides and one slide was stained with Alcian blue and the others were stained using HistGene LCM Frozen Section Staining Kit (Arcturus Bioscience, Mountain View, Calif., USA). The cryostat sections were laser-microdissected with a PixCell II laser-microdissection system (Arcturus Engineering, Mountain View, Calif., USA). Both glands with goblets cells (metaplastic glands) and without (non-metaplastic glands) were isolated. Goblet cells were identified by their characteristic shape with an expanded apical portion and a nucleus at the base of it referring to the slide stained with Alcian blue.

RNA Extraction and Quantitative Polymerase Chain Reaction

Total RNA was extracted from whole biopsy samples using RNeasy Mini kit (Qiagen, Hilden, Germany) and microdissected tissue using a Pico Pure RNA Isolation kit (Arcturus Bioscience). The cDNA syntheses were performed using SuperScriptIII First Strand Synthesis System (Invitrogen, Carlsbad, Calif., USA). Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of *CDX2, MUC5AC, MUC6, MUC2* and *β-actin* mRNA expression was performed using the TaqMan inventoried primers of each gene (Applied Biosystems, Foster City, Calif., USA) and 7500 real-time PCR System (Applied Biosystems) employing TaqMan gene expression assay according to the manufacturer's instruction (Applied Biosystems). Real-time PCR was performed with cDNA for both target genes and the endogenous control using TaqMan Universal PCR Master Mix (Applied Biosystems). Each amplification reaction was performed in triplicate and the average of the threshold cycles was used. The amount of target was obtained by normalization to an endogenous reference (β-actin) and relative to a calibrator. If the microdissection materials contained too small targets for non-metaplastic or metaplastic glands or the average of the threshold cycles for β-actin was >33, the samples were excluded.

Immunohistochemistry

Immunohistochemical staining for CDX2 and mucins was performed using the Vectastain ABC-AP kit (Vector Laboratories, Burlingame, Calif., USA) using previously described methods [20–22]. Four adjacent sections 6-μm thick were cut onto polylysine-coated glass slides. Sections were incubated with mouse monoclonal Cdx2 antibody (1:500, BioGenex, San Ramon, Calif., USA) overnight at 4°C and with diluted primary mouse monoclonal antibodies against MUC2, MUC5AC, and MUC6 (Novocastra Laboratories, Newcastle, UK) at a dilution of 1:200, 1:500, and 1:500, respectively at room temperature for 1 h.

Diagnosis of H. pylori

Venous blood samples were analyzed for specific IgG *H. pylori* antibodies with an enzyme-linked immunosorbent assay (ELISA) kit using the E plate test (Eiken Kagaku, Inc., Tokyo, Japan). Patients were considered to be infected with *H. pylori* if the serum test was positive combined with evidence of chronic gastritis or atrophy with *H. pylori* on histopathological examination. Eradication was confirmed by negative histological examination of gastric biopsies, together with a negative ^{13}C-urea breath test (^{13}C-UBT) following the completion of eradication therapy for 6–8 weeks.

Eradication of H. pylori

Patients were treated with a 7-day regimen consisting of amoxicillin (500 mg tid), clarithromycin (200 mg tid) and a proton pump inhibitor twice daily (total dose = omeprazole 40 mg, lansoprazole 60 mg, or rabeprazole 20 mg), which was the standard approved first-line regimen in Japan. The patients with unsuccessful eradication were retreated with the regimen of changing clarithromycin to metronidazole (250 mg tid).

Statistical Analyses

Values are expressed as the mean ± SD or the median with a 25–75% range, whichever was appropriate depending on whether the data were normally distributed. Mantel-Haenszel χ^2 analysis and the unpaired t test were performed to measure differences in demographic and clinical characteristics. Statistical analyses for significant differences of parameters were performed using the non-parametric Mann-Whitney U test between the two groups and the Wilcoxon signed rank test for paired data. A two-sided p value <0.05 was considered statistically significant. All statistical computations were performed using SPSS (SPSS, Inc., Chicago, Ill., USA).

Results

Twenty-five *H. pylori*-positive patients with ESD for early gastric cancer (cancer group) and 25 controls who were *H. pylori*-positive and matched to the cancer group by gender and age were enrolled in the study. Three patients in the cancer group and 8 patients in the control group were lost to follow-up. Eradication failure occurred in 6 patients in the cancer group and 7 in the control group; 4 cancer patients and 3 controls of these were successfully retreated with the second regimen. One control patient was excluded to match to the cancer group by gender and age. The final study groups consisted of 20 *H. pylori*-positive patients with ESD for early gastric cancer (cancer group) and 12 *H. pylori*-positive controls matched to the cancer group by gender and age. Demographic and clinical characteristics of the study groups are shown in table 1. The locations of the early gastric cancers were: 7 in the lower stomach, 7 in the middle stomach, and 4 in the upper stomach. The remaining 2 patients had malignant lesions located in two different locations.

Table 1. Demographic and clinical characteristics of the patients undergoing eradication

	Controls (n = 12)	Cancer (n = 20)	p values
Age, mean (SD)	65.2 (6.4)	65.7 (9.1)	0.85[a]
Male/female	4/8	6/14	1.0[b]
Current smokers	0 (0%)	4 (20%)	0.27[b]
Regular alcohol intake	5 (41.7%)	10 (50%)	0.73[b]

p values calculated using [a] unpaired t test, [b] Mantel-Haenszel χ^2 analysis.

Whole Biopsy Samples

Using the entire biopsy samples, the *CDX2* mRNA levels positively correlated with *MUC2* mRNA levels ($r = 0.86$, $p < 0.001$). *CDX2* and *MUC2* expression in the cancer group were significantly higher than those in the controls (median *CDX2* 0.75×10^{-2} vs. 0.1×10^{-2}; *MUC2* 0.32×10^{-1} vs. 0.02×10^{-1}, $p = 0.01$). Eradication was associated with a significant decrease in expression of *CDX2* ($p = 0.009$) and *MUC2* ($p = 0.04$) in the cancer group thus reducing the difference between the two groups (fig. 1). *MUC5AC* ($p = 0.01$) and *MUC6* ($p = 0.02$) expressions were significantly increased only in the control group (median *MUC5AC* 4.1–10; *MUC6* 0.57–0.9) and were significantly higher in the controls than those in the cancer group after eradication (fig. 2).

Microdissected Gastric Glands

Using the microdissected gastric glands, expressions of *CDX2* and *MUC2* were significantly higher and *MUC5AC* and *MUC6* expressions were lower in the metaplastic glands than in the non-metaplastic glands both before and after eradication in the total subjects (table 2).

MUC2 expression in the metaplastic glands was significantly lower ($p = 0.03$) in the cancer group than in the controls. However, there was no significant difference in the other genes between the two groups before and after eradication (table 3). *CDX2* expression in the non-metaplastic glands was detectable and disappeared after eradication in each group (table 3; fig. 3).The other genes in the non-metaplastic or metaplastic glands and *CDX2* in the metaplastic glands expressed without significantly change after eradication.

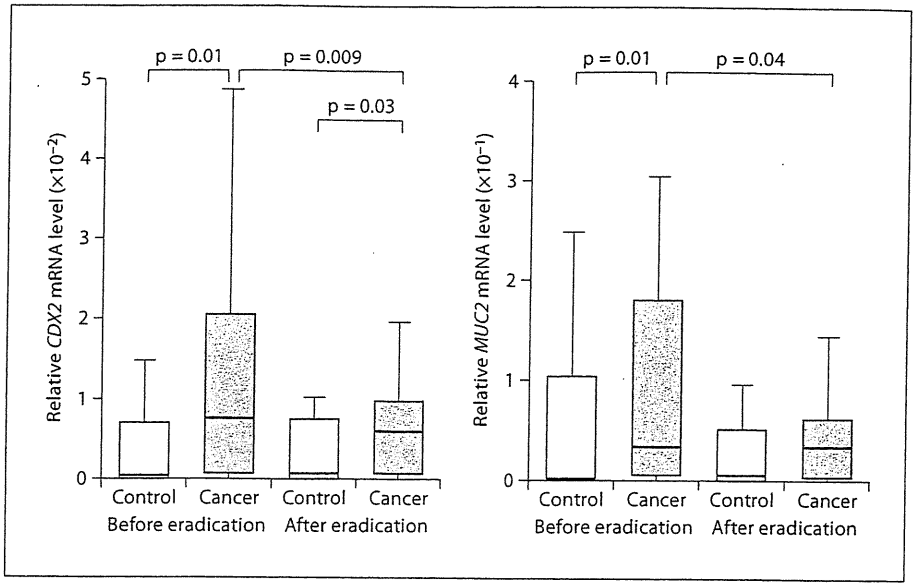


Fig. 1. Comparisons of *CDX2* and *MUC2* expressions between the control group and the cancer group before and after eradication. Horizontal bar = median; box = 25th–75th interquartile range; vertical lines = range of values. mRNA levels are expressed relative to the control gene β-actin. p values were calculated using the non-parametric Mann-Whitney U test between the two groups and the Wilcoxon signed rank test for paired data.

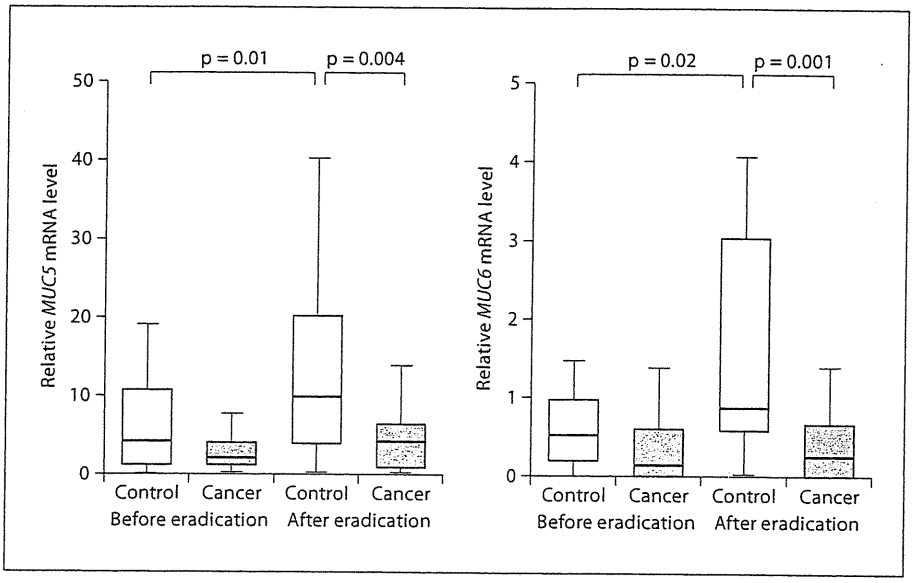


Fig. 2. Comparisons of *MUC5AC* and *MUC6* expressions between the control group and the cancer group before and after eradication. Horizontal bar = median; box = 25th–75th interquartile range; vertical lines = range of values. mRNA levels are expressed relative to the control gene β-actin. p values were calculated using the non-parametric Mann-Whitney U test between the two groups and the Wilcoxon signed rank test for paired data.

Immunohistochemical Staining

In the non-metaplastic glands, MUC5AC expression was detected in foveolar epithelium and mucous neck cells and MUC6 expression was detected in the mucous cells of the neck zone. MUC2 expression displayed a vacuolar staining in the goblet cells. CDX2 as well as MUC2 was detected in the metaplastic glands and was at the adjacent small area in some cancer patients. CDX2 protein expression as well as mucins were associated with mRNA levels, and *H. pylori* eradication decreased CDX2 and MUC2 expression and increased MUC5AC and MUC 6 expressions like as mRNA levels (fig. 4).

Discussion

MUC2 and CDX2 expressions were significantly higher in the fundic regions of gastric mucosa of the cancer patients compared to the non-cancer controls. This difference primarily reflected the higher proportion of glands with IM in the cancer patients compared to the *H. pylori*-infected controls. Interestingly, eradication increased *MUC5AC* and *MUC6* mRNA levels only in the controls. In addition, the difference between those gastric phenotypic genes expression in the two groups also became significant, and the results of immunohistochemi-

Table 2. Comparisons of mRNA levels in the non-metaplastic and metaplastic glands before and after eradication

	Non-metaplastic glands	Metaplastic glands	p values
Before			
CDX2	0 (0–18)	50 (30–120)	<0.00001
MUC2	0 (0–25)	650 (420–970)	<0.00001
MUC5AC	11,080 (4,360–19,595)	840 (50–4,270)	<0.00001
MUC6	70 (10–210)	0 (0–50)	0.01
After			
CDX2	0	20 (10–120)	<0.00001
MUC2	0	335 (130–1,440)	<0.00001
MUC5AC	12,160 (6,490–33,728)	445 (220–1,755)	<0.00001
MUC6	90 (45–365)	0 (0–45)	<0.00001

Values are expressed relative to the control gene β-actin as the median with a 25–75% range. p values were calculated using the non-parametric Mann-Whitney U test.

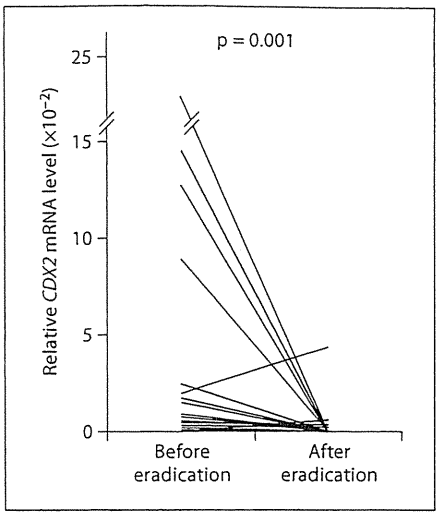


Fig. 3. *CDX2* expression in the non-metaplastic glands of the total subjects before and after eradication. mRNA levels are expressed relative to the control gene β-actin. p values were calculated using the Wilcoxon signed rank test for paired data.

cal stainings were associated with mRNA levels. These results confirm and extend our previous results using immunostainings [20–22]. In our previous study, residual corpus gastritis after eradication was more frequently detected in the cancer group than in the controls and in the mucosa with incomplete IM than in those without incomplete IM. Ravizza [23] reported that complete IM in the antrum regressed 2 or 3 years after eradication, however, incomplete IM remained unchanged. These results suggest that eradication therapy prior to development of incomplete IM is the best strategy to heal the gastritis and to regain the gastric phenotype which should also minimize gastric cancer risk.

Here we show that *CDX2* expression in the non-metaplastic glands was detectable and disappeared after eradication. We isolated the glands with goblets cells (metaplastic glands) and without (non-metaplastic glands) identified by Alcian blue staining. It is possible that adjacent glands without goblet cells are part of metaplastic glands which are possibly incomplete metaplasia glands. Gastric glands without goblet cells expressing CDX2 possibly represent the extension of intestinalization of gastric glands and is consistent with prior clinical studies using immunostaining [24–26]. CDX2 expression was detectable in gastric mucosa infected with *H. pylori* and without obvious metaplastic glands [24, 25], and a small amount of CDX2 expression has also been described in isolated gastric-type glands [24, 25]. These results are consistent with the notion that aberrant *CDX2* expression may be a key event in the initiation of intestinalization of gastric glands. It remains to be determined whether non-metaplastic glands expressing CDX2 are involved in carcinogenesis or are simply markers for an increased risk of developing gastric cancer.

Wong et al. [27] reported a beneficial effect from eradication only in the subgroup of patients without atrophy, IM or dysplasia, suggesting that the benefit of *H. pylori* eradication diminishes once atrophy or IM are present. In contrast, a Japanese study group recently reported a marked decrease in cancer risk following eradication. Their trial included 544 patients with endoscopic treatment for early gastric cancer, and the hazard ratio for metachronous gastric cancer was 0.339 (95% CI 0.157–0.729; p = 0.003) at 3-year follow-up [28]. We selected the patients with recent history of ESD for early gastric cancer as the high-risk group for gastric cancer. We showed that *CDX2* aberrant expression at the metaplastic glands without goblet cells disappeared after eradication even in the cancer group. We speculated that eradication may prevent extension of intestinalization even in the high-risk group for gastric cancer. It is reported that CDX2 expression and intestinal trans-differentiation in the gastric mucosa can be suppressed by an increase in acid secretion that often follows *H. pylori* eradication presumably due to removal of the postulated acid inhibitory factors [29–32]. Further studies are required to elucidate the

Table 3. Comparisons of mRNA levels in the non-metaplastic and metaplastic glands between the control group and the cancer group before and after eradication

		Control	Cancer	p values*
Non-metaplastic glands				
Number of samples[1]	before	10	24	
	after	13	31	
CDX2	before	0 (0–10)	10 (0–20)	0.32
	after	0	0	0.54
	p values	0.02	0.01	
MUC2	before	0 (0–35)	0 (0–38)	
	after	0	0	0.96
	p values	0.40	0.11	0.67
MUC5AC	before	6,390 (3,545–27,780)	15,530 (9,415–28,650)	0.51
	after	16,260 (6,440–42,490)	11,745 (6,490–34,570)	
	p values	0.40	0.43	0.48
MUC6	before	150 (40–550)	60 (3–208)	0.22
	after	110 (40–600)	90 (40–320)	0.95
	p values	0.40	0.19	
Metaplastic glands				
Number of samples[1]	before	5	19	
	after	5	15	
CDX2	before	110 (60–415)	40 (20–123)	0.23
	after	20 (20–140)	20 (10–120)	0.56
	p values	0.07	0.28	
MUC2	before	1,150 (750–5,190)	510 (180–855)	0.03
	after	390 (140–1,620)	280 (150–1,425)	0.59
	p values	0.14	0.20	
MUC5AC	before	1,170 (0–5,510)	840 (95–3,898)	0.66
	after	200 (100–670)	470 (330–8,890)	0.94
	p values	0.27	0.35	
MUC6	before	0 (0–105)	10 (0–105)	0.23
	after	20 (0–160)	0 (0–25)	0.11
	p values	0.47	0.13	

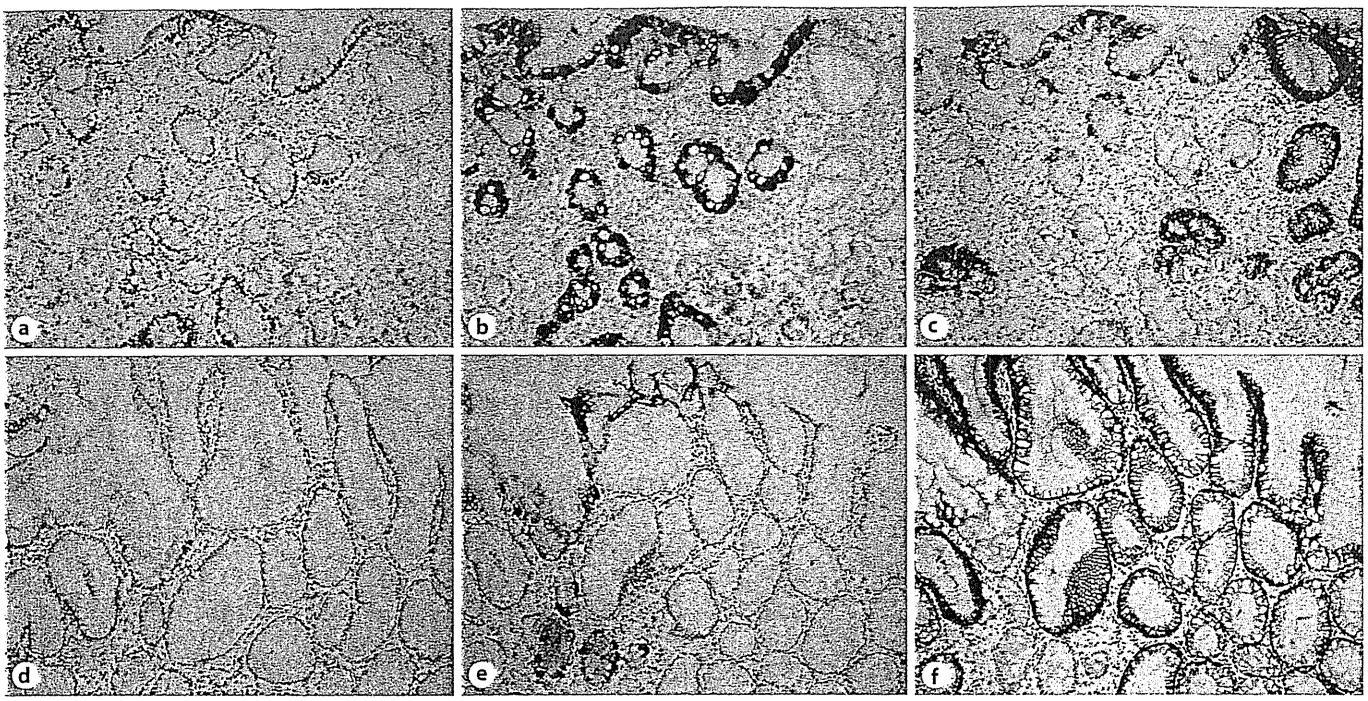
Values are expressed relative ($\times 10^{-3}$) to the control gene β-actin as the median with a 25–75% range.

* p values for the comparisons between the cancer group and the controls were calculated using the non-parametric Mann-Whitney U test. p values for the comparisons between before and after eradication were calculated using the Wilcoxon signed rank test.

[1] Number of samples corrected by laser capture containing enough non-metaplastic or metaplastic glands in the cancer group and the controls. The samples contained too small targets for non-metaplastic or metaplastic glands were excluded.

molecular mechanisms how eradication suppress aberrant *CDX2* expression.

MUC2 and CDX2 were repressed in the metaplastic glands isolated from patients who had undergone mucosal resection of early gastric cancer compared to the controls. Tsukamoto et al. [25] analyzed *MUC2* and *CDX2* mRNA levels in isolated gastric glands from surgically resected antral mucosa and demonstrated that *MUC2* and *CDX2* expressions were progressively upregulated with intestinalization from the gastric type to the gastric/intestinal-mixed type to the intestinal type. In our previous immunohistochemical studies, there is a significant association between types of IM and atrophic scores or serum pepsinogen levels [33]. The most incomplete IM (types II and III) preserving gastric mucin was the gastric and intestinal mixed (GI) type, whereas the complete type expressing MUC2 and CD10 was the intestinal (I) type. Incomplete or gastric/intestinal-mixed type IM was detectable in the mucosa of gastric cancer patients significantly more frequently (58 vs. 38%, $p < 0.001$) than in the controls [33].



Color version available online

Fig. 4. CDX2 (**a**, **d**), MUC2 (**b**, **e**) and MUC5AC (**c**, **f**) immunohistochemical staining of serial sections of the patient with gastric cancer in the corpus greater curve before (**a–c**) and after eradication (**d–f**). Orig. magnif. ×40.

Moreover, CDX2 expression increased in patients in the ascending order of those without IM, those with complete IM and those with incomplete IM ($p < 0.001$) [34]. Although both *MUC5AC* and *MUC6* gene expressions were not significantly different between the two groups, the lower levels of *MUC2* and *CDX2* mRNA in the cancer group of the present study may reflect a higher proportion of those with gastric/intestinal-mixed type IM.

In summary, we first indicated that *CDX2* aberrant expression was detected at the gastric glands without goblet cells in the corpus and disappeared after *H. pylori* eradication. *H. pylori* eradication reversed the gastric phenotype only in the control group. We propose that *CDX2* expression is a feature denoting the early phases of intestinalization of gastric glands and eradication may prevent extension of intestinalization even in the high-risk group for gastric cancer. Depending on the extent and severity of corpus gastritis/atrophy, *H. pylori* eradication can reverse corpus atrophy and have the greatest benefit in reducing gastric cancer risk.

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SURGERY TODAY

Original Article

Survival of Patients Treated by an Autonomic Nerve-Preserving Gastrectomy for Early Gastric Cancer

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Abstract

Purpose. Autonomic nerve preservation in a gastrectomy for gastric cancer improves the postoperative quality of life. We retrospectively examined the survival of patients treated by an autonomic nerve-preserving gastrectomy in comparison to the survival of the patients treated by a conventional gastrectomy.

Methods. The survival of 385 patients treated by an autonomic nerve-preserving gastrectomy for clinical early gastric cancer (the ANP group) was compared with that of 285 patients treated by a conventional gastrectomy (non-ANP group).

Results. Among the ANP group, the numbers of patients with tumor invasion to the mucosa, submucosa, and muscularis propria were 210, 166, and 9, respectively, whereas the numbers of patients with lymph node metastasis grades of N0, N1, and N2 were 360, 21, and 4, respectively. The overall 5-year survival rate of the ANP group was 94.7%, which was superior to that of the non-ANP group (90.4%; $P = 0.003$). The 5-year survival rates of patients with lymph node metastasis were 94.9% and 91.8% in the ANP and non-ANP groups, respectively ($P = 0.733$). Only 3 patients in the ANP group died from gastric cancer.

Conclusions. The survival of patients treated by an autonomic nerve-preserving gastrectomy was equivalent to that of patients treated by a conventional gastrectomy, thus suggesting that an autonomic nerve-preserving gastrectomy could be a useful procedure for the treatment of early gastric cancer.

Key words Gastric cancer · Gastrectomy · Autonomic nerve preservation · Survival

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Introduction

Function-preserving surgery for early gastric cancer is now widely performed in Japan.[1] There are various types of function-preserving operations, including those involving a reduced extent of gastrectomy, autonomic nerve preservation, sphincter preservation, and formation of a neostomach.[2] Because the preservation of the vagus nerve has been demonstrated to improve the postoperative quality of life in patients who undergo either a vagotomy and/or gastrectomy,[3–14] we have performed an autonomic nerve-preserving gastrectomy for early gastric cancer since December 1994. Although autonomic nerve preservation has been considered to maintain the curability of patients,[15,16] the long-term survival rate after an autonomic nerve-preserving gastrectomy has not been fully assessed to date. We retrospectively examined the survival of patients after an autonomic nerve-preserving gastrectomy for early gastric cancer.

Patients and Methods

Between December 1994 and July 2003, 385 patients were treated by an autonomic nerve-preserving gastrectomy for clinical early gastric cancer at our institute (ANP group). The indications for this operation included tumor invasion into the mucosal or submucosal layer (T1), and the absence of lymph node involvement and distant metastases according to clinical and surgical findings (N0/M0). All patients underwent gastrointestinal fiberscopy, a gastrointestinal series, and computed tomography for the preoperative evaluation. If regional lymph node metastasis was suspected by the intraoperative findings, a frozen-section analysis of the lymph node was performed and the patients with positive nodes were excluded from the study. With regard to the specific procedures, a distal gastrectomy was