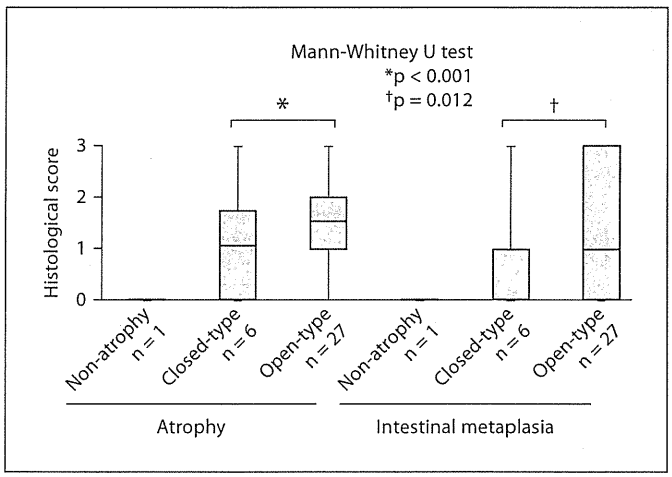

Fig. 3. Histological scores of atrophy and IM in patients who had *H. pylori*-negative gastric cancer. Mean scores of atrophy and IM in 5 sites of background gastric mucosa are shown. Box plots according to endoscopic atrophy show the 25th and 75th percentile with the lowest and highest values, respectively. Scores of atrophy and IM were significantly higher in the endoscopic open-type atrophy group.

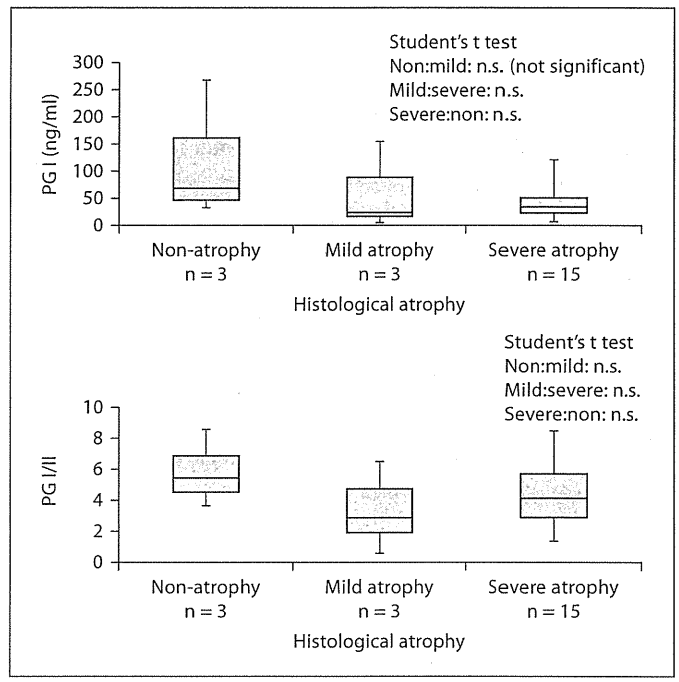

Fig. 4. Correlation between serum PG and histological atrophy in patients who had *H. pylori*-negative gastric cancer. PG I and PG I/II ratio are shown according to degrees of histological atrophy and IM. There were no significant differences in the values of PG I and PG I/II ratio. Horizontal bar: median; box: 25th–75th interquartile range; vertical lines: range of values.

Endoscopic Atrophy and Serological Atrophy of H. pylori*-Negative Gastric Cancer*

Although mild histological atrophy and severe histological atrophy were correlated with endoscopic atrophy, two-thirds of the patients with histological atrophy were negative for serological atrophy. Only 1 cancer in the 34 *H. pylori*-negative gastric cancers occurred in the gastric mucosa without histological atrophy, endoscopic atrophy or serological atrophy (fig. 2).

Mean histological scores of atrophy and IM according to endoscopic atrophy are shown in figure 3. The degrees of endoscopic atrophy were correlated with severity of histological atrophy and IM.

Excluding 13 patients who were using antacids, PG levels were measured in 21 patients of 34 *H. pylori*-negative patients. PG I and PG I/II levels according to histological atrophy and endoscopic atrophy are shown in figures 4 and 5, respectively. There were no significant differences in the values of PG I and PG I/II according to degrees of histological atrophy and endoscopic atrophy.

A Case of H. pylori*-Negative Gastric Cancer without Atrophy*

Figure 6 shows *H. pylori*-negative gastric cancer in a patient without histological, endoscopic or serological atrophy. The tumor was located in the posterior wall of the antrum and was depressed type (a), and the gastric mucosa was smooth with no endoscopically atrophic change (b, c, d). Pathological diagnosis of the tumor, which was completely resected by ESD, was intestinal-type cancer. PG I and II levels were 55.7 and 10.8 ng/ml, respectively, and there was no histological atrophy or IM in the background mucosa.

Discussion

Results of retrospective studies conducted in Japan have indicated that the incidence rate of *H. pylori*-negative gastric cancer is about 2–10% [13–15]. Recently, Yoon et al. [23] reported that the incidence rate was at least 5.4% among South Korean patients. However, advanced gastric cancers, in which the rate of *H. pylori* positivity was lower than that of early gastric cancer, were included into those studies [24, 25]. Therefore, to evaluate the frequency of *H. pylori*-negative gastric cancer, samples obtained from patients with early stage cancer would be more suitable.

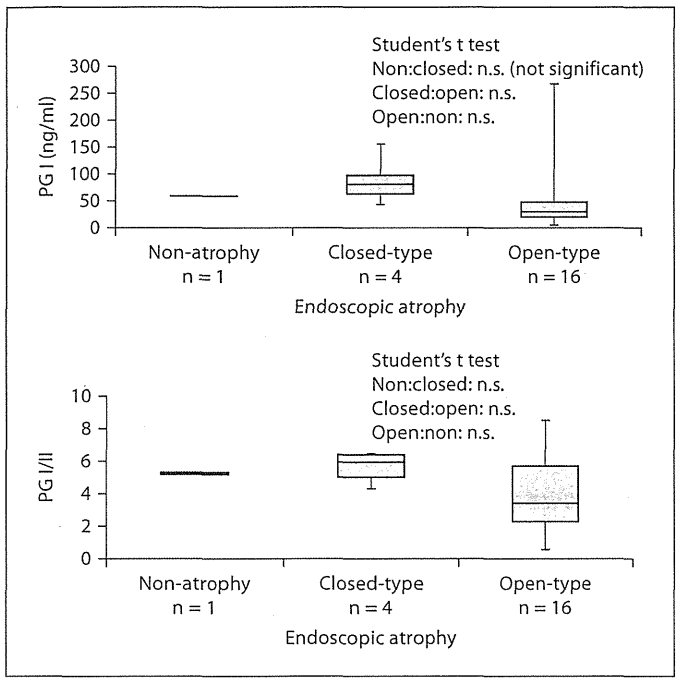

Fig. 5. Correlation between serum PG and endoscopic atrophy in patients who had *H. pylori*-negative gastric cancer. PG I and PG I/II ratio are shown according to endoscopic atrophy. There were no significant differences in the values of PG I and PG I/II ratio between closed-type atrophy and open-type atrophy. Horizontal bar: median; box: 25th–75th interquartile range; vertical lines: range of values.

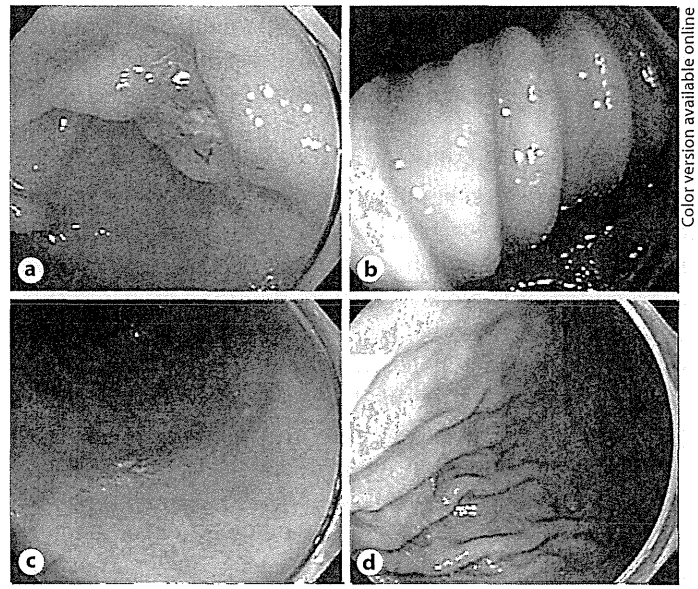

Fig. 6. A case of *H. pylori*-negative gastric cancer without atrophy. **a** A depressed lesion of 15 mm in size is observed in the posterior wall of the antrum. **b** A regular arrangement of collecting venules is seen in the lesser curvature of the angle. **c** Endoscopic atrophy is not seen in the lesser curvature of the corpus. **d** Gastric mucosa in the greater curvature of the corpus is smooth without rugal hypertrophy.

In this study, current or past infection with *H. pylori* was determined by five tests. Histological atrophy and IM, serological atrophy and endoscopic atrophy were analyzed to exclude patients with spontaneous elimination patients as much as possible. Finally, there was only 1 case (0.42%) of early gastric cancer that occurred in patients without current or past *H. pylori* infection and without atrophic mucosa, and the frequency was lower than that in previous studies [13–15, 23].

Recently, Matsuo et al. [26] reported that the frequency of *H. pylori*-negative gastric cancer in many samples of gastric cancer was 0.66%. In that study, *H. pylori*-negative gastric cancer was defined by (1) *H. pylori* antibody, (2) microscopic observation, (3) endoscopic atrophy, and (4) UBT or RUT. Although serological atrophy was not used for their analysis, their data and our data were similar in the low frequency of gastric cancer in patients without *H. pylori* infection and gastric atrophy.

Endoscopic atrophy is commonly used in Japan, but there is no worldwide consensus. The frequency of *H. pylori* infection in patients with atrophic gastritis according to the Kimura-Takemoto classification was 92.7% in Japan, and the severity of endoscopic gastric atrophy according to this classification could help to predict histological atrophy and IM in Vietnam [27, 28]. Recently, image-enhanced endoscopy technology has been developed, and narrow-band imaging with magnifying endoscopy and autofluorescence imaging endoscopy are used for staging atrophic changes in the gastric mucosa [29, 30]. In particular, autofluorescence imaging endoscopy may be useful for objective endoscopic evaluation of atrophic gastritis. Unfortunately, we used conventional endoscopy for evaluating endoscopic atrophy in this series, though our endoscopic atrophy was correlated with histological atrophy and IM.

Uemura et al. [31] reported that gastric cancer in the Japanese population developed only in persons infected with *H. pylori* and intestinal-type gastric cancer did not develop in persons with no or mild endoscopic atrophy. Autoimmune gastritis is very rare in Japan, a *H. pylori* infection might therefore be the most important factor of atrophic gastritis. Although 2 of our cases had diffused

endoscopic atrophy, there was no histological atrophy or serological atrophy. False-negative results of *H. pylori* tests or spontaneous disappearance is suggested in these cases.

Recently, the combination of a serum PG test and a *H. pylori* antibody test has been incorporated into the gastric cancer screening program in Japan [32]. Although the serum PG test might be more accurate and is less invasive than endoscopic biopsies, results obtained by using this test can be affected by age, sex, antacids and infection status of *H. pylori* [22]. *H. pylori* eradication decreases the values of serum PG I and II and increases PG I/II ratio [33]. If the patients in our ESD series had received this screening examination, at least 15 (6.3%) of the patients without a history of eradication would have been included in the lowest risk group of gastric cancer that includes patients who were negative for both *H. pylori* antibodies and PG tests. For reduction of false-negative results of this check-up program, further analysis of PG levels of *H. pylori*-negative gastric cancer is necessary.

Finally, our results must be interpreted in consideration of the following limitations: the sample size was very small and most of our subjects had intestinal-type gastric cancer resected by ESD. The present study should be considered as a preliminary study for countries in which *H. pylori* infection is common and the incidence of gastric cancer is high.

In summary, the prevalence of gastric cancer without current or past *H. pylori* infection and without gastric mucosal atrophy is very low in the Japanese ESD series.

Disclosure Statement

The authors have no conflicts of interest to disclose.

References

1 Nakamura K, Ueyama T, Yao T, Xuan ZX, Ambe K, Adachi Y, Yakeishi Y, Matsukuma A, Enjoji M: Pathology and prognosis of gastric carcinoma. Findings in 10,000 patients who underwent primary gastrectomy. Cancer 1992;70:1030–1037.

2 Gotoda T, Kondo H, Ono H, Saito Y, Yamaguchi H, Saito D, Yokota T: A new endoscopic mucosal resection procedure using an insulation-tipped electrosurgical knife for rectal flat lesions: report of two cases. Gastrointest Endosc 1999;50:560–563.

3 Oka S, Tanaka S, Kaneko I, Mouri R, Hirata M, Kawamura T, Yoshihara M, Chayama K: Indications for the use of endoscopic mucosal resection for early gastric cancer in Japan: a comparative study with endoscopic submucosal dissection. Gastrointest Endosc 2006;64:877–883.

4 Nasu J, Doi T, Endo H, Nishina T, Hirasaki S, Hyodo I: Characteristics of metachronous multiple early gastric cancers after endoscopic mucosal resection. Endoscopy 2005; 37:990–993.

5 Nakajima T, Oda I, Gotoda T, Hamanaka H, Eguchi T, Yokoi C, Saito D: Metachronous gastric cancers after endoscopic resection: how effective is annual endoscopic surveillance? Gastric Cancer 2006;9:93–98.

6 Fukase K, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, Terao S, Amagaki K, Hayashi S, Asaka M, Japan Gast Study Group: Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet 2008;372:392–397.

7 Maehata Y, Nakamura S, Fujisawa K, Esaki M, Moriyama T, Asano K, Fuyuno Y, Yamaguchi K, Egashira I, Kim H, Kanda M, Hirahashi M, Matsumoto T: Long-term effect of *Helicobacter pylori* eradication on the development of metachronous gastric cancer after endoscopic resection of early gastric cancer. Gastrointest Endosc 2012;75:39–46.

8 Asaka M, Kato M, Takahashi S, Fukuda Y, Sugiyama T, Ota H Uemura N, Murakami K, Satoh K, Sugano K, Japanese Society for Helicobacter Research: Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. Helicobacter 2010;15:1–20.

9 Fukuda S, Tanaka M, Soma Y, Shimoyama T, Mikami T, Crabtree JE, Saito H, Munakata A Yoshida Y: Histological analysis of gastritis and *Helicobacter pylori* infection in patients with early gastric cancer: a case-control study. J Gastroenterol Hepatol 2000;15: 1370–1376.

10 Kato M, Asaka M, Shimizu Y, Nobuta A, Takeda H, Sugiyama T: Relationship between *Helicobacter pylori* infection and the prevalence, site and histological type of gastric cancer. Aliment Pharmacol Ther 2004; 20(suppl 1):85–89.

11 Kokkola A, Kosunen TU, Puolakkainen P, Sipponen P, Harkonen M, Laxen F, Virtamo J, Haapiainen R, Rautelin H: Spontaneous disappearance of *Helicobacter pylori* antibodies in patients with advanced atrophic corpus gastritis. APMIS 2003;111:619–624.

12 Kang HY, Kim N, Park YS, Hwang JH, Kim JW, Jeong SH, Lee DH, Jung HC, Song IS: Progression of atrophic gastritis and intestinal metaplasia drives *Helicobacter pylori* out of the gastric mucosa. Dig Dis Sci 2006;51: 2310–2315.

13 Kushima R, Matsubara A, Kakinoki R, Takemura S, Baba M, Ishida K, Okabe H, Saito Y, Hattori T: Comparative histopathological study of *H. pylori*-positive and negative gastric carcinomas (in Japanese). Stomach Intest 2007;42:967–980.

14 Kakinoki R, Kushima R, Matsubara A, Saito Y, Okabe H, Fujiyama Y, Hattori T: Re-evaluation of histogenesis of gastric carcinomas: a comparative histopathological study between *Helicobacter pylori*-negative and *H. pylori*-positive cases. Dig Dis Sci 2009;54: 614–620.

15 Kato S, Matsukura N, Tsukada K, Matsuda N, Mizoshita T, Tsukamoto T, Tatematsu M, Sugisaki Y, Naito Z, Tajiri T: *Helicobacter pylori* infection-negative gastric cancer in Japanese hospital patients: incidence and pathological characteristics. Cancer Sci 2007;98: 790–794.

16 Nishikawa K, Sugiyama T, Kato M, Ishizuka J, Kagaya H, Hokari K, Asaka M: A prospective evaluation of new rapid urease tests before and after eradication treatment of *Helicobacter pylori*, in comparison with histology, culture and ^{13}C-urea breath test. Gastrointest Endosc 2000;51:164–168.

17 Ohara S, Kato M, Asaka M, Toyoda T: Studies of ^{13}C-urea breath test for diagnosis of *Helicobacter pylori* infection in Japan. J Gastroenterol 1998;33:6–13.

18 Dixon MF, Genta RM, Yardley JH, Correa P: Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161–1181.
19 Fujioka T, Tokieda M: Validity of serum anti-*Helicobacter pylori* antibody using immunoassay for the diagnosis in eradication of *Helicobacter pylori* (in Japanese). Jpn J Med Pharm Sci 2000;43:573–579.
20 Kimura K, Takemoto T: An endoscopic recognition of the atrophic border and its significance in chronic gastritis. Endoscopy 1969;3:87–97.
21 Miki K, Morita M, Sasajima M, Hoshina R, Kanda E, Urita Y: Usefulness of gastric cancer screening using the serum pepsinogen test method. Am J Gastroenterol 2003;98:735–739.
22 Miki K: Gastric cancer screening using the serum pepsinogen test method. Gastric Cancer 2006;9:245–253.
23 Yoon H, Kim N, Lee HS, Shin CM, Park YS, Lee DH, Jung HC, Song IS: *Helicobacter pylori*-negative gastric cancer in South Korea: incidence and clinicopathologic characteristics. Helicobacter 2011;16:382–388.
24 Klaamas K, Held M, Wadstrom T, Lipping A, Kurtenkov O: IgG immune response to *Helicobacter pylori* antigens in patients with gastric cancer as defined by ELISA and immunoblotting. Int J Cancer 1996;67:1–5.
25 Caruso ML, Fucci L: Histological identification of *Helicobacter pylori* in early and advanced gastric cancer. J Clin Gastroenterol 1990;12:601–602.
26 Matsuo T, Ito M, Takata S, Tanaka S, Yoshihara M, Chayama K: Low prevalence of *Helicobacter pylori*-negative gastric cancer among Japanese. Helicobacter 2011;16:415–419.
27 Mihara M, Haruma K, Kamada T, Komoto K, Yoshihara M, Sumii K, Kajiyama G: The role of endoscopic findings for the diagnosis of *Helicobacter pylori* infection: evaluation in a country with high prevalence of atrophic gastritis. Helicobacter 1999;4:40–48.
28 Quach DT, Le HM, Nguyen OT, Nguyen TS, Uemura N: The severity of endoscopic gastric atrophy could help to predict Operative Link on Gastritis Assessment gastritis stage. J Gastroenterol Hepatol 2011;26:281–285.
29 Tahara T, Shibata T, Nakamura M, Yoshioka D, Okubo M, Arisawa T, Hirata I: Gastric mucosal pattern by using magnifying narrow-band imaging endoscopy clearly distinguishes histological and serological severity of chronic gastritis. Gastrointest Endosc 2009;70:246–253.
30 Inoue T, Uedo N, Ishihara R, Kawaguchi T, Kawada N, Chatani R, Kizu T, Tamai C, Takeuchi Y, Higashino K, Ishi H, Tatsuta M, Tomita Y, Toth E: Autofluorescence imaging videoendoscopy in the diagnosis of chronic atrophic fundal gastritis. J Gastroenterol 2010;45:45–51.
31 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ: *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;345:784–789.
32 Mizuno S, Miki I, Ishida T, Yoshida M, Onoyama M, Azuma T, Habu Y, Inokuchi H, Ozasa K, Miki K, Watanabe Y: Prescreening of a high-risk group for gastric cancer by serologically determined *Helicobacter pylori* infection and atrophic gastritis. Dig Dis Sci 2010;55:3132–3137.
33 Ohkusa T, Takashimizu I, Fujiki K, Araki A, Honda K, Shimoi K, Sakurazawa T, Horiuchi T, Suzuki S, Ariake K, Ishii K: Changes in serum pepsinogen, gastrin, and immunoglobulin G antibody titers in *Helicobacter pylori*-positive gastric ulcer after eradication of infection. J Clin Gastroenterol 1997;25:317–322.

Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/j.1440-1746.2012.07285.x

GASTROENTEROLOGY

Identification of a high risk gastric cancer group using serum pepsinogen after successful eradication of *Helicobacter pylori*

Masahira Haneda,* Mototsugu Kato,† Saori Ishigaki,* Mio Suzuki,* Masakazu Takahashi,* Manabu Nakagawa,* Shouko Ono,† Yasuaki Mori,* Katsuhiro Mabe,* Shouichi Nakagawa,* Takahiko Kudo,* Yuichi Shimizu* and Masahiko Asaka‡

Departments of *Gastroenterology and ‡Cancer Prevention, Hokkaido University Graduate School of Medicine, and †Division of Endoscopy, Hokkaido University Hospital, Sapporo, Japan

Key words
gastric cancer screening, gastric carcinoma, *Helicobacter pylori* eradication, pepsinogen I/II.

Accepted for publication 8 August 2012.

Correspondence
Dr Mototsugu Kato, Division of Endoscopy, Hokkaido University Hospital, North 14, West 5, Kita-ku, Sapporo, Hokkaido 060-8468, Japan. Email: m-kato@med.hokudai.ac.jp

Abstract

Background and Aim: Pepsinogen (PG) method is widely used to identify high risk groups of gastric cancer. It is very useful before *Helicobacter pylori* eradication, but after eradication the method becomes useless because the PGI, PGII, PGI/II ratios change. Therefore, we aimed to identify a high risk group for gastric cancer using serum pepsinogen after successful eradication of *H. pylori*.

Methods: A total of 261 participants were enrolled after successful eradication of *H. pylori* in Hokkaido University Hospital from 1995 to 2010. Participants with renal failure, taking proton pump inhibitors, and those with advanced gastric cancer were excluded. Serum levels of PGI and II were measured using chemiluminescent immunoassay method.

Results: Receiver operating characteristic curves using cancerous and non-cancerous data in post-eradication determined the optimal cut-off value of PGI/II as 4.5. The sensitivity and the specificity were 65.9% and 79.3%, respectively. The usual PG method includes 48.9% of cancer cases, and the PGI/II $\leq$ 4.5 in post-eradication includes 65.9% of them, and it includes approximately half of the high risk group of diffuse type cancer. PGI/II $\leq$ 4.5 in post-eradication included many gastric cancer cases detected after eradication (12/16 = 75%).

Conclusion: In the identification of a high risk group for gastric cancer, we suggest that the optimal cut-off value of PGI/II after successful eradication of *H. pylori* is 4.5. PGI/II $\leq$ 4.5 in post-eradication includes more gastric cancer cases compared with the traditional PG method, and 75% of gastric cancer cases detected after eradication.

Introduction

Gastric mucosal atrophy resulting from chronic inflammation caused by persistent infection with *Helicobacter pylori* is well known to be strongly associated with the development of differentiated gastric cancer.[1,2] Moreover, gastric cancer risk is known to be increased as atrophic gastritis, based on endoscopic findings, increases.[3,4] Histological studies have also found that gastric cancer risk is incrementally increased by progression of mucosal atrophy, separately, of the gastric corpus and antrum.[5,6] Atrophy involving the entire stomach, referred to as multifocal atrophic gastritis, has been revealed to have the highest risk for development of gastric cancer among background gastric mucosal factors.[7]

In Japan, barium radiography of the stomach is performed as a part of gastric cancer screening for secondary prevention. However, the morbidity and mortality of gastric cancer have not been reduced, for various reasons such as a decreased screening rate and low detection rate of early gastric cancer, showing the limits of the present gastric cancer screening system.[8] Thus, the pepsinogen (PG) method, which is superior for detecting gastric mucosal atrophy, was devised as a more efficient and less invasive gastric cancer screening method and applied to the opportunistic and organized population-based forms of screening.[9–13]

Pepsinogen is the inactive precursor of pepsin specifically produced in the stomach, of which 99% is secreted into the gastric lumen and 1% into the blood stream. PG is comprised mainly of two biochemically and immunologically different isozymes (PGI and PGII). PGI is secreted only from the oxyntic mucosa,[14] PGII from the fundic, pyloric and proximal duodenal glands.[15] Serum PGI levels and PGI/II ratios are known to correlate with the extent of mucosal atrophy in the gastric corpus and gastric acid secretion ability. The PG method is considered to provide useful indices reflecting morphological and functional states of the gastric mucosa and is also referred to as a serological gastric

biopsy.[16,17] In Japan, in which the frequency of mucosal atrophy in the gastric corpus is high, the PG method is extremely suitable. Recent prospective cohort studies also confirmed that measurement of serum PG levels before eradication of *H. pylori* is useful for assessing gastric cancer risk[18–20] and Yanaoka *et al.* reported that atrophy-negative subjects with pepsinogen I of > 70 ng/mL and pepsinogen I/II ratio of < or = 3.0 (reflecting putative inflammation-based high pepsinogen II level) are at high risk for cancer, particularly diffuse-type cancer, with a cancer incidence rate comparable with atrophy-positive subjects.[20] In 2007, a case-control study on the effect of gastric cancer screening using the PG method on mortality reduction showed for the first time that this method is effective for reducing mortality from gastric cancer.[21] Furthermore, the ABC method[18] using both anti-*H. pylori* antibody and serum PG levels allows classification of gastric cancer risk into the following groups based on these levels: Group A is negative for both PG method results and the antibody, Group B is negative for PG method results and positive for the antibody, Group C is positive for both PG method results and the antibody, and Group D is positive for the PG method results and negative for the antibody. According to reports on cohort studies[22] undergoing comprehensive medical examinations[23] etc., based on ABC method results, Groups A, B, C and D represent low, moderate, high, and very high risk, respectively. In cases with classification changing from Group B or C before eradication of *H. pylori* to Group A after eradication, odds ratios are reduced. However, it is known that gastric cancer risk is still higher compared to that in Group A cases before eradication.[22] If no pre-eradication PG level is available, the ABC method is not applicable to the conventional risk classification.

Regarding serological assessment of gastric cancer risk, many studies used serum PG levels before eradication of *H. pylori* as described above, whereas there have been no reports on risk classification of gastric cancer using serum PG levels after eradication of *H. pylori*. The *H. pylori* infection rate in middle-aged and older people in Japan is still higher than in Europe and the United States. Because eradication of *H. pylori* does not result in complete disappearance of gastric cancer risk, using post-eradication serum PG levels for risk classification of gastric cancer may be extremely important.

In this study, we aimed to examine the usefulness of serum PG levels after eradication of *H. pylori* by classifying gastric cancer risk based on these levels.

Methods

Patients. Among patients visiting the outpatient unit of Hokkaido University Hospital between January 1995 and December 2010, we enrolled 261 patients who underwent successful eradication after diagnosis of *H. pylori* infection.

Patients taking proton pump inhibitors,[23] those with a history of upper gastrointestinal tract surgery,[24,25] those with chronic renal failure defined by serum creatinine of 2.0 mg/dL or higher,[26] and those with advanced gastric cancer were excluded because serum PG levels might be affected by their conditions.[27]

Participants included 47 cases of early gastric cancer (gastric cancer group) and 213 non-cancer cases (control group). The gastric cancer group was composed of 34 men and 13 women with a median age of 71 years (64–76). In all 47, early-stage cancer had been confirmed by histopathologic examination of resected samples and endoscopically treated. Cases with advanced gastric cancer were excluded because this disease extensively destroys the normal gastric mucosal structure and may thus affect serum PG levels. The histological type in the gastric cancer group was differentiated cancer in 44 participants and undifferentiated cancer in three.

Table 1 Background of subjects in the study of a high risk gastric cancer group using serum pepsinogen after successful eradication of *Helicobacter pylori*

Total number	261
Age median (range)	57 (45–67)
M : F	142:119
Disease	
GU/DU (including scar)	112 (44%)
Atrophic gastritis	43 (16%)
Hyperplastic polyp	19 (7%)
Nodular gastritis (NG)	35 (13%)
Early gastric cancer	47 (18%)
MALT lymphoma	5 (2%)

DU, duodenal ulcer; GU, gastric ulcer; MALT, mucosa-associated lymphoid tissue.

The control group was composed of 142 men and 119 women with a median age of 54 years (44–62). Endoscopic diagnoses in the control group revealed gastroduodenal ulcer (including scarring) in 112 participants, atrophic gastritis in 43, nodular gastritis in 35, gastric hyperplastic polyp in 19, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma in five (Table 1).

In 16 participants who underwent measurement of serum PG levels after eradication of *H. pylori* at our hospital, gastric cancer was detected after successful eradication. The median time from eradication to detection of cancer was 39.1 months (12.3–69.5). The diseases at the time of eradication included gastric ulcer in six participants, gastric polyp in two, MALT lymphoma in two, and gastric cancer in four.

This study was approved by the ethics committee of Hokkaido University School of Medicine. We adequately explained the study to each participant and obtained his or her written consent.

Measurement of serum samples. Fasting blood samples were collected immediately before endoscopy from all participants. The samples were immediately centrifuged at 4°C, and the serum was frozen and stored at –20°C. Serum PGI and PGII levels were measured by chemiluminescent immunoassay (CLIA) using ARCHITECT analyzer, an automatic CLIA analyzer manufactured by Dainabot (Abbott Japan, Matsudo, Japan). The positive levels were set at PGI $\leq$ 70 and PGI/II $\leq$ 3.0 for the conventional PG method. Serum PG was checked in 226 subjects at 3 months after eradication, in four subjects at 4–6 months, 31 subjects at 7–12 months.

Endoscopic examination. We checked the endoscopic examination at 1, 6 or 12 months and then once every year after eradication therapy.

Methods of* H. pylori *infection diagnosis and eradication. *Helicobacter pylori* infection was assessed by microscopy using Giemsa staining, measurement of serum *H. pylori*-immunoglobulin G antibody levels, rapid urease test, urea breath test, and culture test. Participants were judged to be positive for *H. pylori* based on a positive result on any of these tests. After eradication therapy with a combination of a proton pump inhibitors and several antibiotics, eradication was judged to be successful based on negative results for all of the above tests.

Statistical analysis. The data of continuous variables were expressed as medians (interquartile range).

Friedman test was done to compare the long term value of serum pepsinogen after eradication. The Wilcoxon signed rank test was used to determine a statistically significant difference in continuous variables between two related groups. The Mann–Whitney *U*-test was used to determine a statistically significant difference in continuous variables between two unrelated groups. Receiver operating characteristic (ROC) analysis was used to set cut-off values for the PGI/II ratio in the gastric cancer and non-cancer cases. The detectability of gastric cancer with new cut-off values was analyzed through sensitivity and specificity.

Results

***Changes in serum PG levels after eradication of* H. pylori.** In all participants, changes in mean serum PG levels before *versus* after eradication were examined. Before eradication, the mean serum PGI level was 59.7 (40.5–79.5) ng/mL, the mean serum PGII level was 19.3 (13.1–27.6) ng/mL, and the mean PGI/II ratio was 3.1 (2.1–3.9). After eradication, these values were 38.2 (25.2–49.8) ng/mL, 6.9 (5.0–8.9) ng/mL and 5.6 (4.1–7.2) ng/mL, respectively. The serum PGI and PGII levels were significantly reduced, whereas the PGI/II ratio was increased (Table 2) (Wilcoxon signed rank test, $P < 0.001$). These results are similar to those of previous reports.[28,29] Compared to before eradication, serum PG levels change significantly after eradication.

Table 2 Successful eradication of *Helicobacter pylori* decreased both serum pepsinogen (PG)I and PGII levels and increased PG I/II ratio

n = 261	PGI Median (range)	PGII Median (range)	PGI/II Median (range)
Pre-eradication (ng/mL)	59.7 (40.5–79.5)	19.3 (13.1–27.6)	3.1 (2.1–3.9)
Post-eradication (ng/mL)	38.2* (25.2–49.8)	6.9* (5.0–8.9)	5.6* (4.1–7.2)

*$P < 0.001$, Wilcoxon signed rank test.
PG, pepsinogen.

Time-trend of serum PG values for long-term after* H. pylori *eradication. We investigated time-trend of serum pepsinogen value using samples at 1, 3, 6 or 12 months and every year after eradication treatment. In following up 40 patients, the values of Serum PGI, PGII, PGI/II ratio were not significantly changed from 3 months to 36 months after successful eradication in our study (Fig. 1) (Friedman test, *N.S.*).

ROC analysis using PGI/II ratio post-eradication. The ROC curves of post-eradication PGI/II ratios in the gastric cancer and non-cancer cases are shown in Figure 2. Based on the ROC curve, the optimal cut-off value for the PGI/II ratio appeared to be 4.5. Screening with the PGI/II ratio ≤ 4.5 had sensitivity of 65.9% and specificity of 79.3% for gastric cancer.

Examination of gastric cancer risk using the PGI/II ratio of post-eradication. The gastric cancer and non-cancer cases were examined with a cut-off value of 4.5 for the serum PGI/II ratio of post-eradication. In the non-cancer cases, the conventional PG method detected 29.1% as positive, whereas with the new cut-off value used post-eradication 20.7% were detected as positive. The proportion of positive non-cancer cases was reduced by the new cut-off value. Especially, only 6.1% of the low-risk for gastric cancer group (serum PG > 70 and PGI/II > 3) were included at risk of gastric cancer after eradication (Fig. 3). The conventional PG method in pre-eradication identified 47.9% of gastric cancer cases, whereas the PGI/II ratio ≤ 4.5 in post-eradication identified 65.9% of these cases (Fig. 4). Furthermore, it was suggested that use of the ratio in post-eradication cases may

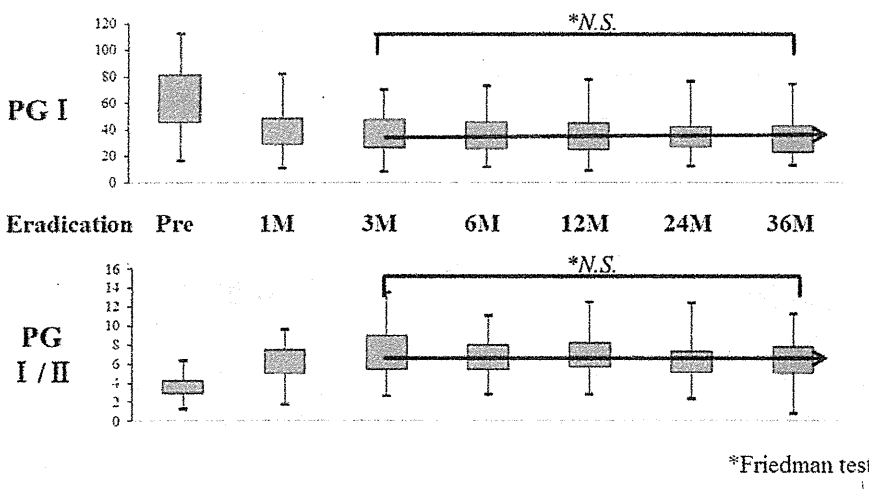

Figure 1 Time-trend of serum pepsinogen (PG) values for long-term after *Helicobacter pylori* eradication. In following up 40 patients, the values of Serum PGI, PGII, PGI/II ratio were not significantly changed from 3 months to 36 months after successful eradication in our study.

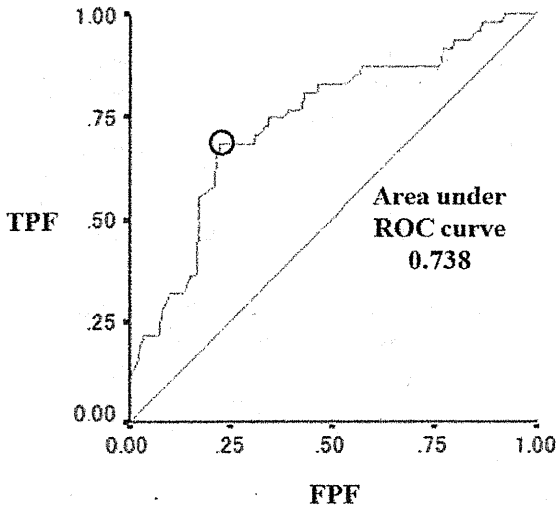

Figure 2 Receiver operating characteristic (ROC) curve and cut-off value using cancerous and non-cancerous data in post-eradication. Cut-off value of PGI/II was determined as 4.5. The sensitivity and the specificity were 65.9% and 79.3%.

identify approximately half of cancers difficult to identify by the conventional PG method, such as those in Group γ (PGI/II ratio ≤ 3 and PGI > 70 ng/mL pre-eradication) who are considered to be at risk for undifferentiated cancer[20] and those with serum PGII levels ≥ 30 ng/mL in pre-eradication[30] (Figs 4 and 5).

Out of 16 participants who were diagnosed as having gastric cancer after eradication at our hospital, 12 (75%) were identified as being at risk by examination with a cut-off value of PGI/II ratio ≤ 4.5 (Fig. 6).

The odds ratio for gastric cancer was 7.44 (95% confidence interval: 3.74–14.8) in participants with serum PGI/II ratios ≤ 4.5 in post-eradication. The gastric cancer risk was significantly higher in this subset than in those with PGI/II ratios > 4.5.

Discussion

Measurement of serum PG levels has often been reported to be useful not only for gastric cancer screening, but also for assessing its risk and identifying patients at high risk for gastric cancer. However, because serum PG levels change after eradication of *H. pylori*, the conventional PG method cannot be applied after successful eradication. We investigated time-trend of serum pepsinogen value after eradication treatment. The values of serum

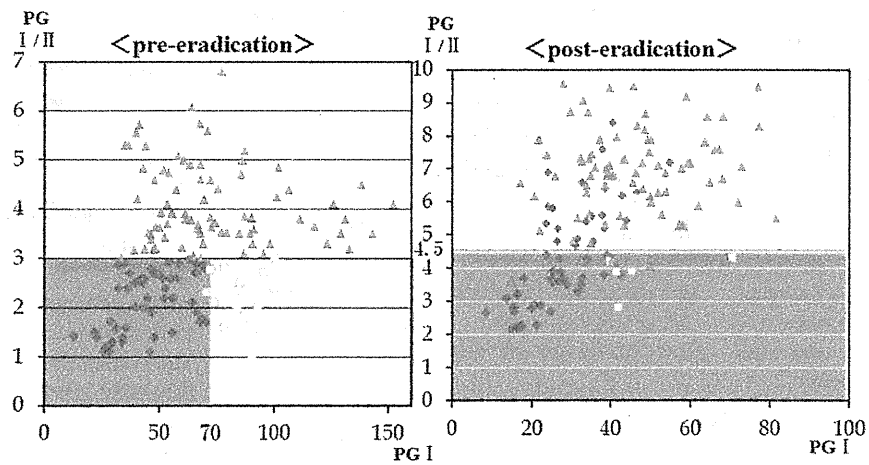

Figure 3 Distribution of non-cancerous cases in pre-eradication and post-eradication. PGI/II ≤ 4.5 includes 20.7% of non-cancer group, whereas traditional PG method includes 29.1%. Especially, only 6.1% of low risk group of cancer (PGI/II ≥ 3 in pre-eradication) was included. ■, PGI/II ≤ 3.0 and PGI ≤ 70 ng/mL (positive PG method group); ▲, PGI/II > 3.0 and PGI > 70 ng/mL (low risk group); ▒, PGI/II ≤ 3.0 and PGI > 70 ng/mL (γ group).

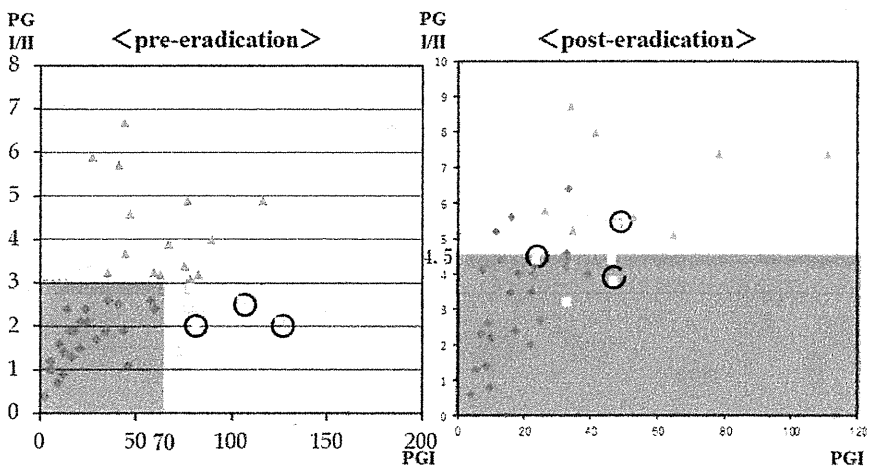

Figure 4 Distribution of gastric cancer cases in pre-eradication and post-eradication. PGI/II ≤ 4.5 included the high risk group of diffuse type cancer (4/8 = 50%) comparing with 0% in traditional PG method. And the traditional PG method in pre-eradication identified 47.9% of gastric cancer cases, whereas the PGI/II ≤ 4.5 in post-eradication identified 65.9% of these cases. ■, PGI/II ≤ 3.0 and PGI ≤ 70 ng/mL (positive PG method group); ▲, PGI/II > 3.0 and PGI > 70 ng/mL (low risk group); ▒, PGI/II ≤ 3.0 and PGI > 70 ng/mL (γ group); ○, actual diffuse type gastric cancer.

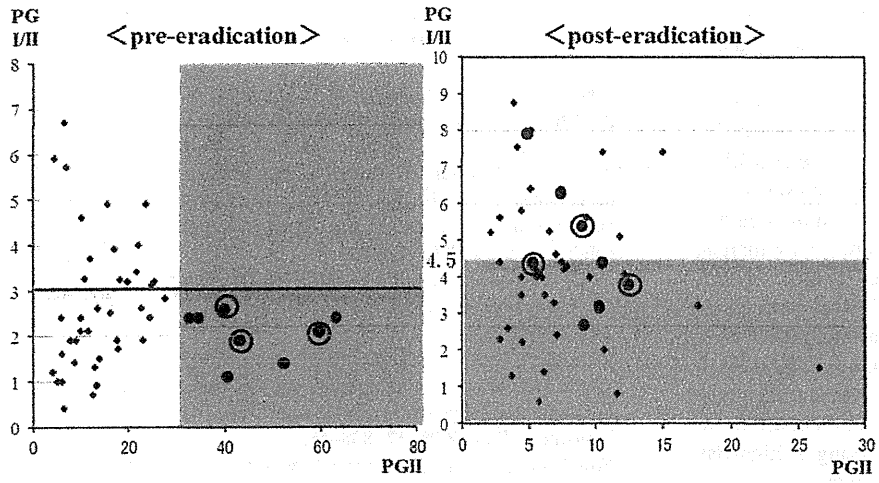

Figure 5 Distribution of gastric cancer cases in pre-eradication and post-eradication. PGI/II ≤ 4.5 included the high risk group of diffuse type cancer(5/8 = 62.5%). ◇, PGII < 30 ng/mL; ●, PGII ≥ ng/mL; ○, actual diffuse type gastric cancer.

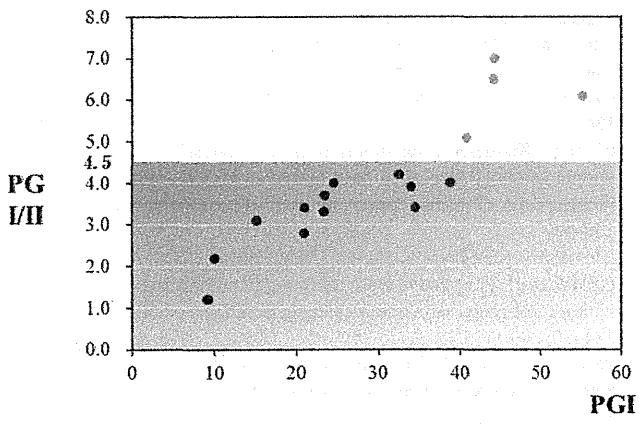

Figure 6 Distribution of gastric cancer cases detected after eradication. PGI/II ≤ 4.5 included gastric cancer cases detected after eradication (12/16 = 75%). ●, PGI/II ≤ 4.5; ○, PGI/II > 4.5.

PGI, PGII, and PGI/II ratio were not significantly changed from 3 months to 36 months after successful eradication in our study.

According to the report by Ito *et al.* the *H. pylori* infection rate was 42.6% in subjects undergoing their initial comprehensive medical examinations, and the rate of eradicated subjects was 8.3%. However, in those who had undergone several examinations, the infection rate was reduced to 16.9%, and the rate of eradicated subjects was increased to 36.7%. These results show that approximately two-thirds of subjects positive for *H. pylori* had undergone eradication therapy.[31] Based on this report, it is anticipated that patients who have achieved eradication (classified into Group E) will increase among those undergoing medical examinations. There has been no report on identification of a subgroup at risk for gastric cancer based on serum PG levels after eradication of *H. pylori* in Group E. The risk classification of gastric cancer based on a PGI/II ratio ≤ 4.5 in post-eradication, as reported in this study, is intended for patients who have achieved eradication (Group E) for whom the conventional method is not indicated in primary screening of the general population for gastric cancer. When the ABC method, which uses both anti-*H. pylori* antibody and serum PG levels in pre-eradication,[22] is applied, the classification using a PGI/II ratio ≤ 4.5 may be useful for setting a cut-off value for high-risk gastric cancer cases who received successful eradication treatment that is confirmed by interview.

If subjects in whom eradication of *H. pylori* has been successful are identified by interview during medical examination, we propose separately treating them as Group E and classifying them into high-risk and low-risk for gastric cancer subgroups based on the PGI/II ratio cut-off value of 4.5, which was determined in this study, for follow-up.

Helicobacter pylori is considered to act as not only an initiator, but also a promoter of gastric cancer. It has been reported that the incidence and growth rate of gastric cancer may be reduced after eradication.[32] Thus, the PGI/II ratio of 4.5 may contribute to determining the optimal surveillance interval. For example, follow-up endoscopy may be performed once every 2 years for those with PGI/II ratios ≤ 4.5 post-eradication or once every 3 years for those with PGI/II ratios > 4.5. Because there has been no report on setting appropriate intervals for endoscopic surveillance after eradication of *H. pylori*, it seems that follow-up endoscopy is often performed annually. We consider the new cut-off value determined in this study to be a useful guide for setting appropriate intervals for endoscopic surveillance in post-eradication.

However, it should be noted that, even if a high-risk group for gastric cancer is identified by serum PG levels among subjects in whom eradication of *H. pylori* has been successful, a low-risk group post-eradication will continue undergoing endoscopic surveillance. In those in whom eradication of *H. pylori* has been successful, endoscopic surveillance should be performed regularly, given that neoplastic cells arising during persistent infection may persist after eradication.

In this study, selection of the control group was biased, and the general population might not have been represented. This is a potential limitation of case-control studies. In order to confirm our results, that is, whether a high-risk group for gastric cancer is identified by a PGI/II ratio ≤ 4.5 in post-eradication, prospective cohort studies on the incidence of gastric cancer are needed. Moreover, the gastric cancer group in the present study was composed mainly of patients with differentiated cancer, with only a few patients with undifferentiated cancer. The association between

gastric mucosal atrophy and undifferentiated cancer is known to be weaker than that of differentiated cancer.[33,34] Thus, further studies on undifferentiated cancer may also be needed.

When serum PG levels after eradication of *H. pylori* were used to identify a high-risk group for gastric cancer, the optimal cut-off value of the PGI/II ratio was considered to be 4.5. The classification using a PGI/II ratio ≤ 4.5 had better sensitivity and specificity for identification of patients with gastric cancer than the conventional PG method. It was suggested that the former may include more than half of patients with undifferentiated gastric cancers, which had been difficult to identify. Moreover, 75% of participants who were diagnosed as having gastric cancer after eradication at our hospital were included in the high-risk group. In the future, it may be necessary to accumulate further cases and conduct a prospective multicenter study. The present study may contribute to effective gastric cancer surveillance by identifying a high-risk group for gastric cancer after eradication of *H. pylori*.

References

1 Correa P, Haenszel W, Cuello C *et al.* Gastric precancerous process in a high risk population: cohort follow-up. *Cancer Res.* 1990; **50**: 4737–40.
2 Siurala M, Varis K, Wiljasalo M. Studies of patients with atrophic gastritis: a 10–15-year follow-up. *Scand. J. Gastroenterol.* 1966; **1**: 40–8.
3 Svendsen JH, Dahl C, Sevendsen LB, Christiansen PM. Gastric cancer risk in achlorhydric patients. A long-term follow-up study. *Scand. J. Gastroenterol.* 1986; **21**: 16–20.
4 Uemura N, Okamoto S, Yamamoto S *et al. Helicobacter pylori* infection and the development of gastric cancer. *N. Engl. J. Med.* 2001; **345**: 784–9.
5 Cassaro M, Rugge M, Gutierrez O, Leandro G, Graham DY, Genta RM. Topographic patterns of intestinal metaplasia and gastric cancer. *Am. J. Gastroenterol.* 2000; **95**: 1431–8.
6 Sipponen P, Stolte M. Clinical impact of routine biopsies of the gastric antrum and body. *Endoscopy* 1997; **29**: 671–8.
7 Rugge M, Meggio A, Pennelli G *et al.* Gastritis staging in clinical practice: the OLGA staging system. *Gut* 2007; **56**: 631–6.
8 Faraji EI, Frank BB. Multifocal atrophic gastritis and gastric carcinoma. *Gastroenterol. Clin. North Am.* 2002; **31**: 499–516.
9 Mario F, Cavallaro LG. Non-invasive tests in gastric disease. *Dig. Liver Dis.* 2008; **40**: 523–30.
10 Miki K, Morita M, Sasajima M, Hoshina R, Kanda E, Urita Y. Usefulness of gastric cancer screening using the serum pepsinogen test method. *Am. J. Gastroenterol.* 2003; **98**: 735–9.
11 Kitahara F, Kobayashi K, Sato T, Kojima Y, Araki T, Fujino MA. Accuracy of screening for gastric cancer using serum pepsinogen concentrations. *Gut* 1999; **44**: 693–7.
12 Varis K, Sipponen P, Laxen F *et al.* Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia. Helsinki Gastritis Study Group. *Scand. J. Gastroenterol.* 2000; **35**: 950–6.
13 Broutet N, Plebani M, Sakarovitch C, Sipponen P. Megraud F and the Eurohepygast Study Group. Pepsinogen A, pepsinogen C, and gastrin as markers of atrophic chronic gastritis in European dyspeptics. *Br. J. Cancer* 2003; **88**: 1239–47.
14 Samloff IM. Cellular localization of group I pepsinogens in human gastric mucosa by immunofluorescence. *Gastroenterology* 1971; **61**: 185–8.
15 Samloff IM, Liebman WM. Cellular localization of the group II pepsinogens in human stomach and duodenum by immunofluorescence. *Gastroenterology* 1973; **65**: 36–42.
16 Miki K, Ichinose M, Shimizu A *et al.* Serum pepsinogens as a screening test of extensive chronic gastritis. *Gastroenterol. Jpn* 1987; **22**: 133–41.
17 Borch K, Axelsson CK, Halgreen H, Damkjaer N, Ledin T, Szesci PB. The ratio of pepsinogen A to pepsinogen C: a sensitive test for atrophic gastritis. *Scand. J. Gastroenterol.* 1989; **24**: 870–6.
18 Watabe H, Mitsushima T, Yamaji Y *et al.* Predicting the development of gastric cancer from combining *Helicobacter pylori* antibodies and serum pepsinogen status: a prospective endoscopic cohort study. *Gut* 2005; **54**: 764–8.
19 Oishi Y, Kiyohara Y, Kubo M *et al.* The serum pepsinogen test as a predictor of gastric cancer: the Hisayama study. *Am. J. Epidemiol.* 2006; **163**: 629–37.
20 Yanaoka K, Oka M, Mukoubayashi C *et al.* Cancer high-risk subjects identified by serum pepsinogen tests: outcomes after 10-year follow-up in asymptomatic middle-aged males. *Cancer Epidemiol. Biomarkers Prev.* 2008; **17**: 838–45.
21 Yoshihara M, Hiyama T, Yoshida S *et al.* Reduction in gastric cancer mortality by screening based on serum pepsinogen concentration: a case-control study. *Scand. J. Gastroenterol.* 2007; **42**: 760–4.
22 Sasazuki S, Inoue M, Iwasaki M *et al.* Effect of *Helicobacter pylori* infection combined with CagA and pepsinogen status on gastric cancer development among Japanese men and women; a nested case-control study. *Cancer Epidemiol. Biomarkers Prev.* 2006; **15**: 1341–7.
23 Ohata H, Kitauchi S, Yoshimura N *et al.* Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. *Int. J. Cancer* 2004; **109**: 138–43.
24 Biemond I, Rieu PNMA, Jansen JBMJ, Joosten HJM, Lamers CBHW *et al.* Prospective study of the effect of gastrectomy with and without bile reflux on serum pepsinogens. *Digestion* 1989; **44**: 124–30.
25 Peetsalu A, Tamm A, Harkonen M *et al.* The effect of vagotomy and antrectomy on serum pepsinogens 1 and 2. *Scand. J. Gastroenterol.* 1990; **25**: 455–61.
26 Nakahama H, Tanaka Y, Shirai D *et al.* Elevated serum pepsinogens in chronic renal failure patients. *Nephron* 1995; **70**: 211–16.
27 Shirai N, Furuta T, Sugimoto M *et al.* Serum pepsinogens as an early diagnostic marker of *H. pylori* eradication. *Hepatogastroenterology* 2008; **55**: 486–90.
28 Hunter FM, Correa P, Fontham E *et al.* Serum pepsinogens as an early diagnostic markers of response to therapy for *Helicobacter pylori* gastritis. *Dig. Dis. Sci.* 1993; **38**: 2081–6.
29 Pérez-Paramo M, Albillos A, Calleja JL *et al.* Changes in gastrin and serum pepsinogens in monitoring of *Helicobacter pylori* response to therapy. *Dig. Dis. Sci.* 1997; **42**: 1734–40.
30 Ito M, Yoshihara M, Takata S *et al.* Serum screening for detection of the high-risk group for early-stage diffuse type gastric cancer in Japanese. *Gastroenterology* 2011 (in press).
31 Ito M. Actual medical practice and challenge of *Helicobacter pylori* in the metropolitan area. *Helicobacter Res.* 2010; **14**: 314–18. (In Japanese).
32 Kato M, Asaka M, Ono S *et al.* Eradication of *Helicobacter pylori* for primary gastric cancer and secondary gastric cancer after EMR. *J. Gastroenterol.* 2007; **42**: S16–20.
33 Correa P. Human gastric carcinogenesis: a multistep and multifactorial process First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res.* 1992; **52**: 6735–40.
34 Palli D, Masala G, Del Giudice G *et al.* CagA+ *Helicobacter pylori* infection and gastric cancer risk in the EPIC-EURGAST study. *Int. J. Cancer* 2007; **120**: 859–67.

J Gastroenterol
DOI 10.1007/s00535-012-0731-8

ORIGINAL ARTICLE—ALIMENTARY TRACT

Multi-center randomized controlled study to establish the standard third-line regimen for *Helicobacter pylori* eradication in Japan

Kazunari Murakami · Takahisa Furuta · Takashi Ando · Takeshi Nakajima · Yoshikatsu Inui · Tadayuki Oshima · Toshihiko Tomita · Katsuhiro Mabe · Makoto Sasaki · Takanori Suganuma · Hideyuki Nomura · Kiichi Satoh · Shinichiro Hori · Syuuji Inoue · Takeshi Tomokane · Mineo Kudo · Tomoki Inaba · Susumu Take · Toshifumi Ohkusa · Shojiro Yamamoto · Shigeaki Mizuno · Toshiro Kamoshida · Kenji Amagai · Junichi Iwamoto · Jun Miwa · Masaaki Kodama · Tadayoshi Okimoto · Mototsugu Kato · Masahiro Asaka · For the Japan GAST Study Group.

Received: 4 September 2012 / Accepted: 4 December 2012

Abstract
Backgrounds The present study sought to establish a standard third-line eradication regimen for *Helicobacter pylori* in Japan.
Methods Subjects were 204 patients with *H. pylori* infection in whom the standard Japanese first- and second-line eradication therapies had proven unsuccessful. Patients were randomly assigned to one of the following third-line eradication therapy groups: (1) LA group: lansoprazole (LPZ) 30 mg 4 times a day (qid) + amoxicillin (AMPC) 500 mg qid for two weeks; (2) LAL group: LPZ 30 mg twice a day (bid) + AMPC 750 mg bid + levofloxacin

K. Murakami and M. Kato contributed equally to this work.

K. Murakami (✉) · M. Kodama · T. Okimoto
Department of Gastroenterology, Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hasama-machi, Yuhu, Oita 879-5593, Japan
e-mail: murakam@oita-u.ac.jp

T. Furuta
Center for Clinical Research, Hamamatsu University School of Medicine, Hamamatsu, Japan

T. Ando
Gastroenterological Department, Social Insurance Kyoto Hospital, Kyoto, Japan

T. Nakajima
Endoscopy Division, National Cancer Center Hospital, Tokyo, Japan

Y. Inui
Inui Clinic of Internal Medicine, Takasaki, Japan

T. Oshima · T. Tomita
Division of Upper Gastroenterology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

K. Mabe · M. Kato (✉)
Division of Endoscopy, Hokkaido University Hospital, 1-11, Nishi-5, Kita-14, Kita-ku, Sapporo, Hokkaido 060-8648, Japan
e-mail: m-kato@med.hokudai.ac.jp

M. Sasaki
Department of Gastroenterology, Aichi Medical University School of Medicine, Nagakute, Japan

T. Suganuma
Japanese Foundation for Cancer Research Cancer Institute Hospital, Tokyo, Japan

H. Nomura
Department of Internal Medicine, Shin-Kokura Hospital, Kitakyushu, Japan

K. Satoh
Division of Gastroenterology, Department of Internal Medicine, Jichi Medical University, Shimotsuke, Japan

S. Hori
National Hospital Organization Shikoku Cancer Center, Matsuyama, Japan

S. Inoue · T. Tomokane
National Hospital Organization Kochi National Hospital, Kochi, Japan

M. Kudo
Department of Gastroenterology, Sapporo Hokuyu Hospital, Sapporo, Japan

Published online: 11 January 2013

(LVFX) 300 mg bid for one week; (3) LAS group: LPZ 30 mg bid + AMPC 750 mg bid + sitafloxacin (STFX) 100 mg bid for one week. Patients for whom these therapies failed underwent a crossover fourth-line eradication regimen. Drug sensitivity was also tested for AMPC, clarithromycin (CAM), MNZ, LVFX, and STFX.

Results Drug resistance rates prior to third-line eradication therapy were 86.4 % for CAM, 71.3 % for MNZ, 57.0 % for LVFX, 8.2 % for AMPC, and 7.7 % for STFX. Intention-to-treat analysis of third-line eradication therapy eradication rates showed a significantly higher rate in the LAS group (70.0 %) compared with the LA group (54.3 %; $p < 0.05$) and the LAL group (43.1 %; $p < 0.001$). The significantly lower rate in the LAL group than the LAS group was caused by bacterial resistance to LVFX.

Conclusions The findings suggest that triple therapy with PPI, AMPC, and STFX for one week would be an effective standard third-line eradication regimen for *H. pylori* in Japan.

Keywords *Helicobacter pylori* eradication therapy · Drug resistance · Levofloxacin · Sitafloxacin · Lansoprazole

Introduction

Helicobacter pylori infection is known to cause various upper gastrointestinal (GI) diseases, from atrophic gastritis and gastroduodenal ulcer to GI mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer [1, 2]. Accordingly, the development of *H. pylori* eradication therapies has been pursued on a global scale. One standard eradication therapy combines a proton pump inhibitor (PPI) with clarithromycin (CAM) and amoxicillin (AMPC), and this triple therapy has been covered under Japan's national health insurance (NHI) scheme since 2000. However, the subsequent increase in bacterial resistance to CAM in Japan caused a decline in the eradication rate of first-line therapy [3], leading to the approval of a second-line eradication therapy substituting metronidazole (MNZ) for CAM in 2007. This second-line therapy has enjoyed a success rate of approximately 90 % [4, 5], but 2–3 % of patients still fail to respond to both first- and second-line therapy. Despite this, however, there is currently no standard eradication therapy in Japan for those failing to respond to second-line therapy, giving rise to expectations for the development of an effective third-line eradication regimen.

While some consider that a third-line eradication therapy should be determined by selecting antibiotics based on tests of the sensitivity of *H. pylori* to each drug [6], clinical drug sensitivity testing is difficult to implement, and previous studies have shown that antibiotics selected on the basis of these results do not always ensure high eradication rates [7, 8].

Consequently, a number of studies have examined the feasibility of a third-line eradication regimen combining a PPI with AMPC (for which *H. pylori* resistance does not tend to develop) for two weeks, or multidrug therapy substituting a new quinolone antibiotic for CAM. AMPC's minimum inhibitory concentration (MIC) for *H. pylori* suggests that the two-week combined regimen of AMPC/PPI would be effective in treating strains that are resistant to CAM and MNZ [3]. In the latter regimen, the most common multidrug combination therapy is a triple regimen

T. Inaba
Department of Gastroenterology, Kagawa Prefectural Central Hospital, Takamatsu, Japan

S. Take
Department of Internal Medicine, Fukuwatari Municipal Hospital, Okayama, Japan

T. Ohkusa
Department of Gastroenterology and Hepatology, The Jikei University Kashiwa Hospital, Kashiwa, Japan

S. Yamamoto
Division of Gastroenterology and Hematology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

S. Mizuno
Department of Gastroenterology and Hepatology, Nihon University, School and Medicine, Tokyo, Japan

T. Kamoshida
Hitachi General Hospital, Hitachi, Japan

K. Amagai
Ibaraki Prefectual Central Hospital and Cancer Center, Ibaraki, Japan

J. Iwamoto
Tokyo Medical University, Ibaraki Medical Center, Ibaraki, Japan

J. Miwa
Toshiba General Hospital, Tokyo, Japan

M. Asaka
Cancer Preventive Medicine, Hokkaido University School of Medicine, Hokkaido, Japan

of PPI, AMPC, and levofloxacin (LVFX), with a reported eradication rate of 60–70 % [9, 10]. Meanwhile, sitafloxacin (STFX) is another new quinolone antibacterial agent with anticipated efficacy due to its low MIC for *H. pylori*, even for LVFX-resistant strains [11]. When combined with PPI and AMPC, STFX is expected to have a good eradication effect, even when used in third-line eradication therapy.

These various study findings suggest that two-week administration of PPI/AMPC and one-week administration of PPI/AMPC/LVFX are both viable options for standard third-line eradication therapy in Japan. However, further studies are required to identify even more effective regimens, and one-week administration of PPI/AMPC/STFX is a strong candidate. Although several types of PPIs are covered under Japan's NHI program for *H. pylori* eradication therapy, lansoprazole (LPZ) was selected in the present study as a potent inhibitor of gastric acid secretion [12]. In Japan, LPZ/AMPC/CAM and LPZ/AMPC/MNZ are covered by the NHI as first-line and second-line eradication therapies, respectively, with the former regimen having a good eradication rate [13, 14].

The aim of the present study was to establish a third-line eradication therapy best suited to use in Japan by comparing the eradication effect and safety of a novel third-line triple therapy substituting STFX for CAM against those of a two-week regimen of LPZ/AMPC and a one-week regimen of LPZ/AMPC/LVFX, both of which are reported to have achieved a degree of success. Moreover, patients failing to respond to third-line eradication therapy underwent a fourth-line eradication therapy based on a crossover regimen, and the efficacy and safety of this therapy was assessed. The correlation between drug resistances and third-line eradication rate was also determined based on the results of drug sensitivity testing and evaluation of drug MICs.

Subjects and methods

Study design

This was a Japanese multicenter, randomized, controlled, comparative study among three groups (Fig. 1). Invitations to join the study were sent to all members of the Japan GAST Study Group (JGSG). Patients were randomly assigned by computer to one of the following third-line eradication therapy groups: (1) LA group: LPZ 30 mg four times a day (qid) + AMPC 500 mg qid for two weeks; (2) LAL group: LPZ 30 mg twice a day (bid) + AMPC 750 mg bid + LVFX 300 mg bid for one week; (3) LAS group: LPZ 30 mg bid + AMPC 750 mg bid + STFX 100 mg bid for one week (Fig. 1).

H. pylori testing was performed at 8 (±4) weeks after completing the third-line eradication therapy, with patients who failed to respond to therapy subsequently undergoing a crossover fourth-line eradication regimen whereby those for whom LAL and LAS failed were treated with LA and those for whom LA failed were given LAS. Patients whose drug compliance fell below 85 % or who experienced a serious adverse drug reaction (ADR) or GI bleeding during the study treatment were not allowed to continue the study. Study protocols were approved by the institutional review board at each institute and complied with provisions of the Declaration of Helsinki.

Study design

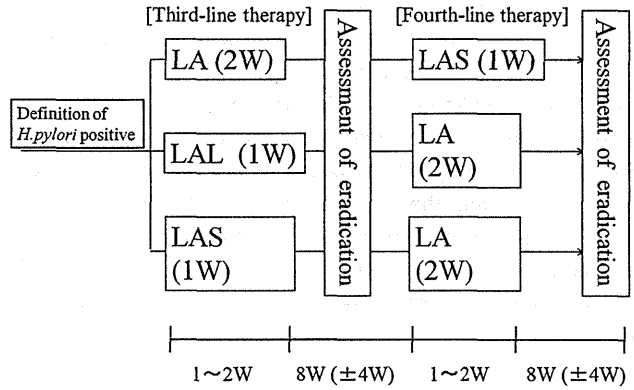

Fig. 1 Study design. Patients were randomly assigned to one of three third-line therapy groups: *LA group* lansoprazole (LPZ) 30 mg qid + amoxicillin (AMPC) 500 mg qid for two weeks, *LAL group* LPZ 30 mg bid + AMPC 750 mg bid + levofloxacin (LVFX) 300 mg bid for one week, *LAS group* LPZ 30 mg bid + AMPC 750 mg bid + sitafloxacin (STFX) 100 mg bid for one week. *H. pylori* testing was performed at 8 (±4 weeks) after completion of third-line eradication therapy, with patients failing to respond to therapy subsequently undergoing a crossover fourth-line eradication regimen whereby those for whom LAL and LAS failed were treated with LA and those for whom LA failed were given LAS

Calculation of the sample size

In the present study, we set the minimum number of registered patients in each group at 50, and the basis for this is given below.

When the study was being designed, there was no published literature on the eradication effects of the third-line eradication regimens investigated herein; there were only conference reports on single-center studies conducted on a small number of subjects. Based on the experience of the research representatives, the eradication rate was estimated to be 50–70 % in the LAL group and 60–80 % in the LA and LAS groups. It was thought that only 2–3 % of patients receiving eradication therapy failed to respond to both first- and second-line eradication therapies, and the number of patients who could be enrolled during the two-year patient registration period was limited. We therefore set the number of patients by assuming the largest possible

difference in eradication rate. Applying the chi-squared test to a 3 × 2 contingency table, a total of 49 patients per group were required to satisfy the conditions of a significance level of 5 % and a statistical power of 80 %. Taking into account dropouts, we set the minimum number of registered patients at 50 per group and 150 in total. We also decided to investigate the outcomes of fourth-line eradication in the event that no significant intergroup differences were seen in the primary endpoints. In addition, differences in eradication rate among the three groups were investigated by applying the chi-squared test to a 3 × 2 contingency table.

Subjects

A total of 204 patients were registered (Fig. 2) from 23 institutes in the period from September 2009 to August 2011. The study targeted *H. pylori*-infected patients in whom first-line (PPI/AMPC/CAM) and second-line (PPI/AMPC/MNZ) eradication therapy had failed, and who gave their written informed consent to participate. The number of times the first- and second-line eradication regimens were administered was not taken into account.

Patients who met any of the following criteria were excluded from the study: (1) age <20 years or ≥81 years; (2) antibiotic treatment(s) after confirmation of the failure of second-line eradication therapy; (3) PPI treatment(s) within one week of study commencement; (4) habitual steroid or non-steroidal anti-inflammatory drug (NSAID) use, with the most recent use occurring within one month

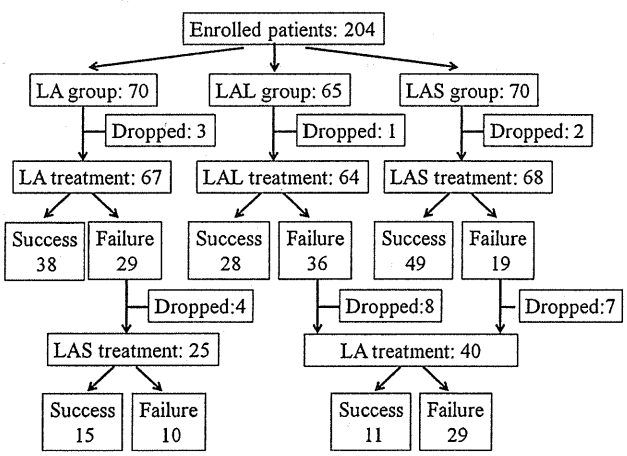

Fig. 2 Assignment of patients and subsequent study flow. The study enrolled 204 patients at 23 sites. Patients were randomly assigned to the LA group ($n = 70$), LAL group ($n = 65$), and LAS group ($n = 70$). Six of the patients taking each third-line treatment did not visit the hospital and dropped out. Third-line therapy was administered to 67, 64, and 68 patients, respectively. Fourth-line therapy was administered to a total of 65 patients for whom third-line therapy failed. Specifically, LAS was administered to 25 patients for whom LA failed, and LA was given to 40 for whom LAL or LAS failed. Nineteen patents taking the fourth-line treatment did not visit the hospital and dropped out

of study commencement; (5) previous medical history of serious renal disorder, liver disorder, or drug hypersensitivity; (6) active peptic ulcer(s); (7) history of gastric ulcer surgery; (8) ineligibility for eradication therapy as determined by a physician; (9) history of allergy to drugs used in this study; (10) serious coexisting illness that could interfere with the study.

Assessment of *H. pylori* infection and eradication

The presence of *H. pylori* prior to third-line eradication therapy was confirmed with the culture method using tissue samples from the greater curvature of the antrum and the greater curvature from the upper part of the gastric corpus. The culture method was used so that sensitivity testing of the study antibiotics could be done at the same time. When *H. pylori* testing was done using the rapid urease test (RUT), the tissue samples were cryopreserved.

Eradication assessment using the UBT was performed at 8 (±4) weeks after the completion of eradication therapy. The use of PPIs, bismuth preparations, and drugs with antiurease activity such as ecabet sodium was prohibited two weeks prior to third-line eradication testing with the urea breath test (UBT), in order to prevent these agents from affecting the test results.

Drug sensitivity testing

The sensitivity of *H. pylori* to each antibiotic was tested using the agar dilution method. The breakpoint was set at 0.5 μg/mL for AMPC, 1 μg/mL for CAM, 16 μg/mL for MNZ, 1 μg/mL for LVFX, and 1 μg/mL for STFX, and a strain was deemed to be resistant when its MIC value was equal to or exceeded the breakpoint.

Endpoints

The primary endpoint was the third-line eradication therapy eradication rate in the intention to treat (ITT) and per protocol (PP) groups. The secondary endpoints were a comparison of combined third- and fourth-line eradication therapy eradication rates and ADR onset when no intergroup differences were observed in the eradication rates of the respective third-line eradication therapies, and a comparison of the correlations between the sensitivity of *H. pylori* to each antibiotic and the third-line eradication rate.

Statistical analysis

Statistical analysis of intergroup differences in eradication rates was performed using a chi-squared test, and a hazard ratio of ≥5 was deemed significant.

Results

Study subjects and flow

The study enrolled 204 patients at 23 sites around Japan from September 2009 to August 2011. The assignment of patients and subsequent study flow are shown in Fig. 2. Patients were randomly assigned to the LA group ($n = 70$), LAL group ($n = 65$), and LAS group ($n = 70$) (ITT analysis set). Six patients taking each third-line treatment did not visit the hospital and dropped out. Third-line therapy was administered to 67, 64, and 68 patients, respectively (PP analysis set). Fourth-line eradication therapy was administered to a total of 65 patients for whom third-line therapy failed. Specifically, LAS was administered to 25 patients for whom LA failed, and LA was given to 40 for whom LAL or LAS failed. Nineteen patents taking the fourth-line treatment did not visit the hospital and dropped out.

The patient characteristics of the PP analysis set are presented in Table 1. Age (mean ± SD) was 60.3 ± 13.3 years, 55.3 % of patients were men, and reasons for *H. pylori* eradication were *H pylori*-positive gastritis in 73 patients (36.7 %), follow-up of endoscopic stomach cancer treatment in 36 (18.1 %), gastric ulcer in 43 (21.6 %), duodenal ulcer in 31 (15.6 %), and gastric/duodenal ulcer in 6 (3.0 %). Similar patient characteristics were seen in each of the three groups, with no major deviations in age, sex, or reason for eradication therapy.

Third-line therapy eradication rates

Drug compliance for third-line eradication therapy exceeded 90 % in 98.5 % of the LA group, 98.4 % of the LAL group, and 94.1 % of the LAS group, and there were no significant intergroup differences in drug compliance.

ITT analysis of third-line therapy eradication rates (95 % CI) showed that the rate was significantly higher in the LAS group (70.0 %) than in the LA group (54.3 %; $p < 0.05$) and the LAL group (43.1 %; $p < 0.001$) (Fig. 3). In PP analysis (95 % CI), the eradication rate was 56.7 % in the LA group, 43.7 % in the LAL group, and 72.1 % in the LAS group; the rate was significantly higher in the LAS group than in the LAL group ($p < 0.001$) (Fig. 4).

Fourth-line therapy eradication rates

The fourth-line eradication rate (95 % CI) was significantly higher among LAS-treated patients (25 patients from the LA group for whom third-line therapy failed), at 60.0 %, than for LA-treated patients (40 patients from the LAS and

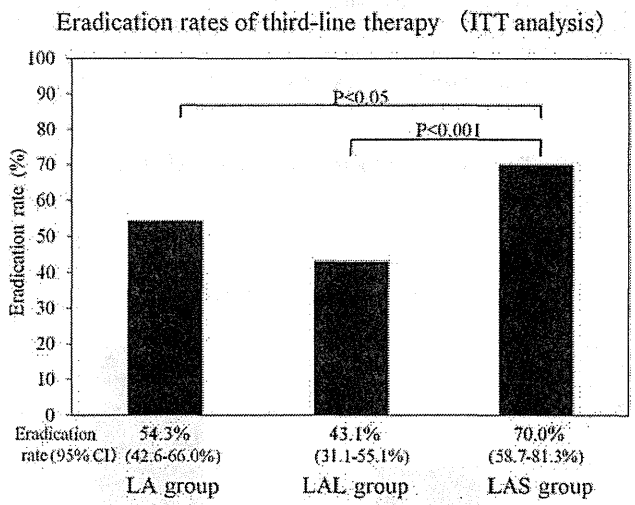

Fig. 3 ITT analysis of third-line therapy eradication rates. The eradication rate was significantly higher in the LAS group (70.0 %) than in the LA group (54.3 %; $p < 0.05$) and the LAL group (43.1 %; $p < 0.001$)

Table 1 Background of patients in this study (PP analysis)

	Total patients	LA ($n = 67$)	LAL ($n = 64$)	LAS ($n = 68$)
Age (mean ± SD)	60.3 ± 13.3	60.3 ± 12.7	61.7 ± 13.1	59.1 ± 14.1
% Male	55.3 %	44.8 %	65.6 %	55.9 %
Disease (*n*)				
Gastritis	73 (36.7 %)	25 (37.3 %)	20 (31.3 %)	28 (41.2 %)
After endoscopic therapy for gastric cancer	36 (18.1 %)	11 (16.4 %)	16 (25.0 %)	9 (13.2 %)
Gastric ulcer	43 (21.6 %)	15 (22.4 %)	13 (20.3 %)	15 (22.1 %)
Duodenal ulcer	31 (15.6 %)	12 (17.9 %)	8 (12.5 %)	11 (16.2 %)
Gastro-duodenal ulcer	6 (3.0 %)	2 (3.0 %)	1 (1.6 %)	3 (4.4 %)
Others	10 (5.0 %)	2 (3.0 %)	6 (9.4 %)	2 (2.9 %)

LA group LPZ 30 mg 4 times a day (qid) + AMPC 500 mg qid for 2 weeks

LAL group LPZ 30 mg twice a day (bid) + AMPC 750 mg bid + LVFX 300 mg bid for 1 week

LAS group LPZ 30 mg bid + AMPC 750 mg bid + STFX 100 mg bid for 1 week

LPZ lansoprazole, *AMPC* amoxicillin, *LVFX* levofloxacin, *STFX* sitafloxacin

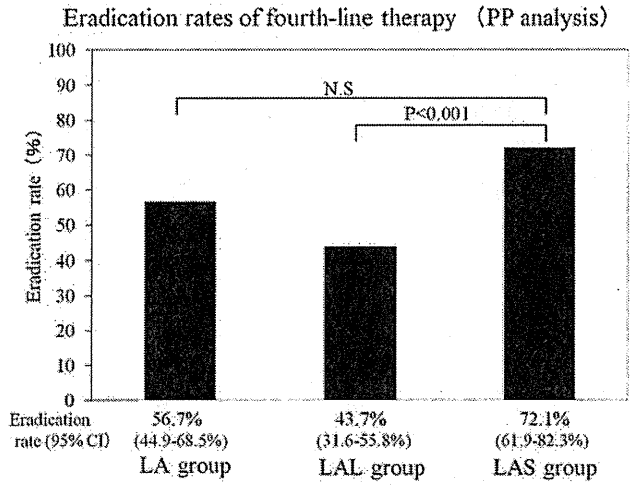

Fig. 4 PP analysis of third-line therapy eradication rates. The eradication rate was 56.7 % in the LA group, 43.7 % in the LAL group, and 72.1 % in the LAS group; the rate was significantly higher in the LAS group than in the LAL group ($p < 0.001$)

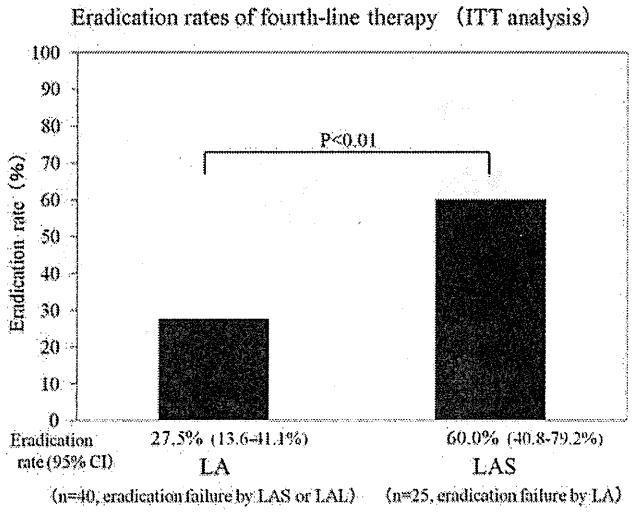

Fig. 5 ITT analysis of fourth-line eradication rates. The eradication rate was significantly higher in the LAS group (25 patients from the LA group for whom third-line therapy failed), at 60.0 %, than in the LA group (40 patients from the LAS and LAL groups for whom third-line therapy failed), at 27.5 % ($p < 0.01$)

LAL groups for whom third-line therapy failed), at 27.5 % ($p < 0.01$) (Fig. 5). There was no significant difference in fourth-line eradication rates with the LA regimen between the LAS group [25.0 % (3/12)] and the LAL group [28.6 % (8/28)]. Drug compliance for fourth-line therapy exceeded 90 % in all patients treated with LA or LAS.

Safety

The adverse event (AE) incidence for third-line eradication therapy was 11.9 % in the LA group, 17.2 % in the LAL group, and 16.2 % in the LAS group. The most frequent AE was diarrhea/soft stool, with incidences of 3.0, 10.9, and 16.2 % in the LA, LAL, and LAS groups, respectively. No AEs warranting discontinuation of the study were observed.

The AE incidence for fourth-line eradication therapy was 3.7 % in LA-treated patients and 4.0 % in LAS-treated patients; all of which were due to diarrhea/soft stool.

Table 2 Rates of resistance to antimicrobials

	AMPC	CAM	MNZ	LVFX	STFX
Cases for sensitivity testing	110	110	108	107	104
Cases of resistant strains	9	95	77	61	8
Rates of resistance (%)	8.2	86.4	71.3	57.0	7.7

Supposed breakpoints: AMPC, 0.5 μg/mL; CAM, 1 μg/mL; MNZ, 16 μg/mL; LVFX, 1 μg/mL; and STFX, 1 μg/mL

AMPC amoxicillin, *CAM* clarithromycin, *MNZ* metronidazole, *LVFX* levofloxacin, *STFX* sitafloxacin

Drug sensitivity testing and resistance

The number of drug sensitivity tests and the number of resistant strains detected (resistance rate) are shown in Table 2. This study targeted patients for whom first- and second-line eradication therapy failed, so many bacterial strains were found to be resistant to each of the study antibiotics. For each antibiotic, the following resistance rates were observed at the supposed breakpoints: AMPC, 8.2 % at 0.5 μg/mL; CAM, 86.4 % at 1 μg/mL; MNZ, 71.3 % at 16 μg/mL; LVFX, 57.0 % at 1 μg/mL; and STFX, 7.7 % at 1 μg/mL. As such, resistance rates were high for all drugs, but CAM-, MNZ-, and LVFX-resistant strains were particularly prevalent.

Drug resistance and third-line therapy eradication rates

A comparison of the correlations between drug resistance and eradication rate in the three third-line eradication regimens is shown in Table 3. Significant differences in the eradication rates for LVFX between sensitive and resistant strains in the LAL group ($p < 0.01$) and the LAS group ($p < 0.05$) can be seen. The eradication rate in the LAL group for LVFX-R was particularly low at 11 %, and that in the LAS group for LVFX-S was especially high at 89 %.

Discussion

The present study compared the efficacy and safety of three types of third-line eradication regimens in *H. pylori*-infected patients in whom first- and second-line therapies had already failed. One-week administration of LPZ/

Table 3 Drug resistance and eradication rates (%)

	LA	LAL	LAS	Total
AMPC				
R	50 % (1/2)	0 % (0/2)	80 % (4/5)	56 % (5/9)
S	50 % (16/32)	44 % (14/32)	70 % (26/37)	55 % (56/101)
CAM				
R	48 % (15/31)	39 % (12/31)	73 % (24/33)	54 % (51/95)
S	67 % (2/3)	67 % (2/3)	67 % (6/9)	67 % (10/15)
MNZ				
R	50 % (14/28)	38 % (9/24)	72 % (18/25)	53 % (41/77)
S	50 % (3/6)	56 % (5/9)	69 % (11/16)	61 % (19/31)
LVFX				
R	45 % (9/20)	11 % (2/18)	57 % (13/23)	39 % (24/61)
S	57 % (8/14)	71 %** (10/14)	89 %* (16/18)	74 %** (34/46)
STFX				
R	0 % (0/3)	0 % (0/3)	50 % (1/2)	13 % (1/8)
S	52 % (14/27)	46 % (14/30)	72 % (28/39)	58 %* (56/96)

AMPC amoxicillin, *CAM* clarithromycin, *MNZ* metronidazole, *LVFX* levofloxacin, *STFX* sitafloxacin, *R* resistant strain, *S* sensitive strain

* $p < 0.05$, ** $p < 0.01$

p values are for comparisons of the eradication rates of sensitive strains with the eradication rates of resistant strains in each regime

AMPC/STFX proved to be significantly more effective at eradicating *H. pylori* than the two-week LPZ/AMPC and one-week LPZ/AMPC/LVFX regimens. The LPZ/AMPC/STFX regimen was also well tolerated, suggesting that it would be highly useful in eradication therapy.

In Japan, both first- and second-line eradication therapy have a failure rate of approximately 3 %, based on supposed eradication rates of 70 and 90 %, respectively. However, in a study by Rokkas et al. [10] of 540 *H. pylori*-positive patients, ITT and PP analysis yielded first-line eradication rates of 70.3 and 76 %, respectively, and second-line eradication rates of 69.1 and 73.5 %, respectively, with 30 patients (5.5 %) receiving third-line eradication therapy. Although that study used different eradication regimens to those employed in Japan, the results suggest that the need for third-line eradication therapy in clinical practice may be greater than we imagined.

Among the third-line eradication regimens evaluated in this study, the two-week LPZ/AMPC regimen had a poor eradication rate: just 54.3 % in ITT analysis and 56.7 % in PP analysis. *H. pylori* does not tend to develop resistance to AMPC, so this antibiotic is more effective at eradicating the bacteria than CAM or MNZ [3]. Nevertheless, the results of sensitivity testing in the present study indicated that *H. pylori* resistance to AMPC was in fact relatively high (8.2 %) and the eradication rate was low, implying that a different antibiotic with more potent antibacterial activity was required to increase the eradication rate.

Furthermore, the eradication rate of the one-week LPZ/AMPC/LVFX regimen was even worse than that of the two-week LPZ/AMPC regimen: 43.1 % in ITT analysis and 43.7 % in PP analysis. LVFX has found widespread use in Japan for the treatment of respiratory and urinary tract infections, leading to concerns that *H. pylori* is becoming less susceptible to the drug. In the present study, the LVFX resistance rate reached 57 % in sensitivity testing, lending weight to these concerns of increased resistance. The *gyr*A mutation has been identified as one of the mechanisms responsible for acquired resistance to LVFX [15]. In overseas clinical studies of third-line eradication regimens based on LVFX, Gisbert et al. [9] reported eradication rates of 60 % in ITT analysis and 66 % in PP analysis, while Rokkas et al. [10] reported a rate of 70 % in both ITT and PP analyses, all of which exceed the eradication rates observed in the present study. The eradication rate of LVFX-resistant strains in the LAL group was also markedly diminished at 11 %. Third-line eradication regimens based on LVFX may be standard overseas, but in Japan, where resistance to LVFX is increasing, a different regimen is required for third-line therapy.

Meanwhile, STFX has a low MIC for *H. pylori* [11] and, when combined with PPI and AMPC, is anticipated to have a good eradication effect, even in third-line eradication therapy. Even in the present study, the eradication rate of the one-week LPZ/AMPC/STFX regimen was significantly higher than those of the other two regimens in ITT analysis at 70.0 %, and was significantly higher than that of the one-week LPZ/AMPC/LVFX regimen in PP analysis at 72.1 %. Moreover, the drug sensitivity test results showed that STFX resistance peaked at 7.7 %, compared to 57 % for LVFX. Eradication rates in the LAS group were also affected by LVFX resistance, but a favorable rate of 89 % was seen in LVFX-susceptible strains.

Although limited to observational studies without an established control group, good eradication results have recently been reported in Japan for STFX-based third-line eradication regimens, with Hirata et al. [16] reporting 75 % in ITT analysis and 80 % in PP analysis, and

Matsuzaki et al. [17] reporting 78.2 and 83.6 %, respectively. These findings imply that third-line therapy using this regimen could achieve an eradication rate of at least 70 %, even in patients with CAM- and MNZ-resistant *H. pylori* infections.

Recent drug sensitivity tests have reported that STFX demonstrated an MIC of ≤0.5 mg/mL for 105 strains of *H. pylori*, including 44 strains with the *gyr*A mutation [18], and that it can even exhibit antibacterial activity in LVFX-resistant *gyr*A mutant strains [17–19]. Furthermore, a study by Yamamoto et al. [20] evaluating the sensitivity of 105 strains of *H. pylori* (including CAM-, MNZ-, AMPC-, and LVFX-resistant strains) to ten types of antibiotics found that STFX had the lowest MIC50 and MIC90 values at 0.015 and 0.06 μg/mL, respectively.

Meanwhile, the issue of a viable fourth-line eradication regimen for use in cases of third-line therapy failure remains to be addressed. In the present study, LA therapy for patients for whom third-line LAS and LAL treatment failed produced a peak eradication rate of only 27.5 %. The number of LAS eradication therapy failures may have been small, but further research is still needed to identify an effective regimen.

The findings of the present study suggest that triple therapy with PPI, AMPC, and STFX for one week would be an effective standard third-line eradication regimen for *H. pylori* in Japan. We postulate that a first-line eradication therapy consisting of LPZ/AMPC/CAM for one week, a second-line eradication therapy of LPZ/AMPC/MNZ for one week, and a third-line therapy of LPZ/AMPC/STFX for one week might have a cumulative eradication rate of more than 99 %. However, a much better third-line regimen, with an eradication rate exceeding 90 %, needs to be established in the future.

Conflict of interest Mototsugu Kato received lecture fees from Takeda Pharmaceutical Company. Hideyuki Nomura received lecture fees from Daiichi Sankyo Co. Ltd. and MSD. Takahisa Huruta received research grants from Takeda Pharmaceutical Co., Ltd., AstraZeneca KK, Eisai Co., Ltd., and Daiichi Sankyo Co. Ltd.

References

1. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med. 2001;345:784–9.
2. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet. 1993;342:575–7.
3. Rimbara E, Noguchi N, Tanabe M, Kawai T, Matsumoto Y, Sasatsu M. Susceptibilities to clarithromycin, amoxycillin and metronidazole of *Helicobacter pylori* isolates from the antrum and corpus in Tokyo, Japan, 1995–2001. Clin Microbiol Infect. 2005;11:307–11.
4. Matsuhisa T, Kawai T, Masaoka T, Suzuki H, Ito M, Kawamura Y, et al. Efficacy of metronidazole as second-line drug for the treatment of *Helicobacter pylori* infection in the Japanese population: a multicenter study in the Tokyo Metropolitan Area. Helicobacter. 2006;11:152–8.
5. Shimoyama T, Fukuda S, Mikami T, Fukushi M, Munakata A. Efficacy of metronidazole for the treatment of clarithromycin-resistant *Helicobacter pylori* infection in a Japanese population. J Gastroenterol. 2004;39:927–30.
6. Malfertheiner P, Mégraud F, O'Morain C, Hungin AP, Jones R, Axon A, et al. Current concepts in the management of *Helicobacter pylori* infection—the Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther. 2002;16:167–80.
7. Beales IL. Efficacy of *Helicobacter pylori* eradication therapies: a single centre observational study. BMC Gastroenterol. 2001;1:7.
8. Gomollón F, Sicilia B, Ducóns JA, Sierra E, Revillo MJ, Ferrero M. Third line treatment for *Helicobacter pylori*: a prospective, culture-guided study in peptic ulcer patients. Aliment Pharmacol Ther. 2000;14:1335–8.
9. Gisbert JP, Castro-Fernández M, Bermejo F, Pérez-Aisa A, Ducons J, Fernández-Bermejo M. Third-line rescue therapy with levofloxacin after two *H. pylori* treatment failures. Am J Gastroenterol. 2006;101:243–7.
10. Rokkas T, Sechopoulos P, Robotis I, Margantinis G, Pistiolas D. Cumulative *H. pylori* eradication rates in clinical practice by adopting first and second-line regimens proposed by the Maastricht III consensus and a third-line empirical regimen. Am J Gastroenterol. 2009;104:21–5.
11. Sánchez JE, Sáenz NG, Rincón MR, Martín IT, Sánchez EG, Martínez MJ. Susceptibility of *Helicobacter pylori* to mupirocin, oxazolidinones, quinupristin/dalfopristin and new quinolones. J Antimicrob Chemother. 2000;46:283–5.
12. Hongo M, Ohara S, Hirasawa Y, Abe S, Asaki S, Toyota T. Effect of lansoprazole on intragastric pH. Comparison between morning and evening dosing. Dig Dis Sci. 1992;37:882–90.
13. Miwi H, Murai T, Sato K, Ohkura R, Yamada T, Nagahara A, et al. Comparison of the efficacy of 400 mg and 800 mg of clarithromycin used with lansoprazole and amoxicillin in eradication regimens for *Helicobacter pylori* infection in a Japanese population. J Gastroenterol. 2000;35:536–9.
14. Asaka M, Sugiyama T, Kato M, Satoh K, Kuwayama H, Fukuda Y, et al. A multicenter, double-blind study on triple therapy with lansoprazole, amoxicillin and clarithromycin for eradication of *Helicobacter pylori* in Japanese peptic ulcer patients. Helicobacter. 2001;6:254–61.
15. Miyachi H, Miki I, Aoyama N, Shirasaka D, Matsumoto Y, Toyoda M, et al. Primary levofloxacin resistance and gyrA/B mutations among *Helicobacter pylori* in Japan. Helicobacter. 2006;11:243–9.
16. Hirata Y, Ohmae T, Yanai A, Sakitani K, Hayakawa Y, Yoshida S, et al. Sitafloxacin resistance in *Helicobacter pylori* isolates and sitafloxacin-based triple therapy as a third-line regimen in Japan. Int J Antimicrob Agents. 2012;39:352–5.
17. Matsuzaki J, Suzuki H, Nishizawa T, Hirata K, Tsugawa H, Saito Y, et al. Efficacy of sitafloxacin-based rescue therapy for *Helicobacter pylori* after failures of first- and second-line therapies. Antimicrob Agents Chemother. 2012;56:1643–5.
18. Murakami K, Okimoto T, Kodama M, Tanahashi J, Fujioka T, Ikeda F, et al. Sitafloxacin activity against *Helicobacter pylori* isolates, including those with gyrA mutations. Antimicrob Agents Chemother. 2009;53:3097–9.
19. Suzuki H, Nishizawa T, Muraoka H, Hibi T. Sitafloxacin and garenoxacin may overcome the antibiotic resistance of *Helicobacter pylori* with gyrA mutation. Antimicrob Agents Chemother. 2009;53:1720–1.
20. Yamamoto T, Takano T, Higuchi W, Nishiyama A, Taneike I, Yoshida K, et al. *Helicobacter pylori* eradication by sitafloxacin-lansoprazole combination and sitafloxacin pharmacokinetics in Mongolian gerbils and its in vitro activity and resistance development. Antimicrob Agents Chemother. 2011;55:4261–6.

20122100 9A(2/2)

厚生労働科学研究費補助金

がん臨床研究事業

ピロリ菌除菌による胃癌予防の経済評価に関する研究

平成２４年度　総括・分担研究報告書

研究代表者　加藤　元嗣

平成２５（２０１３）年　５月

２／２冊