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Eradication of *Helicobacter pylori* prevents cancer development in subjects with mild gastric atrophy identified by serum pepsinogen levels

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A longitudinal cohort study was conducted in *Helicobactor pylori*-infected middle-aged Japanese males to evaluate the preventive effects of *H. pylori* eradication on the development of gastric cancer according to the extent of chronic atrophic gastritis (CAG). The extent of CAG was monitored by baseline serum pepsinogen (PG) levels. We followed 3,656 subjects with persistent *H. pylori* infection and 473 subjects with successful *H. pylori* eradication for cancer development for a mean (SD) of 9.3 (0.7) years. Groups with and without extensive CAG were categorized based on PG test-positive criteria to detect extensive CAG of PG I $\leq$ 70 ng/ml and PG I/II ratio $\leq$ 3.0. During the study period, 5 and 55 gastric cancers developed in *H. pylori*-eradicated and the noneradicated subjects, respectively, indicating no significant reduction in cancer incidence after *H. pylori* eradication. Among the noneradicated subjects, 1,329 were PG test-positive and 2,327 were PG test-negative. Gastric cancer was confirmed in 30 and 25 subjects, respectively. Among subjects whose infection was eradicated, 155 were PG test-positive and 318 were PG test-negative. Of these subjects, gastric cancer was confirmed in 3 and 2 subjects, respectively. Significant reduction in cancer incidence after eradication was observed only in PG test-negative subjects ($p < 0.05$; log-rank test). The results of this study strongly indicate that cancer development after eradication depends on the presence of extensive CAG before eradication and that *H. pylori* eradication is beneficial to most PG test-negative subjects with mild CAG as defined by the aforementioned criteria.

Key words: pepsinogen; *Helicobacter pylori*; eradication; cancer prevention; gastric cancer

Helicobacter pylori is recognized as a major pathogenic factor for persistent inflammation in the human stomach.[1,2] Chronic active gastritis induced by *H. pylori* is involved in the development of peptic ulceration[3] and is also considered a critical component that triggers the first stage of a series of precancerous processes, that is, the atrophy-metaplasia-dysplasia-cancer sequence.[4] In 1994, the International Agency for Research on Cancer (IARC) classified *H. pylori* infection as a definite (class I) carcinogen.[5] Nowadays, it is widely accepted that *H. pylori* is a major risk factor for developing gastric cancer together with its precursor lesions based on extensive evidence derived from clinico-epidemiologic studies[6–15] and experiments with *H. pylori*-infected animals.[16–22] Therefore, it appears quite reasonable to conclude that gastric cancer is theoretically preventable by the eradication of *H. pylori*.

Evidence from the murine model of *H. pylori* infection provides strong support for the reversibility of *H. pylori*-induced mucosal changes in the stomach with early bacterial eradication, and that eradication therapy, even in longstanding *H. pylori* infection, would prevent stomach carcinogenesis.[23–25] To date, several randomized and nonrandomized human intervention trials have been conducted to prove the effectiveness of *H. pylori* eradication in the control of gastric cancer.[15,26–31] However, unlike the case with peptic ulcer disease in which *H. pylori* eradication can significantly modify the natural history by preventing recurrence,[32–35] it remains unclear whether the eradication of *H. pylori* is an effective strategy for reducing the incidence of and mortality from gastric cancer.

Several previous studies revealed that the more advanced the stage of *H. pylori*-related chronic atrophic gastritis (CAG)—that is, the extent of gastric atrophy together with intestinal metaplasia—the greater the cancer risk.[36–40] Thus, the evaluation of CAG is especially important in the analysis of cancer prevention by *H. pylori* eradication. However, previous studies have not assessed the extent of coexisting CAG[15,31] or have assessed it only with endoscopic findings[29,30] and/or histopathology on endoscopic biopsy.[26–28] Both of these methods of diagnosis of gastric atrophy depend on subjective judgment without a gold standard; the reported intra- and inter-observer agreement among these methods are not satisfactory.[41,42] A randomized controlled trial would be an alternative approach. However, this type of trial would not be ethically feasible with today's knowledge about *H. pylori* infection and the development of cancer. Furthermore, the only 2 randomized controlled trials of *H. pylori* eradication in a high-risk area of China had inconsistent results, whereas 1 indicated a possibility of a beneficial effect of eradication in an early phase of *H. pylori* infection,[26] the other showed that *H. pylori* eradication did not lead to a significant reduction in cancer regardless of the severity of coexisting CAG.[28] These 2 studies stratified the extent of CAG based on the histopathology of endoscopic biopsy samples. Since CAG, including intestinal metaplasia, is a multifocal process, a certain degree of random error in the evaluation of CAG will also occur in the analysis of endoscopic biopsy samples. Meanwhile, a considerable number of studies, including ones by us, indicated that serum pepsinogen (PG) levels give an objective and reliable measure for the extent of CAG.[43–45]

Our objective in this study was to elucidate the preventive effects of *H. pylori* eradication according to the extent of *H. pylori*-related CAG, using PG levels to ascertain the degree of CAG. We performed long-term follow-up (mean follow-up period ± SD, 9.3 ± 0.7 y) on the development of gastric cancer in a cohort of *H. pylori*-infected subjects, with and without eradication, in whom serum PG levels were measured.

Abbreviations: CAG, chronic atrophic gastritis; ELISA, enzyme-linked immunosorbent assay; *H. pylori*, *Helicobacter pylori*; IgG, immunoglobulin G; PG, pepsinogen; RIA, radioimmunoassay; SD, standard deviation.

A part of this study was presented at the AGA 2007 conference in Washington, D.C., and was selected as a poster of distinction.

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Subjects and methods

Study subjects

The study was conducted in Wakayama City, Wakayama Prefecture, located in the southwestern part of the main island of Japan. The gastric cancer mortality rate in Wakayama Prefecture is among the highest in Japan. It ranked fourth highest in 2005, with a mortality rate of 53.0/100,000 person-years, whereas for all of Japan the mortality rate for gastric cancer was 39.9/100,000 person-years. Between April 1994 and the end of March 1995, 8,420 factory workers (8,236 males, 184 females) aged 40–60 years underwent an annual multiphasic health screening program. This type of screening program is a common activity in various workplaces throughout Japan. It is done to detect incident diseases in their early stages. Thus, subjects were symptom-free and those who had symptoms requiring prompt medical care were excluded. Therefore, the subjects could be considered to represent the healthy Japanese population.

The study subjects underwent a series of screening tests and procedures: an interview to ascertain the general state of health, physical examination, chest radiography, electrocardiogram, laboratory blood tests, urinalysis, fecal occult blood test, and barium X-ray with digital radiography (DR). Subjects who had a previous history of gastric cancer or adenoma, surgical resection of the stomach, *H. pylori* eradication, or renal failure and those who had been prescribed medication that might affect gastrointestinal function, such as proton pump inhibitors, adrenocortical steroids, or non-steroidal anti-inflammatory drugs, were excluded from the study. Furthermore, the subjects with *H. pylori* infection were selected for the study using serum-specific antibody titers as described in the following section. Thus, 5,776 subjects with *H. pylori* infection (5,645 males, 131 females) were eligible for this study. Some of these subjects had been investigated in a previous cohort study.[46]

Diagnosis of extensive CAG and H. pylori *infection*

The aliquots of separated sera from fasting blood samples collected as routine laboratory tests for the aforementioned general health check-up were stored at −20°C and used for the measurement of serum PG levels and anti-*H. pylori* IgG titers. Serum PG (PG I and II) levels were measured using a modification (RIA-beads Kit, Dainabott, Tokyo, Japan) of our previously reported RIA.[47] Subjects with extensive CAG were diagnosed based on the PG test-positive criteria of PG I $\leq$ 70 ng/ml and PG I/II ratio $\leq$ 3.0.[48,49] These criteria offer a sensitivity of 70.5% and a specificity of 97% in the diagnosis of extensive CAG, using pathological diagnosis as the gold standard.[48] Serum anti-*H. pylori* IgG titers were measured using ELISA (MBL Inc., Nagoya, Japan).[50] Subjects with antibody titers >50 U/ml were classified as *H. pylori*-infected and those with antibody titers <30 U/ml were regarded as infection negative. Subjects with a titer level that was $\geq$30 U/ml and $\leq$50 U/ml were considered indeterminate. The infection-negative and indeterminate subjects were excluded from the study. The sensitivity and specificity of the ELISA test used in this study were 93.5 and 92.5%, respectively.[50]

Treatment of H. pylori *and follow-up*

From April 1994 to March 1997, 5,776 *H. pylori*-infected subjects were informed of their infection and were advised to visit the clinic at their workplace. At the clinic they were told about the possible role of *H. pylori* infection in gastroduodenal disorders, including peptic ulcer disease and gastric cancer, and also about the possible side effects of *H. pylori* eradication therapy. As a result, 852 *H. pylori*-infected subjects (728 males, 124 females) underwent *H. pylori* eradication with dual therapy consisting of the proton pump inhibitor omeprazole 20 mg twice a day and amoxicillin 750 or 500 mg twice a day for 2 weeks, or with triple therapy consisting of the proton pump inhibitor omeprazole 20 mg twice a day, amoxicillin 750 mg twice a day, and clarithromycin 200 mg twice a day for 1 week. *H. pylori* status was assessed by serum *H. pylori* antibody level at the annual health check-up. All subjects were followed until the end of the study period of 10 years, which was the end of March 2004. Subjects underwent the aforementioned health screening program annually and were also screened to identify incident gastric cancer as described in the following section. The incident day of gastric cancer was defined as the day of the health check-up when the cancer was detected. Duration of the observation period was calculated for each subject from the time of the baseline survey to the diagnosis of gastric cancer. The ethics committee of the workplace approved the protocol, and informed consent was obtained from all participating subjects.

Screening for gastric cancer

Cancer screening was performed by double-contrast barium X-ray with digital radiography (DR) and by serum PG as filter tests. For upper gastrointestinal barium X-ray, a remote controlled X-ray fluoroscope (TU-230XB, Hitachi Medical Corporation, Tokyo, Japan) and real-time DR (DR-2000H, Hitachi Medical Corporation, Tokyo, Japan) were used. The double-contrast upper gastrointestinal X-ray series used 150 ml of high-concentration barium at 200%, and 11 films were taken for each subject as described previously.[46] Subjects were also screened using the serum PG filter test. Those with positive barium X-ray findings and/or PG test-positive result were further examined by upper gastrointestinal endoscopy (XQ-200, Olympus, Tokyo, Japan).

Resected specimens of gastric cancer obtained by endoscopy or surgery were assessed histopathologically and classified according to Lauren's classification into intestinal or diffuse type.[51] Location of the cancer in the stomach was classified as cardia or noncardia based on clinical and histopathological records.

Statistical analysis

Data were analyzed using SPSS 11.0 (SPSS, Chicago, IL) and STATA (STATA Corp., College Station, TX). Differences were tested for significance using the *t* test for comparisons between 2 groups, analysis of variance (ANOVA) for comparisons among multiple groups, and Scheffe's LSD test for comparisons of pairs of groups. The chi-square test and Fisher's exact test were used to compare categorical variables. Long-term effects of *H. pylori* eradication on gastric cancer development were analyzed by the Kaplan-Meier method, and statistical differences between curves were tested by the log-rank test.

Results

Among the 5,776 *H. pylori*-positive subjects, 852 received eradication therapy. Of these subjects, 743 (87.2%) (643 males, 100 females) became *H. pylori*-negative after eradication. The remaining 4,924 subjects (4,917 males, 7 females) did not receive eradication therapy (Fig. 1). Since there was an imbalance in the number of female subjects included in the groups with and without eradication, all results of the female subjects were excluded from the analysis. Among the 743 successfully eradicated subjects, 169 either dropped out or became *H. pylori*-positive during the follow-up period. Among the 4,924 subjects with persistent *H. pylori infection*, 1,253 dropped out, underwent eradication therapy after March 1997, or experienced spontaneous resolution of their infection. These subjects were also excluded from the analysis. In addition, we excluded the 1 case of gastric cancer in the *H. pylori*-eradicated subjects and the 8 cases in the noneradicated subjects that had developed in the first year of the study since the cancers might have existed at the start of the study. Thus, to ensure completeness of the diagnosis of *H. pylori* infection or gastric cancer development during the study period, only data from the survivors in March 2004-that is, the 473 subjects with successful eradication and 3,656 subjects with persistent infection-were analyzed.

As shown in Table I, there were no significant differences in baseline characteristics between the subjects with and without

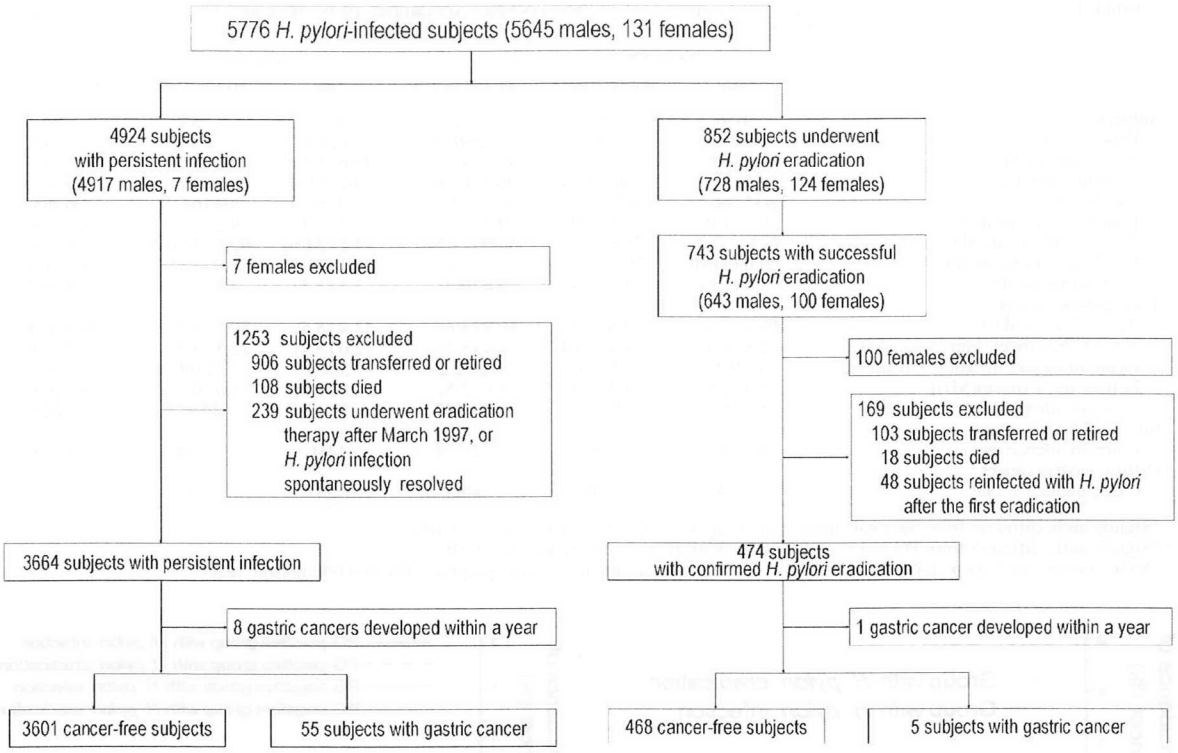

FIGURE 1 – Schematic flow of the study subjects.

eradication in terms of age, current smoking, and current drinking habits. Of the 3,656 subjects with persistent *H. pylori* infection, 36.4% (n = 1,329) had extensive CAG based on serum PG levels using the aforementioned PG test-positive criteria of PG I $\leq$ 70 ng/ml and PG I/II ratio $\leq$ 3.0.[48,49] This figure is not significantly different from that in the 473 *H. pylori*-eradicated subjects, that is, 32.8% (n = 155). Furthermore, the mean levels of serum PG I and PG II and the PG I/II ratio did not differ significantly between the *H. pylori*-eradicated and noneradicated subjects, indicating that the extent of coexisting gastric atrophy was similar in the 2 groups (Table I).

During the study period, 55 subjects developed gastric cancer among the 3,656 subjects with persistent *H. pylori* infection, and 5 subjects developed cancer among the 473 subjects cured of the infection. The Kaplan-Meier analysis revealed that cancer development in the subjects with persistent infection occurred steadily throughout the study period (Fig. 2) In the *H. pylori*-eradicated subjects, on the other hand, cancer development was null until 4 years after the start of the follow-up, and the first cancer was detected 5 years after eradication. Thereafter, cancer development was observed to increase steadily to the level of the noneradicated subjects by the end of the study period. The cancer incidence rates in the *H. pylori*-eradicated and noneradicated subjects were 117/100,000 person-years and 157/100,000 person-years, respectively. There was not a significant reduction in cancer development according to *H. pylori* eradication in the cohort as a whole (p = 0.55; log-rank test).

Macroscopically, 2 cancers (3.3%) that developed in the noneradicated subjects were located in the cardia, and the other 58 cancers (96.7%) were located elsewhere in the stomach. The mean size of the cancers was smaller in the *H. pylori*-eradicated subjects than in the noneradicated subjects, although the difference was not statistically significant. Among the cancers that developed in the noneradicated subjects, 9.1% (5/55) were detected at an advanced stage and were invading the muscularis propria, 16.4% (9/55) were submucosal, and 74.5% (41/55) were mucosal. In contrast, the cancers in the *H. pylori*-eradicated subjects were all mucosal.

As for the 2 histopathological types, the intestinal-type was the dominant phenotype regardless of *H. pylori* eradication, and there was no significant difference in the incidence rate of this type of cancer between the *H. pylori*-eradicated and noneradicated subjects (94/100,000 person-years vs. 103/100,000 person-years). In contrast, the proportion of diffuse-type cancer was significantly reduced by eradication; the proportions were 20% (1/5) in the *H. pylori*-eradicated subjects and 34.5% (19/55) in the noneradicated subjects ($p < 0.05$). The incidence rate of this type of cancer was significantly lower in the *H. pylori*-eradicated subjects (23/100,000 person-years) compared with the noneradicated subjects (54/100,000 person-years) ($p < 0.05$) (Table I).

Next, we analyzed the effects of *H. pylori* eradication on cancer development according to the extent of coexisting gastric atrophy as evaluated by serum PG levels. The study subjects were classified into PG test-positive and PG test-negative groups based on the aforementioned criteria. In both the *H. pylori*-eradicated and noneradicated subjects, the PG test-positive group had more smokers and more alcohol drinkers than the negative group, but the difference was significant only in the noneradicated subjects ($p < 0.05$). The serum PG levels (PG I, PG II) and the PG I/II ratio, did not differ significantly between the *H. pylori*-eradicated and noneradicated subjects, irrespective of the positivity of the PG test, except that the mean PG I level of the PG-negative group was significantly higher in the *H. pylori*-eradicated subjects than in the noneradicated subjects (Table I).

Figure 3 shows the cumulative incidence of cancer in the *H. pylori*-eradicated and noneradicated subjects stratified by PG test criteria (PG I $\leq$ 70 ng/ml, PG I/II ratio $\leq$ 3.0). In the PG test-positive group with extensive CAG, gastric cancer was confirmed

TABLE I – EFFECT OF *H. PYLORI* ERADICATION ON GASTRIC CANCER DEVELOPMENT ACCORDING TO PG TEST RESULTS, BASED ON POSITIVE CRITERIA OF PG I LEVEL ≤ 70 mg/l AND PG I/II RATIO ≤ 3.0

	Group with *H. pylori* infection			Group with *H. pylori* eradication		
	Total	PG test-negative	PG test-positive	Total	PG test-negative	PG test-positive
Subjects	3,656	2,327	1,329	473	318	155
Person-years	35101.5	22,436	12665.5	4,263	2,918	1,345
Age, y [mean(SD)]	49.8 (4.6)	49.5 (4.7)	50.4 (4.3)	49.6 (5.5)	49.3 (5.6)	50.9 (5.5)
Current smoking, *n* (%)	2,087 (57.1)	1,242 (53.4)	845 (63.6)**	262 (55.4)	169 (53.1)	93 (60.0)
Alcohol use, *n* (%)	2,432 (66.5)	1,479 (63.6)	953 (71.7)**	310 (65.5)	204 (64.2)	106 (68.4)
Follow-up, y [mean(SD)]	9.6 (1.0)	9.6 (0.9)	9.5 (1.1)	9.1 (1.4)	9.2 (1.3)	8.7 (1.6)
PG I, ng/ml [mean(SD)]	62.0 (32.5)	75.8 (31.1)	37.9 (17.5)	74.2 (34.6)	92.5 (57.0)**	36.8 (18.2)
PG II, ng/ml [mean(SD)]	20.0 (11.0)	20.3 (12.4)	19.5 (7.8)	23.1 (14.6)	24.9 (16.5)	19.5 (8.4)
PG I/II [mean(SD)]	3.4 (1.6)	4.3 (1.4)	2.0 (0.7)	3.4 (1.5)	4.1 (1.3)	1.9 (0.7)
Total gastric cancer						
Age, y [mean(SD)]	50.8 (3.9)	50.6 (4.3)	50.9 (3.6)	53.4 (5.5)	50.5 (3.5)	55.3 (6.4)
Size of the cancer, mm [mean(SD)]	12.8 (8.3)	14.4 (10.4)	11.4 (5.3)	12.0 (5.7)	12.5 (3.5)	11.7 (7.6)
Depth of cancer invasion (m:sm:mp)[1]	41:9:5	15:7:3	26:2:2	5:0:0	2:0:0	3:0:0
Follow-up, y [mean(SD)]	5.9 (2.5)	4.3 (2.4)	5.6 (2.5)	6.0 (1.0)	5.0 (0)	6.7 (0.6)
Cases/incidence rate[2]	55/157	25/111	30/237	5/117	2/69**	3/223
Intestinal gastric cancer						
Cases/incidence rate[2]	36/103	14/62	22/174	4/94	1/34	3/223
Diffuse gastric cancer						
Cases/incidence rate[2]	19/54	11/49	8/63	1/23*	1/34	0

*Significantly different from the total subjects in group with *H. pylori* infection ($p < 0.05$).
**Significantly different from PG test-negative group with *H. pylori* infection ($p < 0.05$).
[1]Abbreviations used are as follows: m, mucosa; sm, submucosa; mp, muscularis propria.–[2]Per 100,000 person-years.

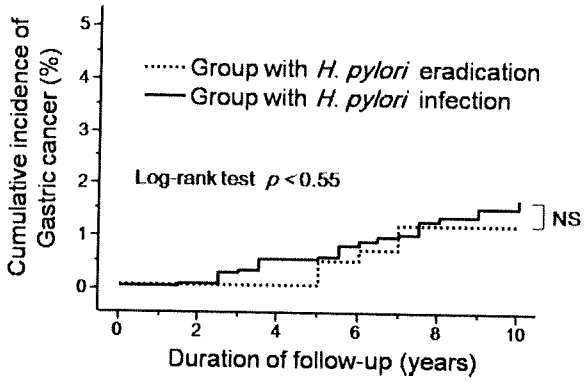

FIGURE 2 – Kaplan-Meier analysis of gastric cancer development in subjects with persistent *H. pylori* infection and in those whose infection was eradicated. The cancer incidence rates of the former and the latter were 157/100,000 person-years and 117/100,000 person-years, respectively. There was no significant difference in cancer development between the 2 groups ($p = 0.55$; log-rank test).

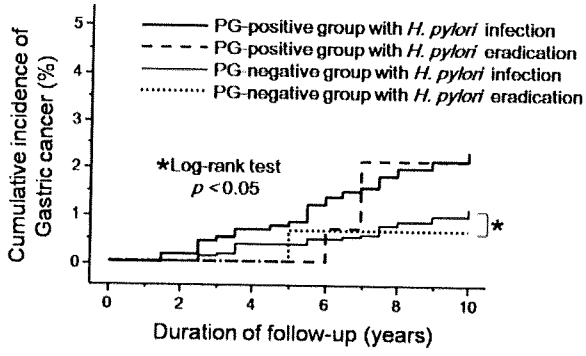

FIGURE 3 – Kaplan-Meier analysis of gastric cancer development in the PG test-positive group with extensive CAG and PG test-negative group with mild CAG according to *H. pylori* infection status. The positive criteria for PG test to detect extensive CAG were PG I level ≤ 70 μg/l and PG I/II ratio ≤ 3.0. Among the subjects with persistent *H. pylori* infection, the cancer incidence rates for the PG test-positive and PG test-negative groups were 237/100,000 person-years and 111/100,000 person-years, respectively. Meanwhile, comparing the *H. pylori*-eradicated subjects, the cancer incidence rates of the PG test-positive and PG test-negative groups were 223/100,000 person-years and 69/100,000 person-years, respectively. Eradication of *H. pylori* significantly reduced cancer development in the PG test-negative group with mild CAG ($p < 0.05$; log-rank test), but not in the PG test-positive group with extensive CAG.

in 3 and 30 subjects among the *H. pylori*-eradicated and noneradicated subjects, respectively, thus, putting the cancer incidence rate at 223/100,000 person-years and 237/100,000 person-years, respectively. Likewise, in the PG test-negative group with mild CAG, cancer was confirmed in 2 and 25 subjects among the *H. pylori*-eradicated and noneradicated subjects, respectively, and the cancer incidence rates were 69/100,000 person-years and 111/100,000 person-years, respectively. Significant reduction in cancer development according to eradication was observed only in the PG test-negative group ($p = 0.04$; log-rank test).

Eradication tended to decrease the development of both intestinal-type and diffuse-type cancer in the PG test-negative group, both showing a p value of less than 0.1. The effect of eradication on the incidence rate of intestinal-type cancer was of marginal significance ($p = 0.06$). In the PG test-positive group, eradication led to an increase in the incidence rate of intestinal-type cancer, although it was not a statistically significant difference. In contrast, the development of diffuse-type cancer in the PG test-positive group was null after eradication (Table I).

In addition to the PG test-positive criteria of PG I ≤ 70 ng/ml and PG I/II ratio ≤ 3.0, which are the most widely used criteria to detect subjects with extensive CAG in Japan,[48,49] stricter criteria of PG I ≤ 50 ng/ml and PG I/II ratio ≤ 3.0, and PG I ≤ 30 ng/ml and PG I/II ratio ≤ 2.0 are used to detect subjects with more extensive atrophy.[52] In this study, the accuracy of cancer detection among the *H. pylori*-eradicated subjects using the criteria PG I ≤ 50 ng/ml and PG I/II ratio ≤ 3.0 was better and more balanced than those by the other 2 sets of criteria, showing a sensitivity of 60% and specificity of 76.7%. The accuracy of cancer detection by the criteria of PG I ≤ 70 ng/ml and PG I/II ratio ≤ 3.0 had a sensitivity of 60% and specificity of 67.5%. Furthermore, the sensitivity and specificity for the criteria of PG I ≤ 30 ng/ml and PG I/II ratio ≤ 2.0 were 20 and 89.3%, respectively. Therefore, the

TABLE II – EFFECT OF *H. PYLORI* ERADICATION ON GASTRIC CANCER DEVELOPMENT ACCORDING TO PG TEST RESULTS, BASED ON POSITIVE CRITERIA OF PG I LEVEL ≤ 50 mg/l AND PG I/II RATIO ≤ 3.0

	Group with *H. pylori* infection			Group with *H. pylori* eradication		
	Total	PG test-negative	PG test-positive	Total	PG test-negative	PG test-positive
Subjects	3,656	2,690	966	473	361	112
Person-years	35101.5	25943.5	9,158	4,263	3319	944
Age, y [mean(SD)]	49.8 (4.6)	49.5 (4.6)	50.8 (4.3)	49.6 (5.5)	49.1 (5.1)	51.0 (4.6)
Current smoking, *n* (%)	2,087 (57.1)	1,471 (54.7)	616 (63.8)**	262 (55.4)	201 (55.7)	61 (54.5)
Alcohol use, *n* (%)	2,432 (66.5)	1,743 (64.8)	689 (71.3)	310 (65.5)	235 (65.1)	75 (67.0)
Follow-up, y [mean(SD)]	9.6 (1.0)	9.6 (0.9)	9.5 (1.2)	9.1 (1.4)	9.2 (1.3)	8.7 (1.5)
PG I, ng/ml [mean(SD)]	62.0 (32.5)	73.6 (29.6)	29.8 (12.9)	74.2 (34.6)	88.6 (54.6)**	27.8 (12.4)
PG II, ng/ml [mean(SD)]	20.0 (11.0)	21.1 (12.0)	16.9 (6.5)	23.1 (14.6)	25.2 (15.7)	16.5 (6.6)
PG I/II [mean(SD)]	3.4 (1.6)	4.0 (1.5)	1.8 (0.7)	3.4 (1.5)	3.9 (1.3)	1.8 (0.7)
Total gastric cancer						
Age, y [mean (SD)]	50.8 (3.9)	50.3 (4.3)	51.4 (3.4)	53.4 (5.5)	50.5 (3.5)	55.3 (6.4)
Size of the cancer, mm [mean(SD)]	12.8 (8.3)	13.4 (9.2)	11.9 (6.9)	12.0 (5.7)	12.5 (3.5)	11.7 (7.6)
Depth of cancer invasion (m:sm:mp)[1]	41:9:5	19:7:4	22:2:1	5:0:0	2:0:0	3:0:0
Follow-up, y [mean(SD)]	5.9 (2.5)	6.2 (2.5)	5.3 (2.5)	6.0 (1.0)	5.0 (0)	6.7 (0.6)
Cases/incidence rate[2]	55/157	30/116	25/273	5/117	2/60**	3/318
Intestinal gastric cancer						
Cases/incidence rate[2]	36/103	15/58	21/230	4/94	1/30	3/318
Diffuse gastric cancer						
Cases/incidence rate[2]	19/54	15/58	4/43	1/23*	1/30	0

*Significantly different from the total subjects in group with *H. pylori* infection ($p < 0.05$).
**Significantly different from PG test-negative group with *H. pylori* infection ($p < 0.05$).
[1]Abbreviations used are as follows: m, mucosa; sm, submucosa; mp, muscularis propria.–[2]per 100,000 person-years.

effect of eradication on cancer development in the PG test-positive and PG test-negative groups based on the criteria of PG I ≤ 50 ng/ml and PG I/II ratio ≤ 3.0 was also analyzed post hoc. As shown in Table II, the analysis revealed a more pronounced reduction in the cancer incidence rate in the PG test-negative group after eradication regardless of histopathological type of cancer, with an incidence rate in the *H. pylori*-eradicated and the noneradicated subjects of 60/100,000 person-years and 116/100,000 person-years, respectively ($p = 0.04$; log-rank test). At the same time, there was no significant reduction, but rather an increase, in the cancer incidence rate in the PG test-positive group after eradication, with an incidence rate of 318/100,000 person-years and 273/100,000 person-years in the *H. pylori*-eradicated and noneradicated subjects, respectively (Table II). In contrast, the results of the analysis using the other criteria of PG I ≤ 30 ng/ml and PG I/II ratio ≤ 2.0 revealed that the effect of eradication was not remarkable in the control of cancer development in the PG test-positive and PG test-negative groups (not shown).

Discussion

Previous studies analyzing the effect of *H. pylori* eradication on the development of gastric cancer have not sufficiently assessed the degree of background CAG, which is a major risk factor for gastric cancer.[36–40,46] In this study, we monitored the extent of coexisting CAG in the study subjects using serum PG levels. As shown by the serum PG levels in the *H. pylori*-eradicated and noneradicated subjects, there was no significant difference in the extent of coexisting CAG between the 2 groups. The results of this longitudinal cohort study demonstrated that the preventive effect of *H. pylori* eradication on cancer development is not evident in an asymptomatic middle-aged population in a high-risk gastric cancer area of Japan. This is in accord with the results of 2 previous randomized controlled studies in another country with a high risk of gastric cancer (China).[26,28] It is highly probable that *H. pylori* eradication targeted to middle-aged subjects in cancer high-risk areas does not lead to reduction in cancer development at the population level.

It is noteworthy that there was a marked delay in cancer development in the *H. pylori*-eradicated subjects, and the cancers that developed were less advanced compared with those in the subjects with persistent infection; all the cases were early mucosal cancer that could be treated by endoscopic resection. This is consistent with the results of animal experiments demonstrating the role of *H. pylori* infection as a promoter in stomach carcinogenesis.[20–22] It also indicates that the initiated cells in the *H. pylori*-infected stomach mucosa, once established as a result of long-lasting inflammation, tend to remain in the deranged structure of the mucosa even after eradication. Without the promoter action, their proliferation is markedly suppressed; however, they grow steadily, leading in time to clinically detectable cancer. Therefore, if the study period were shorter than 5 years, the results would have been quite different, indicating the effectiveness of eradication in the control of cancer in the general population. The difference between our results and some of the previous studies will probably be explained by the relatively short follow-up periods of the other studies, that is, a mean of around 3 years.[29–31]

In this study, we found that *H. pylori* eradication led to a reduction in cancer development in the PG test-negative group with mild CAG, defined by the aforementioned PG test-positive criteria. On the basis of the serum PG levels, the extent of CAG was similar in the *H. pylori*-eradicated and the noneradicated subjects in the PG test-positive group. However, in the PG test-negative group, the mean serum PG I level was significantly higher in the *H. pylori*-eradicated subjects than in the noneradicated subjects. The results of previous studies demonstrated that serum PG levels are elevated in *H. pylori*-related nonatrophic gastritis, reflecting the severity of histological changes due to mucosal inflammation.[53,54] *H. pylori* eradication reverses the serum PG elevation.[55,56] In general, the inflammatory process in the nonatrophic stomach is reported to induce a series of molecular events leading to the development of cancer, especially the diffuse type.[10,57,58] Thus, in the PG test-negative group, the basal level of activity of gastritis and cancer risk are considered to be no less in the *H. pylori*-eradicated subjects than in the noneradicated subjects. *H. pylori* eradication significantly reduced the development of cancer in the PG test-negative group with mild CAG, though it was not sufficient to eradicate gastric cancer in all subjects. In contrast, the effect of eradication was not so remarkable in the PG test-positive group with extensive CAG.

These results are in line with the results of some of the previous randomized and nonrandomized studies in which the extent of CAG was evaluated based on endoscopic findings or histopathology of endoscopic biopsy specimens, demonstrating that eradication is not beneficial in preventing cancer development in a subgroup of subjects with premalignant lesions, that is, CAG,

intestinal metaplasia, and dysplasia.[26,29] The results also appear to indicate that most, if not all, of the PG test-positive subjects are beyond the point of no return in stomach carcinogenesis. Previous observations regarding the long-term effect of *H. pylori* eradication indicate that the regression of extensive atrophy together with intestinal metaplasia occurs slowly as a function of the square of the *H. pylori*-free time, and complete recovery of intestinal metaplasia takes more than 12 years once the infection has been eradicated.[59] On the other hand, eradication in the early stage of *H. pylori*-related gastritis without intestinal metaplasia will lead to recovery or even normalization of the structure and function of the gastric mucosa within a short period of time.[59] It is presumed that the carcinogenic potential of the *H. pylori*-eradicated stomach mucosa does not return to the basal level of uninfected mucosa until complete regression of gastric atrophy and intestinal metaplasia is attained. Thus, as this results confirm, it is highly probable that cancer incidence in the PG test-positive group with extensive CAG does not decrease as markedly within a period of 10 years after eradication compared with the negative group with mild CAG. Further long-term morphological and genetic studies will be required to clarify whether *H. pylori* eradication will eventually lead to the complete regression of *H. pylori*-related gastritis, including intestinal metaplasia, and also to complete eradication of gastric cancer. From the viewpoint of cancer prevention, our results indicate that cancer-susceptible subjects with extensive CAG remain at high risk even after complete eradication, and that they should be the target of regular follow-up for a period of at least 10 years, even after complete eradication. It is possible, however, that incident cancer in the *H. pylori*-eradicated subjects will be slow growing and of lower malignant potential and, as a result, clinically easier to control.

Between the 2 histopathological types of gastric cancer, the intestinal-type is the most common and tends to develop with the sequence of gastritis-atrophy-metaplasia-dysplasia-cancer. The effect of eradication was slight and insignificant on the incidence of this type of cancer in the total cohort. However, in the PG test-negative group with mild CAG, a substantial reduction in the incidence of this type of cancer was observed, although the significance of the reduction was marginal ($p < 0.057$) and was probably due to the small number of subjects. In contrast, diffuse cancer tends to develop directly from nonatrophic gastric mucosa without passing through the sequence. Eradication significantly reduced the incidence of this type of cancer. These results are also compatible with previous observations that a series of events induced by *H. pylori* infection from histopathological changes reflecting acute inflammation to molecular changes of altered DNA methylation of CpG islands in various genes, including E-cadherin, which is considered to be deeply involved in the development of diffuse-type cancer in nonatrophic stomach, disappeared immediately after successful eradication.[59–61]

In conclusion, *H. pylori* eradication is considered to be an effective strategy for reducing the risk of gastric cancer in middle-aged subjects with mild CAG. Serum PG levels give an objective measure for eradication. Most, if not all, of the subjects identified by negative PG test criteria would benefit from *H. pylori* eradication. Although further studies are required to determine the best criteria for indicating eradication, PG I level $\leq$ 50 μg/l and PG I/II ratio $\leq$ 3.0 appear to be the best among the 3 most widely-used sets of criteria for PG tests in Japan. Using the criteria, the number needed to treat (NTT) for 10 years to prevent 1 case of gastric cancer was 179, whereas for the second best set of criteria of PG I level $\leq$ 70 μg/l and PG I/II ratio $\leq$ 3.0 the NTT was 238. In Japan, 60 million people are infected with *H. pylori*, and PG test-negative subjects constitute a major proportion among middle-aged subjects.[46,62,63] Although the annual incidence rate of cancer in PG test-negative, *H. pylori*-infected middle-aged subjects is by no means low, that is, around 0.1%,[46] the cost-effectiveness of such measures is unknown. We have recently reported on a group of subjects with especially high cancer risk among PG test-negative subjects.[52] This group of subjects constitutes about 10% of all PG test-negative subjects and shows a high annual cancer incidence rate of over 0.2%, a comparable level to that in PG test-positive subjects with extensive CAG, whereas the annual cancer incidence rate of other PG test-negative subjects is around 0.05%. The authors believe that this group is an especially good target for eradication. Finally, it appears that serum PG levels will probably be useful in developing strategies for the prevention of gastric cancer with *H. pylori* eradication.

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Preventive effects of etodolac, a selective cyclooxygenase-2 inhibitor, on cancer development in extensive metaplastic gastritis, a *Helicobacter pylori*-negative precancerous lesion

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The present study investigated the preventive effects of etodolac, a selective cyclo-oxygenase (COX)-2 inhibitor, on metachronous cancer development after endoscopic resection of early gastric cancer. Among 267 early gastric cancer patients who underwent endoscopic resection, 47 patients with extensive metaplastic gastritis were selected based on endoscopic findings and our previously described criteria of serum pepsinogen (PG) test-positive and *Helicobacter pylori* antibody-negative conditions. Nonrandomized etodolac treatment (300 mg/day) was administered to 26 patients (Group A), while the remaining 21 patients were untreated (Group B). No significant differences in age, sex distribution, lifestyle factors or extent of metaplastic gastritis at baseline were identified between groups. Patients were followed for metachronous cancer development with endoscopy every 6–12 months for up to 5 years. Mean (standard deviation) follow-up period was 4.2 (0.9) years. In Group B, 5 cancers developed (incidence rate = 6,266/100,000 person-years), significantly more than the 1 cancer in Group A (incidence rate = 898/100,000 person-years; $p < 0.05$). Long-term etodolac treatment did not influence the extent of metaplastic gastritis as revealed by endoscopic findings or by serum PG levels, but effectively reduced metachronous cancer development in patients with extensive metaplastic gastritis. These results strongly suggest that chemoprevention of cancer in the metaplastic stomach is possible by controlling COX-2 expression.

Key words: gastric cancer, pepsinogen, *Helicobacter pylori*, chronic atrophic gastritis, cancer prevention, chemoprevention, COX-2 inhibitor, intestinal metaplasia

Abbreviations: *H. pylori*: *Helicobacter pylori*; CAG: chronic atrophic gastritis; COX: cyclo-oxygenase; ESD: endoscopic submucosal dissection; ELISA: enzyme-linked immunosorbent assay; PG: pepsinogen; NSAIDs: nonsteroidal anti-inflammatory drugs

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Helicobacter pylori triggers chronic inflammation of the infected stomach mucosa and is considered a major risk factor for gastric cancer and associated precursor lesions.[1] As postulated in the multistep model of gastric cancer development by Correa, long-lasting inflammation in the stomach mucosa leads to a cascade of molecular and morphological changes of stomach carcinogenesis, representing the gastritis-atrophy-metaplasia-dysplasia-cancer sequence.[2] This sequence of stomach carcinogenesis is now widely accepted to be strongly promoted by *H. pylori* and is affected by a variety of genetic and environmental factors that may act synergistically.[3] *H. pylori* eradication thus appears to be the most promising approach for the control of gastric cancer development, and the results of animal experiments have revealed that eradication of *H. pylori*, especially in the early stage, is effective for preventing stomach carcinogenesis.[4–6] However, current data indicate that *H. pylori* eradication does not lead to complete eradication of gastric cancer[7–12] and might be effective only in subjects without chronic atrophic gastritis (CAG) together with intestinal metaplasia.[7,10] Moreover, patients with extensive intestinal metaplasia—that is, metaplastic gastritis—should not be treated with eradication

therapy, as bacterial load decreases with the progression of intestinal metaplasia, eventually resulting in spontaneous eradication.[13,14] Alternative chemopreventive measures are thus needed for the prevention of stomach cancer in subjects with metaplastic gastritis.

Cyclo-oxygenase (COX)-2 is an inducible enzyme overexpressed in sites of inflammation and neoplastic tissues. This overexpression leads to enhancement of cell proliferation and migration, suppression of apoptosis, stimulation of neovascularization and alteration of intercellular adhesion, all of which are involved in carcinogenesis.[15] In the stomach, *H. pylori* infection triggers mucosal COX-2 upregulation, and this enhanced expression level is maintained throughout the progression of the aforementioned premalignant lesions to cancer.[16,17] Furthermore, selective inhibition of COX-2 has been shown to prevent the progression of premalignant gastric lesions[18] and the development of gastric cancer in *H. pylori*-infected Mongolian gerbils.[19,20] Treatment with COX-2 inhibitors thus appears to have beneficial preventive effects on *H. pylori*-associated stomach carcinogenesis. We conducted a prospective follow-up study in a group of patients with metaplastic gastritis who underwent endoscopic resection for early gastric cancer to determine whether administration of etodolac, a selective COX-2 inhibitor, prevents metachronous gastric cancer development.

Material and Methods

Study subjects

Between February 2003 and January 2005, a total of 267 patients with early gastric cancer underwent curative endoscopic resection such as endoscopic mucosal resection or endoscopic submucosal dissection (ESD) in Wakayama Medical University Hospital.[21–26] All patients were inhabitants from around the Wakayama area. In Japan, where the incidence of gastric cancer is high, treatment of mucosal gastric cancer without lymph node metastasis is usually achieved with endoscopic resection, preserving the stomach. Although institutional differences in indications for endoscopic resection exist, lesions with preoperative endoscopic diagnoses of intestinal-type intramucosal cancer without ulcer findings, intestinal-type intramucosal cancer $\leq$3 cm in diameter with ulcer findings and intestinal-type minute invasive submucosal (<500 μm below muscularis mucosa) cancer $\leq$3 cm in diameter are considered to be indicated for endoscopic resection.[25,26] In these 267 patients, the extent of coexisting CAG was evaluated endoscopically and by the results of 2 serum tests, pepsinogen (PG) and *H. pylori* antibody level, as described in the following section.

Serologic diagnosis of metaplastic gastritis, etodolac treatment and follow-up

Sera for analyses were obtained from fasting blood samples collected from the 267 patients before endoscopy, stored at −20°C and used for the measurement of serum PG levels and *H. pylori* antibody titers. Serum PG (PGI and PGII) levels were measured using a modification (RIAbeads Kit; Dainabott, Tokyo, Japan) of our previously reported radio-immunoassay.[27] Patients with extensive CAG were diagnosed using the previously described "PG test-positive" criteria: PGI $\leq$ 70 ng/mL and PGI/II ratio $\leq$ 3.0.[28,29] These criteria have a sensitivity of 70.5% and a specificity of 97% for the diagnosis of extensive CAG using pathological diagnosis as the gold standard.[28] Serum anti-*H. pylori* immunoglobulin G titers were measured using enzyme-linked immunosorbent assay (ELISA) (MBL, Nagoya, Japan).[30] Subjects with antibody titers >50 U/mL were classified as positive (*H. pylori*-infected) and those with antibody titers $\leq$50 U/mL were regarded as negative. The sensitivity and specificity of the ELISA test used in the present study were 93.5 and 92.5%, respectively.[30]

As described previously,[31,32] the natural course of *H. pylori* infection can be classified into the following 4 groups based on the results of the 2 serum tests for PG and *H. pylori* antibody: (*i*) healthy subjects without *H. pylori* infection are PG test-negative and *H. pylori* antibody-negative; (*ii*) with the establishment of *H. pylori* infection, the antibody test becomes positive; (*iii*) as infection persists, gastric atrophy advances and the PG test also becomes positive; and (*iv*) as gastric atrophy together with intestinal metaplasia becomes extensive, this leads to a reduction in *H. pylori* load and eventually to spontaneous eradication, so the antibody test again becomes negative. Subjects with metaplastic gastritis can thus be diagnosed serologically based on a PG test-positive, *H. pylori* antibody-negative condition.

Among the 267 patients, those with a previous history of gastric cancer or adenoma; severe liver, kidney or cardiopulmonary disease; past history of gastrointestinal bleeding or peptic ulcer disease; or long-term use of adrenocortical steroids or nonsteroidal anti-inflammatory drugs (NSAIDs) were excluded from the study. In addition, patients who had a previous history of *H. pylori* eradication or renal failure and those who had been prescribed proton pump inhibitors were also excluded from the study. The remaining subjects comprised 47 patients with metaplastic gastritis diagnosed both endoscopically and serologically.

All patients received a full explanation of etodolac treatment after endoscopic mucosal resection and were given the option to undergo this treatment. Patients who consented to treatment received etodolac at 300 mg/day. Patients who rejected this option but provided consent to participate in the study were followed as controls. Patients with and without etodolac treatment were endoscopically followed for metachronous cancer development at 1 week, 4 weeks, 8 weeks and 6 months after resection. Thereafter, patients underwent regular follow-up endoscopy every 6 months. Other than endoscopic follow-up, all patients were reviewed regularly every 1–2 months by clinicians for general health condition, and patients receiving etodolac treatment were monitored for adverse events by interview and clinical laboratory evaluations. Compliance was monitored by pill counts at the time

Epidemiology

Table 1. Profiles of subjects in groups A and B

	Group A with etodolac treatment	Group B without etodolac treatment
Number of subjects (male:female)	26 (22:4)	19 (17:2)
Follow-up, years [mean (SD)]	4.3 (1.1)	4.2 (0.7)
Person-years	111.4	79.8
Age, years [mean (SD)]	71.3 (10.2)	70.6 (7.4)
Alcohol drinking, *n* (%)	10 (38.4)	7 (36.8)
Smoker, *n* (%)	9 (34.6)	7 (36.8)
Serum PG levels at the start of the study		
PGI, mg/ml [mean (SD)]	21.4 (18.4)	20.1 (18.2)
PG I/II [mean (SD)]	1.7 (0.8)	1.5 (0.7)
Serum PG levels at the end of the study		
PGI, mg/ml [mean (SD)]	18.2 (14.1)	18.0 (12.4)
PG I/II [mean (SD)]	1.5 (0.8)	1.6 (0.7)
Total gastric cancer developed		
Case/incidence rate[1]	1/898	5/6266[2]
Details of the resected cancers		
Size, mm [mean (SD)]	31.5 (13.6)	32.4 (17.4)
Location [upper/middle/lower (%)][3]	14/10/2 (54/38/8)	11/7/1 (58/37/5)
Macroscopic type [IIa/IIb/IIc (%)]	18/3/5 (69/12/19)	12/2/5 (63/11/26)
Depth of invasion, *n* of mucosal cancer (%)	26 (100)	19 (100)
Histopathology type, *n* of intestinal type (%)	26 (100)	19 (100)
Synchronous multiple cancer cases, *n* (%)	2 (8)	1 (5)
Method of endoscopic resection, *n* of ESD (%)	26 (100)	19 (100)

[1]Per 100,000 person-years. [2]$p < 0.05$ (*vs.* Group A with etodolac treatment). [3]Location and macroscopic type of the cancer were determined according to the Japanese Classification of Gastric Carcinoma (Ref. 34).

of each review. Written informed consent was obtained from all participating patients. The Committee on Ethics at Wakayama Medical University approved all study protocols.

Evaluation of cancer histopathology

Resected specimens of gastric cancer obtained by endoscopy were assessed histopathologically and classified according to Lauren's classification into intestinal or diffuse type.[33] Location and macroscopic type of the cancer in the stomach were classified based on clinical and histopathological records according to the classifications of the Japanese Gastric Cancer Association.[34]

Statistical analysis

Data were analyzed using SPSS 11.0 software (SPSS, Chicago, IL) and STATA software (STATA, College Station, TX). Differences were tested for significance using the Mann–Whitney *U*-test for comparisons between 2 groups. The chi-square test and Fisher's exact test were used to compare categorical variables. Long-term effects of etodolac on gastric cancer development were analyzed by the Kaplan–Meier method, and statistical differences between curves were tested by the log-rank test. For all comparisons, p values less than 5% ($p < 0.05$) were considered statistically significant.

Results

Among the 47 patients with endoscopically and serologically diagnosed metaplastic gastritis who underwent endoscopic resection for early gastric cancer, 26 received etodolac treatment (Group A) and the remaining 21 did not receive any treatment (Group B). These 2 groups of patients were followed and development of gastric cancer was investigated. During the first year of the study, 2 patients in Group B developed cancer. One cancer case was detected 8 weeks after resection and the other 6 months later. In both cases, the cancerous lesions were able to be retrospectively identified on endoscopic images from before resection. These cancers were considered to be synchronous cancers and were thus excluded from the study, and the remaining 45 patients were analyzed. Table 1 shows baseline characteristics for the 2 groups. No significant differences in age, sex distribution, or lifestyle factors at baseline were apparent between groups. In addition, the extent of CAG together with intestinal metaplasia at the time of mucosal resection as evaluated by endoscopic findings was similar between groups, as were serum PG levels. Furthermore, comparison of clinicopathological features (size, location, macroscopic type, depth of invasion, histopathological type, *etc.*) of the resected cancers revealed

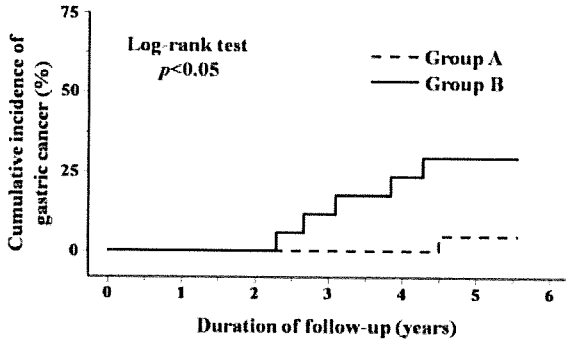

Figure 1. Kaplan–Meier analysis of metachronous cancer development in patients with early gastric cancer resected endoscopically. Group A received etodolac treatment (300 mg/day), while Group B did not receive any treatment. Both groups were followed for up to 5 years. Cancer incidence rates were 898/100,000 person-years for Group A and 6,266/100,000 person-years for Group B, showing a significant difference in cancer development rates between groups ($p = 0.05$; log-rank test).

no significant differences between groups. All cancers in these 47 patients were resected by ESD.

Patients were followed for up to 5 years. Mean (standard deviation) follow-up period was 4.2 (0.9) years. By the end of the study period, cancer development was observed in 1 Group A patient and 5 Group B patients. As shown by Kaplan–Meier analysis, cancer development in the Group A patient was observed 5 years after the start of the study. In contrast, cancer development occurred steadily throughout the study period in Group B patients (Fig. 1). Cancer incidence rates in Groups A and B were 898/100,000 person-years and 6,266/100,000 person-years, respectively, representing a significant difference ($p < 0.05$, log-rank test). Cancers that developed in these patients were all intestinal-type mucosal cancers on histopathology, and sizes were <10 mm in diameter. All these lesions were thus resected endoscopically.

The extent of CAG together with intestinal metaplasia as revealed by endoscopic findings did not change significantly in Group B patients during the study period. In addition, the difference between serum PG levels for each patient at the start compared to the end of the study period was not significantly different and was within the range of interassay variation. Etodolac treatment did not exert any influence on the extent of metaplastic gastritis in Group A patients and did not induce any other specific change in endoscopic findings except for a single case of gastric erosion observed in the prepyloric antrum of 1 patient. Serum PG levels of Group A patients were also unaltered by etodolac treatment. This medication was well tolerated by all patients during the study period.

Discussion

In the present study, long-term treatment with a selective COX-2 inhibitor, etodolac, effectively inhibited metachronous cancer development in curatively treated, early gastric cancer patients with metaplastic gastritis. These results are in line with the results of our previous animal experiment using *H. pylori*-infected Mongolian gerbils,[19] indicating that etodolac can prevent stomach carcinogenesis involving the CAG-metaplasia-dysplasia-cancer sequence. Essentially, the same results have also been reported with the use of another selective COX-2 inhibitor, celecoxib.[20] Furthermore, previous epidemiologic studies have demonstrated that long-term nonselective inhibition of COXs (COX-1 and COX-2) by NSAID treatment is effective for preventing gastric cancer.[35,36] However, evaluation of the preventive effects of selective COX-2 inhibition on gastric cancer by 2 epidemiologic studies investigating the regression of intestinal metaplasia as a primary parameter (a surrogate parameter for cancer prevention) revealed conflicting results. One randomized controlled study indicated that rofecoxib treatment had no significant effect on the regression of intestinal metaplasia,[37] whereas the other nonrandomized study indicated a beneficial effect of celecoxib.[38] The contradictory outcomes of these studies could be partially explained by the differential effects of selective COX-2 inhibitors according to dose, type of drug and duration of exposure,[39] but also, and more importantly, by differences in severity of the target lesion—the extent of coexisting CAG together with intestinal metaplasia—among study patients. The present results indicate that the extent of premalignant lesions as revealed by serum PG levels did not change significantly during the study period despite etodolac treatment. Since the study period was not long, further long-term investigations are warranted to determine the inhibition of progression and/or regression of metaplastic gastritis by COX-2 inhibition. Nonetheless, our results strongly indicate that COX-2 is deeply involved in the growth of initiated cells in the metaplastic stomach and that etodolac treatment leads to a marked delay in cancer development.

We selected early gastric cancer patients with metaplastic gastritis as a target for treatment with a selective COX-2 inhibitor. Several previous studies have demonstrated that the more advanced the stage of *H. pylori*-related CAG, the greater the cancer risk.[31,32,40–44] Subjects with metaplastic gastritis, an end result of long-lasting *H. pylori* infection, are thus considered to be at particularly high risk of gastric cancer. Indeed, our previous longitudinal cohort study found that a group of middle-aged male subjects with metaplastic gastritis based on 2 serum tests—negative results for *H. pylori* antibody and positive results on the PG test—displayed an annual cancer incidence rate of about 0.87%, meaning that 1 cancer developed in 11.5 subjects during every 10-year period.[31,32] Subjects selected for the present study were early cancer patients curatively treated with endoscopic resection and thus appear to constitute a subgroup at even higher risk for gastric cancer among subjects with metaplastic gastritis. A few previous studies have reported an annual incidence of metachronous cancer after endoscopic resection of about 1.3–4.0%,[45,46] while the annual cancer incidence rate in the present study was

>6.0% for the control group. This high incidence rate is probably due to the fact that, in those previous studies, the extent of CAG together with intestinal metaplasia in background stomachs of study patients was not evaluated. Subjects were thus probably heterogeneous in the degree of CAG, which was probably milder as a whole compared to that in subjects in the present study. The observed high incidence rate of metachronous cancer development is evidence for a strategy for cancer prevention in these subjects other than regular and strict follow-up by endoscopy. As described above, subjects with metaplastic gastritis, an *H. pylori*-negative lesion, cannot be treated with *H. pylori* eradication. Furthermore, high expression levels of COX-2 in intestinal metaplasia have been observed even after *H. pylori* eradication.[47] Treatment with a selective COX-2 inhibitor thus appears to represent a reasonable option for subjects with metaplastic gastritis, rather than regular follow-up for cancer.

Although the present study revealed preventive effects of a selective COX-inhibitor, etodolac, on metachronous cancer development in curatively treated gastric cancer patients with metaplastic gastritis, the study shows some limitations. First, the present study was prospectively conducted, but treatment with etodolac was not randomized. However, randomization was not feasible, as most eligible, high-risk cancer subjects were unwilling to remain untreated for long periods, particularly with the knowledge of the results of previous epidemiologic studies that long-term use of NSAIDs, including aspirin, is associated with a reduced risk of gastric cancer.[35,36] In addition, the number of subjects was small because the incidence of serologically diagnosed metaplastic gastritis is quite low, comprising <1% of the middle-aged Japanese population[31,32] and <20% of total gastric cancer cases. Considering the fact that *H. pylori* eradication does not completely eradicate cancer[7–12] and that eradication might be effective in the control of cancer development only among subjects with mild CAG,[7,10] posteradication subjects with extensive CAG and intestinal metaplasia should be considered another possible target for treatment with selective COX-2 inhibitors. However, special attention should be paid to recent evidence that long-term use of COX-2 inhibitors is associated with increased cardiovascular risk, including not only thrombotic events, but also hypertension, congestive heart failure, and arrhythmic events.[48–50] Since the reported cardiovascular toxicity of COX-2 inhibitors is variable among the different drugs and with the dose of each particular drug and, based on past data, etodolac treatment at a dose of 300 mg/day appears to be relatively low in cardiotoxicity,[51,52] the present results warrant a prospective randomized trial. Further careful study using the present dose of etodolac and avoiding inclusion of patients with increased risk of cardiovascular complications would contribute greatly to determining the effectiveness and safety of long-term chemopreventive treatment.

In conclusion, the present results strongly indicate that selective COX-2 inhibitors provide a potent strategy for tertiary cancer prevention in curatively treated gastric cancer patients with metaplastic gastritis, an *H. pylori*-negative premalignant lesion.

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A second-look endoscopy after endoscopic submucosal dissection for gastric epithelial neoplasm may be unnecessary: a retrospective analysis of postendoscopic submucosal dissection bleeding

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Background: Endoscopic submucosal dissection (ESD) is one of the curative endoluminal surgical procedures for gastric epithelial neoplasms. There has been little research on bleeding after gastric ESD.

Objective: To investigate cases of post-ESD bleeding and to verify whether a second-look endoscopy after ESD is effective in the prevention of delayed bleeding.

Design: A retrospective study with consecutive data.

Setting: A single tertiary referral center.

Subjects: A total of 454 gastric epithelial neoplasms (386 early gastric cancers and 68 gastric adenomas).

Interventions: ESD and second-look endoscopy.

Main Outcome Measurements: Predictors on post-ESD bleeding by univariate analysis, incidence of post–ESD bleedings, and the timing of those before and after second-look endoscopy.

Results: Post–ESD bleeding occurred in 26 (5.7%) lesions. Gross type (flat or depressed type) was the only factor influencing post-ESD bleeding. All cases of post-ESD bleeding occurred within 14 days after ESD (median 2; range 0-14), and bleeding tended to occur from the lower and upper stomach earlier and later, respectively. In 19 lesions with delayed bleeding more than 24 hours after ESD, the maximum delayed bleeding rates before and after the second-look endoscopy were 2.8% and 2.5%, respectively.

Limitations: A retrospective, single-center analysis.

Conclusions: A second-look endoscopy after gastric ESD may contribute little to the prevention of delayed bleeding. (Gastrointest Endosc 2010;71:241-8.)

Endoscopic submucosal dissection (ESD) has been increasingly established as a promising endoluminal technique for GI epithelial neoplasms. Unlike conventional techniques such as EMR, ESD consists of circumferential mucosal incision and submucosal dissection, which have made it possible, even in a large tumors or tumors with ulcerative findings, to resect in en bloc fashion with tumor-free margins.[1-5] By using ESD, we have performed curative resection for node-negative early gastric cancer (EGC)[6] instead of open surgery.

One of the major concerns about gastric ESD is postoperative bleeding. It occurs in approximately 5% of the patients who undergo gastric ESD.[7-10] In almost all cases, endoscopic hemostasis is effective in stopping bleeding if an emergency endoscopy is properly performed. Therefore, it is necessary to determine the nature of the post-ESD bleeding and the appropriate management. In practice, a second-look endoscopy is routinely performed the next day or later after ESD in most hospitals in Japan to check for the possibility of postoperative bleeding. However, performing a second-look endoscopy without any signs of bleeding has not yet been validated, and, to the contrary, bleeding even after the confirmation of hemostasis sometimes occurs. Because there have been few reports concerning post-ESD bleeding or its

Abbreviations: EGC, early gastric cancer; ESD, endoscopic submucosal dissection; GA, gastric adenoma; PPI, proton pump inhibitor.

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prevention,[10] we retrospectively reviewed our gastric ESD cases to verify the clinicopathological features of post-ESD bleeding and to confirm whether a second-look endoscopy was necessary for preventing delayed bleeding.

PATIENTS AND METHODS

A total of 476 lesions with a histologic diagnosis of gastric epithelial neoplasms (406 EGCs and 70 gastric adenomas [GAs]) were consecutively treated with ESD in our hospital from December 2003 to November 2008. Among them, 454 lesions (386 EGCs and 68 GAs) were included after exclusion of 14 lesions in a remnant stomach after gastrectomy or in a gastric tube after esophagectomy and 8 lesions in which perforation occurred during ESD caused by their specific physiological conditions.

ESD was principally indicated for possible node-negative EGCs according to the criteria of Gotoda et al[6] based on endoscopic findings including chromoendoscopy with biopsy. In cases of possible node-positive EGCs, ESD was only indicated when the lesions were considered to be technically removable from the gastric wall and patients desired to undergo ESD after sufficient information was provided by both endoscopists and surgeons. In cases of GAs, ESD was only indicated when it was possible that the lesion contained foci of cancer or the patients strongly desired the lesion to be resected. All patients provided written informed consent before undergoing treatment.

ESD procedures

The ESD technique was described elsewhere.[1-5] In brief, after circumferential marking, submucosal injection was performed below the tumor to create a submucosal fluid cushion. Next, circumferential cutting of the mucosal layer with muscularis mucosa approximately 5 mm outside the marking was performed. Subsequently, the submucosal layer beneath the tumor was dissected to detach it from the gastric wall. A mixture of 10% glycerin, 5% fructose, and 0.9% saline solution (Glyceol; Chugai Pharmaceutical Co, Tokyo, Japan) containing 0.005% indigo carmine and 0.0005% epinephrine was used as submucosal fluid.[11] Hyaluronic acid was additionally used when the lesion could not rise enough by using Glyceol alone.[12,13] The Flexknife (KD-630L; Olympus, Tokyo, Japan) was selected as the main electrosurgical knife.[3,14] Other knives, such as the IT knife (KD-610L; Olympus),[1,2] Hookknife (KD-620LR; Olympus),[4] Splash-needle (DN-2618A; Pentax, Tokyo, Japan),[5] and a needle-knife, were used in some cases instead of the Flexknife, according to the tumor characteristics and/or operator preference. Hemostatic forceps (HDB2422 W; Pentax) were used for bleeding during ESD or for visible vessels on the mucosal defect after removal.[15]

Capsule Summary

What is already known on this topic

- Endoscopic submucosal dissection (ESD) facilitates en bloc resection with tumor-free margins in gastric cancers but is associated with a postoperative bleeding rate of 5%.

What this study adds to our knowledge

- In a retrospective study of 454 cases of gastric ESD, postoperative bleeding occurred in 26, all within 14 days.
- Delayed bleeding rates before and after the second-look endoscopy were 2.8% and 2.5%, respectively.

The patient was usually allowed to eat a light meal the day after ESD. In principle, a second-look endoscopy was performed once within 1 week to check whether there was a recent hemorrhage or a possible bleeding spot (a nonbleeding visible vessel) that should be treated on the mucosal defect. The day of the second-look endoscopy was decided by the operator according to the patient's condition or the operation day once during the hospitalization. If the hospitalization was uneventful, patients were discharged within 1 week after ESD. Principally, the patients receiving anticoagulants and/or antiplatelet drugs were instructed to stop taking them for 1 week before and 1 week after ESD. When bleeding or nonbleeding visible vessels were seen (sometimes in removing adherent clots by forceps or water jet) on the second-look endoscopy, prophylactic hemostasis was performed. Clipping with hemostatic clips (HX-610-135 or HX-610-090L; Olympus) was performed for large nonbleeding vessels, and thermocoagulation with hemostatic forceps was performed for bleeding vessels or small nonbleeding vessels or in locations where it was difficult to place a clip because of consolidation of the ulcer bed. Between the day of ESD and the first day of feeding (2 days in most cases), 20 mg omeprazole twice daily was administered intravenously. Thereafter, from the day of feeding to at least 2 weeks after ESD, according to the decision of physicians in charge, one of the following proton pump inhibitors (PPIs) was administered orally: 10 mg rabeprazole, 20 mg omeprazole, or 30 mg lansoprazole once daily (these were thought to be equally effective for acid suppression).[16] A follow-up endoscopy was performed 2 months after ESD. Patients were also asked to contact the physicians in case of hematemesis or melena, even after discharge. When perforation or post-ESD bleeding occurred, the schedules for discharge including food intake were changed according to the patient's condition. In cases of possible post-ESD bleeding, the patient underwent an emergency endoscopy, and endoscopic hemostasis was performed on bleeding spots or nonbleeding visible vessels, mainly by clipping or thermocoagulation.

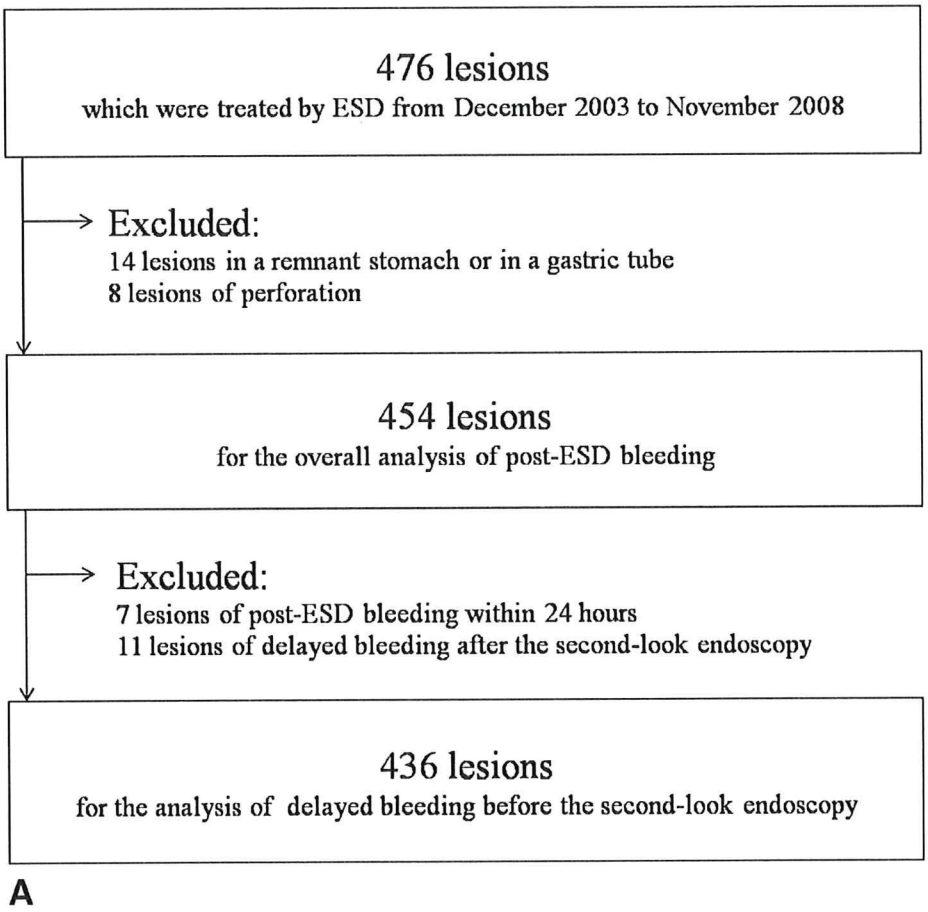

Figure 1. A, Flowchart showing the inclusion in the analysis of delayed bleeding before the second-look endoscopy. **B,** Flowchart showing the inclusion in the analysis of delayed bleeding after the second-look endoscopy. (Continued on next page)

Data analysis

Post-ESD bleeding was defined as massive bleeding from the mucosal defect after ESD, as diagnosed by the emergency endoscopy, which was performed because of hematemesis or melena. To investigate factors influencing post-ESD bleeding, the following variables were analyzed: age, sex, comorbidities (hypertension, diabetes mellitus, heart disease, chronic renal failure, and liver cirrhosis), and the use of anticoagulants and/or antiplatelet drugs (patient-related factors); the location (upper third, middle third, or lower third), circumference (anterior wall, posterior wall, lesser curve, or greater curve), gross type (0-I/IIa, 0-IIb/IIc, or combined type), ulcerative findings in the submucosal layer (endoscopically present or absent), the resection style (en bloc or piecemeal), tumor size (maximum diameter of the resected tumor actually measured), tumor depth (mucosal tumor or submucosal invasive tumor), and histologic type (intestinal type, diffuse type, or adenoma) (lesion-related factors); the period of ESD (early [2003-2005] and late [2006-2008]); and the operator's experience with gastric ESD ($\leq$30 cases, 31-100 cases, and $>$100 cases) (operator-related factors).

Delayed bleeding was defined as post-ESD bleeding diagnosed more than 24 hours after ESD. From the consecutive cases, rates of overall post-ESD bleeding and delayed bleeding before and after the second-look endoscopy were investigated to determine the usefulness of the second-look endoscopy. The maximum follow-up duration was 56 days when almost all the artificial ulcers were considered to be cured.[17] Flow charts for inclusion in each analysis are shown in Figure 1.

Statistical analysis

Univariate analysis was performed by using the Student *t* test for age and tumor size; the Fisher exact test for probability for sex, comorbidities, the use of anticoagulants and/or antiplatelet drugs, ulcerative findings, resection style, tumor depth, and the period of ESD; and the χ^2 test for location, circumference, gross type, histologic type, and the operator's experience. Statistical significance was set at a P value of $<.05$, and if there were more than 1 predictor with a significant difference by univariate analysis, multivariate analysis by using a logistic regression

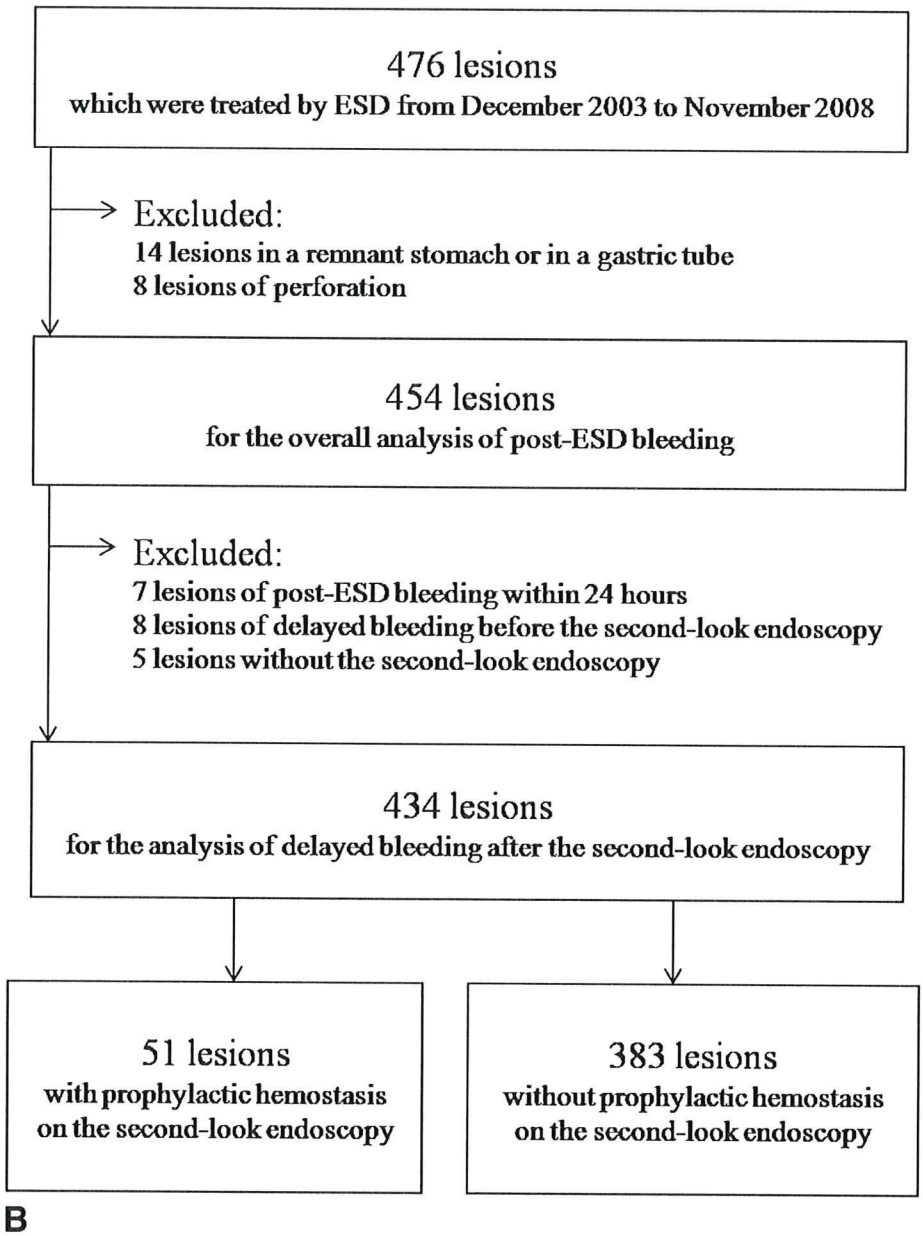

Figure 1 (*Continued*)

model was planned. The analyses of post-ESD bleeding were performed by using the Kaplan-Meier method.

RESULTS

Post-ESD bleeding occurred in 26 (5.7%) of 454 lesions. All the occurrences of bleeding were successfully managed with only endoscopic treatment, and no surgical intervention was required. No post-ESD bleeding was followed by rebleeding. In 8 (1.8%) lesions, a blood transfusion was performed.

The univariate analysis of variables for post-ESD bleeding is shown in Table 1. Only gross type (0-IIb/IIc) was considered the predictor for post-ESD bleeding with a significant difference ($P = .0376$). Multivariate analysis was not performed because there was only 1 predictor demonstrated by the univariate analysis.

All of the post-ESD bleedings occurred within 14 days after ESD (median 2; range 0-14), as shown in Figure 2. All cases of bleeding could be classified into 2 groups: the early bleeding group (16 cases) and the late bleeding group (10 cases), as shown in Figure 3. The early bleeding group had more occurrences of bleeding in the lower

TABLE 1. Univariate analysis of predictors on post-endoscopic submucosal dissection bleeding

Factors	Post-ESD bleeding		*P* value
	Occurred (26 cases)	Did not occur (428 cases)	
Patient-related			
Age (y), mean ± SD	70.6 ± 9.1	68.2 ± 9.2	.1945
Sex (male/female)	19/7	328/100	.6398
Comorbidities			
Hypertension (present/absent)	8/18	97/331	.3423
Diabetes mellitus (present/absent)	2/24	37/391	>.9999
Heart disease (present/absent)	4/22	35/393	.2651
Chronic renal failure (present/absent)	1/25	5/423	.2994
Liver cirrhosis (present/absent)	3/23	15/413	.0766
Anticoagulants/platelets (used/not used)	5/21	52/376	.3533
Lesion-related			
Location (U/M/L)	6/8/12	88/147/193	.9172
Circumference (AW/GC/LC/PW)	5/3/11/7	77/72/168/111	.9184
Gross type (I-IIa/ IIb-IIc/combined)	4/21/1	157/236/35	.0376*
Ulcerative findings (present/absent)	5/21	47/381	.2028
Resection style (en bloc/piecemeal)	24/2	419/9	.1262
Tumor size, mm (mean ± SD)	22.7 ± 17.2	18.7 ± 13.1	.1402
Tumor depth (mucosa/submucosa)	21/5	353/75	.7925
Histologic type (intestinal/diffuse/adenoma)	25/0/1	348/13/67	.1550
Operator-related			
Period of ESD (early/late)†	12/14	212/216	.8408
Operator experience (<30/31-100/>100)	8/6/12	144/84/200	.9005

ESD, Endoscopic submucosal dissection; *SD*, standard deviation; *U*, upper third; *M*, middle third; *L*, lower third; *AW*, anterior wall; *GC*, greater curve; *LC*, lesser curve; *PW*, posterior wall.
*Significantly different (IIb/IIc vs I/IIa).
†Early, 2003-2005; late, 2006-2008.

third of the stomach, and the late bleeding group had more occurrences of bleeding in the upper third (Table 2).

The median duration between ESD and the second-look endoscopy was 4 days (range 1-8), and it was performed most frequently the day after ESD. Among 19 cases of delayed bleeding, 8 and 11 occurred before and after the second-look endoscopy, respectively. No bleeding was found only on the second-look endoscopy. In 11 cases of delayed bleeding after the second-look endoscopy, red dots were seen in 5, black dots in 3, and clean ulcers without clots in 3 on the second-look endoscopy. Bleeding spots could be retrospectively matched to the dots on the ulcer on the second-look endoscopy in 7 cases, but there were no signs identified before bleeding in 4 cases.

Of 51 and 383 cases with and without prophylactic hemostasis, respectively, delayed bleeding after the second-look endoscopy occurred in 1 and 10 cases, respectively. As shown in Figures 4 and 5, the maximum delayed bleeding rates before and after the second-look endoscopy were 2.8% and 2.5%, respectively.

DISCUSSION

Previous prospective, randomized trials revealed the efficacy of a second-look endoscopy after endoscopic hemostasis for bleeding peptic ulcers,[18-20] and this is the reason why we routinely perform a second-look endoscopy after gastric ESD. This study, however, found that