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【 ORIGINAL ARTICLE 】

Risk Factors for Gastric Cancer after the Eradication of *Helicobacter pylori* Evaluated Based on the Background Gastric Mucosa: A Propensity Score-matched Case-control Study

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Abstract:

Objective The eradication of *Helicobacter pylori (H. pylori)* reduces the risk for gastric cancer (GC) development, but it cannot prevent GC completely. We investigated the risk factors of early GC development after the eradication of *H. pylori,* based on the histological characteristics of gastric mucosa.

Methods Sixty-one patients who underwent endoscopic submucosal dissection for early GC after successful *H. pylori* eradication (Group A) and 122 patients without developing a gastric neoplasm over 3 years after successful *H. pylori* eradication (Group B) were analyzed. We compared the histological findings of the patients enrolled in Group A and Group B before and after the propensity score-matching.

Results Comparing the characteristics of two the groups, Group A consisted predominantly of males, had significantly more elderly patients, and the years after successful eradication tended to be longer. We performed score matching for these three factors to reduce the influence of any confounding factors. After matching, the scores of inflammation for Group A (n=54) was significantly higher than those of Group B (n =54) at the greater curvature of the antrum, the lesser curvature of the corpus, and the greater curvature of the corpus. According to a multivariate analysis, inflammation of the greater curvature of the antrum and lesser curvature of the corpus were found to be independent risk factors. The risk ratio and 95% CI were 5.92 (2.11-16.6) (p<0.01), and 3.56 (1.05-13.2) (p=0.04), respectively.

Conclusion A continuous high level of inflammation of the background gastric mucosa may be a risk factor for gastric cancer onset after *H. pylori* eradication.

Key words: background gastric mucosa, gastric cancer, inflammation, *Helicobacter pylori*, propensity score matching

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Introduction

It is well known that the eradication of *Helicobacter pylori (H. pylori)* reduces the risk for gastric cancer (GC) development (1-5). In Japan, the eradication of *H. pylori*-associated gastritis has been included in national health insurance coverage since February 2013, aiming to suppress the new onset of GC. It is already known that *H. pylori* eradication does not completely prevent GC development in all individuals (6). GC after *H. pylori* eradication is very interesting in that it develops and advances in different envi-

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ronments and conditions, in comparison to *H. pylori*-positive GC. Therefore, it is important to characterize the clinicopathological features of GC after *H. pylori* eradication and identify its developmental differences with GC with ongoing *H. pylori* infection.

Several studies have been reported regarding the risk factors for the development of GC after eradication, regarding the gastric mucosa, gastric mucosal atrophy and intestinal metaplasia (IM) (7-10). Kodama et al. performed a histological evaluation of the background gastric mucosa before the eradication of *H. pylori*, using the updated Sydney scoring system. They reported that inflammation, as well as the atrophy scores and IM scores at the greater curvature of the corpus were significantly higher in the GC group who underwent the successful eradication of *H. pylori* than in the matched non-GC group, and they mentioned that inflammation might promote the onset of GC (8).

However, the histological features of the background gastric mucosa at the time of GC detection after the eradication of *H. pylori* are not well known. Thus, we performed a retrospective analysis to investigate the risk factors for early GC development after *H. pylori* eradication, based on the histological characteristics of the gastric mucosa. Especially, we focused on chronic inflammation, and compared GC patients after successful eradication and non-GC patients.

Materials and Methods

Patients

We enrolled 426 patients who underwent endoscopic submucosal dissection (ESD) for early initial GC at Okayama University Hospital between January 2013 and June 2017, excluding postoperative stomach cancer, stomach tube cancer, and cases with a history of endoscopic resection for gastric cancer. Six patients who tested negative for *H. pylori* infection without any previous definite eradication and four patients who were histologically diagnosed with gastric carcinoma with lymphoid stroma, were excluded. For gastric carcinoma with lymphoid stroma it was considered to be difficult to evaluate the background mucosa accurately because of the dense infiltration of lymphocytes and macrophages from mucosa to submucosa.

Additionally, we excluded 209 patients who were *H. pylori* positive and 42 patients with insufficient eradication data. One hundred and sixty-five patients had achieved successful eradication before the detection of their initial GC.

GC of patients who have eradicated within 1 year might have been cancer undetected prior to successful eradication, and thus might have been affected by *H. pylori* infection (8, 11, 12). In the present study, we defined GC after successful *H. pylori* eradication as that which was detected and diagnosed at least 1 year after the therapy. Therefore, we also excluded 94 patients without a previous history of eradication and 10 patients who were discharged after eradication for less than 1 year. Finally, the remaining 61 patients who underwent ESD with post *H. pylori* eradication were analyzed in the present study and defined as Group A (GC group) (Fig. 1). In cases with multiple lesions, the largest lesion was selected.

In the control group, we enrolled 434 patients who underwent a histologic examination from the background gastric mucosa after *H. pylori* eradication. However, patients who had not received follow-up endoscopy over 3 years (n=162), had a history of gastric cancer (n=136), and developed GC during the follow-up (n=14) were excluded. Finally, we enrolled 122 patients. This group was defined as Group B (a non-GC group) (Fig. 1).

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Okayama University Hospital. Informed consent was acquired by the opt-out method.

Evaluation of H. pylori status

In most of the cases of group A, *H. Pylori* eradication had been performed by another institution. Therefore, we checked that the success of the eradication was clearly described in the medical records, and verified before ESD when anti-*H. pylori* antibody titers was <3 (Eiken E-plate test) and the histologic examination from the background gastric mucosa were negative. On the other hand, for Group B, *H. pylori* eradication was confirmed by a urea breath test (cutoff value, 2.5 per mil) and a histologic examination.

Endoscopic atrophy

We evaluated endoscopic gastric atrophy according to the Kimura-Takemoto classification (13), and classified the results into three grades: mild (C-1 and C-2 patterns), moderate (C-3 and O-1 patterns), and severe (O-2 and O-3 patterns).

Histological analysis

Three biopsy specimens were obtained from the greater curvature of the antrum, the lesser curvature of the corpus, and the greater curvature of the corpus. The gastric mucosa samples were evaluated according to the updated Sydney system, with the degree of inflammation (mononuclear cell infiltration), neutrophil activity, atrophy, and IM classified and scored as 'normal,' 0; 'mild,' 1; 'moderate,' 2; and 'marked,' 3; according to a visual analogue scale (14). In Group B (control group), biopsy specimens were obtained at every follow up endoscopy, and the newest data was enrolled. In Group A (cancer group), biopsy specimens were obtained at the time of preoperative endoscopy (approximately 1-2 months before ESD).

Furthermore, we conducted a pilot study using an immunohistochemical analysis to elucidate the type of mononuclear cell infiltration. Sections were immunohistochemically stained using an automated Bond Max stainer (Leica Biosystems, Melborne, Australia). The following primary antibodies were used: CD79a (JCB79a, dilution 1:50; Dako, Glostrup, Denmark), CD3 (LN10, dilution 1:100; ABCAM,

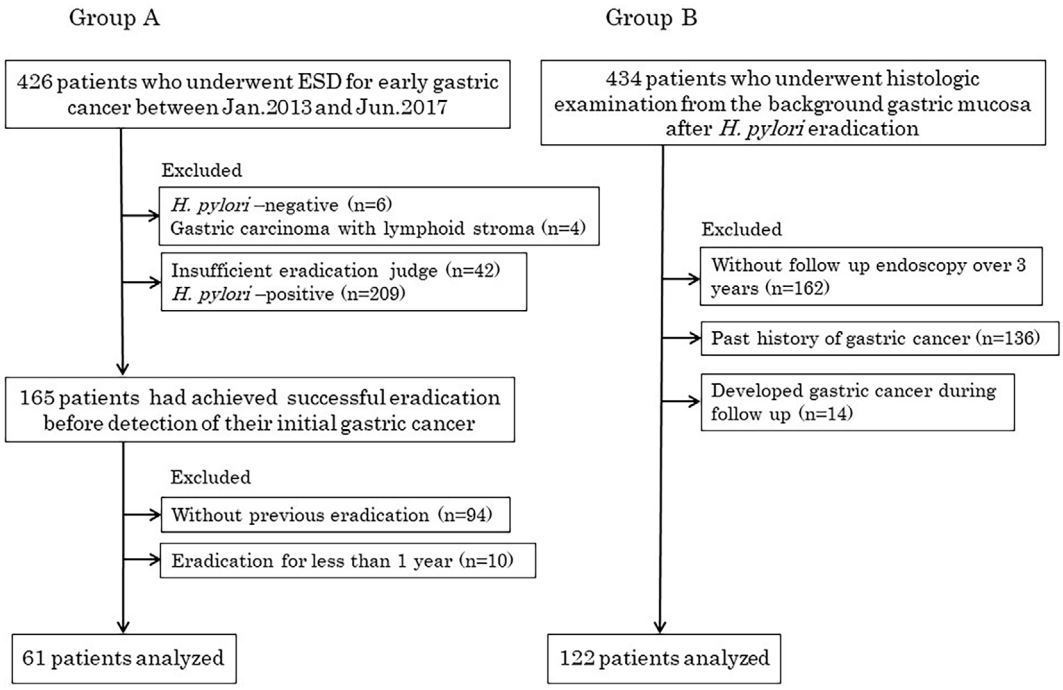

Figure 1. **Flowchart of patients.**

Cambridge, UK).

Experienced pathologists from Okayama University Hospital performed the histological evaluations. We compared the scores from the histological evaluations of the gastric mucosa between the two groups.

Statistical analysis

All data are presented as the mean ± standard deviation for continuous variables and as numbers for categorical variables. The continuous variables were evaluated using the Mann-Whitney *U*-test. The categorical variables were evaluated with the chi-square test and Fisher's exact tests.

We performed a multivariate analysis to assess the strength and independence of mucosal inflammation. A multivariate analysis was performed using a logistic regression analysis. In order to perform the analysis, we changed each score grade (from normal to marked by updated Sydney system) to two groups (normal vs. mild-marked).

We used a propensity score-matching analysis to adjust any significant differences in the baseline clinical characteristics of the patients and to reduce those that were considered to be potentially confounding factors (15, 16), including age, gender, and years after successful eradication. The propensity score model was well calibrated and clearly distinguished between Group A and Group B (*c*-statistic = 0.69). The *c*-statistic was calculated by measuring the receiver operating characteristic curve to assess the validity of the model. Before and after propensity score-matching, we compared the histological findings of the enrolled patients in Group A and Group B within a caliper of width of 0.2 of the standard deviation of the logit of the propensity score.

The statistical analysis was performed using the JMP® 13 software package (SAS Institute, Cary, USA), and a value of $p<0.05$ was considered to indicate statistical significance.

Results

Characteristics of the gastric cancer group after successful eradication

Table 1 shows the lesion characteristics of GC group (Group A) after successful eradication. Of the 61 cases of Group A, 35 GCs were located in the middle third of the stomach, thus being the most numerous. We also found that the tumor size was small (14.7±1.1 mm), the macroscopic type of tumor was mostly flat/depressed, and the histological type was mostly differentiated-type adenocarcinoma with intramucosal invasion. These characteristics did not differ from the characteristics of GC after eradication as previously reported (7, 12, 17, 18).

Comparison of the clinical characteristics

Comparing the characteristics of Group A and Group B, we found that Group A consisted predominantly of males (44 males vs. 17 females), while patients from Group B had an equal gender distribution (61 males vs. 61 females) ($p<0.01$). There were also significantly more elderly patients (median age, 71 years vs. 68 years, $p<0.01$). Although the difference was not significant, the years after successful eradication were longer in Group A than in Group B (4.64±4.4 vs. 4.16±2.2, $p=0.33$) (Table 2). There may have been some bias, because of the difference depending on gender, age, and the years after successful eradication. Therefore, we reconsidered the histologic evaluation of the background gastric mucosa by propensity score-matching the age, gender, and years after *H. pylori* eradication.

After propensity score-matching, 54 patients with GC and 54 patients without GC were included in each group. There was no significant difference in age, gender, and the years after successful eradication between the two groups (Table 2).

Comparison of the histological findings of the background gastric mucosa

We compared the scores in the background gastric mucosa between Group A and Group B using the updated Sydney scoring system. In the background gastric mucosa evaluation, neutrophils were not described because almost all of them had disappeared after *H. pylori* eradication in both groups. Inflammation (mononuclear cell infiltration) (0.77±0.56 vs. 0.33±0.47, p<0.01), atrophy (1.17±0.90 vs. 0.58±0.77, p<0.01), and IM (0.80±0.85 vs. 0.38±0.71, p<0.01) scores at the greater curvature of the antrum were significantly higher in Group A than in Group B. Similarly, inflammation (0.89±0.45 vs. 0.62±0.57, p<0.01), atrophy (1.59±0.99 vs. 0.84±0.96, p<0.01), and IM (1.52±1.16 vs. 0.77±0.95, p<0.01) scores at the lesser curvature of the corpus were significantly higher in Group A than in Group B. Furthermore, the inflammation (0.57±0.59 vs. 0.31±0.47, p<0.01) and atrophy (0.41±0.72 vs. 0.17±0.42, p=0.02) scores at the greater curvature of the corpus were significantly higher in Group A (Table 3).

Inflammation of the background gastric mucosa of the GC group after eradication was significantly higher than the scores for the matched Group B with the greater curvature of the antrum (0.78±0.57 vs. 0.28±0.45, p<0.01), the lesser curvature of the corpus (0.89±0.42 vs. 0.64±0.59, p<0.01), and the greater curvature of the corpus (0.59±0.60 vs. 0.35±0.48, p=0.03), respectively.

Other significant differences were observed in the atrophy of the greater curvature of the antrum (1.13±0.87 vs. 0.64±0.77, p<0.01), the lesser curvature of the corpus (1.62±1.02 vs. 0.92±1.01, p<0.01), and in IM of the greater curvature of the antrum (0.78±0.82 vs. 0.46±0.77, p=0.02), the lesser curvature of the corpus (1.57±1.19 vs. 0.85±0.98, p<0.01) (Table 4).

The risk factors for the development of GC from the histological findings of the background gastric mucosa are shown in Table 5. According to a multivariate analysis, not only atrophy of the lesser curvature of the corpus, but also inflammation of the greater curvature of the antrum and lesser curvature of the corpus, were found to be independent risk factors. The risk ratio and 95% CI were 6.05 (1.28-35.5) (p=0.03), 5.92 (2.11-16.6) (p<0.01), and 3.56 (1.05-13.2) (p=0.04), respectively.

We present a typical case of GC that was detected 10 years after eradication. It was difficult to determine the border line of the lesion in this case (Fig. 2a), because the metaplasia of the mucosa was remarkable (Fig. 2b). The histological examination confirmed the presence of an epithelium with a low degree of atypicality (ELA) in the surface layer of a low cancerous lesion (Fig. 2c), which Masuda et al. reported as the characteristic feature in patients who have undergone successful eradication (19). We examined the background gastric mucosa before ESD. Inflammation was observed in all three biopsy specimens as well as around the lesion (Supplementary material), with the tissue of the lesser

Table 1. Lesion Characteristics of Gastric Cancer Group after Successful Eradication.

	n=61
Location	
Upper third	5
Middle third	35
Lower third	21
Tumor size (mm, mean±SD)	14.7±1.1
Macroscopic type	
Protruding	9
Depressed/Flat	52
Histological type	
Differentiated	55
Undifferentiated/mixed	6
Depth	
M/SM1†	58
SM2‡	3
Curability of endoscopic resection	
Curative	54
Non curative	7

Data are presented as numbers or mean±SD, SD: standard deviation, M: mucosal invasion
†SM1; submucosal invasion depth<500 μm from muscularis mucosa layer
‡SM2; submucosal invasion depth ≥500 μm from muscularis mucosa layer

Table 2. Comparison of the Clinical Characteristics between Gastric Cancer Group (Group A) and Non-gastric Cancer Group (Group B) before and after Propensity-score Matching.

	All patients				Propensity-matched patients			
	Group A (n=61)	Group B (n=122)	p value	ASD	Group A (n=54)	Group B (n=54)	p value	ASD
Gender (male/female)	44/17	61/61	<0.01	0.421	37/17	34/20	0.68	0.111
Age (years, median)	71 (53-85)	68 (29-87)	<0.01	0.601	70 (53-83)	71 (51-83)	0.62	0.107
Years after successful eradication (years, mean±SD)	4.6±4.4	4.2±2.2	0.33	0.115	4.5±4.4	4.6±2.5	0.87	0.028

Data are presented as numbers, mean±SD or median (range), SD: standard deviation, ASD: absolute standardized difference

Table 3. Comparison of the Histological Findings between Group A and B.

	Group A (n=61)	Group B (n=122)	p value
Greater curvature of the antrum			
Inflammation	0.77±0.56	0.33±0.47	<0.01
Atrophy	1.17±0.90	0.58±0.77	<0.01
Intestinal metaplasia	0.80±0.85	0.38±0.71	<0.01
Lesser curvature of the corpus			
Inflammation	0.89±0.45	0.62±0.57	<0.01
Atrophy	1.59±0.99	0.84±0.96	<0.01
Intestinal metaplasia	1.52±1.16	0.77±0.95	<0.01
Greater curvature of the corpus			
Inflammation	0.57±0.59	0.31±0.47	<0.01
Atrophy	0.41±0.72	0.17±0.42	0.02
Intestinal metaplasia	0.35±0.76	0.18±0.53	0.10

Data are presented as mean±standard deviation.

Table 4. Comparison of the Histological Findings between Group A and B (after Matching).

	Group A (n=54)	Group B (n=54)	p value
Greater curvature of the antrum			
Inflammation	0.78±0.57	0.28±0.45	<0.01
Atrophy	1.13±0.87	0.64±0.77	<0.01
Intestinal metaplasia	0.78±0.82	0.46±0.77	0.02
Lesser curvature of the corpus			
Inflammation	0.89±0.42	0.64±0.59	<0.01
Atrophy	1.62±1.01	0.92±1.01	<0.01
Intestinal metaplasia	1.57±1.19	0.85±0.98	<0.01
Greater curvature of the corpus			
Inflammation	0.59±0.60	0.35±0.48	0.03
Atrophy	0.44±0.75	0.19±0.49	0.06
Intestinal metaplasia	0.40±0.79	0.17±0.47	0.11

Data are presented as mean±standard deviation.

Table 5. Univariate and Multivariate Analysis of the Histological Findings.

	Group A (n=54)	Group B (n=54)	Univariate analysis p value	Risk ratio (95%CI)	Multivariate analysis p value
Greater curvature of the antrum					
Inflammation	39	15	<0.01	5.92 (2.11-16.6)	<0.01
Atrophy	35	21	<0.01		
Intestinal metaplasia	31	18	0.01		
Lesser curvature of the corpus					
Inflammation	46	30	<0.01	3.56 (1.05-13.2)	0.04
Atrophy	44	27	<0.01	6.05 (1.28-35.5)	0.03
Intestinal metaplasia	39	26	<0.01		
Greater curvature of the corpus					
Inflammation	30	19	0.03	2.31(0.93-5.96)	0.07
Atrophy	15	9	0.15		
Intestinal metaplasia	13	7	0.13		

curvature of the corpus being the most remarkable of these three specimens (Fig. 3a). We performed an immunohistochemical analysis, and revealed that CD79 was strongly positive (Fig. 3b), while CD3 was partially positive (Fig. 3c). We performed the same analysis on several other cases, and these results were also recorded.

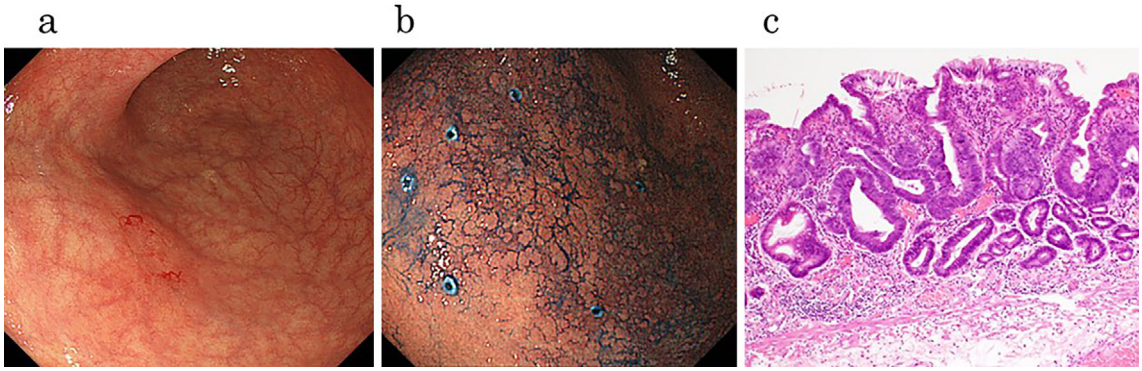

Figure 2. **Endoscopic and pathological features of gastric cancer after successful *H. pylori* eradication therapy. (a) Conventional endoscopic view showed a flat reddish lesion, but there was no marked difference between the cancerous area and surrounding non-cancerous area. (b) Chromoendoscopy by indigo carmine was used and it was still difficult to determine the border line of the lesion because of the metaplasia of mucosal background. (c) Histological examination confirmed epithelium with a low degree of atypicality (ELA) in the surface layer of a low cancerous lesion.**

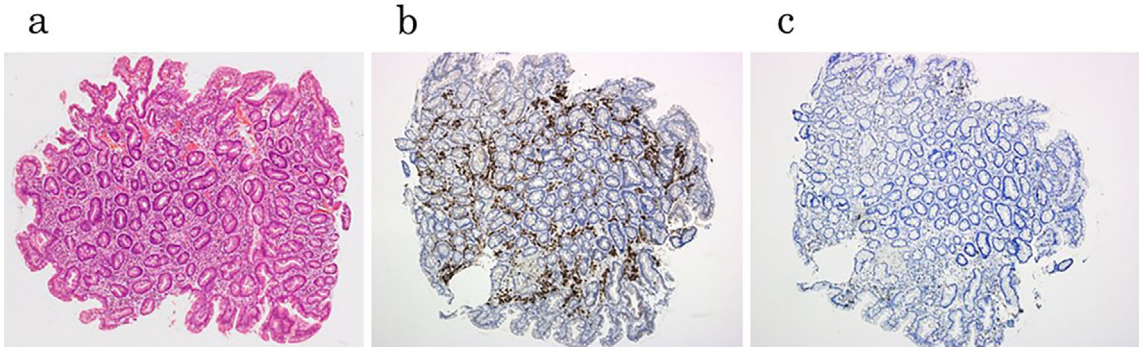

Figure 3. **Immunohistochemical analysis of the tissue of lesser curvature of the corpus. (a) Inflammatory cells were found by Hematoxylin and Eosin staining. (b) CD79a was strongly positive. (c) CD3 was partially positive.**

Discussion

Many studies have been conducted to investigate the risk factors for GC after the eradication of *H. pylori*. Age at eradication (6, 8), severe atrophic gastritis, and intestinal metaplasia especially of the corpus (1, 20, 21), among others, have been evaluated. In this study, significant differences were observed in atrophy and IM of the greater curvature of the antrum, the lesser curvature of the corpus. These results are compatible with previous reports that recommended early eradication before the occurrence of atrophy and IM progression. Furthermore, we have focused on the histological characteristics of the background gastric mucosa after *H. pylori* eradication. Only the inflammation score of every background gastric mucosa specimen of the GC group was significantly higher than that of the non-GC group. On the other hand, the significant difference in atrophy and IM in the greater curvature of the corpus disappeared after propensity score matching. We consider the reason for this phenomenon to be due to the fact that the cases with atrophy and IM in the greater curvature of the corpus were relatively rare.

Moreover, the degree of mononuclear cell infiltration in the IM mucosa is considered to be greater than in the non-metaplastic mucosa because inflammation of the gastric mucosa thought to have a close association with atrophy and IM. Therefore, we added a multivariate analysis, and thereby identified that inflammation of the greater curvature of the antrum and lesser curvature of the corpus tended to be independent risk factors.

Chronic inflammation is characterized by the infiltration of mononuclear cells. It has been reported that the inflammation score of the gastric mucosa showed significant degradation after the eradication of *H. pylori* infection from about 6 months to 5 years (8, 22). We also showed that the inflammation of the greater curvature of the antrum, and the lesser curvature and the greater curvature of the corpus was significantly higher in the GC group compared to the age, sex, and the years after successful eradication in the matched non-GC group. Therefore, we consider that the continuous mononuclear cell infiltration of the gastric mu-

cosa could be a risk factor for GC after successful *H. pylori* eradication.

Several reasons can be considered as to why mononuclear cell infiltration remains after successful eradication and it may therefore become a risk factor for the onset of GC. Kodama et al. reported that the background gastric mucosa of the GC group had greater mononuclear cell infiltration than the non-GC group before the eradication of *H. pylori,* and they hypothesized that the continuous inflammation after eradication might promote the onset of GC (8). In this study, mononuclear cell infiltration at the time when GC was detected was significantly higher than that of the non-GC group. Thus, we demonstrated that continuous inflammation before and after eradication can promote the onset of GC.

Continuous chronic inflammation with mononuclear cell infiltration could accelerate the levels of DNA hypermethylation in the gastric mucosa, and could thereby promote the onset of GC. Reports on GC studies have shown that aberrant DNA methylation plays an important role, and the degree of accumulation of aberrant DNA methylation correlates highly with GC risk (23, 24).

Once *H. pylori* has been eradicated, the DNA methylation level decreases somewhat but it does not disappear completely (25, 26). Persistent methylation levels after eradication are considered to reflect methylation in the stem cells (27) and the degree of residual DNA methylation could closely correlate with GC risk (28).

Schneider et al. demonstrated that chronic inflammation, as measured by the level of infiltration of mononuclear cells, was a significant factor for methylation after adjusting the other variables (29). From this study, it is possible that, in the case of the GC groups, chronic inflammation remained even after *H. pylori* eradication, and they also demonstrated higher DNA methylation levels than the non-GC groups.

This cohort study had a single center retrospective design. Therefore, there are several limitations associated with this study. First, the patients' background of each group such as BMI, history of alcohol intake, smoking, family history, and so on were not considered. Sun et al. demonstrated that GC risk was affected by lifestyle factors, which may regulate the expression of inflammatory cytokines and promote gastric carcinogenesis (30). There has been no report on the relationship between GC developing after eradication and lifestyle factors. However, chronic inflammation caused by lifestyle factors may have affected our results as chronic inflammation of the background gastric mucosa is an important factor in GC development.

Second, there was not sufficient data available on the background gastric mucosa before *H. pylori* eradication in the GC group. Therefore, it is difficult to compare between the two groups before eradication. Third, we could not adequately assess and provide details on the presence of mononuclear cells. Nonetheless, we conducted a pilot study using an immunohistochemical analysis to elucidate the type of mononuclear cell infiltration, and as a result, CD79a was strongly positive. CD79a serves as a pan-B cell marker. In animal models, CD79a invasion has been reported to be observed in the gastric mucosal model of *H. pylori* infection (31). Several studies have reported that the spontaneous activation of B cells promoted de novo epithelial carcinogenesis by initiating chronic inflammation (32-34). Our immunostaining results suggest the possibility that the development of GC after *H. pylori* eradication is connected to the persistence of chronic inflammation after eradication. In the future, a more detailed study, based on an immunohistochemical analysis of mononuclear cell infiltration, should be conducted.

Finally, because of the short period of time for the development of new gastric cancer after eradication therapy, this study may have sometimes identified the tumor progression of a preexisting precursor rather than tumor initiation after eradication therapy. Take et al. described in a recent manuscript that the risk of gastric cancer developing after eradication of *H. pylori* was greater in the second decade of follow-up than in the previous 10-year period, and endoscopic surveillance for gastric cancer should thus be continued beyond 10 years after the eradication of *H. pylori* infection (35). Therefore, to investigate the association between persistent mucosal inflammation and new tumor development, a long-term cohort study is needed.

Conclusion

In conclusion, this study suggested that the high level of mononuclear cell infiltration of the background gastric mucosa may be a risk factor for GC onset after eradication, in addition to gastric atrophy and intestinal epithelialization. In the future, more intensive endoscopic follow-up is needed for patients who have been identified as a high-risk group based on their background gastric mucosa after the eradication of *H. pylori*.

The authors state that they have no Conflict of Interest (COI).

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