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<th>Cutoff Pepsinogen Level for Predicting Unintendedly Eradicated Cases of Helicobacter pylori Infection in Subjects with Seemingly Normal Pepsinogen Levels</th>
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<td>Author(s)</td>
<td>Kishikawa, H; Kimura, K; Ito, A; Arahata, K; Takarabe, S; Kaida, S; Miyauchi, J; Miura, S; Kanai, T; Nishida, J</td>
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Cutoff pepsinogen level for predicting unintendedly eradicated cases of *Helicobacter pylori* infection in subjects with seemingly normal pepsinogen levels

Short running title: Pepsinogen level to predict unintendedly eradication of H. pylori

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Key words: *Helicobacter pylori*, pepsinogen, serum antibody, risk stratification, gastric cancer screening.
Abstract

Backgrounds/Aims: In the ABC method, which is a method for risk stratification of gastric cancer using serum anti-*H. pylori* antibody and pepsinogen (PG) test, subjects with normal PG and seronegative for *H. pylori* are named as “Group A” and are regarded as having a low risk of gastric cancer. These “Group A” subjects include unintentionally eradicated cases at relatively high risk, and this study aimed to identify these subjects.

Methods: Of the 109 subjects, 76 were classified as uninfected Group A subjects with negative histologic *H. pylori* infection and no histologic and endoscopic atrophy, and 33 subjects were classified serologically as Group A after successful eradication, which are serologically equal to the unintendedly eradicated cases in Group A. The usefulness of measuring PG levels to detect post-eradication cases was validated by using a receiver-operating characteristic (ROC) curve analysis.

Results: The area under the ROC curve for PGI level was 0.736 ± 0.06 (p < 0.01; cutoff value, 37.0 ng/ml; sensitivity, 77.6%; specificity, 72.7%), and that for the PGI/II ratio was 0.660 ± 0.06 (p < 0.01; cutoff value, 5.1; sensitivity, 84.2%; specificity, 43.4%).

Conclusion: PGI levels of ≤37 ng/ml and PG-I/II ratios of ≤5.1 effectively identified unintendedly eradicated cases in Group A.

Introduction

Numerous epidemiological and basic studies have shown that *Helicobacter pylori* infection-induced gastric mucosal atrophy is a significant risk factor in gastric cancer development [1-4]. The basic histopathological lesions of Correa’s cascade include *H. pylori*-related gastritis, chronic atrophic gastritis, intestinal metaplasia and carcinoma, and this scheme is now widely accepted as a model for stomach carcinogenesis [5]. As gastric mucosal atrophy is mainly induced by *H. pylori* infection, except for autoimmune gastritis, a serum screening method has been developed in Japan to determine high-risk subjects through detection of mucosal atrophy induced by *H. pylori* infection [6-8]. A serum pepsinogen (PG) test using PGI and PGII has been regarded as a reliable marker to assess the extent of atrophic gastritis [9, 10]. PGI is contained by chief cells in the fundic mucosa, and PGII is contained not only in the fundic gland mucosa, but also in the pyloric, cardiac, and duodenal mucosae. Thus, the combination of a low PGI level and a low PGI/II ratio is an indicator of histological and functional status of gastric mucosal atrophy [10]. In most cases, a positive result for serum *H. pylori*. 

*Helicobacter pylori* antibody indicates current *H. pylori* infection. Thus, a gastric cancer screening method using serum anti-*H. pylori* antibody and PG tests has been applied in some health check-up settings in Japan for screening high risk subjects of gastric cancer, and the method is called as the “ABC method” [6-8, 11, 12].

In the ABC method, the subjects are classified into 4 groups based on the results of the above-mentioned 2 serologic tests: Group A (seronegative for *H. pylori* and normal PG); Group B (seropositive for *H. pylori* and normal PG); Group C (seropositive for *H. pylori* and atrophic PG); and Group D (seronegative for *H. pylori* and atrophic PG). The risk of gastric cancer is highest in Group D, followed by Groups C, B, and A. Therefore, groups B, C, and D are advised to undergo endoscopy. Subjects in Group A are regarded as “*H. pylori* infection-negative subjects” who are at low risk for gastric cancer; thus, endoscopy is not often recommended for them [6-8].

In the ABC screening method, subjects with a previous history (as determined by medical interview) of eradication of *H. pylori* infection are strictly excluded, but unintentionally eradicated cases are inevitably included. Recently, several reports have suggested that subjects with unintended *H. pylori* eradication account for up to 10% of subjects without a history of eradication [11, 13, 14]. Our previous report demonstrated that all 10 cases of gastric neoplasia (2.9%) classified into Group A (345 subjects) using the ABC method showed endoscopic signs of atrophy, indicating a post-eradication status. Moreover, these post-eradication subjects were relatively high-risk subjects compared with the *H. pylori*-uninfected subjects [15]. Unintended *H. pylori* eradication could occur after exposure to antibiotics for the treatment of other infectious diseases, and the recent inappropriate use or abuse of antibiotics may increase the unintended eradication rate [16]. These findings suggest that a certain rate of “unexpectedly eradicated cases” with negative seroconversion is incorrectly classified into Group A using the ABC method. These patients are not regarded as candidates for endoscopy, despite an elevated risk for gastric cancer. However, the risk factors to identify these relatively high-risk subjects in Group A have not yet been completely elucidated. We particularly focused on serum PG levels as a marker to identify these subjects based on previous reports suggesting that serum PG levels remain unchanged after more than 6 months of successful eradication [17, 18].

In the present study, the characteristics of a virtual population serologically classified as Group A composed of (1) *H. pylori*-uninfected subjects and (2) subjects with successful eradication of infection showing normal PG level and negative *H. pylori* antibody titer, mimicking unexpectedly eradicated cases, were examined. This study aimed to identify cutoff values of PG associated with these post-eradication cases
among subjects with a normal PG levels and negative H. pylori antibody titer to detect the post-eradication cases classified into Group A and increase the efficacy of a mass screening system using the ABC method.

**Methods**

**Patients**

Between 2007 and 2016, 863 outpatients who visited the Department of Gastroenterology of the Tokyo Dental College, Ichikawa General Hospital, to undergo upper gastrointestinal endoscopy were prospectively analyzed. A total of 46 consecutive H. pylori-seropositive subjects who underwent successful eradication therapy were enrolled, and endoscopic and serological evaluations were performed after at least 12 months. Successful H. pylori eradication was defined as a negative $^{13}$C-urea breath test performed more than 8 weeks after completion of eradication. Of the 46 subjects, 7 with a positive H. pylori antibody titer after the eradication therapy and 6 with PG test-positive atrophic gastritis after the eradication therapy were excluded. A total of 33 patients (71.7%) who were seronegative for H. pylori and had normal PG test results (serologically defined as Group A) after eradication were included in the final analysis. Patients were endoscopically diagnosed with peptic ulcers (n = 11) and adenomas (n = 2). Based on the atrophic border scale, 4 (12%) had no atrophic gastritis and 29 (88%) had closed or open type atrophic gastritis [19]. Of the 33 subjects, 15 (45.5%) were originally classified into Group B (positive H. pylori and normal PG) and 18 (54.5%) were originally classified into Group C (positive H. pylori and atrophic PG).

On the other hand, a total of 378 consecutive outpatients classified into Group A (negative H. pylori antibody titer and normal PG) using the ABC method were included in the study. Of the 378 subjects, 76 consecutive uninfected subjects with (1) negative results of histological H. pylori infection, (2) no histological atrophy of the gastric gland, and (3) no endoscopic atrophy were evaluated. Endoscopic exam revealed three patients with peptic ulcers and 73 normal subjects. Based on the atrophic border scale, all subjects had no atrophic gastritis [19].

From the two subgroups of subjects, a virtual H. pylori seronegative population (n = 109) that consisted of post-eradication subjects serologically classified into Group A (normal PG and negative H. pylori antibody titer) after successful eradication (n = 33) and uninfected Group A subjects (n = 76) was created, and predictors to identify post-
eradicat
[295x789]ion
[85x729]subjects (mimicking unintendedly eradicated cases) were evaluated.
The reasons for performing endoscopy were dyspepsia, annual endoscopic follow-up, screening in asymptomatic subjects, and abnormalities on barium X-ray examinations.
Exclusion criteria were as follows: (1) use of histamine-2 receptor antagonists, proton pump inhibitors, or potassium-competitive acid blockers within the preceding 2 months; (2) H. pylori eradication therapy before the study as recalled by the patient; (3) presence of viral diseases, such as acute respiratory diseases; (4) pregnancy or lactation; (5) presence of renal (serum creatinine level >1.5 mg/dL) and/or liver dysfunction (total bilirubin level >1.5 mg/dL, AST, and ALT level >50 IU/mL); or (6) a history of gastric cancer or any type esophageal or gastric surgery [15, 20].
This study was approved by the Tokyo Dental College Ichikawa General Hospital Ethics Committee (No. 101-B/2007, 283/2012, and I-283R/2015) and was conducted according to the principles of the Second Declaration of Helsinki. All patients provided their written, informed consent prior to enrollment.

Endoscopic and Histologic Examinations
Gastrointestinal endoscopy was performed using electrical panendoscopes (type XQ260, H290; Olympus, Tokyo, Japan). All endoscopies were performed by a single experienced gastroenterologist (HK). Endoscopic gastric mucosal atrophy was graded using the endoscopic atrophic border of Kimura and Takemoto [19]. The extent of atrophy was divided into normal and atrophic (open type or closed-type atrophic gastritis), as previously described [15]. At each endoscopy to evaluate “uninfected Group A” subjects, 2 biopsy specimens were taken from both the greater curvature of the antrum and the mid-corpus, and were stained with hematoxylin-eosin or Giemsa stain. Gastritis scores in gastric mucosa were evaluated by a specialist using the updated Sydney system [21].

Evaluation of H. pylori Serology, Serum PGI, PGI/II Ratio, and Gastrin Level
Fasting blood samples were collected immediately before endoscopy. Measurements of serum PG-I and PG-II levels were performed using a commercial radioimmunoassay (RIA) kit, as previously described [15, 20]. An “atrophic” PG test was defined as when the criteria of both PGI ≤70 ng/mL and PGI/II ratio ≤3 being satisfied, and a “normal” PG test was defined as these criteria were not being satisfied [6-8, 11]. The reported sensitivity and specificity of these criteria to detect extensive atrophy are 70.5% and
97%, respectively [22]. Gastrin level was measured using RIA (Gastrin RIA Kit II, Fujirebio Diagnostics Co. Ltd, Tokyo, Japan).

Serum *H. pylori* antibody titer was measured using a direct enzyme immunoassay kit (E Plate “Eiken” Hp antibody; Eiken Kagaku Co. Ltd, Tokyo, Japan). Subjects with an antibody concentration <10 U/mL were classified into the infection-negative group according to the manufacturer’s recommended cutoff value; this cutoff value has been widely used in previous studies [6-8, 11, 23, 24]. Using these criteria, subjects with a normal PG test and negative status for *H. pylori* antibody titer were classified into Group A [6-8, 11].

**pH Measurement**

Gastric juice samples were collected through a sterile tube during endoscopy, and their pH was measured with a glass electrode, as previously described [19].

**Statistical Analysis**

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) v 22 (SPSS Inc., Chicago, IL, USA) for Windows. Data of continuous variables are expressed as median (interquartile range). The Mann-Whitney U test was conducted to determine significant differences in continuous variables between different groups. The chi-squared test or Fisher’s exact test was used for categorical variables to compare uninfected cases and post-eradication cases.

The cutoff values for PGI level and the PGI/II ratio were evaluated using receiver operating characteristic (ROC curves). Area under the ROC curve (AUROC) analysis was performed to assess the utility and the goodness-of-fit of PG for predicting post-eradication cases (n = 33) from healthy subjects who had never had the infection (n = 76). A 2-sided $p$ value of $< 0.05$ was considered significant.

**Results**

**Baseline Characteristics of the Study Population**

In the 33 *H. pylori*-positive subjects who underwent successful eradication treatment for 12-36 months (median 24.0 months), changes in serum PG levels, serum *H. pylori* antibody titer, and fasting gastric juice pH before and after eradication therapy were examined. After eradication, the serum median PGI level significantly decreased from
51.9 ng/ml (29.7 ng/mL–68.6 ng/mL) to 30.1 ng/mL (24.3 ng/mL–40.3 ng/mL), whereas the PGI/II ratio significantly increased from 2.2 (1.7–3.3) to 5.9 (4.7–6.7). The serum *H. pylori* antibody titer, serum gastrin level, and fasting gastric juice pH significantly decreased from 36.0 U/ml (23.0–70.0 U/ml) to 6.0 U/ml (3.0–7.0 U/ml), 132.5 pg/ml (82.0–218.5 pg/ml) to 95.0 pg/ml (76.0–127.0 pg/ml), 6.6 (2.2–7.1) to 1.8 (1.5–2.9), respectively (Table 1, Fig 1a-e).

The serum PGI level (*p* < 0.001) and PGI/II ratio (*p* < 0.01) of the 76 uninfected Group A subjects were significantly higher than those of the 33 post-eradication subjects classified into Group A after eradication. However, the serum gastrin level (*p* < 0.01), *H. pylori* antibody titer (*p* < 0.01), and fasting gastric pH (*p* < 0.01) of uninfected Group A subjects were significantly lower (Table 1, Fig 1a-e).

**ROC Analysis**

From the virtual *H. pylori*-seronegative population (n = 109), ROCs for PGI level and the PGI/II ratio were constructed to assess different areas under the curve and determine the best threshold values to differentiate post-eradication cases (n = 33, mimicking unintendedly eradicated cases in Group A) from those that were true-negative for *H. pylori* infection (n = 76). *H. pylori* antibody titer was excluded as a variable because of its instability due to the chronologically progressive decrease of *H. pylori* antibody titers after eradication [25, 26]. Serum gastrin level and gastric juice pH were excluded as they could not be evaluated in serum gastric cancer screening using the conventional ABC method. Age was also not used as a variable because the subjects were outpatients and *H. pylori* was eradicated as a part of medical therapy, thus introducing bias into the evaluation. Based on the ROC curve analysis, the optimal cutoff values for PGI and the PGI/II ratio were 37.0 ng/ml and 5.1, respectively (Fig. 2). The AUROC for PGI level was 0.736 ± 0.06 (95% CI=0.621–0.851, *p* < 0.01; cutoff value =37.0 ng/ml, sensitivity = 77.6%, 1-specificity = 27.3%), and that for the PGI/II ratio was 0.660 ± 0.06 (95% CI=0.546–0.774, *p* < 0.01; cutoff value = 5.1, sensitivity = 84.2%, 1-specificity = 57.6%).

**Discussion**

Low PGI (≤37 ng/mL) and a low PGI/II ratio (≤5.1) were significant predictors for unintendedly eradicated cases among subjects seronegative for *H. pylori* with normal PG test results (defined as Group A using the ABC method). We consider that these findings can resolve the critical disadvantage of using the ABC method for serum
screening of gastric neoplasia cases, which is the incorporation of post-eradication subjects with relatively high-risk for gastric carcinoma into Group A [6, 8, 11, 15]. Based on our results, the subjects with a low PGI (≤37 ng/mL) or PGI/II ratio (≤5.1) should be regarded as a higher-risk population within Group A and thus should undergo endoscopic surveillance. The prevalence of these high-risk subjects (PGI of ≤37 ng/mL or low PGI/II ratio of ≤5.1) in Group A was 35.8% (135/379 subjects in group A, unpublished data).

Because the majority of subjects with neoplasia cases assumed to be classified into Group A were originally classified as post-eradication subjects, as previously reported [11, 15], the discrimination of post-eradication cases serologically classified into Group A is important [11]. Moreover, the risk of gastric cancer in *H. pylori*-uninfected subjects is extremely low in Japan [24]. Thus, the purpose of gastric cancer screening using serum test is to detect strictly *H. pylori* infected subjects and *H. pylori* post-eradication subjects because eradication merely reduces the incidence of gastric cancer to 54% [28]. In this study, 18 of 33 (54.5%) post-eradication subjects were classified into Group C using the ABC method before eradication, suggesting that they were originally a relatively high-risk group, and periodic endoscopic surveillance should be performed even after eradication.

Kitamura et al. [28] recently reported that a cutoff PGI/II ratio of ≤5 shows high sensitivity and accuracy to diagnose *H. pylori*-induced gastritis. Kim et al. [29] reported a similar result; they found that the optimal cutoff value of serum PG-I/II ratio for predicting histologically confirmed atrophic gastritis of the antrum is 4.9. The present study demonstrated that a similar cutoff PGI/II ratio of ≤5.1 could also be applied to a limited population of *H. pylori* seronegative subjects to identify subjects with *H. pylori*-induced gastritis in Group A.

Boda et al. [11] reported that a discriminant function using serum gastrin and PGs could effectively discriminate gastric neoplasia cases classified into Group A from “*H. pylori*-uninfected” Group A subjects. The concept employed in their study to identify “high-risk subjects in Group A” was similar, but slightly different, from that used in the present study. They intended to predict limited cases of unintentionally eradicated subjects with gastric neoplasia, and they did not consider other post-eradication cases. In the present study, we identified subjects without neoplasia but suspected unintended eradication in Group A. Thus, the present strategy is better as a screening strategy. Furthermore, the present study showed that a simple method using serum PG levels alone could be easily used in gastric cancer screening by the ABC method. However, the previous strategy using gastrin levels seems impractical, because gastrin is not a test
item in the ABC method [6-8, 12].

For the purpose of accuracy testing, we intentionally excluded *H. pylori* antibody titer as a potential predictor of post-eradication cases because of the progressive decrease of the antibody titer after eradication [25, 26], thus making it unsuitable as a predictor. However, we previously demonstrated that a high-negative *H. pylori* antibody titer (within the normal range for *H. pylori* serology but equal to the minimum determination limit or higher) is a statistically significant predictor to discriminate gastric neoplasia cases in Group A [15]; a high-negative *H. pylori* antibody titer itself may be a potential candidate predictor of post-eradication cases.

The median gastric pH significantly decreased after successful eradication in the present study. This significant improvement of acidity after eradication seems to be consistent with previous work by Furuta et al. [30]. They showed that fasting gastric juice pH of *H. pylori* positive gastric ulcer changes significantly from 6 to 2 after eradication. Reflux esophagitis is well known to occur in approximately 10% of subjects after eradication therapy [31, 32], suggesting that significant improvement of gastric acidity should be observed shortly after eradication in cases without histological improvement of atrophy.

Several limitations of this study should be considered. First, age may be a candidate predictor for unintended eradication cases because elderly patients have greater chances of exposure to antibiotics than younger patients. Second, because the median time after eradication in our population is approximately 2 years, the appropriateness of these criteria (cutoff value) for subjects after a long period following eradication has not been evaluated yet. [33]. Although the time course changes of PG levels after eradication have not been elucidated, at least low PGI level (≤37 ng/ml) may be used as a predictor of post-eradication cases because of the following reasons. First, PGI level generally does not increase after eradication [17, 18, 34]. Second, low PGI level is not associated with increased acid secretion after eradication, suggesting that PGI level would decrease synchronously with recovery from atrophy [35].

In summary, 2 predictors, PGI level ≤37 ng/ml and PG-I/II ratio ≤5.1, effectively identified post-eradication cases incidentally classified into group A using the ABC method. Endoscopy should be performed in these cases, even in Group A subjects, who are regarded as low risk of gastric cancer. The results obtained in this present study are particularly important to increase the accuracy of gastric cancer screening using the ABC method.
Acknowledgments
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Disclosure statement
All authors declare that there are no potential conflicts of interest.

References


Table 1. Baseline characteristics of the study population

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<tr>
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<th>Uninfected Group A subjects (n = 76)</th>
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<tr>
<td></td>
<td>before eradication</td>
<td>after eradication</td>
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<tr>
<td>Male, n (%)</td>
<td>18 (54.5)</td>
<td>38 (50.0)</td>
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<td>Age, years</td>
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<tr>
<td>Median</td>
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<td>Normal/closed or open type</td>
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<td>76/0*</td>
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<td>ABC classification (Groups A/B/C/D)</td>
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<tr>
<td>Median</td>
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<td>95†</td>
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<tr>
<td>Range</td>
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<tr>
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<tr>
<td>Range</td>
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*p < 0.001 vs. negatively seroconversion cases.
†p < 0.01 vs. before eradication.
‡p < 0.001 vs. before eradication.
§p < 0.01 vs. after eradication.
∥Gastrin was tested in 18 subjects serologically classified into Group A after eradication and in 38 uninfected subjects.
¶Fasting gastric juice pH was tested in 22 subjects classified serologically as Group A after eradication and 68 uninfected cases.
**Figure legends**

**Figure 1.**
Comparison of variables between before and after eradication in subjects classified into Group A after eradication, and a comparison between the post-eradication subjects and uninfected subjects.

The top and bottom of each box represent the first and third quartiles, respectively, and the line across each box represents the median. The upper and lower whiskers show scores outside the middle 50%. Outliers are plotted as individual points. The significance of differences between all pairs of groups was determined using the Mann-Whitney U test (*p < 0.05).

**Figure 2.**
Receiver operating characteristic (ROC) curves for the performance of pepsinogen (PG)-I level (2a) and the PG I/II ratio (2b) for distinguishing the post-eradication subjects from uninfected subjects.
Figure 2b