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Predictors of Gastric Neoplasia in Cases Negative for *Helicobacter pylori* Antibody and with Normal Pepsinogen

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Abstract. *Background/Aim: Individuals negative for Helicbacter pylori antibody and with a normal pepsinogen test (group A) are regarded as being at low risk in serum gastric cancer screening known as the ABC method, and endoscopy is not recommended; however, this group may include 2-10% of gastric cancer cases. Patients and Methods: A total of 345 individuals who underwent upper gastrointestinal endoscopy and were classified by ABC as group A (H. pylori antibody titer <10 U/ml, and pepsinogen-I >70 ng/ml or I/II ratio >3) were enrolled, and predictors of gastric neoplasia were investigated. Results: Ten gastric neoplasia cases (gastric cancer and adenoma) were found to be included. Multiple logistic regression analyses identified H. pylori antibody titer ≥3 U/ml (odds ratio=14.4, 95% confidence interval=2.7-76.9; p<0.01) and pepsinogen-I/II ratio ≤4.3 ng/ml (odds ratio=10.0, 95% confidence interval=2.1-47.9; p<0.01), but not age as independent predictive factors of neoplasia. Conclusion: Endoscopy should be considered in individuals with H. pylori antibody titer of ≥3 U/ml and a pepsinogen-I/II ratio of ≤4.3 in those classed as group A by ABC method.*

Many epidemiological and experimental studies agree with the association between gastric carcinogenesis and *Helicobacter pylori* infection (1-5). *H. pylori*-related gastritis extends from the antrum to the corpus, and results in extensive atrophic gastritis with intestinal metaplasia, a precursor of non-cardia gastric cancer (6, 7). There is an general agreement that the serum pepsinogen level is associated with progression of atrophic gastritis (8, 9). *H. pylori* antibody reflects *H. pylori* infection status quantitatively; the reported sensitivity for *H. pylori* infection is over 90% (10, 11). Thus, the screening method of the combination of serum pepsinogen and serum *H. pylori* antibody titer is advocated for gastric cancer screening, and is called the "ABC method" (12-14). Several prospective studies have confirmed that the ABC method provides good gastric cancer screening (12-15).

With the ABC method, the risk for the development of gastric cancer is stratified into four categories: group A: *H. pylori*-negative (titer <10 U/ml), normal pepsinogen (pepsinogen-I >70 ng/ml or I/II ratio >3); group B: *H. pylori*-positive, normal pepsinogen; group C: *H. pylori*-positive, abnormal pepsinogen; and group D: *H. pylori*-negative, abnormal pepsinogen. The risk of gastric cancer is the highest in group D, followed in order by groups C, B, and A (12). However, several investigators have recently reported that even about 2-10% of gastric cancer cases were classified into group A, suggesting that a certain percentage of those in group A are not true *H. pylori*-negative cases, and some develop gastric cancer (16-20). Thus, it is important to identify the patients at high risk for gastric cancer among those with a normal pepsinogen and negative *H. pylori* antibody titer. Recently, several studies have pointed-out the relationship between the *H. pylori* antibody titer and gastric cancer risk, especially in subjects with advanced atrophy (21-24). However, the risk factors among those at low risk have not yet been fully elucidated.

In the present study, the aim was to identify clinically useful factors associated with gastric neoplasia cases among individuals negative for serum *H. pylori* antibody and having a normal pepsinogen status (group A by the ABC method).

Patients and Methods

Between 2007 and 2015, a total of 734 outpatients who attended the Department of Gastroenterology of the Tokyo Dental College, Ichikawa General Hospital for upper gastrointestinal endoscopy

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were prospectively enrolled. The reasons for performing endoscopy were as follows: dyspepsia, annual endoscopic follow-up, screening purpose in asymptomatic individuals, abnormalities on barium X-ray examinations, and positive ABC screening method.

A blood sample was drawn before the endoscopic examination. Measurement of serum pepsinogen-I, pepsinogen-II, and *H. pylori* IgG antibodies was contracted out to LSI Medience Co., Ltd. (Tokyo, Japan), as described previously (24, 25). The criterion of pepsinogen-I ≤70 ng/ml and pepsinogen-I/II ratio ≤3 has been widely applied to mass screening for gastric cancer; atrophy-positive is defined when the criterion is fulfilled, and atrophy-negative is defined when the criterion is not fulfilled (13, 14, 26, 27). The reported sensitivity and specificity of these criteria to detect extensive atrophy is 70.5% and 97%, respectively (27).

The IgG antibody titer was measured with a direct Enzyme immunoassay (EIA) kit (E Plate "Eiken" Hp antibody; Eiken Kagaku Co. Ltd, Tokyo, Japan). Individuals with antibody concentration <10 U/ml were categorized as the *H. pylori* infection-negative group according to the manufacturer's recommended cutoff value, and this cutoff value has been widely used in previous studies (5, 16, 22, 27). The reported sensitivity and specificity of this kit using the cutoff value were 91.2% and 97.4%, respectively, based on the results of stool antigen test as gold standard (28), and other investigators reported sensitivity of 100% and specificity of 80%, using rapid urease test, culture and histology as a gold standard (29).

Exclusion criteria were as follows: i) use of histamine-2 receptor antagonists or proton pump inhibitors within the preceding two months; ii) *H. pylori* eradication therapy before the study as recalled by the patient; iii) presence of viral diseases such as acute respiratory diseases; iv) pregnancy or lactation; or v) a history of severe renal or liver dysfunction.

Gastrointestinal endoscopy was performed using electrical panendoscopes (type XQ260; Olympus, Tokyo, Japan). All endoscopies were performed by a single experienced gastroenterologist (HK). Gastric neoplasia was defined as gastric cancer and adenoma. All gastric neoplasias were treated endoscopically or surgically, and specimens obtained were fixed with buffered formalin and stained with hematoxylin and eosin. Pathologic diagnoses were reviewed by experienced pathologist (JM) and judged according to the criteria of the Japanese Classification of Gastric Carcinoma (30). Endoscopic gastric mucosal atrophy was diagnosed by an endoscopic scoring system using the endoscopic atrophic border of Kimura and Takemoto and the extent of atrophy was divided into normal and open type or closed type (31).

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.

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 are expressed as means±standard deviation (SD). Comparisons between groups were performed using the *t*-test for continuous variables and the chi-square test and Fisher's exact test for categorical variables to compare gastric neoplasia cases and no-gastric neoplasia cases.

Factors associated with gastric neoplasia cases were then evaluated using receiver operating characteristic curves (ROC), and area under the receiver operating characteristic curve (AUROC) analysis was used to assess the utility and the goodness-of-fitness of parameters in the diagnosis of gastric neoplasia in group A. Comparison of ROC curves was obtained using the Hanley-McNeil method. Using significant factors, univariate and multiple logistic regression analyses were performed. A two sided *p*-value of less than 0.05 was considered significant.

Results

Study participants. Figure 1 shows the exclusion process for identifying individuals with a negative *H. pylori* status (<10 U/ml) and a negative pepsinogen test (atrophy-negative: pepsinogen-I >70 ng/ml, or pepsinogen-I/II ratio >3). After excluding those who met the exclusion criteria, a total of 734 individuals, including 43 gastric neoplasia cases, were enrolled in our study, and 357 with a positive *H. pylori* antibody titer (≥10 U/ml; groups B and C), and 32 pepsinogen test-positive and *H. pylori*-negative with extensive atrophic gastritis (pepsinogen-I ≤70 ng/ml, pepsinogen-I/II ratio ≤3 and *H. pylori* antibody titer <10 U/ml; group D) were excluded. Consequently, 193 (26.1%) were classified as group B, 164 (23.2%) as group C, and 32 (3.7%) as group D, according to the ABC method. Finally, 345 patients (47.1%) were included for the final analysis and diagnosed as belonging to group A.

Baseline characteristics and comparisons between H. pylori-negative individuals with low and high antibody titers. Table I shows the baseline characteristics of the study subjects. A total of 345 individuals (170 men (49.3%), mean age 57.7±15.2 years) were examined for the study. The reasons for endoscopic examination were dyspepsia in 202 patients, annual endoscopic follow-up in 48, screening purpose in 25, abnormalities on barium X-ray examinations in 65, and positive result for ABC screening method in five. The mean pepsinogen-I concentration was 50.4±22.3 ng/ml, and the mean pepsinogen-I/II ratio was 6.8±1.8.

These various parameters were compared between cases with gastric neoplasia and those without. The mean age and the *H. pylori* antibody titer were significantly lower in the cases without gastric neoplasia than in gastric neoplasia cases ($p<0.01$). The mean pepsinogen-I/II ratio ($p<0.01$) was significantly higher in cases without gastric neoplasia. However, there were no significant differences in pepsinogen-I levels. The prevalence of endoscopically atrophic cases (both of closed type and open type) was significantly higher in those with gastric neoplasia (10/10) than in those without (57/335).

Table I also shows the clinical characteristics of 10 cases of *H. pylori*- and atrophy-negative gastric neoplasia. Four patients underwent endoscopy for dyspepsia, three for abnormal barium-X ray result, two for annual health check-up, and one due to positive ABC screening method. Thus, six asymptomatic cases and four dyspeptic cases are included. Eight patients were men. Histologically, eight out of the 10

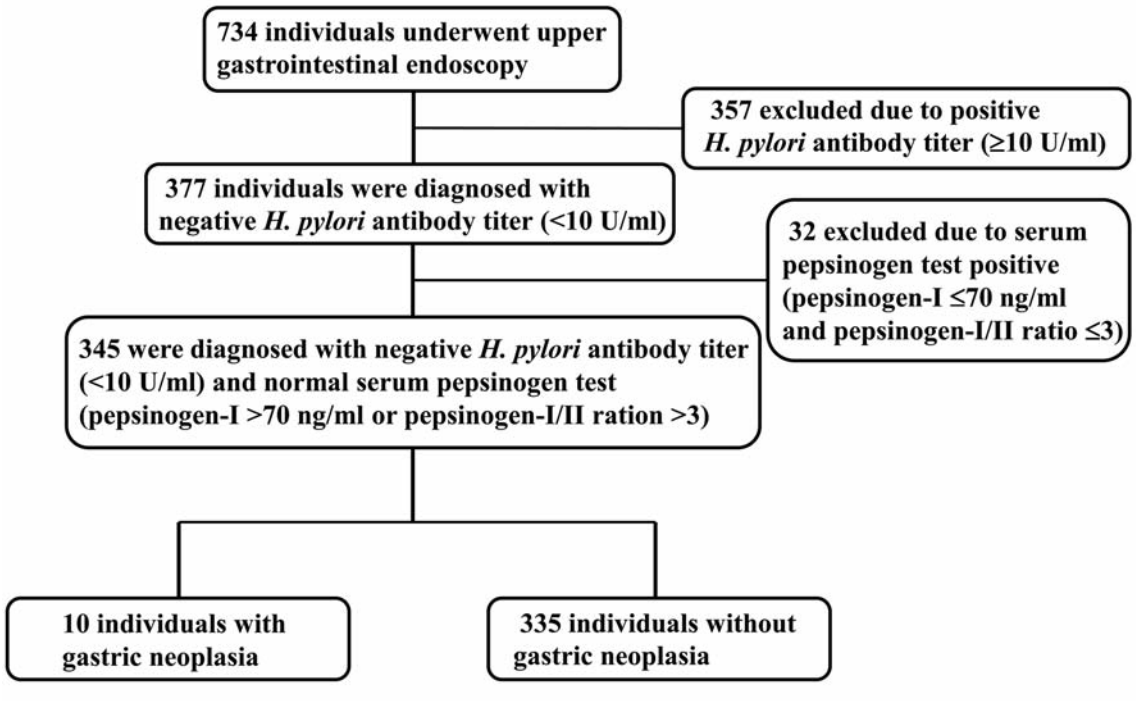

Figure 1. *Study flow diagram*.

Table I. *Baseline study population characteristics with and without gasric neoplasia*.

Characteristic	All, N=345	Gastric neoplasia N=10	No gastric neoplasia N=335	*p*-Value
Male (N%)	170 (49.3%)	8 (80.0%)	162 (48.4%)	0.06
Age (years, mean±SD)	57.7±15.2	70.9±6.4	57.3±15.2	<0.01*
Pepsinogen I (ng/ml, mean±SD)	50.4±22.3	37.6±19.3	50.8±22.3	0.06
Pepsinogen I/II ratio (mean±SD)	6.8±1.8	4.9±1.4	6.8±1.7	<0.01*
H. pylori antibody titer (U/ml, mean±SD)	0.7±1.9	4.3±3.1	0.6±1.7	<0.01*
Endoscopic atrophic border (normal/close type or open type)	67/345	0/10	57/335	<0.01*
Pathology				
Adenoma/intestinal/diffuse		1/8/1		
Deapth of invasion				
M/SM-MP		7/3		
Treatment				
ESD or EMR/Surgery		7/3		

*A *p*-value of less than 0.05 was considered significant in the comparison between cases with gastric neoplasia and those without. Intestinal: intestinal-type, diffuse: diffuse-type gastric cancer according to the Japanese Classification of the Gastric Cancer. M: Mucosa, SM: submucosa, and MP: muscularis mucosa, ESD: endoscopic submucosal dissection, EMR: endoscopic mucosal resection.

had well-differentiated adenocarcinoma, one had poorly differentiated adenocarcinoma, and one had gastric adenoma. In seven cases, the depth of invasion was confined to the mucosa, and in three cases it was at the submucosal layer or deeper. Seven cases were resected endoscopically, and three underwent curative surgery.

Assessment of the predictive accuracy for gastric neoplasia cases in group A. ROC analysis was performed to evaluate screening utility and identify a cutoff point for *H. pylori* antibody titer, pepsinogen-I/II ratio, and age, which were significantly different between gastric neoplasia cases and cases without gastric neoplasia. These three factors were all

Table II. *Univariate and multivariate analyses to identify potential risk factors for gastric neoplasia in subjects negative for Helicobacter pylori antibody and with normal pepsinogen levels.*

	No. (%) of individuals	Gastric neoplasia prevalence	OR (95% CI)			
			Univariate	Multivariate		
				Model 1	Model 2	Model 3
H. pylori antibody titer (U/ml)						
≥3	51 (14.8%)	8/51 (15.7%)	27.1 (5.6-132.2)	14.9 (2.80-79.4)		14.4 (2.7 -76.9)
<3	294 (85.2%)	2/294 (0.7%)	1	1		1
p-Value			<0.001*	<0.01*		<0.01*
Pepsinogen I/II ratio						
≤4.3	22 (6.4%)	6/22 (27.3%)	29.9 (7.7-116.7)	13.3 (3.0-59.1)	21.2(5.0-90.5)	10.0 (2.1-47.9)
>4.3	323 (93.6%)	4/323 (1.2%)	1	1	1	1
p-Value			<0.001*	<0.01*	<0.01*	<0.01*
Age (years)						
≥71	76 (22.0%)	6/76 (7.9%)	5.7 (1.6-20.7)		2.6 (0.6-11.1)	2.3 (0.5 -10.9)
<71	269 (78.0%)	4/269 (1.5%)	1		1	1
p-Value			<0.001*		0.203	0.279

*A *p*-value less than 0.05 was considered significant. Model 1 includes logistic regression analysis for *H. pylori* antibody titer and pepsinogen-I/II ratio as predictors of gastric neoplasia cases. Model 2 incorporates age and pepsinogen-I/II ratio. Model 3 includes all three variables. In Model 2 and Model 3, age did not have any statistically significant association with gastric neoplasia. OR: Odds ratio; CI: confidence interval.

significant for predicting gastric neoplasia. The AUROC for *H. pylori* antibody titer in predicting gastric neoplasia in group A was 0.846±0.08 [95% confidence interval (CI)=0.698-0.993, $p<0.01$; cutoff value=1.5 U/ml, sensitivity=80%, specificity=87.5%]. The AUROC for the pepsinogen-I/II ratio was 0.809±0.07 (95% CI=0.673-0.945, $p<0.01$; cutoff value=4.35, sensitivity=60%, specificity=95.5%), and that of age was 0.774±0.06 (95% CI=0.664-0.883, $p<0.01$; cutoff value=70.5 years, sensitivity=60%, specificity=79.1%). To set the cutoff value of *H. pylori* antibody titer to 1.5 U/ml is equivalent to lowering the cutoff value from 10 U/ml to 3 U/ml because results greater than 0 U/ml are expressed continuously from 3 to 100 U/ml. Thus, practically, a high antibody titer for those who were *H. pylori*-negative (≥3 U/ml), a low pepsinogen-I/II ratio (≤4.3), and older age (≥71 years) were associated with gastric neoplasia cases on the ROC analysis.

Factors associated with gastric neoplasia on univariate and multivariate logistic regression analyses. To further explore the associations with gastric neoplasia cases in group A, univariate and multivariate logistic regression analyses incorporating *H. pylori* antibody titer (≥3 U/ml or not), pepsinogen-I/II ratio (≤4.3 or not), and age (≥71 year or not) were performed (Table II). Univariate analysis revealed that *H. pylori*-negative antibody titer ≥3 U/ml ($p<0.001$), pepsinogen-I/II ratio ≤4.3 ($p<0.001$) and age ≥71 years ($p<0.001$) as predictors of gastric neoplasia in group A. We performed multivariate logistic regression analysis using these three variables. The evaluation including serum *H. pylori* antibody titer and pepsinogen-I/II ratio showed that these two factors retained an independent association with gastric neoplasia cases (model 1). In model 2 incorporating age and pepsinogen-I/II ratio, age did not show any significant trend towards association with gastric neoplasia. Logistic regression analysis incorporating these three factors indicated that serum *H. pylori* antibody titer [odds ratio (OR)=14.4, 95% CI=2.7-76.9, $p<0.0$ 1] and pepsinogen-I/II ratio (OR=10.0, 95% CI: 2.1-47.9; $p<0.01$) were independent predictors, however, age did not show any significant association. In model 2 and model 3, age lost significant association with gastric neoplasia. Thus, serum *H. pylori* antibody titer and pepsinogen-I/II ratio, independently predicted gastric neoplasia, and the AUROC of model 1 was statistically better than that of both model 2 and 3 (Table III).

Table III. *Assessment of the fit of multivariate analysis models.*

	AUROC	Standard Error	*p*-Value	95% Confidence interval
Model 1	0.872	0.056	<0.01‡	0.762-0.983
Model 2	0.734*	0.085	<0.05‡	0.567-0.900
Model 3	0.792**	0.06	<0.01‡	0.674-0.910

AUROC: Area under the receiver operating characteristic curve; *$p<0.05$ *vs.* Model 1. **$p<0.01$ *vs.* Model 1. ‡A *p*-value less than 0.05 was considered significant.

Discussion

Although barium radiography has been the most common gastric cancer screening method in Japan, the ABC method is now widely incorporated into the gastric cancer screening program in Japan (12, 14, 32, 33).

In the present study, *H. pylori* infection was determined by a commonly used direct EIA kit based on *H. pylori* strains from Japanese individuals. This EIA kit using 10 U/ml as a cutoff value has been widely applied to large studies analyzing over 1,000 participants (5, 22, 27, 34), and the accuracy of this kit to detect current *H. pylori* infection has been well established. However, in the present study, it was found that even within the normal range for *H. pylori*-negative individuals, a relatively high serum *H. pylori* antibody titer (≥3 U/ml) is useful for detecting gastric neoplasia in individuals classed as group A by ABC, in which endoscopy is not usually recommended.

Considering that more than 99% of gastric cancer cases are associated with *H. pylori* infection (2, 5), gastric neoplasia cases in group A are not considered 'true-negative' *H. pylori* cases but as cases with past infection. We consider that the development of gastric neoplasia in group A may possibly be related to the gastric mucosa after unexpected eradication of *H. pylori*. Use of antibiotics has increased significantly, and such increased use of antibiotics may also induce unexpected eradication of *H. pylori* globally (35). Although a significant decrease in the antibody titer occurs after eradication, seroreversion 18 months after eradication is reported to occur in as many as 45% (36, 37). Thus, complete seronegativity cannot usually be obtained even after successful eradication therapy. We hypothesized that in spontaneously eradicated cases, the antibody titer decreased significantly but did not actually reach zero, which is why lowering the cutoff value of the antibody titer is effective for detecting neoplasia in group A.

We also clarified that a low pepsinogen-I/II ratio (≤4.3) is useful for detecting gastric neoplasia in group A. Characteristically, because of the increase in the pepsinogen-I/II ratio after successful eradication therapy (38, 39), most cases with atrophic pepsinogen (typically pepsinogen-I/II ratio <3.0) are classified as 'seemingly normal pepsinogen' after eradication therapy but the pepsinogen-I/II ratio usually does not achieve high values. Thus, low-normal pepsinogen-I/II ratios in group A may represent advanced atrophy after eradication. The present finding that a pepsinogen-I/II ratio of 4.3 or less is associated with gastric neoplasia suggests that these cases were originally of advanced atrophy and should be regarded as high-risk.

In the present study a population of 734 individuals with 43 cases of gastric neoplasia, 389 (53.0%) were classified into groups B-D, including 33 of gastric neoplasia, who required further examination by endoscopy; suggesting that 33 out of 43 (76.7%) cases became candidates for endoscopic examination. If individuals with an *H. pylori* antibody titer ≤3 U/ml or a pepsinogen-I/II ratio ≤4.3 are regarded as 'positive' in group A, candidates for further endoscopic examination increased significantly from 76.7% (33 out of 43) to 97.7% (42 out of 43 gastric neoplasia cases).

The prevalence of gastric neoplasia cases (2.9 %) among group A individuals in this study is higher than that of previous studies (14, 40). One possible reason for this high prevalence is that our study population included symptomatic cases and abnormal barium X ray cases, although the study subjects of previous reports were based on asymptomatic individuals undergoing a health checkup. This finding suggests that high-risk gastric neoplasia cases may be included in group A if the ABC method is applied to outpatients. However, because the serological characteristics of asymptomatic group A cases and those of outpatient cases are nearly identical (pepsinogen I/II ratio in asymptomatic cases and our outpatients was 3.6 and 3.8, respectively), we consider that this cutoff point is applicable to both these groups (40). Because the ABC method is a non-invasive method for stratifying the risk of future gastric carcinogenesis, we consider that it can be adapted to screening asymptomatic individuals as well as outpatient cases.

Several limitations should be considered when interpreting the results of this study. Firstly, there were only 10 gastric neoplasia cases in group A in this study, and it is important to further clarify the risk factors for gastric neoplasia in group A in a larger, multi-institutional study. The second is the validity of dividing individuals with 'negative' antibody titer into two sub-groups. However, the cutoff value to detect those with current as well as past infection should be established to identify those at high risk of gastric cancer in group A. We have demonstrated that in group A, an antibody titer lower than the recommended cutoff value has important meaning for the identification of those at high risk, suggesting that lowering the cutoff value can be justified in this limited population.

In conclusion, two predictors, a high *H. pylori*-negative antibody titer (≥3 U/ml) and a low pepsinogen-I/II ratio (≤4.3) are important factors for effectively detecting neoplasia in cases classed as group A by the ABC method. Since group A is usually regarded as consisting of individuals with a healthy stomach, the identification of high-risk cases in group A is an urgent problem. Thus, we consider that the present findings are especially important in countries using the ABC method for gastric cancer screening.

Conflicts of Interest

The Authors have no competing interests.

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