chronological changes in PG I and II values over a period of several years as our study has. We could find only one study [41] that showed the same trends in serological atrophy as our study did. A large-scale study of Japanese subjects reported that very few cases of atrophy of the gastric mucosa were observed among those without *H. pylori* infection [42], further confirming that a decrease in *H. pylori* prevalence may result in a decrease in gastric mucosal atrophy. Studies reporting a decrease in *H. pylori* prevalence over the years may be considered as indicating a concomitant decrease in gastric mucosal atrophy.

The prevalence of gastric mucosal atrophy seems to have clearly decreased in Japan, along with the prevalence of *H. pylori* infection, with the decrease in the younger population being more conspicuous. Although there may be some limitations to the conclusions discussed above, these findings would seem to explain the decline we observed in gastric cancer incidence. The change in frequency of serological atrophy of the gastric mucosa was more rapid compared with the change in gastric cancer incidence, and this may have occurred because there was a time lag between the beginning of gastric cancer in the atrophic mucosa and the clinical diagnosis of the cancer when it has developed.

Future incidence of gastric cancer

In Japan, there seems to be a decreasing birth cohort effect for *H. pylori* infection, as well as for serological atrophy of the gastric mucosa. The decreasing birth cohort effect is expected to continue in the future. In the observation period of this study, a decline in the incidence of gastric cancer was observed, and it was most marked in those aged 20–39 years. It is expected that the marked decline in the incidence of gastric cancer in the younger population may extend to the older population in the future, as the young population with a low prevalence of *H. pylori* infection gets older.

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□ ORIGINAL ARTICLE □

Comparison of Serum IgA and IgG Antibodies for Detecting *Helicobacter pylori* Infection

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Abstract

Objective **Although the diagnostic utility of serum IgG antibodies to *Helicobacter pylori* (*H. pylori*) is well established, the usefulness of IgA-based tests is less well documented. The aim of this study was to evaluate two commercially available ELISAs, both for IgG and IgA.**

Patients and Methods **Rapid urease test and histology analysis were performed in 183 patients. A patient was considered to be *H. pylori*-positive when either biopsy test was positive, and considered to be noninfected when both tests were negative. Intestinal metaplasia was determined by dye endoscopy with methylene blue. ELISA testing was performed using the EPI HM-CAP IgG and PP-CAP IgA assays and EIAgen IgG and IgA assays.**

Results **Sensitivity was 94.7, 93.9, 94.8, and 97.0% for HM-CAP IgG, PP-CAP IgA, EIAgen IgG, and EIAgen IgA, respectively. Although sensitivity was excellent for both IgG and IgA antibodies, specificity of both IgA EIAs was low (PP-CAP 72.6%, EIAgen *H. pylori* IgA 59.2%). Three of 101 *H. pylori*-infected patients were PP-CAP positive and HM-CAP negative and four were EIAgen *H. pylori* IgA positive and EIAgen IgG negative. Of eight noninfected patients in whom intestinal metaplasia was found, PP-CAP IgA results were positive in three of five patients with a HM-CAP IgG negative result and EIAgen IgA was detected in one of four patients with an EIAgen IgG negative result.**

Conclusions **Since some patients have IgA positive but IgG negative results, great care should be taken not to underestimate the prevalence of *H. pylori* infection from the results of IgG serology.**

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Key words: *Helicobacter pylori*, IgG antibody, IgA antibody, atrophic gastritis, intestinal metaplasia

Introduction

Serological tests are commercially available, easy to perform, and inexpensive and therefore have been recommended for the diagnosis of *Helicobacter pylori* (*H. pylori*) infection (1). Many serological tests, mainly immunoglobulin G (IgG) based, have been validated against invasive methods (2). The IgG antibody level to *H. pylori* is usually increased and may, in assays using specific antigens, be a marker for *H. pylori* infection (3). Because the serum IgG test has shown high sensitivity and specificity, the serology test has been used widely in epidemiologic studies (4, 5). Unlike tests that rely on bacterial urease activity, antibody tests can be performed in patients taking proton pump inhibitors or antibiotics but are not generally useful in the immediate follow-up after eradication therapy (3, 6, 7). Most of these studies have reported a drop in titer at six months of approximately 40–50% from pretreatment levels in patients in whom the bacteria was eradicated (3, 6–9).

Serum antibodies to *H. pylori*, IgG, IgA, and less frequently, to IgM classes, are detected in infected individuals (10). IgM antibodies can be detected shortly after the infection is acquired, but IgA and IgG titers indicate chronic infection (11). Although the diagnostic utility of serologic detection of IgG antibodies to *H. pylori* is well established, the usefulness of IgA-based tests is less well documented. Kosunen et al (10) have described a subset of *H. pylori* infected patients who are positive for IgA but negative for IgG antibodies to *H. pylori*, making the evaluation of IgA titers the only method of serologic confirmation of treatment. The aim of this study was to evaluate two commercially available ELISAs, both for IgG and IgA, for the diagnosis of *H. pylori* infection.

Materials and Methods

Patients

Between October 2000 and March 2001, a total of 183 pa-

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Table 1. Summary of Individual Test Results in *H. pylori*-infected Patients

HM-CAP IgG	PP-CAP IgA	EIAgen IgG	EIAgen IgA	No. of case
+	+	+	+	81
+	−	+	+	2
−	+	+	+	1
+	+	−	+	1
+	−	+	−	1
−	+	−	+	1
+	−	−	−	1
−	−	−	+	2
+	+	Indeterminate	+	2
Indeterminate	+	Indeterminate	+	1
+	+	Indeterminate	Indeterminate	1
−	+	Indeterminate	+	1
Indeterminate	+	+	+	4
Indeterminate	Indeterminate	+	−	1
+	Indeterminate	+	+	1

tients who underwent upper endoscopy for the evaluation of symptoms suggestive of upper gastrointestinal tract disease were evaluated. The patients were 19 to 85 years old (121 women and 62 men), with a median age of 57.7 years. Patients were excluded if they had previously been treated for *H. pylori*, had undergone previous gastroduodenal surgery, or had used a proton pump inhibitor, antibiotics, or bismuth compounds within the previous month.

Endoscopy

After obtaining informed consent, routine upper endoscopy was performed in the usual manner under local pharyngeal anesthesia with the patient lying in a left lateral position. Two antral biopsies were taken from within 3 cm of the pylorus. One antral biopsy was placed in a rapid urease test (RUT, PyloriTek test, Serim Research Corp., Elkhart, IN), the remaining biopsy specimen was fixed with 10% formalin for histologic examination with Giemsa stain. The extension of intestinal metaplasia was judged by dye endoscopy with methylene blue solution. After conventional observation, 20 ml of a 0.5% solution of methylene blue was sprayed on the entire gastric mucosa. Two minutes after the application of methylene blue, approximately 50 to 100 ml of tap water was vigorously sprayed on the gastric mucosa to wash off excess dye. Positive staining which reflected the presence of the columnar mucosa with intestinal metaplasia was defined as blue staining of noneroded mucosa that persisted despite vigorous water irrigation.

A patient was considered to be infected with *H. pylori* when either of two biopsy tests (RUT and histology) was positive, and considered to be noninfected when both tests gave concordant negative results.

Serology

All patients had blood drawn for serological testing at the time of endoscopy. The serum was separated, divided into aliquots, and stored at −20°C before testing. ELISA testing was performed using the EPI HM-CAP IgG and PP-CAP IgA (Enteric Products, Inc., N.Y.) assays and EIAgen *H. pylori* IgG and IgA (BioChem ImmunoSystems, Inc., P.A.) assays. All assays were performed in accordance with manufacturer's instructions. The assays were performed and quantitative Elisa Values (E.V.) extrapolated for each sample according to manufacturer's instructions. Assay values thus calculated for each kit were interpreted as positive, negative, or indeterminate according to the manufacturer's instructions. The calculated ELISA is read as negative if the ELISA value of HM-CAP IgG and PP-CAP IgA is below 1.8, positive if above 2.2, and indeterminate if it is between 1.8 and 2.2. The indeterminate range of EIAgen *H. pylori* IgG and IgA antibody was between 13.5 and 16.5.

Results

Of 183 patients, 101 were *H. pylori* positive and 82 were *H. pylori* negative by using the results of the biopsy tests as the "gold standard". The average age of those infected by the organism was 59.0 years, whereas that for the noninfected individuals was 56.2 years. Among 101 *H. pylori*-infected patients, both RUT and histology were positive in 90 patients. The remaining 11 patients were found to have *H. pylori* infection by RUT alone (n=6) or by histology alone (n=5). Peptic ulcer diseases were detected in 20 patients (14 gastric ulcers, six duodenal ulcers); all of them were *H. pylori* infected. Two of the infected patients had gastric cancers.

Summary of individual results is shown in Tables 1 and 2. In 81 of 101 *H. pylori*-infected patients, all the tests provided the positive results (Table 1), whereas in only 33 of 82 noninfected patients all four tests were negative (Table 2). Discrepancy between PP-CAP IgA and HM-CAP IgG was found in 27 of 160 patients (16.9%) when patients with at

Table 2. Summary of Individual Test Results in Noninfected Patients

HM-CAP IgG	PP-CAP IgA	EIAgen IgG	EIAgen IgA	No. of case
–	–	–	–	33
–	–	–	+	7
+	–	–	–	1
+	–	–	+	2
–	+	–	+	8
–	+	+	+	2
+	–	+	+	1
+	+	+	+	4
Indeterminate	–	–	–	5
Indeterminate	+	+	+	1
Indeterminate	+	–	+	1
+	Indeterminate	+	Indeterminate	1
–	Indeterminate	–	+	3
–	Indeterminate	–	–	3
–	Indeterminate	–	Indeterminate	2
Indeterminate	+	–	+	1
–	+	–	Indeterminate	4
–	–	–	Indeterminate	4

Table 3. Summary of HM-CAP/PP-CAP and EIAgen *H. pylori* IgG/IgA Results Compared with Results of RUT and Histology

	Indeterminate Results	True Positive	Fale Positive	True Negative	False Negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
EIAgen IgG	5	91	9	73	5	94.8	89.0	91.0	93.6	92.1
EIAgen IgA	12	97	29	42	3	97.0	59.2	77.0	93.3	81.3
HM-CAP IgG	13	90	9	66	5	94.7	88.0	90.9	93.0	91.8
PP-CAP IgA	11	93	20	53	6	93.9	72.6	82.3	89.8	84.9

least one indeterminate result were excluded.

The overall sensitivity, specificity, and positive and negative predictive values are given in Table 3. The HM-CAP IgG titers were above the cut-off value in 90 of 101 *H. pylori*-infected patients and in 9 of 82 noninfected patients. PP-CAP IgA results were positive in 93 of 101 *H. pylori*-infected patients and in 20 of 82 noninfected patients. EIAgen IgG and IgA antibodies were detected in 91 and 97 of the 101 *H. pylori*-infected patients and in 9 and 29 of the 82 noninfected patients, respectively. Sensitivity was 94.7, 93.9, 94.8, and 97.0% for HM-CAP IgG, PP-CAP IgA, EIAgen IgG, and EIAgen IgA, respectively. Although sensitivity was excellent for both IgG and IgA antibodies, specificity of both IgA ELISAs was low (PP-CAP 72.6 %, EIAgen *H. pylori* IgA 59.2 %). Three of 101 *H. pylori*-infected patients were PP-CAP positive and HM-CAP negative and four were EIAgen *H. pylori* IgA positive and EIAgen IgG negative. In such cases, determination of specific IgA was informative, giving positive results, while the IgG titers were less than the cutoff value. Accuracy was higher for IgG ELISAs than for IgA ELISAs because of lower specificity of IgA.

Of eight noninfected patients in whom intestinal metaplasia was found by dye endoscopy, PP-CAP IgA results were positive in three of five patients with a HM-CAP IgG negative result and EIAgen IgA was detected in one of four patients with an EIAgen IgG negative result (Table 4).

Discussion

Serology is the noninvasive technique of choice to detect *H. pylori* infection because it is simple, widely available, and inexpensive. The reported sensitivity and specificity of IgG serology is highly variable, ranging from 30% to 100% (12–14). The HM-CAP IgG, evaluated in our study, had a sensitivity, specificity, positive predictive value, and negative predictive value of 94.7, 88.0, 90.9, and 93.0%, respectively. These values were very similar to those of EIAgen IgG. False-positive results of HM-CAP IgG and EIAgen IgG were obtained in 9 patients. When we compared the sensitivity and specificity of serology against biopsy-based methods such as histology and rapid urease test, defined as the gold standard, failure of the biopsy methods to detect the organism may decrease the serological true-positives and increase the false-positives. False-negative biopsies could occur when the active site of infection was missed because of the patchy

Table 4. Results of Serological Tests in Eight Patients with Extensive Intestinal Metaplasia in Whom *H. pylori* Infection was Determined to be Negative by RUT and Histology

No	Age	Gender	EIAgen IgG	EIAgen IgA	HM-CAP IgG	PP-CAP IgA
1	73	F	−	Indeterminate	−	+
2	67	M	+	+	−	+
3	76	F	−	−	−	−
4	73	F	+	+	+	+
5	78	M	−	+	−	+
6	79	F	+	+	+	+
7	70	F	+	+	+	+
8	76	F	−	−	−	−

distribution of *H. pylori* in the stomach. Multiple biopsy specimens from different areas of the stomach may reduce sampling errors.

In contrast, patients who have had a previous infection with *H. pylori* and whose antibody levels were still elevated may have a negative histology and a positive result of serology. Despite the presence of serum antibodies, failure to detect *H. pylori* in biopsy specimens could have been due to atrophy or intestinal metaplasia of gastric mucosa (14, 15). It has already been reported that this may be a major problem in the elderly (16). Some elderly patients with a negative IgG result may have been infected previously and seroreversion may occur, reflecting that the organism have been eradicated spontaneously by the progression of atrophic gastritis and intestinal metaplasia. Based on these viewpoints, dye endoscopy was performed to evaluate the presence of intestinal metaplasia which was considered to relate to *H. pylori* infection.

Intestinal metaplasia was found significantly more often in the *H. pylori*-positive group than in the *H. pylori*-negative group. Japanese previous reports (17, 18) indicated that the prevalence of IgG antibodies to *H. pylori* was lower in the elderly people compared with those less than 40 years of age. It has been unclear whether the low prevalence of IgG antibodies to *H. pylori* in the elderly reflects spontaneous eradication of the organism. Miwa H et al (19) demonstrated insufficient diagnostic accuracy of imported serological kits for *H. pylori* infection in the Japanese population. However, several studies even in Western countries reported that the accuracy of serological tests in the elderly is unsatisfactory (16).

On the other hand, several studies supporting the clinical utility of IgA serology have appeared. Two studies have noted few patients with confirmed *H. pylori* infection and with only IgA antibodies (20, 21). Aromaa A et al (22) reported that IgA antibodies and low levels of pepsinogen I increase the risk of gastric carcinoma. In addition, IgA antibodies may appear earlier than IgG antibodies in patients who become reinfected (8, 22).

While the sensitivity of all four tests was good the present study, with values above 92%, specificity was very low for the two IgA-based tests. In general, one can expect an IgA ELISA to have lower sensitivity values than an IgG ELISA because most individuals exhibit a predominantly IgG immune response to infection with *H. pylori* (23). However, some investigators have found that about 2% of patients produce an IgA response in the absence of an IgG response (10, 20). Furthermore, Jaskowski et al (24) showed a higher frequency of IgA-positive IgG-negative patients (38/824 cases; 7.2%) with gastrointestinal disorders suggestive of *H. pylori* infection. It is suggested that *H. pylori* infection is excluded by the clinician in the majority of these infected patients solely on the basis of a negative IgG serology result. In our population, three (3.0%) of 101 infected patients was IgA positive and IgG negative. Of eight noninfected patients in whom intestinal metaplasia was found by dye endoscopy, PP-CAP IgA and EIAgen IgA results were positive in three of five patients with a HM-CAP IgG negative result and in one of four patients with an EIAgen IgG negative result, respectively. Since positive serology results are evidence of contact with *H. pylori* but do not necessarily indicate current infection, determination of IgA is informative for such cases. Furthermore the prevalence of atrophic gastritis and intestinal metaplasia is very high in *H. pylori* positive Japanese (17). In conclusion, great care should be taken not to underestimate the prevalence of *H. pylori* infection from the sesults of IgG serology in clinical practice.

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Alimentary Tract

Breath sample collection through the nostril reduces false-positive results of ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection

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Abstract

Background. One of the disadvantages of ^{13}C-urea breath test is possible interference by urease activity not related to *Helicobacter pylori*.
Aims. We design the simple and non-invasive modification to avoid the contamination of $^{13}CO_2$ produced in the mouth.
Patients and methods. One hundred and twenty-nine patients who underwent diagnostic upper endoscopy were enrolled. Within 1 week of the endoscopic procedure, each patient received the modified ^{13}C-urea breath test. Breath samples were collected at baseline and at 1, 3, 5, 10, 15, 20 and 30 min after ingestion of 100 mg ^{13}C-urea solution through the mouth and the nostril at each time point.
Results. The breath $\Delta^{13}CO_2$ value through the nostril at 1 min was already higher in *H. pylori*-positive patients than in *H. pylori*-negative patients. Using 2.5‰ as the cut-off value, the sensitivity and specificity of the modified ^{13}C-urea breath test at 20 min were both 100%, whereas the sensitivity and specificity of the standard ^{13}C-urea breath test were 97.7 and 94%, respectively, using 3‰ as the cut-off value.
Conclusions. The modified ^{13}C-urea breath test in which breath samples are collected through the nostril provides an easy way of avoiding false-positive results for the detection of *H. pylori* infection.

Keywords: ^{13}C-urea breath test; *H. pylori*; Sample collection through the nostril

1. Introduction

^{13}C-urea breath test (UBT) has become the most convenient non-invasive method for the diagnosis of the presence of *H. pylori* infection [1–4]. One of the main disadvantages of UBT is possible interference by urease activity not related to *H. pylori*, as there is bacterial flora in the mouth and the intestine. In order to eliminate the problem of false-positive results in early breath samples, due to urease-producing bacteria other than *H. pylori*, some modifications of UBT have been suggested, such as mouth washing [5,6], or supplying ^{13}C-urea as a rapid-release tablet [7,8]. A shorter time of breath sample collection may also be important for diagnostic value, especially for persons with rapid gastric emptying, and for avoiding false-positive results from the rapid transit of ^{13}C-urea to the colon. An endoscopic UBT, in which ^{13}C-urea solution is sprayed directly into the stomach through a biopsy channel, is one of the useful modifications to avoid the contamination of $^{13}CO_2$ produced in the mouth and the intestine [9–11]. Despite an endoscopic UBT has high diagnostic reliability for the diagnosis of *H. pylori* infection, they are invasive and stressing for the patient. In the present study, we design the more simple and non-invasive modification of UBT.

2. Patients and methods

One hundred and twenty-nine patients who underwent diagnostic upper endoscopy for gastrointestinal symptoms were enrolled in the present study, including 75 females and 54 males, with a mean age of 60.3 (14–79) years. None of

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129 patients enrolled had symptoms such as nose obstruction or nose discharge. To avoid impediments in UBT results, patients were excluded initially due to the presence of any of the following conditions: the ingestion of proton pump inhibitors, H_2-receptor antagonists, antibiotics, or bismuth salts in the previous 2 months; previous gastrointestinal surgery; and having a past history of *H. pylori* eradication therapy. The study was approved by our local ethics committee, and prior written consent was given by all patients included in this study.

At endoscopy, after noting the presence and location of abnormal findings, two biopsy specimens were taken from two sites on the greater curvature of the antrum and the midbody of the stomach. The biopsy specimens were placed in 10% buffered formalin fixative for routine processing, sectioning and staining with haematoxylin and eosin and Giemsa stains. At least one experienced histopathologist, who was blinded to endoscopic findings, evaluated the specimens. *H. pylori* was determined by Giemsa-staining sections. In addition, *H. pylori* IgG antibody concentrations were measured with an ELISA method (HM-CAP). A value of >2.2 was considered positive and a value of <1.9 was considered negative. Patients with a value of not less than 1.9 nor more than 2.2 were excluded in this study. Patients with positive *H. pylori* histology were considered *H. pylori*-positive. Both histology and serology were required to be negative to establish a patient as being without *H. pylori* infection. Patients with positive antibody and negative histology were excluded from analysis.

Within 1 week of the endoscopic procedure, each patient received the modified UBT. After overnight fasting, 100 ml tap water and 100 mg ^{13}C-urea solution were used. Breath samples were taken at baseline and at 1, 3, 5, 10, 15, 20 and 30 min after administration. At each time point breath samples were collected in duplicate through the mouth and the nostril. After an approximately normal inspiration and a 15-s breath-hold, the patient squeezed one nostril with their second finger and placed a sampling port of collection bag into another nostril, forming a tight seal around it with the nostril (Fig. 1). They blew into a collection bag through the nostril like blowing their nose. Although the collection bag used had a one-way breathing valve, using the cap plug assured that sample volume would not be lost due to a leak through the flap-valve. As soon as the collection bag was removed from the nostril, a new one was placed in the mouth and a breath sample was collected through the mouth. These breath samples were analysed on isotope ratio mass spectrometer (ABCAG: Europa Scientific, Crewe, UK), which measures the ratio of the heavy and light isotopes in a sample and compares this to a standard gas. The $^{13}C/^{12}C$ ratio was calculated and expressed as delta over baseline ($\Delta‰$).

The cut-off values of the UBT at each time point were calculated separately according to the sensitivity, specificity and accuracy. The optimal cut-off value of excess $\Delta^{13}CO_2$ for each protocol was determined by the accuracy. The McNemar's test was used to assess statistical differences among sensitivity and specificity of different UBTs. In a time course study, the differences of mean $\Delta^{13}CO_2$ values at each time point between modified UBT and standard UBT were assessed with Student's paired *t*-test. A *P* value of <0.05 was considered significant.

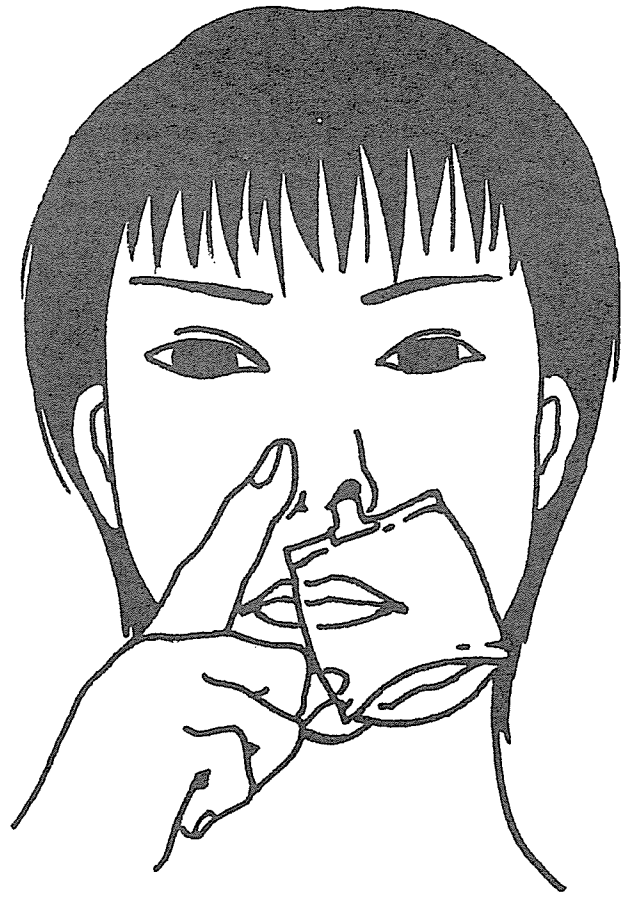

Fig. 1. Schematic drawing showing the breath sample collection technique through the nostril. After an approximately normal inspiration and a 15-s breath-hold, the patient squeezed one nostril with their second finger and placed a sampling port of collection bag into another nostril, forming a tight seal around it with the nostril. They blew into a collection bag through the nostril like blowing their nose.

3. Results

Two patients were excluded from analysis because they had positive serology and negative histology. Of the remaining 127 patients, 42 had *H. pylori* infection. When using the standard UBT, in which breath samples were collected through the mouth, the breath $\Delta^{13}CO_2$ values at 1 min after ingestion of ^{13}C-urea solution did not differ between *H. pylori*-positive patients and *H. pylori*-negative patients (Fig. 2). At 3 min and all subsequent time points, however, the breath $\Delta^{13}CO_2$ values were significantly higher in *H. pylori*-positive patients, compared with those in *H.*

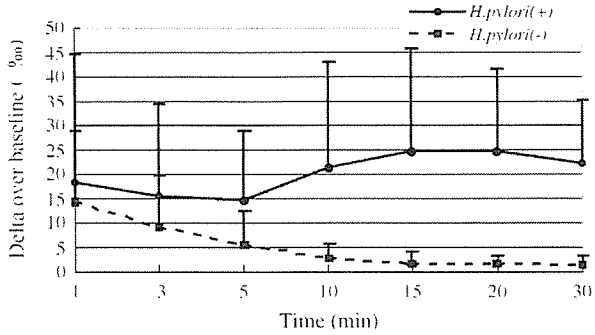

Fig. 2. The mean breath $\Delta^{13}CO_2$ values at various time points collected through the mouth in *H. pylori*-positive and -negative patients. Values are significantly different at 3 min and later ($P < 0.02$).

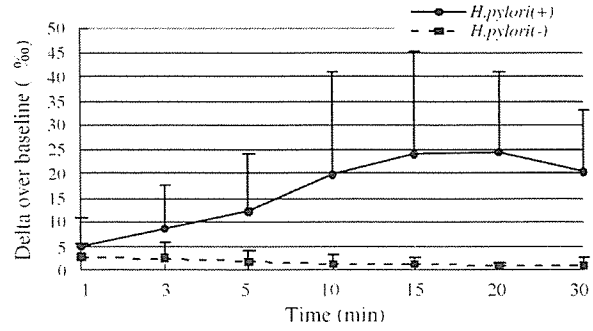

Fig. 3. The mean breath $\Delta^{13}CO_2$ values at various time points collected through the nostril in *H. pylori*-positive and -negative patients. The breath $\Delta^{13}CO_2$ values at all time points were higher in *H. pylori*-positive patients than in *H. pylori*-negative patients ($P < 0.003$).

pylori-negative patients ($P < 0.02$). When breath samples were collected through the nostril, the breath $\Delta^{13}CO_2$ value at 1 min was already higher in *H. pylori*-positive patients than in *H. pylori*-negative patients ($P < 0.003$) (Fig. 3).

Tables 1 and 2 show that the cut-off values for the modified UBT, as well as for the standard UBT, were optimal at each time point. At the 20-min sampling point, the sensitivity and specificity of the modified UBT were both 100% using 2.5‰ as the cut-off value, whereas the sensitivity and specificity of the standard UBT were 97.7 and 94.2%, respectively, using 3‰ as the best cut-off value. At the 15-min sampling point, the optimal cut-off values for the diagnosis of *H. pylori* infection were identified as 3.5‰ in the modified UBT and 5.0‰ in the standard UBT. The sensitivity and specificity of modified UBT and standard UBT at 15 min were 93.0 and 98.8%, and 90.7 and 95.4%, respectively. When shortening the duration of the test and sampling at 10 min, the modified UBT had a sensitivity of 93% and a specificity of 95.3%, using 2.5‰ as the best cut-off value (Table 1). The modified UBT was more accurate than the standard UBT for determining *H. pylori* infection with superior sensitivity and specificity ($P < 0.001$).

4. Discussion

Various modifications of UBT have been reported, including changes in dosage of urea, type of test meals used, timing of sample collection and position of patients [1,7,12–15]. A Japanese standard protocol was proposed in 1998 as having high sensitivity and specificity for UBT [1]. In this method, the patients who have fasted are given 100 mg of ^{13}C-urea in 100 ml water and then their mouth is immediately rinsed with water. After that they are placed in the left lateral decubitus position for 5 min. Breath samples are collected at the baseline and at 20 min after ingestion of ^{13}C-urea. The cut-off value of UBT for the diagnosis of *H. pylori* infection is 2.5‰. If UBT results are not affected by urease activity in the mouth, it is possible to decrease the duration of the test. The shorter the duration of the test, the more convenient it is for the patient. In addition, it becomes possible to eliminate the problem of false-positive results in late breath samples caused by urease-producing bacteria in the intestine.

To avoid interference by oral bacteria, we can change either the administration route of ^{13}C-urea or the collection route of breath samples. Some investigators have reported the endoscopic UBT in which the ^{13}C-urea was directly into

Table 1
Sensitivity, specificity and accuracy of the modified UBT in which breath samples are collected through the nostril at each cut-off value

Cut-off value (Δ‰)	Sensitivity (%) (sampling time)			Specificity (%) (sampling time)			Accuracy (%) (sampling time)		
	10 min	15 min	20 min	10 min	15 min	20 min	10 min	15 min	20 min
1.0	100	100	100	45.3	25.6	64.0	63.6	50.4	76.0
1.5	97.7	100	100	76.7	60.5	87.2	83.7	73.6	91.5
2.0	97.7	97.7	100	89.5	93.0	96.5	92.2	94.6	97.7
2.5	93.0	97.7	100	95.3	95.3	100	94.6	96.1	100
3.0	90.7	95.3	95.3	96.5	96.5	100	94.6	96.1	98.4
3.5	88.4	93.0	95.3	97.7	98.8	100	94.6	96.9	98.4
4.0	88.4	90.7	93.0	97.7	98.8	100	94.6	96.1	97.7
4.5	86.0	88.4	93.0	97.7	98.8	100	93.8	95.3	97.7
5.0	81.4	88.4	90.7	98.8	98.8	100	93.0	95.3	96.9
5.5	81.4	86.0	90.7	98.8	100	100	93.0	95.3	96.9
6.0	81.4	83.7	90.7	98.8	100	100	93.0	94.6	96.9

Table 2
Sensitivity, specificity and accuracy of the standard UBT in which breath samples are collected through the mouth at each cut-off value

Cut-off value (Δ‰)	Sensitivity (%) (sampling time)			Specificity (%) (sampling time)			Accuracy (%) (sampling time)		
	10 min	15 min	20 min	10 min	15 min	20 min	10 min	15 min	20 min
1.0	100	100	97.7	31.4	19.8	47.7	53.5	46.5	65.1
1.5	97.7	100	100	48.8	44.2	79.1	65.1	62.8	86.0
2.0	97.7	100	100	58.1	77.9	86.0	71.3	85.3	90.7
2.5	95.3	97.7	97.7	69.8	82.6	90.7	78.3	87.6	93.0
3.0	93.0	97.7	97.7	74.4	88.4	94.2	80.6	91.5	95.3
3.5	90.7	90.7	93.0	81.4	90.7	96.5	84.5	93.0	95.3
4.0	90.7	90.7	93.0	86.0	91.9	97.7	87.6	91.5	96.1
4.5	86.0	90.7	93.0	88.4	93.0	98.8	87.6	92.2	96.9
5.0	86.0	90.7	93.0	89.5	95.4	100	88.4	93.8	97.7
5.5	83.7	90.7	93.0	91.9	95.3	100	89.1	93.8	97.7
6.0	81.4	88.4	93.0	91.9	95.3	100	88.4	93.0	97.7

the stomach, bypassing the oral cavity [9–11]. These endoscopic UBT show high sensitivity and specificity. Unfortunately, the need for endoscopy makes these tests costly and inconvenient for the patient.

An alternative method in which interference by oral bacteria can be avoided is the modified UBT in which breath samples are not collected through the mouth but through the nostril. In the *H. pylori*-negative patients who have urease-producing bacteria in the mouth, $^{13}CO_2$ produced by oral bacteria should contaminate the exhaled breath and result in positive tests despite $^{13}CO_2$ is not produced in the stomach. This minor change alone was more effective for avoiding interference with oral bacteria than expected in the present study. At 3 min and all subsequent time points, the breath $\Delta^{13}CO_2$ values were significantly higher in *H. pylori*-positive patients, compared with those in *H. pylori*-negative patients (Fig. 2). The breath $\Delta^{13}CO_2$ values at 15 min and thereafter were not affected by the difference in the route of sample collection. From these results, in patients with oral urease activity, urea hydrolysis occurred in the mouth so that, by 15 min, $^{13}CO_2$ excretion had returned to near-baseline values. These suggested that when collecting breath samples through the nostril, the duration of the test might be decreased.

For the standard UBT, in which breath samples are collected through the mouth, the 20-min sample is thought to give accurate results, which are not affected by oral bacteria. In other words, it is difficult to determine the *H. pylori* infection by the standard UBT earlier than 20 min. Actually, the optimal cut-off value of the standard UBT was 5.0‰ at 20 and 15 min, and increased to 5.5‰ at 10 min (Table 2).

In contrast, the optimal cut-off values of the modified UBT were lower than those of the standard UBT at all time points. At the 20-min sampling point, the sensitivity and specificity of the modified UBT were both 100% using 2.5‰ as the cut-off value. Since the mean breath $\Delta^{13}CO_2$ values of modified UBT at 1 min was already higher in *H. pylori*-positive patients than in *H. pylori*-negative patients (Fig. 3), we attempted to calculate sensitivity and specificity at earlier time points. The optimal cut-off value of the modified UBT for the diagnosis of *H. pylori* infection were identified as 3.5‰ at 15 min with high sensitivity and specificity more than 93%. When shortening the duration of the test and sampling at 10 min, the modified UBT had a sensitivity of 93% and a specificity of 95.3%, using 2.5‰ as the best cut-off value (Table 1). Since these results were comparable to those of serological tests [16,17] and previous reports [2,5], we suggest that 10 min after ingestion of ^{13}C-urea is sufficient for the clinical use of UBT, and the shortening of the test duration is a feasible option.

Indeed, the shorter the duration of the test, the more convenient it is for the patient. The sensitivity and specificity of the modified UBT at 20 min were both 100% using 2.5‰ as the cut-off value, whereas both results at 15 min were less than 99% (Table 1). Although it is desired that the duration of the test is decreased, 20-min time point should be selected as the optimal sampling time in the modified UBT.

In the present study, we did not use the test meals according to the Japanese Standard Protocol [1], although test meals, including citric acid, have been used in most of the previous studies because of its slowing effect on gastric emptying [14,15,18].

A delay in gastric emptying can maximise the gastric residence time of ^{13}C-urea and exposure time of the organisms to ^{13}C-urea. Therefore, the accuracy of UBT may be improved by prolonging the contact of the test meal with *H. pylori* urease. If test meals were used in the modified UBT, the accuracy might increase at earlier time-point.

On the other hand, some investigators have reported high sensitivity and specificity of UBT protocols without test meals [1,6]. The disadvantage of a test meal in the UBT has been noted when breath samples from early time-points were used [7]. The aim of the present study is to determine whether the duration of the test is decreased. Then, the modified UBT did not employ a test meal. The necessity for a test meal in the UBT needs further evaluation.

Main indication for UBT is the confirmation of successful *H. pylori* eradication therapy [3]. In Japan, it is currently recommended that UBT be performed no less than 2 months after the completion of eradication therapy. Since $^{13}CO_2$

values in UBT after eradication therapy are likely to become lower, reflecting a low *H. pylori* density, high sensitivity and specificity of UBT are required for determining the results of treatment. In addition, a clear recommendation of the modified UBT for clinical practice is the diagnosis of active *H. pylori* infection in patients with atrophic gastritis because they are also likely to have a low density of *H. pylori* [19]. We will make a further study to evaluate the efficacy of the modified UBT for determining the results of treatment or *H. pylori* status in patients with severe atrophic gastritis, especially in the elderly.

In conclusion, the simple modification, in which breath samples are collected through the nostril, provides an easy way of avoiding false-positive readings without need for mouth washing, or supplying ^{13}C-urea as a rapid-release tablet. This easy procedure is well tolerated by the patient. Considering that a more rapid, non-invasive and simple test is desirable, we believe that the modified UBT is valid for diagnosing active *H. pylori* infection in clinical practice.

Conflict of interest statement

None declared.

List of abbreviations

CO_2, carbon dioxide; UBT, ^{13}C-urea breath test.

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Serum Pepsinogens as a Predicator of the Topography of Intestinal Metaplasia in Patients with Atrophic Gastritis

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The importance of atrophic gastritis with intestinal metaplasia is related to the fact that it increases the risk of gastric cancer development. The aim of this study is to evaluate the diagnostic potential of serum pepsinogens in predicting the topography of intestinal metaplasia. Both dye endoscopy and ^{13}C-urea breath test were carried out in 878 subjects. Serum pepsinogen I, pepsinogen II, and IgG antibody to *Helicobacter pylori* were measured. The overall prevalence of intestinal metaplasia was higher in subjects with lower PG I/II ratios and lower PG I values. Based on ROC curves, a cutoff value for pepsinogen I/II ratio of less than 3.0 would have identified intestinal metaplasia with a sensitivity of 71.7% and a specificity of 66.7% in *Helicobacter pylori*-positive subjects. It is possible that serum pepsinogens could be used as a screening test for high-risk subjects with intestinal metaplasia.

KEY WORDS: intestinal metaplasia; serum pepsinogens; *Helicobacter pylori*; atrophic gastritis.

The clinical importance of atrophic gastritis with intestinal metaplasia is related to the fact that it increases the risk of gastric cancer development. (1–3). In the process of carcinogenesis, at least for the intestinal type of gastric carcinoma, it was proposed that the gastric mucosa evolves through the stages of chronic active gastritis, glandular atrophy, intestinal metaplasia, and dysplasia before developing gastric adenocarcinoma (3). The risk of gastric neoplasias rises with increasing grade and extent of atrophic gastritis (4). Atrophic gastritis is usually diagnosed with endoscopy and biopsies. However, there is significant potential sampling error in identifying intestinal metaplasis by random biopsy because intestinal metaplasia of the gastric mucosa is reported to be patchy. Thus we assessed the topography of intestinal metaplasia using vital staining in this study instead of taking biopsies from the antrum and corpus. The efficacy of using vital staining with methylene blue to help identify areas of intestinal metaplasia in the distal stomach and cardia, including Barrett's esophagus, has been documented (5–8). The results of staining showed a good correlation with the histological grading of intestinal metaplasia (9).

Although an endoscopic examination has a high reliability for the diagnosis of atrophic gastritis, it is invasive and stressful for the patient. In previous clinical studies (10–13), serum pepsinogen (PG) is a known marker of gastric mucosal status, including mucosal atrophy. Very low serum PG I levels and a low PG I:II ratio are accurate predictors of severe gastric atrophy and are frequently found in gastric cancer (14–16). However, the severity and topography of gastritis vary considerably between individuals. Recently, the serum PG method has been the first screening step in Japan, instead of photofluorography (10, 11, 16, 17), because several problems have been noted, such as its cost effectiveness, the risks of X-ray exposure,

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and its low sensitivity (less than 40%) in detecting early gastric cancer (18). This has made it possible to screen large populations without the need for endoscopy. Intestinal metaplasia is usually diagnosed with dye endoscopy or biopsies. Although the PG method has many advantages, there have been no studies of relations between serum PGs and extent of intestinal metaplasia. The aim of this study is to evaluate the diagnostic potential of serum PGs in predicting the topography of intestinal metaplasia.

MATERIALS AND METHODS

Between December 1999 and October 2001, the dye-endoscopic study was carried out in 878 subjects who consecutively underwent upper gastrointestinal endoscopy. They were 322 men and 556 women, with a mean age of 58.1 years (range, 19–90 years). Exclusion criteria included prior gastric surgery, pregnancy, or a history of *Helicobacter pylori* eradication therapy. We also excluded subjects with a history of recent intake of proton pump inhibitors, H2-receptor antagonists, or antibiotics in the preceding month because we considered the possibility of modification to PG levels as a consequence of medication.

Endoscopic procedures were performed by a single endoscopist (Y.U.). Patients first underwent standard upper endoscopy with examination of the esophagus, stomach, and duodenum. Next, a spray of 20 ml of 0.5% methylene blue solution was applied sequentially to the entire gastric mucosa. Immediately after the application of methylene blue, approximately 50 to 100 ml of tap water was sprayed on the gastric mucosa to wash off excess dye. Positive staining was defined as blue-stained mucosa that persisted despite vigous water irrigation.

After methylene blue staining, the pattern of mucosal staining was classified into four groups. For grade A, the positive staining lesion is located at the antrum; for grade B, the positive staining lesion is found from the antrum to the middle part of the lesser curvature aspect; and for grade C, the positive staining lesion is in the antrum and the body of the greater curvature as well as the lesser curvature.

All patients underwent a ^{13}C-urea breath test (UBT) within 2 weeks after endoscopy. After overnight fasting, 20 ml of water containing 100 mg of ^{13}C-urea was administrated to the patient. Patients were kept in the sitting position during testing. Breath samples were collected at baseline and 20 min after ingestion of ^{13}C-urea. ^{13}C was measured as the $^{13}CO_2/^{12}CO_2$ isotope ratio and is expressed as Δ over baseline (‰). Breath samples were analyzed by mass spectrometry. A change in the Δ^{13}C value over baseline of more than 2.5‰ was considered positive (19). *H. pylori* infection was established by a positive UBT.

Blood samples for measurements of PG I, PG II, and IgG antibody to *H. pylori* were taken prior to endoscopy, centrifuged immediately at 4°C, and stored at −20°C until use. Serum PG concentrations were assayed using PG I and PG II Riabead Kits (20) (Dainabot Co Ltd, Tokyo). Serum samples were also examined for *H. pylori* antibody by an enzyme-linked immunosorbent assay (ELISA) using the EPI HM-CAP IgG (Enteric Products, Inc., New York) assays (21). All assays were performed in accordance with the manufacturer's instructions. The assays were performed and quantitative ELISA values (EV) extrapolated for each sample according to the manufacturer's instructions. Assay values thus calculated for each kit were interpreted as positive, negative, or indeterminate. The calculated ELISA is read as negative if the ELISA value of HM-CAP IgG is below 1.8, positive if it is above 2.2, and indeterminate if it is between 1.8 and 2.2.

Data on serum PG levels and age are presented as mean ± SD (standard deviation). Comparisons of groups were made using the paired *t* test. A P value <0.05 was accepted as indicating statistical significance.

RESULTS

A total of 878 patients were included in the study and underwent dye endoscopy and UBT. Of the 878 patients, 47 (5.4%) had an indeterminate result. The remaining 831 patients were classified into four groups according to positivity and negativity for *H. pylori* antibody and UBT: group A (UBT[+] and *H. pylori* antibody[+], group B (UBT[+] and *H. pylori* antibody[−], group C (UBT[−] and *H. pylori* antibody [+]), and group D (UBT[−] and *H. pylori* antibody[−]). Of the 831 subjects, 454 (54.6%) were allocated to group A, 68 (8.2%) to group B, 93 (11.2%) to group C, and 216 (26.0%) to group D.

As shown in Table 1, group B showed a significantly older mean age than the other groups. There was no statistically significant difference in sex distribution among the four groups. Serum PG I level was highest in group A, followed by group B, group D, and group C. Serum PG II levels in groups A to D tended to decrease. Serum PG I/II ratio was lowest in group A.

Overall, intestinal metaplasia was present in 358 (65.4%) of 547 patients with positive serology and in 339 (64.9%) of 522 UBT-positive patients, whereas only 36 (16.7%) of 216 patients who tested negative on both tests had intestinal metaplasia. These differences were statistically significant ($P < 0.001$ by χ^2 analysis). The prevalence of intestinal metaplasia was higher in group A than in the other groups. Despite the fact that the overall rates of intestinal metaplasia were significantly higher in group A than in group C (67.0 vs 58.1%; $P = 0.0055$, $\chi^2 = 7.72$), the proportion of grade C was significantly higher in group C (21/54; 38.9%) than in group A (69/304; 22.7%; $P < 0.01$, $\chi^2 = 6.98$). The difference between group C and group B (10/35; 28.6%; $P = 0.32$, $\chi^2 = 0.99$) was not statistically significant (Table 2).

The overall prevalence of intestinal metaplasia was 52% (455/878) and higher in subjects with lower PG I/II ratios and lower PG I values. Intestinal metaplasia was found in 252 (82%) of 299 subjects with a PG I/II ratio of less than 2.5 and in 58 (88%) of 66 subjects with a PG I value of less than 25 ng/ml (Tables 3 and 4).

Receiver operating characteristic (ROC) analysis was used to determine an optimum cutoff for serum PG I and PG I/II ratio in distinguishing atrophic gastritis with versus without intestinal metaplasia (Figures 1–4). As for PG I,

TABLE 1. CHARACTERISTICS OF THE FOUR GROUPS CLASSIFIED BY POSITIVITY OR NEGATIVITY FOR UBT AND SEROLOGY

	UBT(+)		UBT(−)	
H. pylori *antibody*	H. pylori *antibody*(+)	H. pylori *antibody*(−)	H. pylori *antibody*(+)	H. pylori *antibody*(−)
Group	A	B	C	D
No. of subjects	454	68	93	216
Age (mean ± SD)	57.8 ± 12.8	52.7 ± 13.4	63.1 ± 12.9*	57.8 ± 14.2
Male/female	185/269	27/41	31/62	68/148
PG I (ng/ml)	61.7 ± 28.3	55.0 ± 28.0	42.8 ± 33.8	48.7 ± 22.8
(95% CI)	(59.1–64.2)	(48.2–61.8)	(35.9–49.7)	(45.7–51.8)
PG II (ng/ml)	23.0 ± 10.1	17.0 ± 10.6	13.1 ± 9.15	9.07 ± 5.36
(95% CI)	(22.1–24.0)	14.5–19.6)	(11.2–15.0)	(8.36–9.89)
PG I/PG II	2.83 ± 1.22	3.85 ± 2.10	3.73 ± 2.33	5.57 ± 1.64
(95% CI)	(2.72–2.94)	(3.26–4.36)	(3.25–4.21)	(5.35–5.79)

*$P < 0.001$.

the cutoff value that gave the most favorable sensitivities and specificities was 60 ng/ml in group A, 50 ng/ml in group B, 40 ng/ml in group C, and 35 ng/ml in group D. A cutoff value of 35 ng/ml in group D was the most favorable among the four groups and would have identified intestinal metaplasia with a sensitivity of 75% and a specificity of 80% (Figure 4). As for PG I/II ratio, the most favorable cutoff values in groups A to D were 3, 3, 4, and 5, respectively. A cutoff value for PG I/II ratio of less than 3 would have identified intestinal metaplasia with a sensitivity of 71.7 and 67.6% and a specificity of 66.7 and 73.5% in group A and group B, respectively.

Although cutoff values varied in each group, PG I/II ratio had achieved a higher sensitivity than PG I in all groups. Therefore, PG I/II ratio was considered to be a more useful index for distinguishing atrophic gastritis with intestinal metaplasia than that without intestinal metaplasia.

DISCUSSION

It is now clear that intestinal metaplasia is a part of the spectrum of atrophic gastritis with *H. pylori* infection. Xia et al. (22) showed that the prevalence of intestinal metaplasia was significantly higher at the gastric antrum in patients with *H. pylori* infection compared with uninfected subjects. However, only a portion of infected patients go on to develop intestinal metaplasia, suggesting that factors other than *H. pylori*, such as environmental and host genetic factors, may contribute to the progression from atrophic gastritis to intestinal metaplasia. Previous studies demonstrated the low prevalence of intestinal metaplasia in some ethnic populations, despite a much higher prevalence of *H. pylori* infection (23, 24). This suggests that *H. pylori* alone may be insufficient for the development of intestinal metaplasia.

On the other hand, several authors (25, 26) have demonstrated a reduction in sensitivity of serological tests for *H. pylori* infection in patients with intestinal metaplasia. The use of the serological test may result in a systemic underestimate of *H. pylori* infection effect in any case. As reported by Hala et al. (27), the detection rate of intestinal metaplasia increased from 48 to 75% when the biopsy sites were changed from the anterior and posterior wall of the corpus and antrum to the greater and lesser curvatures and by adding one biopsy from the angular incisura. Thus, sampling errors may affect the prevalence of intestinal metaplasia. For these reasons, in the present study, we assessed the topography of intestinal metaplasia using vital staining and evaluated *H. pylori* infection using a combined method with serology and UBT.

TABLE 2. PREVALENCE OF INTESTINAL METAPLASIA (IM) IN EACH GROUP

	UBT(+)		UBT(−)	
Serology	*Serology*(+)	*Serology*(−)	*Serology*(+)	*Serology*(−)
No. of subjects	454	68	93	216
Grade of IM				
None	150 (33.0%)	33 (48.5%)	39 (41.9%)	180 (83.3%)
Grade A	21 (4.6%)	2 (2.9%)	3 (3.2%)	6 (2.8%)
Grade B	214 (47.1%)	23 (33.8%)	30 (32.3%)	17 (7.9%)
Grade C	69 (15.2%)	10 (14.7%)	21 (22.6%)	13 (6.0%)
Grades A+B+C	304 (67%)	35 (51.5%)	54 (58.1%)	36 (16.7%)

TABLE 3. ASSOCIATION BETWEEN THE PREVALENCE OF INTESTINAL METAPLASIA (IM) AND SERUM PG I/II RATIOS

	IM(+)	*IM*(−)	*No. of patients*	*Incidence of IM(%)*
<1.0	34	3	37	91.9
1.0–1.5	60	8	68	88.2
1.5–2.0	64	14	78	82.1
2.0–2.5	94	22	116	81.0
2.5–3.0	61	28	89	68.5
3.0–3.5	50	28	78	64.1
3.5–4.0	36	32	68	52.9
4.0–4.5	16	50	66	24.2
4.5–5.0	11	44	55	20.0
>5.0	29	194	223	13.0

Sensitivity and specificity of available ELISA tests are sufficient, generally ranging from 94 to 100% and from 87 to 100%, respectively (21, 28–31), and there is a very high concordance between serology and UBT (29). However, high serum IgG levels were found in some patients with no sign of *H. pylori* infection (32). These false positives might result from the fact that patients had a past infection, since IgG titers are known to decline very slowly after eradication of *H. pylori* (30). It has been thought that IgG depends mainly on the complex interaction between bacterial infection and immunological host response (32). A great advantage of serology is the fact that it reflects the evidence of contact with the bacteria without the problem of sampling errors. False-negative results may occur in the early stages of infection, when it is impossible to detect appreciable IgG levels.

Discrepancies between serology and UBT have been reported by several investigators (33,34). They could be due to recent acquisition of infection and, consequently, a delay in development of antibodies. The positive testing for antibodies to *H. pylori* but negative testing for UBT may

TABLE 4. ASSOCIATION BETWEEN THE PREVALENCE OF INTESTINAL METAPLASIA (IM) AND SERUM PG I VALUES

	IM(+)	*IM*(−)	*No. of patients*	*Incidence of IM(%)*
<5	4	0	4	100
5–10	12	2	14	85.7
10–15	23	3	26	88.5
15–20	19	3	22	86.4
20–25	27	9	36	75.0
25–30	29	16	45	64.4
30–35	36	32	68	52.9
35–40	31	43	74	41.9
40–45	29	33	62	46.8
45–50	34	41	75	45.3
50–60	58	72	130	44.6
60–70	41	61	102	40.2
>70	112	108	220	50.9

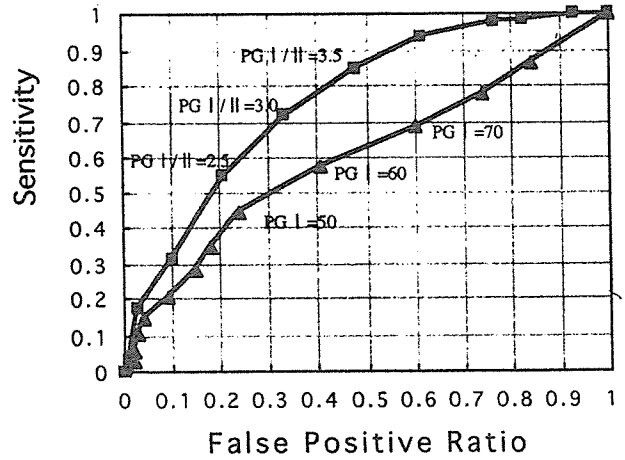

Fig 1. Receiver operating characteristic curves (ROC) of serum pepsinogen (PG) I (▲) and PG I/II ratio (■) in distinguishing subjects with and without intestinal metaplasia in group A.

be due to the use of antimicrobials for other common infections or spontaneous elimination of the infection (35). Malaty et al. (33) showed that the prevalence of *H. pylori* infection varied from 32%, when diagnosis of the infection was based on UBT, to 18%, when the diagnosis was based on serology. Thus, since both UBT and serology have important limitations, the subjects were classified into four groups according to positivity and negativity for *H. pylori* antibody and UBT. In the present study, 68 (23.9%) of 284 subjects with negative serology had positive UBT results and 93 (17.0%) of 547 subjects with positive serology did not have *H. pylori* antibody.

Serology depends on the interaction between bacterial infection and immunological response, whereas the serum PG method is linked mainly to local mucosal damage

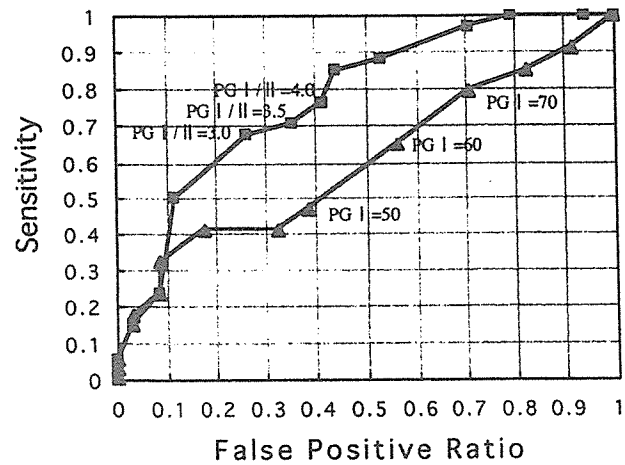

Fig 2. ROC of serum PG I (▲) and PG I/II ratio (■) in distinguishing subjects with and without intestinal metaplasia in group B.

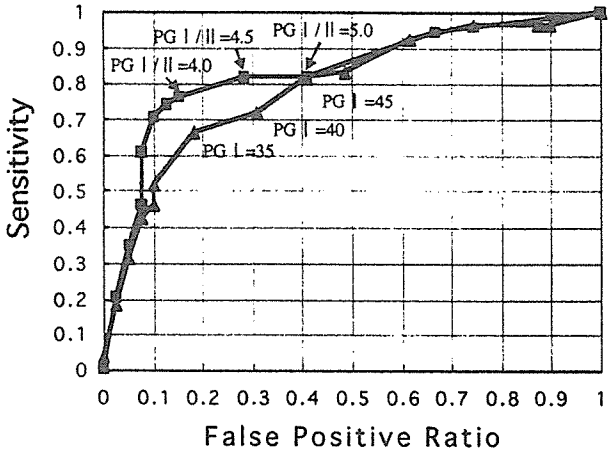

Fig 3. ROC of serum PG I (▲) and PG I/II ratio (■) in distinguishing subjects with and without intestinal metaplasia in group C.

(32). Yamaji et al. (36) demonstrated that 0.14% of patients with gastric cancer had a nagative serology result and concluded that a weak *H. pylori* antibody response meant a high risk for gastric cancer. Although it has been shown that *H. pylori* infection is associated with an increased risk for the development of gastric cancer (37), a reduction in sensitivity of serological tests for *H. pylori* infection in patients with intestinal metaplasia has been demonstrated by several authors (25,26). Thus, it is possible that patients with severe atrophic gastritis and intestinal metaplasia which was considered to be a precancerous lesion could not be detected by serology alone. Actually, in the present study, intestinal metaplasia was present in 358 (65.4%) of 547 patients with positive UBT, whereas only 36 (16.7%) of 216 patients who tested negative on both two tests had intestinal metaplasia.

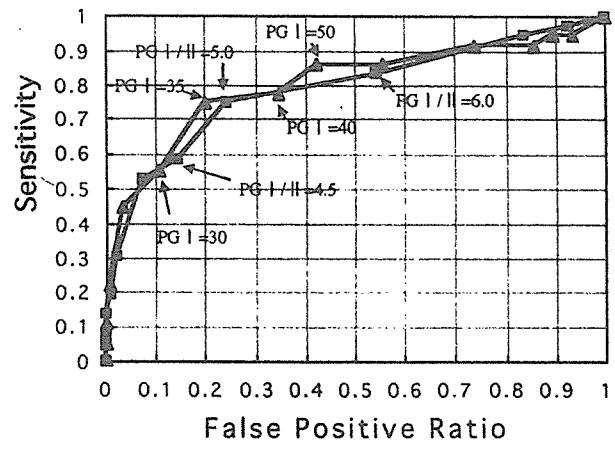

Fig 4. ROC of serum PG I (▲) and PG I/II ratio (■) in distinguishing subjects with and without intestinal metaplasia in group D.

Serum pepsinogen parameters; especially PG I/II and PG I/II ratio, have been proven to be markers for atrophic gastritis (10–13). Therefore, the measurement of serum PGs has recently drawn attention as a candidate for a new screening test for gastric cancer in Japan (11, 16–18). It has been reported that subjects with *H. pylori* infection had significantly higher PG I and PG II concentrations and a significantly lower PG I/II ratio than those without *H. pylori* infection (38) and that these levels are changed by eradication of *H. pylori* (39). In Japan, several studies (38,40) have shown that the prevalence of infection is strongly associated with age and this age-related increase in infection occurs in the elderly. Thus, the absence of serum antibodies in patients with active or previous infection seems to increase in the elderly (40,41). It is possible that patients who had a previous infection and do not have serum antibodies are not detected as a high-risk group for gastric cancer, despite the presence of severe atrophic gastritis. The measurement of serum PGs is able to detect patients with extensive atrophic gastritis, regardless of *H. pylori* status.

In our study, intestinal metaplasia was detected in 358 (65.4%) of 547 patients with serum antibody and in 339 (64.9%) of 522 patients with positive UBT. Thus, because the measurement of serum antibodies alone cannot assess the presence of intestinal metaplasia, we used additional serum markers, PG I and PG I/II ratio, for detecting patients with intestinal metaplasia in this study.

Although several determinations of a suitable cutoff point for gastric cancer screening have previously been reported based on the findings of X-ray methods, using a serum PG I concentration of less than 70 ng/ml and a PG I/II ratio of less than 3.0 as the cutoff point has been widely accepted in Japan (11,17). When the measurement of serum PGs is used for detecting individuals with intestinal metaplasia, we have to determine the suitable cutoff point. Based on ROC curves in this study, the cutoff points of serum PG I and PG I/II ratio for intestinal metaplasia varied among the four groups from 35 to 60 ng/ml and from 3 to 5, respectively.

Although the cutoff values varied in each group, the PG I/II ratio achieved a higher sensitivity than PG I in all groups. Therefore, the PG I/II ratio was considered to be more useful for detecting atrophic gastritis with intestinal metaplasia. Patients with positive UBT results, regardless of the presence of serum antibody, had the lowest cutoff point among the four groups. Consistent with previous reports (11, 17), in which a PG I/II ratio of less than 3.0 was determined as the cutoff point for detecting atrophic gastritis, the suitable cutoff point for PG I/II ratio was 3.0 for detecting intestinal metaplasia. However, patients with negative UBT results had higher cutoff points.

Unexpectedly, intestinal metaplasia was present in 36 (16.7%) of 216 patients who tested negative on both two tests. When using serum antibodies alone for detecting intestinal metaplasia, these patients should be excluded. Using a serum PG I/II ratio of less than 5.0 as the cutoff point, patients who had severe atrophic gastritis with intestinal metaplasia but negative testing for UBT and serology can be detected with a sensitivity of 75.0% and a specificity of 75.6%. As reported previously, if a serum PG I/II ratio of less than 3.0 is used as the cutoff point for gastric cancer, this value provides a sensitivity of only 19.4% and a number of patients with intestinal metaplasia should be overlooked. Thus, it seems that determinations of a suitable cutoff point in the respective groups are essential to intestinal metaplasia screening.

The proportion of grade C intestinal metaplasia was significantly higher in group C (21/54; 38.9%) than group A and was 6.0% (13/216) in group D. The 13 group Δ patients with extensive intestinal metaplasia could not be detected by either UBT or serology. Using a serum PG I/II ratio of less than 5.0 as the cutoff point, 10 (77%) of 13 patients were detected.

Despite the world decline in incidence and mortality, gastric cancer is a leading cause of cancer death in many countries (42). Although the prevalence of *H. pylori* infection in Japan has fallen in recent years (38), those who are infected remain at risk of gastric cancer. *H. pylori* infection was detected in up to 70% of the population by the age of 40 years in Japan (38). Since early life acquisition of *H. pylori* has been considered to increase the risk of developing gastric cancer (43), infected individuals aged 40–50 years, belonging to the age group with the largest number of people in Japan, will be at higher risk of gastric cancer in the near-future. The high prevalence of intestinal metaplasia among *H. pylori*-infected patients suggests that the risk of development of gastric cancer will remain high. Since gastric cancers are potentially curable if they are diagnosed at early stages, screening for intestinal metaplasia is necessary for early detection of gastric cancer.

In conclusion, it is possible for serum PGs to be used as a screening test for high-risk subjects with atrophic gastritis with intestinal metaplasia, rather than as a test for gastric cancer itself. The measurement of serum PGs provides much information on the presence of intestinal metaplasia as well as atrophic gastritis.

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原著

地域住民を対象とした2段階ペプシノゲン法胃がん検診の死亡減少効果の検討

Effect of the Two-step Serum Pepsinogen Test Method on Reducing Stomach Cancer Mortality among the Urban Residents

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Abstract

In 2000, we carried out the two-step serum pepsinogen (PG) test method to detect gastric cancer in the residents of Katsushika city aged 40, 45, 50 and 55 years. In this program 4,490 persons (1, 652 men, 2,838 women) participated, and 17,488 persons (10,069 men, 7,419 women) did not. Eight gastric cancers (PG positive six and PG negative two) were diagnosed and the total incidence value of the cancer was 0.18%. Cohort study was designed to assess the reduction of gastric cancer mortality. Both of the participants group and non-participants group were followed up during 4 years since the day of screening. A total of 82,813.0 person-years were followed up and follow-up rate of participants and non-participants were 93.5% and 90.0% and number of death from gastric cancer were one (scirrhus type) and twenty one respectively. The data was analyzed using Cox regression models. From the document of death certificate, ten gastric cancer deaths in the non-participants excluding both patients suffered from gastric cancer before the program and death within one year after the beginning of the follow-up study were used for analysis. The hazard ratio (95% confidence interval) was 0.587 (0.074-4.628). Our program showed reduction of gastric cancer mortality, but statistically not significant. We concluded that the two-step serum PG test method adopted were effective for three years because there was no gastric cancer death among the participants group during 3-year observation period.

Keyword: pepsinogen, gastric cancer, mortality, cohort study

はじめに

東京都葛飾区（人口約43万）では、平成12年度に40、45、50、55歳の区民の節目健診の採血にあわせ血清ペプシノゲン（以下PG）を測定し、胃がんの高危険群のスクリーニングを行うペプシノゲン法を導入実施した。胃がん検診としては三木らの基準[1)]に基づきPG Ⅰ≦70ng/mlかつPG Ⅰ/Ⅱ≦3.0を基準値とし、陽性者には胃内視鏡による精密検査を、PG陰性者には後日胃X線撮影を行う2段階法を採用した。このPG法胃がん検診が区民の胃がん死亡の減少にどのような影響を与えたかを検討するため、対象者を検診の受診者および非受診者の2群に分け、住民

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