Tohoku J. Exp. Med., 2014, **232**, 293-300

Cutoff Serum Pepsinogen Values for Predicting Gastric Acid Secretion Status

Katsunori Iijima,[1] Tomoyuki Koike,[1] Yasuhiko Abe[2] and Tooru Shimosegawa[1]

[1]Division of Gastroenterology, Tohoku University Graduate School of medicine, Sendai, Miyagi, Japan
[2]Department of Gastroenterology, Yamagata University Faculty of Medicine, Yamagata, Yamagata, Japan

Measurement of the gastric acid secretion is useful for estimating the risk for various diseases in the upper gastro-intestinal tract; however, the procedure causes significant distress to the subjects. Pepsinogens I and II are secreted from the gastric fundic glands, and thus, the serum pepsinogen levels reflect the gastric functional statuses. The aim of this study is to establish appropriate serum pepsinogen cutoff points for predicting the gastric acid secretion status. In a total of 627 Japanese subjects, gastric acid secretion was measured with an endoscopic gastrin test, and the serum pepsinogen values and serum *Helicobacter* (*H.*) *pylori*-IgG antibody were also measured. After checking the correlation between gastric acid secretion and serum pepsinogen, the receiver operating characteristics analyses were employed for determining the most suitable cutoff points of serum pepsinogen for the gastric acid secretion status (i.e., hypochlorhydria, profound hypochlorhydria, and hyperchlorhydria). The pepsinogen I/II ratio and pepsinogen I showed the best correlation with gastric acid secretion in *H. pylori*-positive and *H. pylori*-negative subjects, respectively. The serum pepsinogen I/II ratio (or pepsinogen I in cases of *H. pylori*-negative subjects) was useful to determine the gastric acid secretion status with acceptable to outstanding diagnostic accuracy (the range of the area under the curve: 0.79-0.93). The diagnostic accuracy was further improved after stratifying the subjects by *H. pylori*-infection status. Estimating gastric acid secretion levels by simple measurement of serum pepsinogens will have significant clinical implications in estimating the risks for various diseases of the upper gastrointestinal tract.

Keywords: endoscopic gastrin test; gastric acid secretion; *Helicobacter pylori*; receiver operating characteristics analysis; serum pepsinogen
Tohoku J. Exp. Med., 2014 April, **232** (4), 293-300.

Introduction

Alteration of the gastric secretion level is related to the pathogenesis of various kinds of diseases originating from the upper gastrointestinal (GI) tract. Classically, individuals with hyperchlorhydria, especially those with *Helicobacter pylori* infection, are predisposed to the development of duodenal ulcers, whereas individuals with hypochlorhydria are at the risk of gastric cancer through the development of a premalignant condition, gastric atrophy (El-Omar et al. 1995, 1997) Thus, measurement of the gastric acid secretion level has some clinical implications in estimating the risk for various diseases in the upper GI tract. However, given that the procedure causes significant distress to the subjects and is time-consuming, direct measurement of the gastric acid secretion level has become less prevalent these days.

Pepsinogen (PG) I is secreted exclusively by the fundic glands (Samloff 1971), and PG II is secreted by the fundic glands, pyloric glands, and proximal duodenal mucosa (Samloff and Liebman 1973). Previous studies have shown that the serum PG I level and/or I/II ratio reflect the morphological (Samloff et al. 1982; Borch et al. 1989) and functional statuses (Samloff et al. 1975; Nakanome et al. 1983; Miki et al. 1987; Feldman et al. 1988) of the gastric mucosa. Thus, many studies have reported a significant correlation between serum PG values and gastric acid secretion levels (Samloff et al. 1975; Nakanome et al. 1983; Miki et al. 1987; Haruma et al. 1993; Kinoshita et al. 1997; Feldman et al. 1988; Iijima et al. 2005; Derakhshan et al. 2006), although none has demonstrated specific cutoff serum PG values for individual gastric acid secretion levels, which would help estimate the relative risk for various upper GI diseases.

As a simplified modification of the conventional gastric acid secretion test, we previously developed a rapid, simple endoscopic method for evaluating gastrin-stimulated maximal acid output (endoscopic gastrin test [EGT]) (Iijima

Received February 6, 2014; revised and accepted March 18, 2014. Published online April 9, 2014; doi: 10.1620/tjem.232.293.
Correspondence: Katsunori Iijima, M.D., Division of Gastroenterology, Tohoku University Graduate School of Medicine, Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan.
e-mail: kiijima@med.tohoku.ac.jp

et al. 1998). With this method, we measured gastric acid secretion level in a series of patients with various upper GI diseases such as peptic ulcer, reflux esophagitis, Barrett's esophagus, and superficial gastric or esophageal cancer (Iijima et al. 1998, 2000, 2005, 2010; Koike et al. 2001; Abe et al. 2004; Inomata et al. 2006; Iwai et al. 2013). In this study, we retrospectively analyzed the accumulated database based on our previous studies and investigated the correlation between gastric acid secretion levels and serum PG values. Thereafter, we calculated the most suitable cut-off serum PG values for determining individual gastric acid secretion levels using receiver operating characteristic (ROC) analysis.

Methods

A total of 627 patients who underwent the EGT and measurement of serum PG values between 1995 and 2012 at Tohoku University Hospital were enrolled in this retrospective study. In all the participants, *H. pylori* infection status was determined based on the serum immunoglobulin G (IgG) antibody level. The endoscopic diagnoses were duodenal ulcer in 45 subjects, gastric ulcer in 51, concurrent duodenal and gastric ulcers in 10, reflux esophagitis in 50, Barrett's esophagus and superficial Barrett's cancer in 38, superficial gastric cancer in 13, superficial esophageal squamous cell carcinoma in 86, and chronic gastritis or normal in the rest of the subjects. The exclusion criteria used in the present retrospective study were a history of gastric surgery, a history of *H. pylori* eradication treatment, serious systemic disease (especially renal failure), and current intake of antisecretory agents such as H_2 blockers and proton pump inhibitors. The present retrospective study was approved by the Tohoku University School of Medicine Ethics Committee (2013-1-106).

Endoscopic gastrin test

During the routine endoscopic examination, gastrin-stimulated gastric acid secretory response was estimated using the EGT, which is a modification of the conventional gastrin-stimulated maximal acid output test. The details of the EGT were reported previously. Briefly, the subjects were injected intramuscularly with pentagastrin at a dose of 6 μg/kg (Sigma, St. Louis, MO, USA) after an overnight fast and 15 min before undergoing endoscopy. After inserting the endoscope into the stomach, pooled gastric fluid was aspirated and discarded. Gastric fluid secreted between 20 and 30 min after pentagastrin injection was aspirated and collected under direct visualization during routine endoscopic examination of the stomach and duodenal bulb. The volume of the fluid sample collected over the 10-min period was recorded, and the H^+ concentration was determined by titration. The acid output in the 10-min period was calculated as the product of the volume and H^+ concentration, and the EGT result was expressed as H^+ mEq/10 min. We demonstrated previously that EGT values correlate highly with the peak acid output determined by conventional methods (correlation coefficient, 0.92) and have high reproducibility (coefficient of variation, 5.6) (Iijima et al. 1998).

Determination of H. pylori infection status

H. pylori infection status was determined by the titers of serum IgG antibodies against *H. pylori*, using an E Plate "Eiken" *H. pylori* antibody (Eiken Chemical Co. Ltd., Tokyo, Japan) (Kikuchi et al. 2011), and a titter ≥ 10 U/ml was diagnosed as *H. pylori*-positive.

Serum PG values

Blood samples were obtained from each patient under fasting conditions, and the serum was separated and stored at −20°C. Fasting serum PG I and PG II levels were measured by chemiluminescent enzyme immunoassay using commercial kits (Lumipulse PG I and II, Fujirebio Inc., Tokyo) (Kikuchi et al. 2011).

Statistical analyses

The serum PG values and EGT values were expressed as mean (SD) values. The Pearson correlation coefficients (r) between the EGT values and serum PG findings (PG I and II levels, and the PG I/II ratio) were assessed using a linear regression analysis.

We previously indicated that the mean (SD) of the EGT values in healthy subjects without *H. pylori* infection was 3.6 (1.5) mEq/10 min (Iijima et al. 1998). We defined *hypochlorhydria* as an EGT value lower than mean (−1 SD) and *profound hypochlorhydria* as an EGT value lower than mean (−2 SD). In addition, *hyperchlorhydria* was previously defined as an EGT value higher than mean (1 SD). Using these criteria, we demonstrated the relative risks for various upper GI diseases depending on the gastric acid secretion levels (Iijima et al. 2010, 2011; Iwai et al. 2013). In the present study, we used the same criteria to categorize the gastric acid secretion levels of our subjects.

ROC curves were constructed to extract the corresponding cut-off values for the respective gastric acid secretion statuses (hypochlorhydria, profound hypochlorhydria, and hyperchlorhydria). The area under the curve (AUC) and 95% confidence intervals (CIs) were calculated and compared between the various serum PG parameters (PG I levels, PG II levels, or PG I/II ratios alone, and combinations of the serum PG I levels and PG I/II ratios) to predict 3 demarcations for the gastric acid secretion status. The discriminatory ability of each biomarker was evaluated as follows: < 0.7, no discrimination; 0.71-0.79, acceptable; 0.8-0.89, excellent; and ≥ 0.9, outstanding discrimination (Zweig and Campbell 1993). The resulting cutoff values from each evaluation were applied for determination of sensitivity and specificity. All the statistical analyses were performed using the SPSS software (IBM SPSS Statistics 20.0).

Results

The 627 enrolled subjects comprised 430 males, with a mean age of 58.7 (15.4) years. Of the enrolled subjects, 430 had *H. pylori*-positive test results, whereas the remaining 197 patients had *H. pylori*-negative test results.

Correlation between the gastric acid secretion level and serum PG values

In the analysis for the entire cohort of 627 subjects, the serum PG I level was modestly correlated with the EGT value (r = 0.43, Fig. 1A), whereas no significant correlation was found between the serum PG II level and the EGT value (Fig. 1B). Consequently, the serum PG I/II ratio was well correlated with the EGT value (r = 0.57, Fig. 1C). Thus, among the 3 serum PG-related parameters (PG I level, PG II level, and PG I/II ratio), the PG I/II ratio best reflected gastric acid secretion status.

Then, repeated subgroup analyses revealed that the correlation between serum PG values and gastric acid secretion levels considerably differed depending on the *H. pylori*

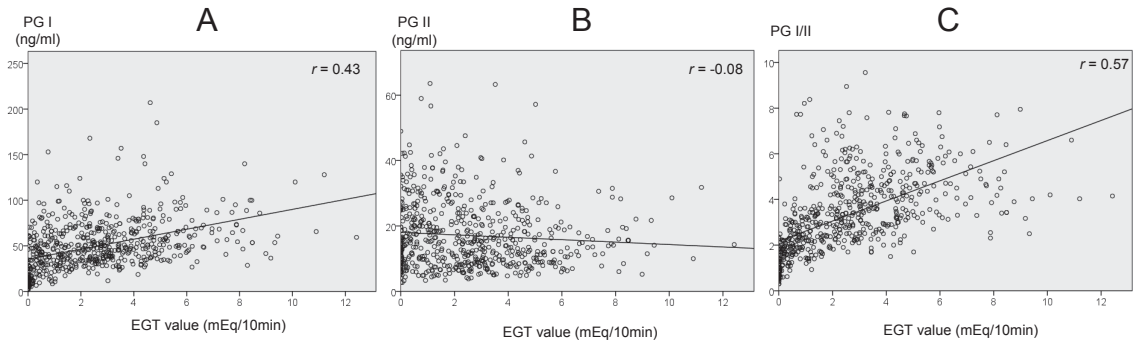

Fig. 1. Correlation of the endoscopic gastrin test value with serum pepsinogens in the entire cohort.
Panels show the correlation of the endoscopic gastrin test (EGT) value with the serum pepsinogen I level (A), pepsinogen II level (B), and pepsinogen I/II ratio (C) in the entire cohort (n = 627). r, Pearson correlation coefficient.

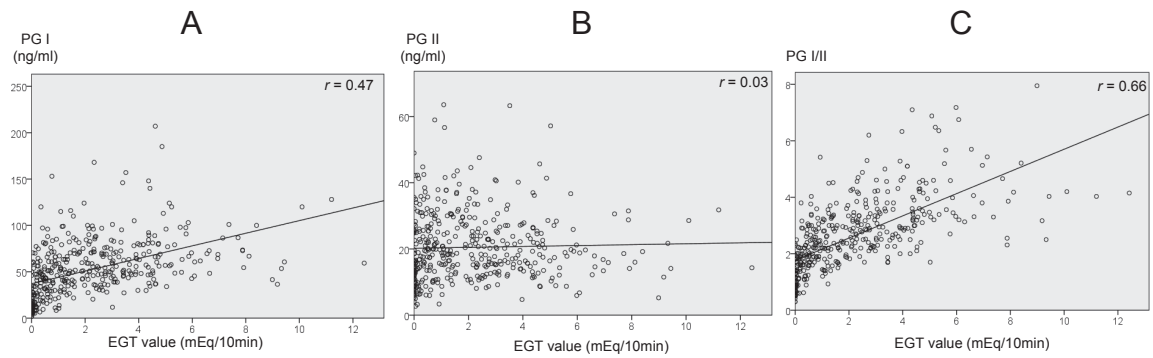

Fig. 2. Correlation of the endoscopic gastrin test value with serum pepsinogens in subjects with *H. pylori* infection.
Panels show the correlation of the endoscopic gastrin test (EGT) value with the serum pepsinogen I level (A), pepsinogen II level (B), and pepsinogen I/II ratio (C) in the subjects with *H. pylori* infection (n = 430). r, Pearson correlation coefficient.

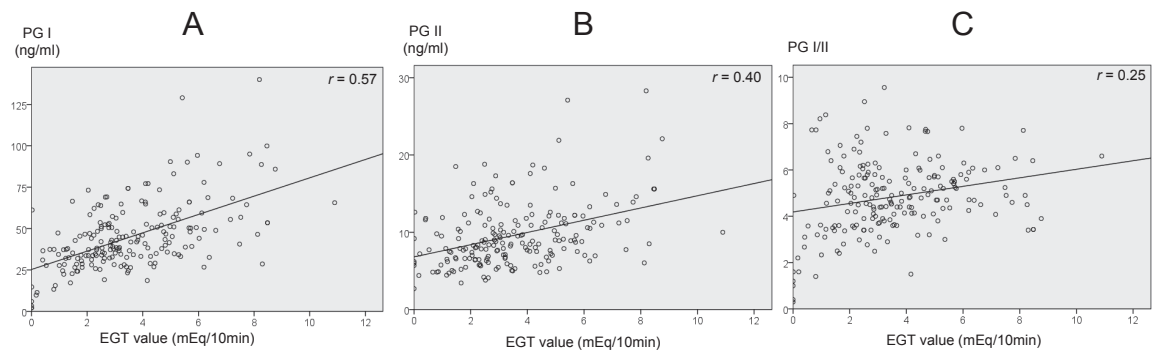

Fig. 3. Correlation of the endoscopic gastrin test value with serum pepsinogens in subjects without *H. pylori* infection.
Panels show the correlation of the endoscopic gastrin test (EGT) with the serum pepsinogen I level (A), pepsinogen II level (B), and pepsinogen I/II ratio (C) in the subjects without *H. pylori* infection (n = 197). r, Pearson correlation coefficient.

infection status. In the subjects with *H. pylori* infection, as in the entire cohort, the PG I levels were modestly correlated with the EGT values (r = 0.44, Fig. 2A), with no correlation between the PG II levels and the EGT values (Fig. 2B); consequently, a good correlation was found between the PG I/II ratio and the EGT value (r = 0.66, Fig. 2C). In contrast, in the subjects without *H. pylori* infection, the PG I level showed relatively high correlation with the EGT value (r = 0.57, Fig. 3A), whereas the PG II level had a modest correlation with the EGT value (r = 0.40, Fig. 3B), resulting in a very weak correlation between the PG I/II ratio and the EGT value (r = 0.24, Fig. 3C). Thus, although the PG I/II ratio best reflected the gastric acid secretion status in the subjects with *H. pylori* infection, the PG I level rather than the PG I/II ratio did so in the subjects without *H. pylori* infection.

Determination of the best cutoff values for individual gastric acid secretion status based on means of the ROC curves

Based on the predefined criteria for classifying the EGT results, 303 (47%) of the 627 enrolled subjects were classified as having hypochlorhydria; in particular, 155 (24%) had profound hypochlorhydria, whereas 66 (10%) had hyperchlorhydria.

Fig. 4 shows the ROC curve for discriminating the hypochlorhydric patients from the other patients in the entire cohort or in the subgroups according to *H. pylori* infection status. In the entire cohort, the AUC (95% CI) values determined for the PG I level, PG II level, and PG I/II ratio were 0.72 (0.68-0.76), 0.44 (0.39-0.48), and 0.86 (0.83-0.89), respectively, indicating that the PG I/II ratio showed the best and excellent diagnostic accuracy for the discrimination of hypochlorhydric patients. The combined evaluation with the PG I level and PG I/II ratio (AUC [95% CI], 0.86 [0.83-0.89]) did not further improve the diagnostic accuracy compared with the evaluation using the PG I level alone (Fig. 4A). The best PG I/II ratio cutoff value for predicting hypochlorhydria in the entire cohort was 3.0, and the corresponding sensitivity and specificity were 76.4% and 78.7%, respectively (Table 1). Meanwhile, the results of the ROC analysis differed according to *H. pylori* infection status, as expected based on the above-mentioned correlation between the serum PG values and gastric acid secretion levels. In particular, although the PG I/II ratio showed the best diagnostic accuracy for the discrimination of hypochlorhydric subjects from the subjects with *H. pylori* infection based on an AUC (95% CI) of 0.87 (0.84-0.90) (Fig. 4B), the PG I level rather than the PG I/II ratio showed the best diagnostic accuracy in the subjects without *H. pylori* infection based on an AUC (95% CI) of 0.80 (0.73-0.88) (Fig. 4C). Accordingly, the best cutoff PG I/II ratio for predicting hypochlorhydria in the subjects with *H. pylori* infection was 2.7, at a corresponding sensitivity of 79.2% and specificity of 76.3% (Table 1). Meanwhile, the best cutoff PG I level for predicting hypochlorhydria in the subjects without *H. pylori* infection was 35 ng/mL, at a corresponding sensitivity and specificity of 70.0% and 76.3%, respectively (Table 1).

Regarding the ROC analysis for profound hypochlorhydria, the PG I/II ratio showed the highest diagnostic accuracy in the entire cohort, according to an AUC (95%

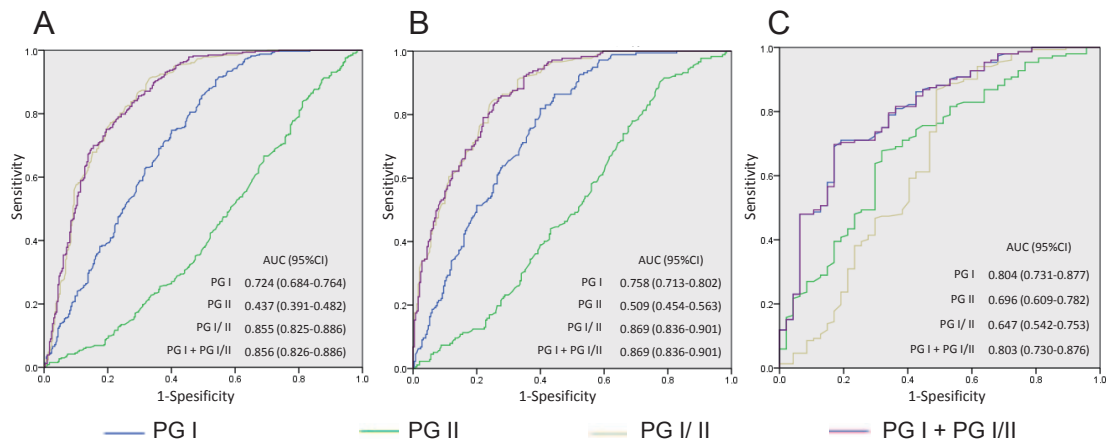

Fig. 4. Receiver operating characteristic analysis of pepsinogen values for hypochlorhydria.
Shown are receiver operating characteristic curves of the serum pepsinogen values to discriminate the subjects with hypochlorhydria from the entire cohort (A), subjects with *H. pylori* infection (B), and subjects without *H. pylori* infection (C).
PG, pepsinogen; AUC (95% CI), area under the curve (95% confidence interval).

Table 1. Serum pepsinogen cutoff points and the corresponding sensitivity and specificity for predicting various gastric acid secretion statuses.

	Diagnosis of hypochlorhydria			Diagnosis of profound hypochlorhydria			Diagnosis of hyperchlorhydrua		
	Cutoff point	Sensitivity	Specificity	Cutoff point	Sensitivity	Specificity	Cutoff point	Sensitivity	Specificity
Entire cohort (n = 627)	PG I/II ≤ 3.0	76.4%	78.7%	PG I/II ≤ 2.3	85.5%	85.3%	PG I/II ≥ 3.9	73.8%	71.2%
H. pylori-positive subjects (n = 430)	PG I/II ≤ 2.7	79.2%	76.3%	PG I/II ≤ 2.2	82.3%	82.5%	PG I/II ≥ 3.3	78.6%	77.2%
H. pylori-negative subjects (n = 197)	PG I ≤ 35 ng/ml	70.0%	76.3%	N.A.	N.A.	N.A.	PG I ≥ 50 ng/ml	70.6%	72.7%

PG, pepsinogen; N.A., not analyzed.

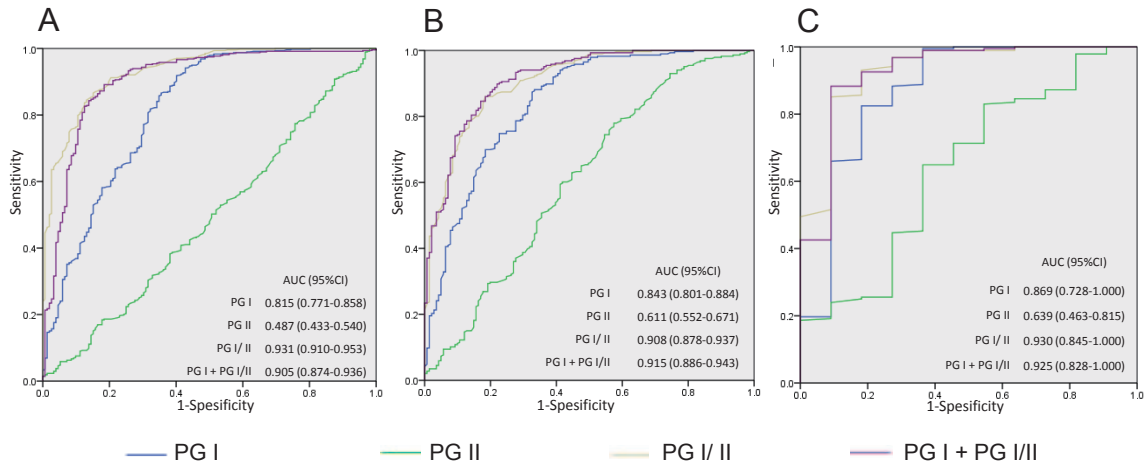

Fig. 5. Receiver operating characteristic analysis of pepsinogen values for profound hypochlorhydria.
Shown are receiver operating characteristic curves of the serum pepsinogen values to discriminate the subjects with profound hypochlorhydria from the entire cohort (A), subjects with *H. pylori* infection (B), and subjects without *H. pylori* infection (C).
PG, pepsinogen; AUC (95% CI), area under the curve (95% confidence interval).

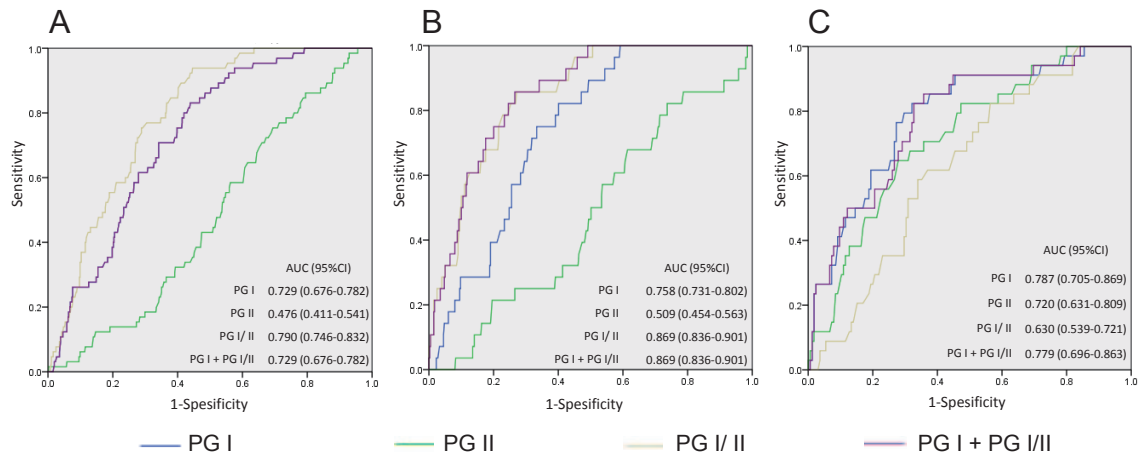

Fig. 6. Receiver operating characteristic analysis of pepsinogen values for hyperchlorhydria.
Shown are receiver operating characteristic curves of the serum pepsinogen values to discriminate the subjects with hyperchlorhydria from the entire cohort (A), subjects with *H. pylori* infection (B), and subjects without *H. pylori* infection (C).
PG, pepsinogen; AUC (95% CI), area under the curve (95% confidence interval).

CI) of 0.93 (0.91-0.94) (Fig. 5A). Such outstanding diagnostic accuracy with the PG I/II ratio was preserved regardless of *H. pylori* infection status; the AUC (95% CI) values for the PG I/II ratio were 0.91 (0.88-0.94) and 0.93 (0.85-1.00) in the subjects with and those without *H. pylori* infection (Fig. 5B and C). The best cutoff PG I/II ratio for predicting profound hypochlorhydria was 2.3 in the entire cohort, and the corresponding sensitivity and specificity were 85.5% and 85.5%, respectively (Table 1). Similarly, the best cutoff PG I/II ratio for predicting profound hypochlorhydria was 2.2 in the subjects with *H. pylori* infection, with a sensitivity of 82.3% and specificity of 82.5% (Table 1). We could not perform the same analysis in the group without *H. pylori* infection because of the small number of subjects with profound hypochlorhydria.

Likewise, the results of the ROC analysis for hyperchlorhydria in the entire cohort indicated that the PG I/II ratio showed the highest diagnostic accuracy, according to an AUC (95% CI) of 0.79 (0.75-0.83) (Fig. 6A). Although the AUC value for hyperchlorhydria in the entire cohort was somewhat lower than the above-mentioned values for hypochlorhydria or profound hypochlorhydria, the diagnostic accuracy was well within the acceptable level. The diagnostic accuracy for discriminating hyperchlorhydric subjects was substantially improved by dividing the subjects according to *H. pylori* infection status. That is, an excellent diagnostic accuracy for discriminating hyperchlorhydric subjects was observed in the group with *H.*

pylori infection, with an AUC (95% CI) of 0.85 (0.79-0.91) using the PG I/II ratio (Fig. 6B), whereas the diagnostic accuracy was preserved in the group without *H. pylori* infection, with an AUC (95% CI) of 0.79 (0.71-0.87) using the PG I level (Fig. 6C). The best cutoff PG I/II ratio for predicting hyperchlorhydria was 3.9 in the entire cohort, and the corresponding sensitivity and specificity were 73.9% and 71.2%, respectively (Table 1). In addition, in the subjects with *H. pylori* infection, a PG I/II ratio of 3.3 was indicated to be the best cutoff value for predicting hyperchlorhydria, with a corresponding sensitivity of 77.2% and specificity of 77.2%. Meanwhile, in the subjects without *H. pylori* infection, a PG I value of 50 ng/mL was judged as the best cutoff value for predicting hyperchlorhydria, with a corresponding sensitivity and specificity of 70.6% and 72.7%, respectively (Table 1).

Discussion

In this retrospective study of 627 subjects, we established reliable cutoff serum PG values to predict gastric acid secretion status with acceptable to outstanding diagnostic accuracy. This is the first study to demonstrate reliable cutoff values for PG-related parameters for predicting gastric acid secretion status.

In this study, gastric secretion statuses *(hypochlorhydria*, *profound hypochlorhydria*, and *hyperchlorhydria*) of enrolled subjects were classified based on the predefined criteria that we previously established among *H. pylori*-negative normal subjects (Iijima et al. 1998). Then, we analyzed the data from heterogeneous patient groups with wide range of gastric secretion as a group, focusing on the correlation between serum PGs and gastric acid secretion regardless of the attendant gastro-duodenal disorder. We consider that the inclusion of various attendant gastro-duodenal disorders would not distort the correlation between gastric acid secretion and serum PGs. Further, we considered that such analysis would be more practical in clinical setting for managing various patients because of the demonstrated usefulness of serum PG as an index for a wide range of gastric acid secretion levels.

Although many previous studies from other institutes reported significant correlations between serum PG values and gastric secretion levels in 30-120 subjects (Samloff et al. 1975; Nakanome et al. 1983; Miki et al. 1987; Haruma et al. 1993; Kinoshita et al. 1997; Feldman et al. 1988; Iijima et al. 2005; Derakhshan et al. 2006), the EGT, a simplified gastric secretory test, enabled us to enroll a much greater number of subjects to analyze such associations in the present analysis.

In the ROC analyses of the entire cohort, the PG I/II ratio consistently showed the best diagnostic accuracy among the serum PG-related parameters for determining the gastric acid secretion status (hypochlorhydria, profound hypochlorhydria, or hyperchlorhydria). Nonetheless, as expected from the differing correlations between the serum PG values and gastric acid secretion levels depending on *H. pylori* infection status, the PG-related parameters showing the best diagnostic accuracy were distinct with or without the presence of infection. While the PG I/II ratio was best for discriminating hypochlorhydria and hyperchlorhydria in the subjects with *H. pylori* infection, the PG I level rather than the PG I/II ratio was best for discriminating the 2 acid secretion statuses in the subjects without *H. pylori* infection. Consequently, we applied the cutoff PG I/II ratio or serum PG I level to predict the acid secretory status in the subjects with or without *H. pylori* infection, respectively. PG I has been demonstrated to be influenced not only by the parietal cell mass but also by gastric mucosal inflammation induced largely by *H. pylori* infection (Iijima et al. 2005). Because the influence of mucosal inflammation on PG I level in the *H. pylori*-free stomachs was unremarkable, PG I mainly reflects parietal cell mass, leading to a better indicator of gastric acid secretion in the subjects without *H. pylori* infection. In contrast, considering that the PG I and PG II levels in the subjects with *H. pylori* infection were elevated by mucosal inflammation despite the acid secretory level (Iijima et al. 2005), taking the ratio of PG I to PG II of each individual seems useful for counterbalancing this relative increase, leaving the PG I/II ratio as a better parameter to predict gastric acid secretion in *H. pylori*-positive subjects. Given that the *H. pylori* infection status was the major factor that influenced correlation between gastric acid secretion and serum PG values (Iijima et al. 2005) and that the infection rate was time-dependent (Fujisawa et al. 1999), setting up different cutoff values depending on infection status will help maintain the diagnostic accuracy of the PG test as a measure of gastric acid secretion level even in future analyses.

The gastric acid secretion level declines with atrophic changes in the fundic mucosa (Samloff et al. 1982; Miki et al. 1987; Borch et al. 1989; Derakhshan et al. 2006), especially in subjects with *H. pylori* infection (Iijima et al. 2004), which can be occasionally recognized endoscopically as a discoloration of the mucosa (Kimura 1972). Hence, suppressed gastric acid secretion could be predictable to some extent for endoscopists. In contrast, no endoscopic sign suggestive of hyperchlorhydria was found, as it usually occurs in the absence of endoscopically recognized atrophic change. Hence, measuring serum PG levels and classifying gastric acid secretion levels using appropriate cutoff values should be considered as a powerful tool for identifying hyperchlorhydric subjects. Identification of hyperchlorhydria may have significant clinical implications, even apart from extreme cases such as the Zollinger-Ellison syndrome. For example, we recently reported that individuals with hyperchlorhydria are at high risk of low-dose aspirin-induced gastropathy (Iijima et al. 2011). Aspirin is now widely administered as an antithrombotic drug for the prevention of cerebrovascular and cardiovascular diseases. Thus, identifying hyperchlorhydric subjects by measuring serum PG values could provide significant benefits given the large numbers of people now taking aspirin as a prophy-

lactic.

There are some limitations of the present study. Firstly, considering that gastric acid secretion levels are well demonstrated to considerably differ between Oriental and Western populations (Feldman et al. 1988), different cutoff values might be required for Western populations. In addition, caution should be exercised in the use of Japanese and European assay kits because of their considerably differing measured serum PG values, as recently reported (Iijima et al. 2009a; Miki and Fujishiro 2009). Secondly, Since *H. pylori* status was determined by serum antibody test alone in the present study, some misclassification could occur in the present study. However, IgG antibody test showed high sensitivity and specificity over 90% for diagnosing *H. pylori* infection (Wilcox et al. 1996; Sasazuki et al. 2006) and it would be practical in clinical settings to measure both *H. pylori* antibody and pepsinogen concentrations using the same serum samples. Finally, the PG cutoff values calculated in the present analysis could not be applied to those with a history of *H. pylori* eradication because the correlation between gastric acid secretion and serum PGs is considerably altered after the therapy (Iijima et al. 2009b). Since we have recently reported that gastric acid secretory capacity is inversely associated with the risk for gastric carcinoma emerging after successful eradication (Iijima et al. 2012), the serum PG values after eradication might be useful for estimation of the cancer risk among eradicated patients. Alternative cut-off values specific to eradicated patients need to be established.

In conclusion, using a large number of subjects, we established reliable cutoff serum PG values for predicting gastric acid secretion status with acceptable to outstanding diagnostic accuracy. Stratification by *H. pylori* infection status further improved the diagnostic accuracy of our parameters. Given that gastric secretion level is a primary determinant for the development of various diseases in the upper GI tract, estimating gastric acid secretion levels by the measurement of serum PG values and subsequent classification with appropriate cutoff values will have significant implications in clinical and epidemiological studies in terms of estimating risks for various diseases of the upper GI tract.

Abbreviations

AUC, area under the curve; CI, confidence intervals; EGT, endoscopic gastrin test; GI, gastrointestinal; PG, pepsinogen; ROC, receiver operating characteristics.

Conflict of Interest

The authors declare no conflict of interest.

References

Abe, Y., Ohara, S., Koike, T., Sekine, H., Iijima, K., Kawamura, M., Imatani, A., Kato, K. & Shimosegawa, T. (2004) The prevalence of Helicobacter pylori infection and the status of gastric acid secretion in patients with Barrett's esophagus in Japan. *Am. J. Gastroenterol*., **99**, 1213-1221.

Borch, K., Axelsson, C.K., Halgreen, H., Damkjaer Nielsen, M.D., Ledin, T. & Szesci, P.B. (1989) The ratio of pepsinogen A to pepsinogen C: a sensitive test for atrophic gastritis. *Scand. J. Gastroenterol*., **24**, 870-876.

Derakhshan, M.H., El-Omar, E., Oien, K., Gillen, D., Fyfe, V., Crabtree, J.E. & McColl, K.E. (2006) Gastric histology, serological markers and age as predictors of gastric acid secretion in patients infected with Helicobacter pylori. *J. Clin. Pathol*., **59**, 1293-1299.

El-Omar, E.M., Oien, K., El-Nujumi, A., Gillen, D., Wirz, A., Dahill, S., Williams, C., Ardill, J.E. & McColl, K.E. (1997) Helicobacter pylori infection and chronic gastric acid hyposecretion. *Gastroenterology*, **113**, 15-24.

El-Omar, E.M., Penman, I.D., Ardill, J.E., Chittajallu, R.S., Howie, C. & McColl, K.E. (1995) Helicobacter pylori infection and abnormalities of acid secretion in patients with duodenal ulcer disease. *Gastroenterology*, **109**, 681-691.

Feldman, M., Richardson, C.T., Lam, S.K. & Samloff, I.M. (1988) Comparison of gastric acid secretion rates and serum pepsinogen I and II concentrations in Occidental and Oriental duodenal ulcer patients. *Gastroenterology*, **95**, 630-635.

Fujisawa, T., Kumagai, T., Akamatsu, T., Kiyosawa, K. & Matsunaga, Y. (1999) Changes in seroepidemiological pattern of Helicobacter pylori and hepatitis A virus over the last 20 years in Japan. *Am. J. Gastroenterol*., **94**, 2094-2099.

Haruma, K., Yoshihara, M., Sumii, K., Tari, A., Watanabe, C., Kodoi, A. & Kajiyama, G. (1993) Gastric acid secretion, serum pepsinogen I, and serum gastrin in Japanese with gastric hyperplastic polyps or polypoid-type early gastric carcinoma. *Scand. J. Gastroenterol*., **28**, 633-637.

Iijima, K., Abe, Y., Kikuchi, R., Koike, T., Ohara, S., Sipponen, P. & Shimosegawa, T. (2009a) Serum biomarker tests are useful in delineation between patients with gastric atrophy and normal, healthy stomach. *World J. Gastroenterol*., **15**, 853-859.

Iijima, K., Abe, Y., Koike, T., Uno, K., Endo, H., Hatta, W., Asano, N., Asanuma, K., Imatani, A. & Shimosegawa, T. (2012) Gastric cancers emerging after H. pylori eradication arise exclusively from non-acid-secreting areas. *Tohoku J. Exp. Med*., **226**, 45-53.

Iijima, K., Ara, N., Abe, Y., Koike, T., Iwai, W., Uno, K., Asano, N., Imatani, A., Ohara, S. & Shimosegawa, T. (2011) Gastric acid secretion level modulates the association between Helicobacter pylori infection and low-dose aspirin-induced gastropathy. *J. Gastroenterol*., **46**, 612-619.

Iijima, K., Koike, T., Abe, Y., Ara, N., Uno, K., Imatani, A., Ohara, S. & Shimosegawa, T. (2009b) Alteration of correlation between serum pepsinogen concentrations and gastric acid secretion after H. pylori eradication. *J. Gastroenterol*., **44**, 819-825.

Iijima, K., Koike, T., Abe, Y., Yamagishi, H., Ara, N., Asanuma, K., Uno, K., Imatani, A., Nakaya, N., Ohara, S. & Shimosegawa, T. (2010) Gastric hyposecretion in esophageal squamous-cell carcinomas. *Dig. Dis. Sci*., **55**, 1349-1355.

Iijima, K., Ohara, S., Koike, T., Sekine, H. & Shimosegawa, T. (2004) Gastric acid secretion of normal Japanese subjects in relation to Helicobacter pylori infection, aging, and gender. *Scand. J. Gastroenterol*., **39**, 709-716.

Iijima, K., Ohara, S., Sekine, H., Koike, T., Kato, K., Asaki, S., Shimosegawa, T. & Toyota, T. (2000) Changes in gastric acid secretion assayed by endoscopic gastrin test before and after Helicobacter pylori eradication. *Gut*, **46**, 20-26.

Iijima, K., Ohara, S., Sekine, H., Koike, T., Kubota, Y., Kato, K., Asaki, S. & Toyota, T. (1998) A new endoscopic method of gastric acid secretory testing. *Am. J. Gastroenterol*., **93**, 2113-2118.

Iijima, K., Sekine, H., Koike, T., Imatani, A., Ohara, S. & Shimosegawa, T. (2005) Serum pepsinogen concentrations as a measure of gastric acid secretion in Helicobacter pylori-

negative and -positive Japanese subjects. *J. Gastroenterol*., **40**, 938-944.

Inomata, Y., Koike, T., Ohara, S., Abe, Y., Sekine, H., Iijima, K., Ariizumi, K., Yamagishi, H., Kitagawa, Y., Imatani, A. & Shimosegawa, T. (2006) Preservation of gastric acid secretion may be important for the development of gastroesophageal junction adenocarcinoma in Japanese people, irrespective of the H. pylori infection status. *Am. J. Gastroenterol*., **101**, 926-933.

Iwai, W., Abe, Y., Iijima, K., Koike, T., Uno, K., Asano, N., Imatani, A. & Shimosegawa, T. (2013) Gastric hypochlorhydria is associated with an exacerbation of dyspeptic symptoms in female patients. *J. Gastroenterol*., **48**, 214-221.

Kikuchi, R., Abe, Y., Iijima, K., Koike, T., Ara, N., Uno, K., Asanuma, K., Asano, N., Imatani, A. & Shimosegawa, T. (2011) Low serum levels of pepsinogen and gastrin 17 are predictive of extensive gastric atrophy with high-risk of early gastric cancer. *Tohoku J. Exp. Med*., **223**, 35-44.

Kimura, K. (1972) Chronological transition of the fundic-pyloric border determined by stepwise biopsy of the lesser and greater curvatures of the stomach. *Gastroenterology*, **63**, 584-592.

Kinoshita, Y., Kawanami, C., Kishi, K., Nakata, H., Seino, Y. & Chiba, T. (1997) Helicobacter pylori independent chronological change in gastric acid secretion in the Japanese. *Gut*, **41**, 452-458.

Koike, T., Ohara, S., Sekine, H., Iijima, K., Abe, Y., Kato, K., Toyota, T. & Shimosegawa, T. (2001) Helicobacter pylori infection prevents erosive reflux oesophagitis by decreasing gastric acid secretion. *Gut*, **49**, 330-334.

Miki, K. & Fujishiro, M. (2009) Cautious comparison between East and West is necessary in terms of the serum pepsinogen test. *Dig. Endosc*., **21**, 134-135.

Miki, K., Ichinose, M., Shimizu, A., Huang, S.C., Oka, H., Furihata, C., Matsushima, T. & Takahashi, K. (1987) Serum pepsinogens as a screening test of extensive chronic gastritis. *Gastroenterol. Jpn.*, **22**, 133-141.

Nakanome, C., Akai, H. & Goto, Y. (1983) Serum group I pepsinogen levels in patients with peptic ulcer and normal subjects. *Tohoku J. Exp. Med*., **139**, 151-158.

Samloff, I.M. (1971) Cellular localization of group I pepsinogens in human gastric mucosa by immunofluorescence. *Gastroenterology*, **61**, 185-188.

Samloff, I.M. & Liebman, W.M. (1973) Cellular localization of the group II pepsinogens in human stomach and duodenum by immunofluorescence. *Gastroenterology*, **65**, 36-42.

Samloff, I.M., Secrist, D.M. & Passaro, E. Jr. (1975) A study of the relationship between serum group I pepsinogen levels and gastric acid secretion. *Gastroenterology*, **69**, 1196-1200.

Samloff, I.M., Varis, K., Ihamaki, T., Siurala, M. & Rotter, J.I. (1982) Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. *Gastroenterology*, **83**, 204-209.

Sasazuki, S., Inoue, M., Iwasaki, M., Otani, T., Yamamoto, S., Ikeda, S., Hanaoka, T. & Tsugane, S. (2006) Effect of Helicobacter pylori infection combined with CagA and pepsinogen status on gastric cancer development among Japanese men and women: a nested case-control study. *Cancer Epidemiol. Biomarkers Prev*., **15**, 1341-1347.

Wilcox, M.H., Dent, T.H., Hunter, J.O., Gray, J.J., Brown, D.F., Wight, D.G. & Wraight, E.P. (1996) Accuracy of serology for the diagnosis of Helicobacter pylori infection: a comparison of eight kits. *J. Clin. Pathol*., **49**, 373-376.

Zweig, M.H. & Campbell, G. (1993) Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin. Chem*., **39**, 561-577.