REVIEW

The Role of Serum Pepsinogen in the Detection of Gastric Cancer

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The incidence of gastric cancer is very high in Japan, Korea, and China. Reducing the morbidity and mortality associated with gastric cancer requires early diagnosis, which can be facilitated by applying gastroscopy more frequently in high-risk groups. A strategy of population screening for gastric cancer is currently being adopted in Korea, Japan, and the Matsu region of Taiwan, but using different screening methods. In addition, the history of pepsinogen (PG) in research as a gastric cancer biomarker has varied, in that the use of serum levels of PGI and PGII and the PGI/PGII ratio as gastric cancer screening tools was introduced in Japan before 1990, but in Korea the first research results were only reported in 2008. This review first evaluates the physiology of PG, followed by the usefulness or limitations of serum PG testing with regard to the detection of gastric cancer. Finally, the factors affecting the efficacy of PG tests as a gastric cancer biomarker (i.e., *Helicobacter pylori* infection status, gender, histopathologic features, and cancer location and depth) are evaluated. It was found that the strategies used to increase the efficacy of PG tests should be individualized in each country according to the seroprevalence of *H. pylori*. **(Gut Liver 2010;4:307-319)**

INTRODUCTION

Gastric cancer is the second leading cause of cancer-related death in the world.[1] However, the risk of gastric cancer varies among the countries and populations in the world, and also in the Asian region. High risk areas include Korea, Japan and China, where the age standardized incidence rate (ASR) is higher than 20 per 100,000. Specifically, in Korea age standardized incidence rate of gastric cancer during 1990-2001 determined by the Cancer Registry at the Korean National Cancer Center in 2002, was 65.6 per 100,000 for men and 25.8 for women.[2] In case of Japan the age-adjusted mortality has decreased from 69.9 to 34.5 per 100 000 in men and from 34.1 to 13.2 per 100,000 in women between 1980 and 2003.[3] Intermediate risk countries (ASR 11-19/100,000) include Malaysia, Singapore and Taiwan, while low risk areas (ASR <10/100,000) include Australia, New Zealand, India and Thailand.[1] The geographic variability in gastric cancer appears to be explained by a synergistic interaction between *Helicobacter pylori* (*H. pylori*) infection and environmental factors such as diet (salt, nitrates and low intake of fresh fruits and vegetables)[4-9] or smoking.[10] When diagnosed at an early stage, 5-year survival rates for gastric cancer exceed 90%.[11,12] In addition, early gastric cancer, depending on the depth of mucosal infiltration and degree of differentiation, may also be suitable for endoscopic mucosal resection or endoscopic submucosal dissection, with lower morbidity, but similar efficacy, when compared to surgery.[13] On the other hand, when diagnosed at an advanced stage, 5-year survival rates are below 50%.[14] Thus, it is necessary to diagnose gastric cancer at an early stage to reduce the morbidity and mortality from gastric cancer. A strategy of population screening for gastric cancer has been adopted in Korea,[15] Japan,[16] and in Taiwan,[17] which is different. That is, in Korea a health check-up program (upper endoscopy or

upper gastrography) designed to detect gastric cancer has been implemented biannually for those over 40 years by the Korean government since 2001,[15] but there has been criticism regarding the cost/benefit of this strategy. In Japan gastric cancer screening using photofluorography has been recommended for population-based and opportunistic screening.[16] In case of Matzu, an offshore island between Taiwan and mainland China endoscopy was performed for participants who had shown positive test results from anti-*H. pylori* (immunoglobulin IgG) and pepsinogen I and II (PG I/II) or reporting a family history of gastric cancer or a personal history of peptic ulcer or other gastrointestinal disease from questionnaire.[17] As the approach in Matzu if serum pepsinogen test could work as serum markers or an indicators which can identify those at high risk selective monitoring, then the costs of gastric cancer screening would be decreased.

There is a precancerous cascade, in which the gastric mucosa undergoes a series of changes resulting in gastritis, atrophy, intestinal metaplasia, and dysplasia, before developing into gastric cancer.[4] *H. pylori* colonizes the gastric mucosa and triggers a series of inflammatory reactions, and considered as important cause of atrophic gastritis (AG).[18] AG caused by *H. pylori* usually begins at the gastric antrum and extends proximally towards the cardia,[19] resulting in decrease of gastric secretory function as the area of fundic gland mucosa also gets smaller. Although AG is a histopathological diagnosis the accurate quantification of the AG extent based on a few endoscopic biopsy samples is very difficult because AG is usually a multifocal process especially in the early stage.[20] Serum PG has been found to be a marker of gastric mucosal status, including mucosal atrophy.[21,22] A low PG I level and a low PG I/II ratio have been associated with severe gastric atrophy, and are frequently found in gastric cancer.[18,23-32] In Japan measures of PG I and PG II levels were found to be a noninvasive and straightforward means of mass screening for gastric cancer, as compared with endoscopy.[25,33,34] Most of these reports used immunoradiometric assay by PG I, II RIA BEAD kits from Dainabot, Tokyo.[24-27,29,31] In contrast to these reports from Japan, the recent study which has been performed in Korea showed that the sensitivity and specificity of PG II ratio ≤3 for detection of gastric cancer was rather low, 59.2% and 61.0%, respectively using Latex enhanced turbidimetric immunoassay (L-TIA) (Shima Laboratories, Tokyo, Japan).[32] In addition, a study from Singapore showed that the prevalence of low PG was highest in Indian subjects although gastric cancer incidence was lowest in this race than Chinese and Malay.[35] These results suggest that the application of PG test should be cautious

in the different condition or race.

The aim of this review is to evaluate the usefulness or limitation of serum PG in the detection of gastric cancer based on the literature. In addition, affecting factors (*H. pylori* infection status, gender, age, histopathologic features, cancer location, and depth) on the efficacy of PG tests were evaluated to find out a way to increase the efficacy of this gastric cancer biomarker.

SERUM PEPSINOGENS

1. Pepsinogen I and II

Two biochemically distinct pepsinogens are produced by gastric mucosa. PG I (also called as PGA) is exclusively produced by chief and mucous neck cells in the fundic glands, while PG II (also called as PGC) is secreted by these cells and also by the cells in the pyloric glands and Brunner's glands.[36,37] Serum PG concentrations have been shown to reflect the morphological and functional status of the gastric mucosa. As the fundic gland mucosa reduces, PG I levels gradually decrease, whereas PG II levels remain constant.[38] Thus, the serum PG I level as well as the PG I/II ratio, were positively correlated with maximal gastric output.[39,40] However, there has been a report that did not show any significant relation between acid secretion and PG levels.[41] For this reason Iijima et al.[42] suggested that PG I was influenced not only parietal cell mass but also by gastric mucosal inflammation induced largely by *H. pylori* infection. *H. pylori*, which causes inflammation and peptic ulcer diseases, was found to be one of the most important factors that affect PG levels as well as gastric secretion. Finally, it was realized that the subjects should be divided into two groups according to their *H. pylori* status.

2. *H. pylori* infection and the effect of *H. pylori* eradication on the pepsinogen levels

Serum PG I and PG II levels are known to increase in the *H. pylori*-associated non-atrophic superficial gastritis. However, as PG II exhibits a greater rise relative to PG I,[43-45] the PG I/II ratio decreases in the presence of *H. pylori*-infection (Fig. 1).[32] The value of PG II is different depending on *H. pylori*-infection. That is, in *H. pylori*-positive subjects there is little correlation between PG II and gastric acid secretion because of the wide variety of PG II levels. In contrast, in *H. pylori*-negative subjects PG II is a relatively constant value and correlates with acid secretion. There have been several reports regarding the increase of PG levels in case of *H. pylori*-infection. Cave et al.[46] showed that *H. pylori* sonicate and *H. pylori* lipopolysaccharide stimulate PG release from isolated rabbit
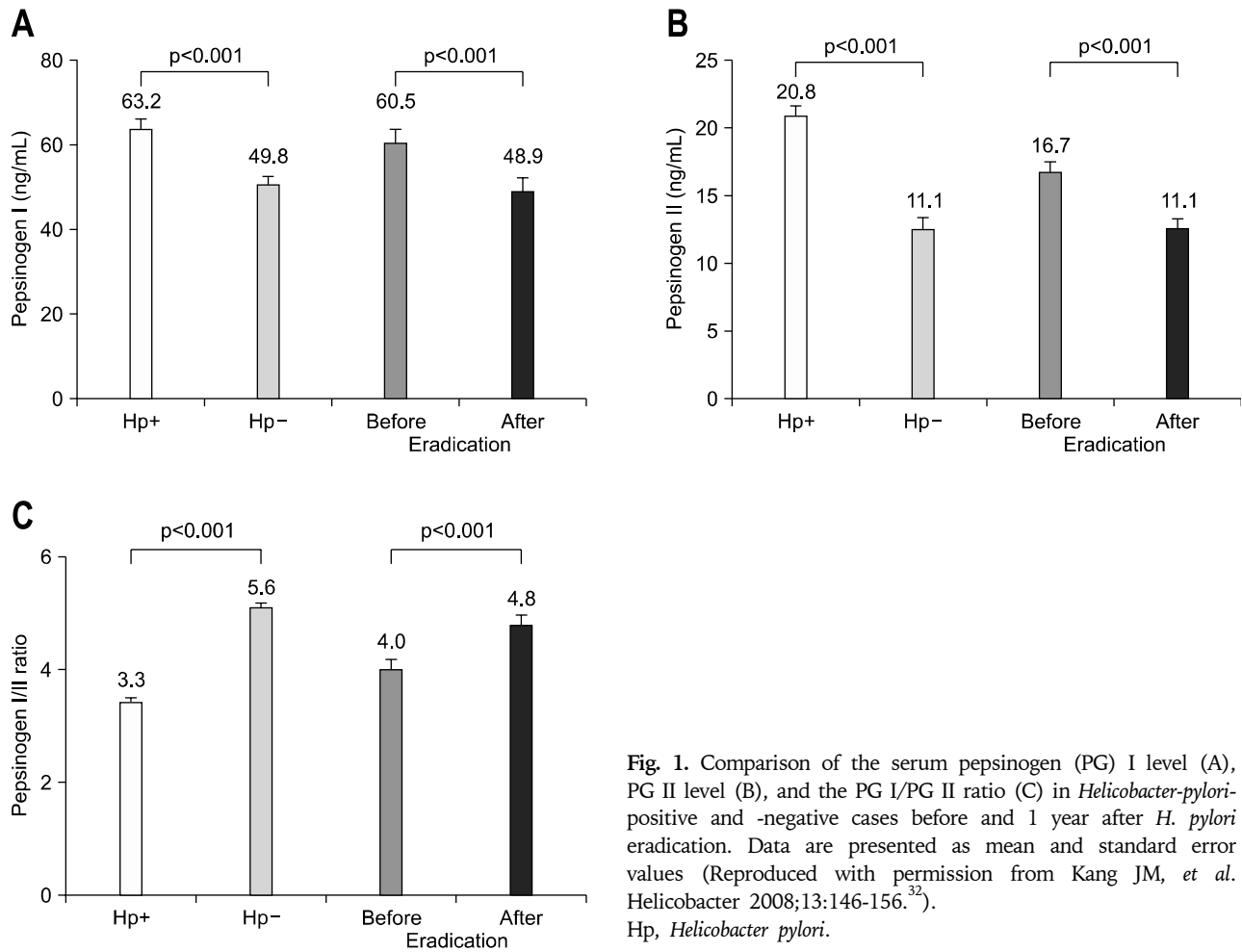
Fig. 1. Comparison of the serum pepsinogen (PG) I level (A), PG II level (B), and the PG I/PG II ratio (C) in *Helicobacter-pylori*-positive and -negative cases before and 1 year after *H. pylori* eradication. Data are presented as mean and standard error values (Reproduced with permission from Kang JM, *et al.* Helicobacter 2008;13:146-156.[32]).
Hp, *Helicobacter pylori*.

gastric glands, suggesting of a direct stimulatory effect of *H. pylori* on chief cells. In addition, purified *H. pylori* lipopolysaccharide increased PG secretion 50-fold while the *E. coli* lipopolysaccharide raised this secretion 12-fold, suggesting the specificity of *H. pylori* lipopolysaccharide on gastric secretion.[47] In addition to this direct effect of *H. pylori* the *H. pylori*-associated gastric inflammation status also increases the PG levels.[44]

These elevated PG levels were found to be decreased 1 year after the eradication of *H. pylori* (Fig. 1A and B).[32] This decrease originates from the decreases of the severity of *H. pylori*-associated gastritis as well as from the clearing of *H. pylori* lipopolysaccharide. Although *H. pylori*-eradication reduced both of PG I and PG II, but PG II exhibited a greater decrease relative to PG I (Fig. 1A and B), thus elevated the PG I to PG II ratio similar to the pre-eradication level (Fig. 1C).[32] A significant decrease in basal PG II levels occurred immediately (one month) after completion of the treatment but the decrease of PG I level occurs progressively for 6 months.[48]

3. The effect of other factors on pepsinogen levels

Other factors such as age, gender, height, body weight, body surface area, smoking, and drinking habits have been suggested to be related with PG I and PG II levels in the literature.[43,49-52] Kim *et al.*[43] showed that male gender was associated with higher PG I than female but there was no correlation in case of PG II or PG I/II ratio in 1,485 healthy subjects (964 [64.9%] *H. pylori* positive cases). However, PG I was not associated with body mass index (BMI). The only independent significant factor associated with gender was smoking, which was more common in men than in women in this study; however, there was no significant correlation between the PG levels and the smoking status. These results suggest that the difference in the PG I levels observed between genders could be related to hormonal effects. For *H. pylori* infection and age, PG I did not show any significant change (Fig. 2A). However, PG II increased two-fold, a change much greater than PG I, in *H. pylori*-positive subjects (Fig. 2B), and this reduced the PG I/II ratio (Fig. 2C). In addition, the PG II
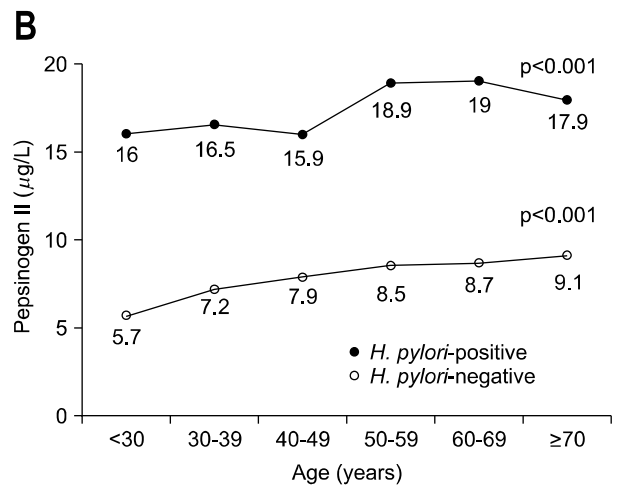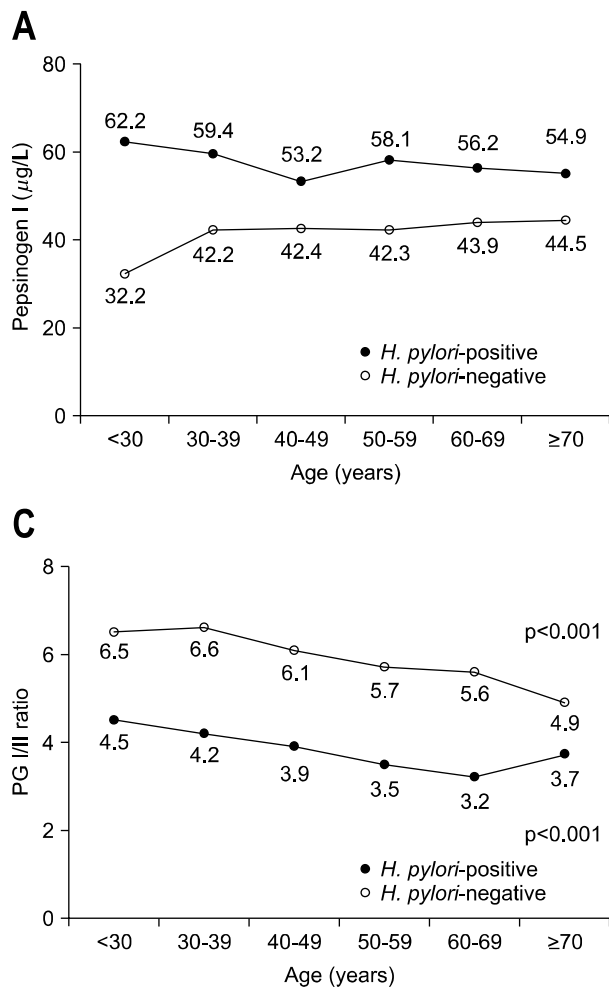
A

B

C

Fig. 2. Distributions of pepsinogen (PG) I (A), PGII (B), and PGI/PGII ratio (C) with patient age according to the presence of *H. pylori* infection (Reproduced with permission from Kim HY, *et al.* Eur J Gastroenterol Hepatol 2009;21:606-612.[43]).

A

$r=0.132$, $p<0.001$

B

$r=-0.229$, $p<0.001$

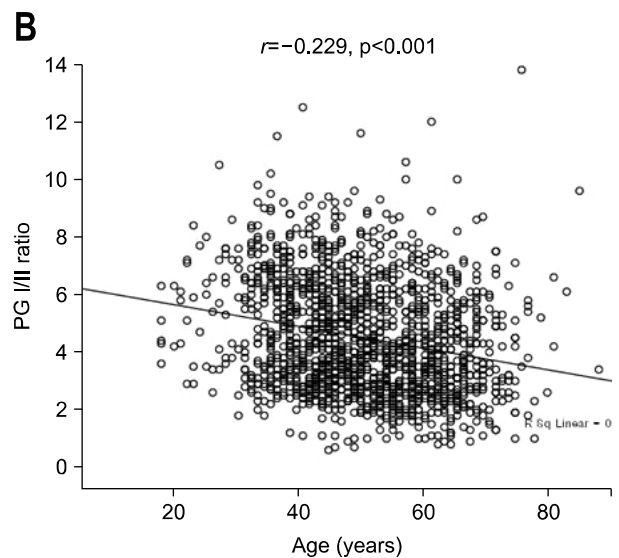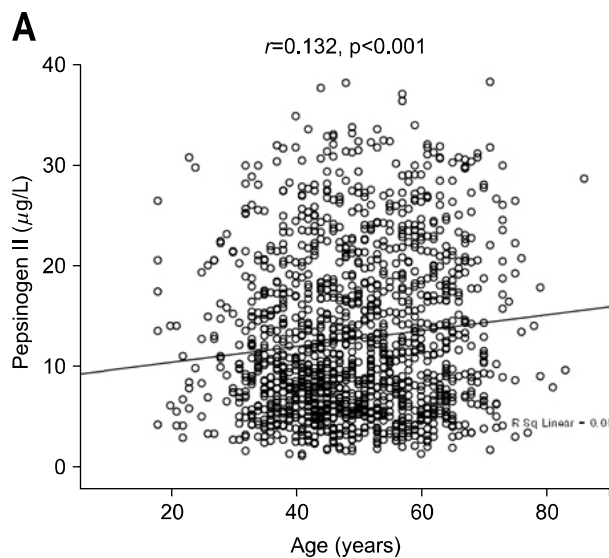**Fig. 3.** Correlations between pepsinogen (PG) II, PG I/II ratio, and patient age. There was a positive correlation between PG II and age (Pearson's correlation coefficient $r=0.132$) (A) and a negative correlation between the PG I/II ratio and age (Pearson's correlation coefficient $r=-0.229$) (B) (Reproduced with permission from Kim HY, *et al.* Eur J Gastroenterol Hepatol 2009;21:606-612.[43]).

Table 1. List of Pepsinogen Test Kits Available in Japan and Korea

Sales agent	Assay system	Launched year
Dainabot Co. (Japan)	IRMA	1992
	CLIA	2000
Wako Pure Chemical Ind. (Japan)	EIA	1997
	CLEIA	2000
Eiken Chemical Co. (Japan)	EIA	1997
	CLEIA	2000
International Reagents Co. (Japan)	EIA	1997
Sanwa Kagaku Kenkyujyo Co. (Japan)	LIA	1999
Kainos Lab. (Japan)	EIA	1999
Azwell (Japan)	ELISA	2000
Kyokuto Pharm. Ind. Co. (Japan)	ELISA	2000
Iatron Lab. (Japan)	LIA	2000
Kyowa Medics Co. (Japan)	ELISA	2001
Shima Lab. Co. (Japan)	LIA (Latex enhanced turbidimetric immunoassay)	2002
Biohit Oyj (Finad)	ELISA	

Reproduced with permission from Miki K, *et al*. Am J Gastroenterol 2003;98:735-739.[33]
CLEIA, chemiluminescent enzyme immunoassay; CLIA, chemiluminescent immunoassay; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; IRMA, immunoradiometric assay; LIA, latex immunoassay.

increased significantly with age regardless of *H. pylori* (Figs. 2B, 3A), different from the PG I observations, resulting in the decrease of PG I/II ratio proportion to age regardless of *H. pylori* (Figs. 2C, 3B). These results suggest that gender and age are affecting factors on the levels of PG I, PG II, and PG I/II ratio, which might be confounding factors for the interpretation of cut off values of PG levels in case of atrophic gastritis or gastric cancer.

4. Measurement method of serum pepsinogen levels

There are several methods of measuring serum pepsinogens (Table 1),[23] which could be another factor for causing different results among reports. The radioimmunoassay method which has been usually used to measure serum PG levels,[53,54] is no longer available. Instead, immunoradiometric assay (PG I/II RIA BEAD; Dainabot Co. Ltd., Tokyo, Japan), a modified method of radioimmunoassay became popular.[24-31] Soon after latex enhanced turbidimetric immunoassay (LIA), a biochemical test, which is easy to perform, and makes it possible to measure serum pepsinogen levels in large numbers, thus suitable for mass screening became available.[32,55] Finally, enzyme-linked immunosorbent assays (ELISA) (Bihit ELISA kit; Biohit, Helsinki, Finland) became used.[56,57] Several determinations of a suitable cut off point for gastric cancer

screening have been reported in case of immunoradiometric assay (IRMA). That is, PG I concentration ≤ 70 ng/mL and PG I/II ratio ≤ 3 has been widely accepted as a cut off value of AG in Japan.[24-27,29] However, in the other countries such as USA and Thailand the cut off value for PG I/II ratio was set as 2 and 3.3, respectively, for gastric cancer diagnosis although they used the same method (Table 2).[28,30] Furthermore, the normal value of pepsinogen levels could be different depending on test methods. For instance the mean PG I level checked by using LIA was found to be 42.2 ng/mL in the 521 *H. pylori*-negative healthy check up subjects in comparison to the 56.3 ng/mL in the 964 *H. pylori*-positive subjects,[43] which is quite lower than the normal value of PG I by IRMA. However, the results of PG I/ II ratio by LIA were similar to other reports using IRMA method.[24-31] That is, the mean PG I/II ratio in 521 *H. pylori*-negative healthy check up subjects was 6.0 in comparison to the 3.7 in the 964 *H. pylori*-positive subjects.[43] In addition, the PG I/II cut off value for atrophic gastritis was 6.0 for *H. pylori*-negative subjects and 3.0 for *H. pylori*-positive individuals.[43] Taken together it might be reasonable to conclude that to increase the efficacy of the PG I/II ratio for the detection of atrophic gastritis or gastric cancer the cut off value of the PG I or PG I/II ratio could be stratified according to the *H. pylori* status or the measurement methods of PG levels in each country.

ATROPHIC GASTRITIS AND INTESTINAL METAPLASIA

1. Serum pepsinogen test as a biomarker of atrophic gastritis and intestinal metaplasia

The clinical importance of atrophic gastritis (AG) with intestinal metaplasia (IM) is related with the fact that it increases the risk of gastric cancer development.[18,58,59] In the process of gastric carcinogenesis, especially, for the intestinal type of gastric carcinomas it has been proposed that the gastric mucosa evolves through the stages if chronic active gastritis, glandular atrophy, IM, and dysplasia before developing into gastric adenocarcinoma.[58] The risk of gastric neoplasia rises with the increasing grade and extent of AG.[60] AG is usually diagnosed by endoscopic biopsies. However, there is a significant potential sampling errors in identifying AG or IM by a random biopsy because the AG or IM of the gastric mucosa could be patchy, especially in the early stage.[20] Instead low serum pepsinogen has been used as a surrogate marker for AG.[21,22,61] In addition, the differential changes in PG levels are indicative of the histological state of the gastric mucosa. That is, as gastritis progresses PG I and PG II

are positively correlated with activity and chronic inflammation of gastritis in the antrum and corpus.[32] However, PG I/II ratios are inversely correlated with activity and chronic inflammation of gastritis in the corpus (Fig. 4A and B, respectively), which was similar in the antrum. This phenomenon was attributed to a greater in-

Table 2. The Hazard Ratios of Serum Pepsinogen Levels and/or *H. pylori* Serology for the Risk of Gastric Cancer

Nation (publication year)	Study method/ follow-up period	Population/ gastric cancer cases	Seum pepsinogen/ *H. pylori* serology assay system	Hazard ratio of pepsinogen or *H. pylori* or sensitivity and specificity of PG test
USA (1993)[18]	Nested case-control study	Unknown/ 136	RIA/ELISA	*H. pylori* IgG in the absence of PG I <50 ng/mL: OR 2.4 PG I <50 ng/mL in the absence of Hp: OR 0.8 (p>0.5)
Finland (1996)[23]	Nested case-control study/13 years	39,368/84	RIA/EIA	PG I ≤49 ng/mL: 2.68 (95% CI, 1.35-5.30)/ *H. pylori* IgA: 2.52 (95% CI, 1.14-5.57) Low PG I and Hp IgA+: 5.96 (95% CI, 2.02-17.57)
Japan (1997)[24]	Nested case-control study/8 years	2,858/45	Immunoradiometric assay (PG I/II RIA BEAD; Dainabot Co. Japan)/Prikaplate G EIA	PG I ≤70 ng/mL and PG I/II ratio ≤3: 3.38 (95% CI, 1.54-7.42) *H. pylori* IgG: 1.84 (95% CI, 0.59-5.72)
Japan (1999)[25]	Cross-sectional study	5,113/13	Immunoradiometric assay (PG I/II RIA BEAD; Dainabot Co. Japan)	Sensitivity and specificity of PG screening for gastric cancer by PG I ≤70 ng/mL and PG I/II ratio ≤3: 84.6% and 73.5%
Japan (2004)[26]	Cohort study of male factory workers/7.7 years	4,655/45	Immunoradiometric assay (PG I/II RIA BEAD; Dainabot Co. Japan)/ELISA by MBL	PG I ≤70 ng/mL and PG I/II ratio ≤3: 3.03 (95% CI, 1.67-5.49)
Japan (2005)[27]	Cohort study health examination program/4.7 years	6,983/43	Immunoradiometric assay (PG I/II RIA BEAD; Dainabot Co. Japan)/ELISA by GAP-IgG kit	"atrophic": PG I ≤70 ng/mL and PG I/II ratio ≤3 Group A: normal PG and Hp− Group B: normal PG and Hp+ Group C: "atrophic" and Hp+ Group D: "atrophic" and Hp− Hazard ratio compared with group A: Group B: 1.1 (95% CI, 0.4-3.4) Group C: 6.0 (95% CI, 2.4-14.5) Group D: 8.2 (95% CI, 3.2-21.5)
USA, Hawaii (2005)[28]	Nested case-control study (non-cardiac cancer)	Unknown/ 299	Immunoradiometric assay (PG I/II RIA BEAD; Dainabot Co. Japan)/ELISA by Pyloristat kit	PG I ≤30 ng/mL: 2.69 (95% CI, 1.81-3.99) PG I/II ratio ≤2: 2.85 (95% CI, 1.85-4.37) *H. pylori* IgG or CagA seropisitivity: 4.86 (95% CI, 5.90-8.13) Either *H. pylori* IgG or CagA who had PG I ≤30 ng/mL: 9.21 (95% CI, 4.95-17.13) Either *H. pylori* IgG or CagA who had PG I/II ratio ≤2: 4.22 (95% CI, 1.75-10.18)
Japan (2006)[29]	Nested case-control study/14 years	123,576/511	Immunoradiometric assay (PG I/II RIA BEAD; Dainabot Co. Japan)/ELISA by Eiken kit (CagA by ELISA kit)	PG I ≤70 ng/mL and PG I/II ratio ≤3: 3.8 (95% CI, 2.7-5.4) *H. pylori* IgG: 11.4 (95% CI, 4.4-29.2) *H. pylori* IgG and PG I ≤70 ng/mL and PG I/II ratio ≤3: 10.1 (95% CI, 5.6-18.2)
Thailand (2006)[30]	Cross-sectional case-control study	167/56	Immunoradiometric assay (PG I/II RIA BEAD; Dainabot Co. Japan)/ELISA by SRL	PG I/II ratio <3.3: 2.3 (95% CI, 1.10-4.80) *H. pylori* IgG: 2.95 (95% CI, 1.4-6.39)
Japan (2006)[31]	Cohort study community program/14 years	2,446/89	Immunoradiometric assay (PG I/II RIA BEAD; Dainabot Co. Japan)/EIA by HM-CAP	PG I ≤30 ng/mL and PG I/II ratio ≤2: for men: 4.56 (95% CI, 2.42-8.60) for women: 5.84 (95% CI, 2.00-17.11) PG I ≤70 ng/mL and PG I/II raio ≤3: for men: 3.42 (95% CI, 1.92-6.11) for women: 1.88 (95% CI, 0.69-5.16) *H. pylori* IgG: 2.84 (95% CI, 1.51-5.33)

Table 2. Continued

Nation (publication year)	Study method/ follow-up period	Population/ gastric cancer cases	Seum pepsinogen/ *H. pylori* serology assay system	Hazard ratio of pepsinogen or *H. pylori* or sensitivity and specificity of PG test
Korea (2008)[32]	Cross-sectional case-control study	1,004/165	Latex enhanced turbidimetric immunoassay (Shima Lab. Japan)/Genedia *H. pylori* ELISA	PG I/II ratio ≤3.0: 2.20 (95% CI, 1.41-3.41) *H. pylori* IgG: 2.17 (95% CI, 1.41-3.41) OR compared with PG I/II ratio >3.0 and Hp-: PG I/II ratio ≤3.0 and Hp-: 2.04 (95% CI, 0.58-7.19) PG I/II ratio >3.0 and Hp+: 3.20 (95% CI, 1.60-6.41) PG I/II ratio ≤3.0 and Hp+: 5.52 (95% CI, 2.83-10.77) *H. pylori* IgG and PG I/II ratio ≤3: 5.5 (95% CI, 2.8-10.8) Sensitivity and specificity of PG screening for gastric cancer by PG I/II ratio ≤3: 59.2% and 61.0%
China (2009)[56]	Case-cohort study/ 15 years (non-cardiac cancer)	29,584/363	Bihit ELISA kit; (Biohit, Helsinki, Finland)	PG I/II ratio <4: 2.72 (95% CI, 1.77-4.20) *H. pylori* IgG and PG I/II ratio ≤3: 5.5 (95% CI, 2.8-10.8)

CI, confidence interval; PG, pepsinogen; Hp, *Helicobacter pylori*; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; OR, odds ratio.

crease in PG II than PG I with increasing activity and chronic inflammation.[32,43-45] However, as disease severity increases further to atrophic gastritis, chief cells are replaced by pyloric glands resulting in decrease of PG I but the level of PG II remains elevated, thus the PG I/II ratio are further reduced reflecting atrophy and intestinal metaplasia (Fig. 4C and D, respectively). As atrophic gastritis is acknowledged to be a precancerous condition or frequently associated findings especially in case of intestinal type of gastric cancer this decrease of PG I concentration (≤70 ng/mL) and PG I/II ratio (≤3) has been widely accepted as a cut off value of gastric cancer in Japan.[24-27,29]

2. Different value of pepsinogen test as a biomarker of atrophic gastritis depending on each country

However, several studies have yielded conflicting results regarding the values of pepsinogens as biomarkers of AG. Broutet *et al*. showed that only PG I/II ratio among PG I, PG II, and PG I/II ratio was a reliable marker of AG in the corpus with a sensitivity of 77% and a specificity of 87% at a cutoff value of 5.6.[62] Similarly, Korean study showed that a PG I of ≤70 ng/mL was found to have a sensitivity of around 80% for detecting corpus AG/IM, but very low specificity, around 30%.[32] In contrast, a PG I/II ratio of ≤3 showed moderate sensitivity (66.8% and 67.5%, respectively) and high specificity (74.7 and 70.1%, respectively) for detecting corpus AG/IM.[32] Furthermore,

in 147 asymptomatic Italian subjects PG I, PG II, and PGI/II ratio were not reliable for predicting antral predominant atrophic gastritis,[63] suggesting that the value of PG levels as a biomarker of AG could be variable in each country or each population.

EFFICACY OF PEPSINOGEN TEST FOR GASTRIC CANCER BIOMARKER

1. Pepsinogen test as a biomarker of gastric cancer

In Japan gastric cancer screening using photofluorography was started in Miyagi prefecture around 1960, and this approach has been adopted nationwide.[16] In 1983, under the Health Service Law for the Aged, gastric cancer screening was introduced for all residents aged 40 years and over.[16] Photofluorography was recommended for gastric cancer screening based on the results of several case-control and cohort studies. However, small asymptomatic cancers are relatively difficult to detect using photofluorography, raising the necessity of compensatory tool. Thus, other methods including endoscopy, serum pepsinogen and *H. pylori* antibody testing have been tried in the clinical setting for opportunistic screening.[16] That is, PG as a marker for atrophic gastritis has been incorporated into gastric cancer screening programs to identify people who would benefit most from gastric cancer screening (Table 2).[24-27,29,31] This trial has been also performed in other countries (Table 2).[18,23,28,30,32] In general all of these
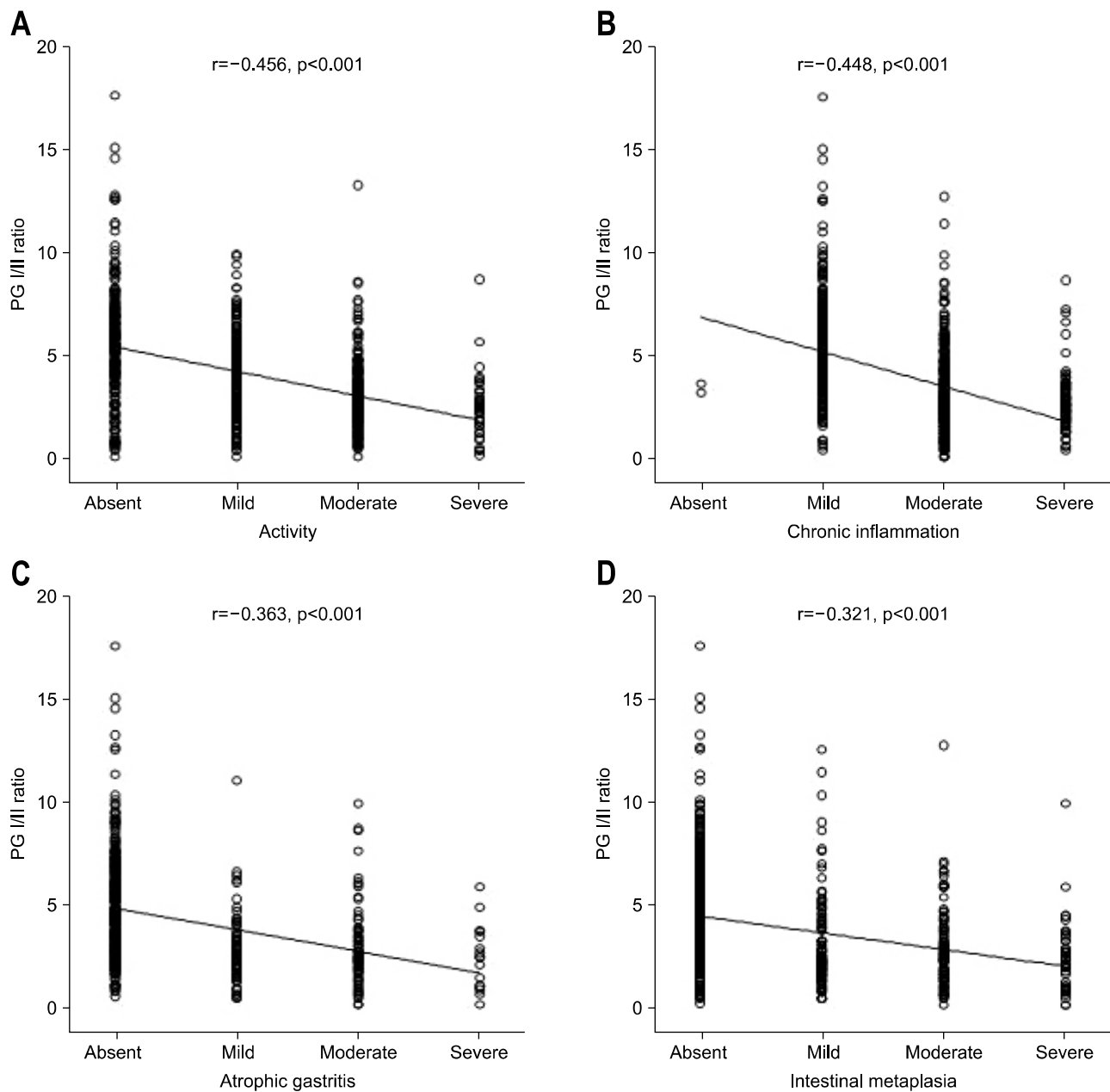
Fig. 4. Correlations between serum pepsinogen (PG) I/II ratio and histological features in the corpus: activity (A), chronic inflammation (B), atrophic gastritis (C), and intestinal metaplasia (D) (Reproduced with permission from Kang JM, *et al.* Helicobacter 2008;13:146-156.32).
r, correlation coefficient.

studies have shown that PG testing is useful in detecting gastric cancer. Miki *et al.*[33] performed a meta-analysis of the sensitivity and specificity results from 42 individual studies. In this meta-analysis PG I level $\leq$70 ng/mL and PG I/II ratio $\leq$3 had a sensitivity of 77% and false positive rate of 27%. The positive predictive value was varied between 0.77% and 1.25%, and the negative predictive value varied between 99.08% and 99.90%. However, studies from several countries the OR of PG I/II ratio has

been reported to be around 2-3, which is lower than those from Japanese reports, from 2.8 to 9.7.[23,24,26,27,31] Specifically, in a case control study from Thailand, the odds ratio (OR) for gastric cancer was 2.3 (95% confidence interval [CI], 1.10-4.80, Table 2) for PG I/II ratio $\leq$3.3.[30] In case of China OR for gastric cancer was 2.72 (95% CI, 1.77-4.20, Table 2) for PG I/II ratio $\leq$4,[56] and in Korea it was 2.20 (95% CI, 1.41-3.41) for PG I/II ratio $\leq$3.0.[32] Taken together gastric cancer was more frequent

and the hazard ratio was significantly higher in the group with a lower PG I/II ratio although the degree was different in each country. These results are suggestive of a dose-response correlation between cancer development and the progression of atrophic gastritis.

2. Different value of pepsinogen test as a biomarker of gastric cancer depending on country

Kitahara *et al.*[25] reported that the sensitivity and specificity of PG I/II ≤3 alone for gastric cancer are 84.6% and 67.2%, respectively, and that those of PG I ≤70 ng/mL and a PG I/II ratio of ≤3.0 are 84.6% and 73.5%, respectively. When PG I ≤70 ng/mL and a PG I/II ratio of ≤3.0 are further categorized into PG I ≤50 ng/mL and a PG I/II ratio of ≤2.0 as strong positive then the hazard ratio for gastric cancer further increased.[31] In contrast to Japan, in Korean study a PG I of ≤70 ng/mL showed sufficient sensitivity (72.4%), but a low specificity (20.2%), and the sensitivity and specificity of a PG I/II ratio cut off of ≤3 were found to be 59.2-61.7% and 61.0%, respectively, in gastric cancer or dysplasia when the latex enhanced turbidimetric immunoassay method (Shima Laboratories) was used.[32] Furthermore there is other example with respect to the utility of serum pepsinogen levels. That is, a study from Singapore examined whether racial differences in the prevalence of *H. pylori* and serum PG could account for a racial difference in gastric cancer incidence.[35] The *H. pylori* seroprevalence was similar between Chinese and Indian subjects, but significantly lower among Malay subjects. The gastric cancer incidence rates correlated with *H. pylori* seropositivity for the Chinese and Malay subjects, but not for the Indian subjects. The prevalence of low PG was highest in Indian subjects even when adjusted for gender and the presence of *H. pylori*. Altogether these results suggest that the usefulness of PG I and PG I/II ratio may different by country, thus these biomarkers should be validated before they are used practically for the screening of gastric cancer.

3. The affecting factors on the efficacy of pepsinogen test as a biomarker of gastric cancer

There might be several reasons for the different values of pepsinogen test as a biomarker of gastric cancer among several countries. The candidates are *H. pylori* infection, gender, histological type of gastric cancer, distribution of gastric cancer, gastric cancer stage or age when the gastric cancer is diagnosed.

1) *H. pylori* infection

H. pylori infection of the gastric mucosa is closely related to the level of serum PG and has been shown to in-

crease the risk of gastric cancer. Thus it is quite natural that *H. pylori* infection affects on the PG test and it is necessary to analyze the PG tests depending on *H. pylori* positivity. Parsonnet *et al.* reported no significant difference in gastric cancer risk between *H. pylori*-negative subjects with a low PG level (PG I <50 ng/mL) and subjects with a normal PG level (PG I ≥50 ng/mL) but most of reports showed a significant association between serum PG levels and development of gastric cancer in *H. pylori*-negative as well as *H. pylori*-positive subjects.[26,31] The difficulty of diagnosis of *H. pylori* infection especially in the background of severe AG/IM might be one of causes of this difference. That is, sometimes it is difficult to exclude the *H. pylori*-false negative subjects in the *H. pylori*-negative group because both AG and IM significantly affected the validity of *H. pylori* diagnostic tests.[64] At first, the sensitivity of the *Campylobacter* like organism test, which is a very simple and practical test for clinicians, is markedly reduced with progression of AG or IM, which drives the bacterium out of the gastric mucosa.[65] As time further goes on after the disappearance of *H. pylori* from mucosa then the serology slowly be converted into negative, and the subjects could be categorized as *H. pylori*-negative.[66] A Japanese report that the hazard ratio was the highest in the group of *H. pylori*-negative and PG-positive (PG I level ≤70 ng/mL and PG I/II ratio ≤3) could be explained from this background (Table 2).[27] However, Korean report did not show similar results. That is, the OR of gastric cancer was highest in case of *H. pylori*-positive and PG I/II ratio ≤3 (Table 2).[32] Taken together these reports suggest that cut off value of PG test or the efficacy of PG test could be variable depending on *H. pylori* prevalence and the severity of AG in each country.

2) Gender

The age-adjusted standardized incidence ratios of gender for gastric cancer are usually reported to be 3-4 times frequent in the men than women.[31,32] In addition, the efficacy of PG test were found to be different between genders. Oishi *et al.*[31] showed that adjusted hazard ratio of PG I level ≤70 ng/mL and PG I/II ratio ≤3 (positive) was 3.42 (95% CI, 1.92-6.11) in men but there was no significance in women 1.88 (95% CI, 0.69-5.16). However, when the cut off value increased up to PG I level ≤50 ng/mL and PG I/II ratio ≤2 (strong positive) then both of genders were showed high hazard ratio (in men 4.13 [95% CI, 2.18-7.82]; in women 5.77 [95% CI, 1.91-17.39]). Several clinical and epidemiological studies have reported that compared with women, men have a higher risk of developing gastric cancer after severe gastric atrophy.[60,67] In the animal experiments, this tolerance of

women toward carcinogenesis has been explained in terms of their sex hormones.[68] Thus, if women proportion is increased among gastric cancer patients then the efficacy of PG test will be decreased. To increase the efficacy of PG test in screening of gastric cancer, it might be necessary to reduce the cut off level of PG I and PG I/II ratio in women than in men.

3) Histology of gastric cancer

Regarding the gastric cancer subtype as determined by the Lauren classification,[69] it has been hypothesized that the intestinal type and diffuse type of gastric cancer have different etiologies because of their different epidemiological features such as gender, age, and geographical patterns. In addition, Correa's hypothesis,[58] which applies to the intestinal type of gastric cancer, implies that there is a different pathophysiology for these two subtypes of gastric cancer. The intestinal type has been more commonly identified in males, and in older age groups, and in high risk areas for gastric cancer. On the other hand, the diffuse type is more frequent in younger age groups, and the gender ratio is close to one.[70,71] Thus, it has been proposed that the intestinal type is more dependent on environmental factors, whereas the diffuse type is more related to host factors such as genetic susceptibility. PG I and PG I/II ratio were found to be more valuable for intestinal type than diffuse type cancer, which is probably related with different grades of AG and IM according to histologic type. It is generally accepted that intestinal type gastric adenocarcinoma arises through a multistep process that from chronic gastritis that progresses through stages of atrophy, intestinal metaplasia, and dysplasia, and finally results in intestinal-type cancer.[58] In contrast, diffuse type gastric cancer does not progress through severe atrophic gastritis.[72] Thus, it appears to be reasonable that pepsinogen, a marker of chronic atrophic gastritis, is closely associated with intestinal type gastric cancer. For instance, Oishi et al.[31] showed that multivariate-adjusted hazard ratio of PG I level ≤ 70 ng/mL and PG I/II ratio ≤ 3 (positive) was 3.75 (95% CI, 2.07-6.80) in intestinal type but there was no significance in diffuse type 1.69 (95% CI, 0.66-4.30). Even the cut off value increased up to PG I level ≤ 50 ng/mL and PG I/II ratio ≤ 2 (strong positive) this was not so changed (intestinal type 5.85 [95% CI, 3.14-10.93]; diffuse type 1.85 [95% CI, 0.51-6.72]). This is also supported by the findings of several clinical and epidemiologic studies.[26,32,73-75] Thus if the ratio of diffuse type among gastric cancers is higher then the efficacy of PG test could be lower, which has been shown in Korean report.[32] That is, in this report the proportions with diffuse or intestinal type turned out to be

42.1% (160 patients) and 57.9% (220 patients) among 380 gastric cancer patients. This is quite different from that found in Japanese studies, which reported that the diffuse type proportion is relatively low at 16-33%.[26,27,31,68]

4) Cancer location

When the gastric cancer is divided into cardiac and non-cardiac cancer PG test was significant only in the non-cardiac cancer in USA[28] and China report,[56] probably because the pathogenesis of gastric cancer is different. Furthermore when the cancer is divided into proximal one third and distal two thirds of the stomach the magnitude of the association was stronger in the distal cancer.[18,28] Oishi et al.[31] also showed that adjusted hazard ratio of PG I level ≤ 70 ng/mL and PG I/II ratio ≤ 3 (positive) was 2.98 (95% CI, 1.69-5.23) in the distal two thirds but there was no significance in proximal one third (OR, 1.91; 95% CI, 0.68-5.35). However, when the cut off value increased up to PG I level ≤ 50 ng/mL and PG I/II ratio ≤ 2 (strong positive) then both of gastric cancer location showed high hazard ratios (in the proximal one third 4.11 [95% CI, 1.42-11.94]; in the distal two thirds 4.71 [95% CI, 2.55-8.70]). This result could be related with the fact that atrophic change in gastric mucosa spreads from the antrum into body side.[19] Thus if the portion of proximal one third gastric cancer is increased it could be one reason of decrease of the efficacy of PG test.

5) Depth of invasion

To be useful as a cancer marker it should be sensitive enough for the detection of precancerous lesion as well as cancer. In addition, it should detect early gastric cancer (EGC) as well as advanced gastric cancer (AGC). In regard to precancerous lesion Korean report showed that the sensitivity and specificity of PG I/II ratio ≤ 3 were similar in both of dysplasia and cancer group.[32] That is, in cancer group the sensitivity and specificity were 59.2% and 61.0%, respectively and in dysplasia group they were 61.7% and 61.0%, respectively.[32] In the regard to gastric cancer, Japanese report[31] showed that adjusted hazard ratio of PG I level ≤ 70 ng/mL and PG I/II ratio ≤ 3 (positive) was 2.70 (95% CI, 1.50-4.87) in EGC and it was 4.42 (95% CI, 1.68-11.64) in AGC. This report confirmed that PG test can predict not only AGC but also EGC.

6) Age

Age was found to be one of the significant risk factors of AG and IM.[59] If gastric cancer develops in the aged persons whose stomach showed severe AG and IM then PG test is very useful. Thus the cancer developing age

could affect the usefulness of PG test as a gastric cancer biomarker. Actually, in the Korean report mean age at diagnosis of gastric cancer cases was 54.9±12.3 years,[32] which was substantially less than the 64 to 66 years of the previous Japanese studies.[25,76] Moreover, as compared with Korean cancer patients, elderly cancer patients in the Japanese studies may have had more severe AG/IM, and that this could be a cause of false negative result for *H. pylori* in Japan. This background might be a reason why the hazard ratio of *H. pylori* −, PG test + group highest in Japan in contrast to the *H. pylori* +, PG test + is highest in Korea.[32] Thus, it is possible that gastric cancers in the Korean population might develop more frequently before conversion to seronegative for *H. pylori* than those in the Japanese population.

COMBINATION OF PEPSINOGEN TEST AND *HELICOBACTER PYLORI* INFECTION IN THE DETERMINATION OF GASTRIC CANCER RISK

In Japan, where the morbidity and mortality from gastric cancer are considerably high, a serum PG test based on the combination of the serum PG I level and the PG I/II ratio has been implemented to screen for gastric cancer.[21,23,25] *H. pylori* infection of the gastric mucosa is closely related to the level of serum PG[77] and has been shown to increase the risk of gastric cancer.[78] However, there have been arguments regarding the value of PG test in the absence of *H. pylori* seropositivity. For instance Parsonnet et al.[18] reported that *H. pylori* in the absence of low PG I (<50 ng/mL) was independently associated with cancer (OR, 2.4; p=0.04) but low PG I in the absence of *H. pylori* infection was not associated with cancer, concluding that infection rather than gastric atrophy is the determining factor in cancer risk. Similarly the OR of gastric cancer in the case of *H. pylori* + and PG I/II ratio >3 was significant (OR, 1.6; 95% CI, 1.60-6.41) based on *H. pylori* − and PG I/II ratio >3 but there was no significance in case of *H. pylori* − and PG I/II ratio ≤ 3 (OR, 2.04; 95% CI, 0.58-7.19) in Korea.[32] These results suggest that *H. pylori* infection could be a more important factor than PG test in the determination of gastric cancer risk in USA or Korea. However, as the seroprevalence is rather high, 59.6% in the age over 16 years in Korea[79] the *H. pylori* infection alone is not helpful for the localization of high risk group for gastric cancer, thus it needs another factor such as PG I/II ratio ≤3. In Japan PG test was very useful for the detection of gastric cancer regardless of *H. pylori* positivity.[31] Furthermore the hazard ratio of *H. pylori* −, PG test + group was highest[27] suggesting that these two tests can also localize the highest risk

group for gastric cancer in Japan, which is different from Korea.

FUTURE DIRECTIONS

Gastric cancer is the leading cause of morbidity and mortality in Japan, Korea and China but mass screening method is different. Two non-invasive tests, serum PG levels and *H. pylori*-positivity, could provide a tool for selecting those at high risk of having gastric cancer, and its adoption would reduce numbers of endoscopy candidates during national gastric cancer screening. However, as the sensitivity and specificity of PG test is different in each country further research is necessary to increase the efficacy of PG test as a gastric cancer biomarker. As the efficacy is different depending on gender, age, cancer histology, location and cancer depth, this research should include targeting of PG test in which the efficacy is maximized by subcategorization in a large population with long-term follow-up. In addition, the limitations of PG test should be clarified.

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