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Method evaluation of pepsinogen I/II assay based on chemiluminescent immunoassays and comparison with other test methods

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ABSTRACT

Background: Serum pepsinogen (PG) I and the PG I/PG II ratio have been used for atrophic gastritis (AG) diagnosis for decades. Low levels of PG I and/or PG I/PG II are closely related to AG and predict the risk of gastric cancer. We evaluated the performance of the chemiluminescent immunoassay-based Architect Pepsinogen I/II assay.
Methods: The evaluation consisted of determination of the precision, linearity, limit of blank (LoB), limit of detection (LoD) and method comparison with Eiken and Biohit assays.
Results: The total CVs were below 5% for both PG I and PG II. Acceptable linearity was observed for PG I and PG II in their respective reportable ranges. The PG I LoB was 0.317 ng/mL and the PG II LoB was 0.418 ng/mL, and LoDs were 0.412 ng/mL and 0.497 ng/mL, respectively. Correlation analysis indicated that results of the Architect assay were comparable to those of the Eiken and Biohit assays, but the three methods lead to different estimations of the cancer risk.
Conclusion: The overall analytical performance of Architect Pepsinogen I/II assay is acceptable for the detection of patients with suspected AG. The categorization results of gastric cancer risk showed some difference among test methods suggesting the need for harmonization among the methods from vendors.

1. Introduction

Gastric cancer is the second most common cancer worldwide following lung cancer in males and breast cancer in females. The incidence of gastric cancer varies by region; it is the second highest in Korea [1,2]. Early diagnosis of gastric cancer allows for the higher 5-year survival rate >90% when compared with more advanced stage. Therefore, much research focuses on early detection to raise the overall survival rate and improve prognosis for gastric cancer [3,4]. The Asia Pacific Working Group on Gastric Cancer has presented serologic, radiographic and endoscopic tests as methods of gastric cancer screening, and recommended the serum pepsinogen (PG) test, especially in high-prevalence countries [4,5].

The gastric mucosa secretes pepsinogens I and II (PG I and PG II), biochemically and immunologically distinct precursors of pepsin. PG I is secreted mainly by gastric chief cells of the fundus mucosa and mucous neck cells [6,7], while PG II-producing cells—including cardiac gland cells, pyloric gland cells and Brunner's gland cells of the proximal duodenal mucosa and fundus mucosa—are widely distributed from the stomach to duodenum [6,8].

The intestinal type gastric cancer occurs through a multistep cascade, beginning with atrophic gastritis of the normal gastric mucosa, followed by intestinal metaplasia and dysplasia [9]. Because atrophic gastritis advances from the pylorus to the oral side, progressing atrophy induces a reduction in the number of gastric cells, accordingly decreased concentrations of PG I and the ratio of PG I/PG II [10].

Several researchers have found that serum PG measurements have significant value in screening for precancerous lesion, atrophic gastritis [11,12]. In Japan, subjects are screened first by serum PG, then those with low concentrations are further tested by gastric endoscopy. The PG assay is inexpensive, non-invasive and can be performed simultaneously with other blood tests, so it is a widely used screening test for atrophic gastritis [5,13–15]. Several serum PG immunoassays are commercially available, based on the principle of immunoassays including enzyme immunoassays (EIA), radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), latex turbidometric immunoassays (LTIA) and chemiluminescent immunoassays (CLIA) [16]. Among these, the Architect Pepsinogen I/II assay that is based on the CLIA is performed using the Architect instrument. The cutoff value of <70 ng/ml

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for PG I and <3.0 in screening test proposed by Japanese Gastric Cancer Association (JGCA) has been used commonly. However, they did not refer to the particular measurement for this cutoff value. Though clinical guidelines for gastric cancer screening suggest a cutoff value of PG and various methods are in clinical use, standard measurement procedures and decision criteria depending on methods have not yet been established. Therefore, differences in PG results based on various assay platforms and population should be determined so that optimal cutoff for each platform need to be established.

2. Methods

2.1. Subjects

From September to October 2013, 87 patients visiting the Gastroenterology Department of the Asan Medical Center and undergoing enteroscopy with biopsy were enrolled in this study. In this study, we included newly diagnosed patients with atrophic gastritis, intestinal metaplasia or gastric cancer confirmed histologically when PG tests were performed. Exclusion criteria included a history of gastric resection, diagnosis of non-atrophic gastritis or other benign disease prior to the serum PG test. We retrospectively reviewed patients' medical records and collected information including gender, age, medical history, endoscopic diagnosis, and pathological findings. The clinical protocol and design of this study were approved by the Institutional Review Board (IRB) of the Asan Medical Center (IRB approval No. 2013-0839).

2.2. Laboratory measurements

Serum PG was measured using the Architect i2000 immunoassay analyzer (Abbott Laboratories) with the Architect Pepsinogen I/II (Abbott) based on the CLIA. For comparison, serum PG was also measured using the Beckman Coulter AU 5822 (Beckman Coulter Inc.) with the Eiken Pepsinogen I/II (Eiken Chemical Co., Ltd.) based on the LTIA principle and the Biohit Pepsinogen I/II ELISA kit (Biohit Oyj). Architect and Eiken Pepsinogen assays were carried out according to the manufacturers' instructions in our laboratory. Biohit assay was performed following the manufacturers' instructions in Green Cross Laboratories.

2.3. Precision study

Precision evaluation was performed based on the Clinical and Laboratory Standards Institute (CLSI) EP5-A2 guideline [17]. The quality control materials with 3 concentrations (derived from human serum) were included in the Architect kit. Two replicates of each control sample were analyzed twice per day for 20 days, with two runs separated by at least 2 h.

2.4. Linearity

We referred to the CLSI EP6-A guideline for linearity evaluation of the method [18]. Patient samples were diluted serially to make five concentrations in accordance with the manufacturers' analytic measurement range (AMR: PG I, 0–200 ng/ml; PG II, 0–100 ng/ml). Four replicates of each concentration were analyzed for all samples. The mean values of each specimen were compared with assigned values. Verification of a manufacturer's linearity claim can be performed with visual inspection of graph of the results, with comparison against a linearity goal. We specified the limit on nonlinearity, which is less than one-fourth the goal for 25% of allowable total error based on CAP linearity survey.

2.5. Comparison of PG assays

Method comparison was performed in accordance with the CLSI EP9-A3 guideline [19].

We collected patient samples which cover clinically relevant concentration range and analyzed the correlations between test methods for the PG I, PG II and PG I/PG II ratio. 87 patient samples from subjects enrolled in this study were analyzed on Architect, Eiken and Biohit Pepsinogen I/II assays. All the samples were repeatedly measured twice for each method.

2.6. Detection limit

Detection limits were evaluated based on the CLSI EP17-A2 guideline [20]. The limit of blank (LoB) was set by calculating the mean and SD of measurements for 20 replicates of blank sample using the zero concentration calibrator. And 1.671 ng/ml for PG I and 0.739 ng/ml for PG II concentrations from patient samples were used to evaluate the limit of detection (LoD).

2.7. Statistical analyses

Data analysis was performed using EP Evaluator Release 8 (David G. Rhoads Assoc.), SPSS version 13.0 (SPSS Inc.) software. Comparisons of serum PG concentrations among disease groups were performed with one-way ANOVA followed by the Bonferroni post hoc test. A $P < 0.05$ were considered to indicate statistical significance.

3. Results

3.1. Differences among subjects

The study population included 65 males and 22 females and the median age was 63 years (range, 16–83 years). Subjects were divided into 3 groups based on endoscopic and histological findings: chronic atrophic gastritis without intestinal metaplasia (CAG without IM), 27 patients (31.0%); chronic atrophic gastritis with intestinal metaplasia (CAG with IM), 37 (42.5%); gastric cancer, 23 (26.4%) [early gastric cancer (EGC), 20 and advanced gastric cancer (AGC), 3] (Table 1). There was no significant difference in age among the groups ($P = 0.496$). The subjects with gastric cancer showed the highest rates of *Helicobacter pylori* infection. As measured by the Architect assay, PG II

Table 1
Demographic findings and pepsinogen levels (mean ± SD) determined by both methods according to endoscopic/histologic findings.

Endoscopic/histologic finding		CAG without IM	CAG with IM	EGC/AGC	*P* value
No. of patients (%)		27 (31.0)	37 (42.5)	23 (26.4)	
Sex (M/F)		(19/8)	(30/7)	(16/7)	
Age (year)		61.3 ± 12.8	63.4 ± 8.0	60.5 ± 9.1	NS
H. pylori positive (%)		11 (40.7)	17 (45.9)	16 (69.6)	0.025
Architect	PG I (ng/ml)	54.65 ± 33.05 (21.07–145.44)	41.64 ± 24.71 (6.60–110.85)	58.30 ± 39.14 (13.87–176.13)	NS
	PG II (ng/ml)	10.01 ± 7.08 (2.00–30.44)	9.44 ± 8.26 (2.07–48.30)	15.44 ± 9.98 (3.28–42.93)	0.022
	PG I/PG II	6.32 ± 2.29 (2.08–10.84)	5.26 ± 2.04 (1.16–8.59)	4.42 ± 2.52 (1.58–11.76)	0.014
Eiken	PG I (ng/ml)	55.82 ± 32.86 (22.74–149.23)	43.28 ± 26.56 (7.21–132.92)	58.77 ± 37.07 (16.48–171.41)	NS
	PG II (ng/ml)	12.76 ± 7.56 (4.10–34.32)	12.18 ± 8.45 (4.88–50.11)	18.55 ± 10.98 (6.48–48.24)	0.022
	PG I/PG II	4.64 ± 1.37 (1.80–7.27)	3.83 ± 1.36 (0.95–6.72)	3.43 ± 1.45 (1.57–6.52)	0.008
Biohit	PG I (ng/ml)	96.72 ± 62.75 (19.46–281.24)	72.36 ± 40.83 (14.53–209.02)	100.25 ± 66.89 (23.09–329.65)	NS
	PG II (ng/ml)	10.87 ± 8.40 (3.52–34.17)	10.18 ± 9.01 (2.71–52.94)	16.95 ± 10.33 (3.08–39.19)	0.018
	PG I/PG II	10.13 ± 3.23 (3.01–14.17)	8.69 + 4.01 (2.19–18.66)	7.05 ± 4.42 (2.55–20.77)	0.025

Abbreviations: AGC, advanced gastric cancer; CAG, chronic atrophic gastritis; EGC, early gastric cancer; IM, intestinal metaplasia; PG, pepsinogen; SD, standard deviation.