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Detection of gastric atrophy by circulating pepsinogens: a comparison of three assays

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Abstract

Background—Circulating levels of pepsinogens have been used in high gastric cancer-risk Asian and European populations to triage endoscopic evaluation for more severe pathology. There are different analytic methods with uncertain correlations. We therefore compared diagnostic performance of three commonly used pepsinogen assays to detect histologically confirmed gastric atrophy.

Methods—We tested plasma samples from adult patients with (n=50) and without (n=755) moderate or severe gastric corpus atrophy, as determined histologically by consensus of three expert pathologists. A single laboratory measured pepsinogens I (PgI) and II (PgII) using

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commercially available assays: two ELISA assays produced by Biohit (Finland) and Vector Best (Russia), and a latex-agglutination assay from Eiken (Japan). Quantitative correlations were assessed by Spearman statistics. Receiver operating characteristic (ROC) curves vs. histological diagnosis were calculated using both the manufacturers' and optimized cutoffs.

Results—Pepsinogen levels were highly correlated among the assays (pairwise Rhos: PgI 0.84, PgII 0.87; all p-values<0.01). Based on manufacturers' cutoffs, sensitivities, specificities and areas under the ROC curve for detecting moderate to severe histological corpus atrophy by PgI/PgII were 44%/91%/0.70, 56%/84%/0.76, and 52%/90%/0.77 for Biohit, Vector Best and Eiken, respectively. Cutoffs optimized by ROC or data mining analyses did not substantially improve test-performance.

Conclusions—Commercial assays for pepsinogen have good relative agreement but are imperfect tests for clinical diagnosis of gastric atrophy.

Impact—Pepsinogen testing alone does not provide sufficient information for gastric cancer risk stratification. Future investigations should focus on other potential markers, in combination with pepsinogens.

Keywords

pepsinogen; stomach; atrophy; gastric cancer; risk stratification

INTRODUCTION

Gastric cancer is the third leading cause of cancer death worldwide [1]. Endoscopic screening may detect precancerous lesions and asymptomatic cancers for early treatment to reduce disease burden. However, it is not clear which populations have sufficiently risk to warrant this invasive procedure. Current clinical guidelines suggest pepsinogen testing for non-invasive identification of individuals at high cancer risk [2] with potential application to risk-stratification both in Asia and Europe [2,3].

Pepsinogens are pepsin pro-enzymes that may be measured in blood as indirect markers of gastric mucosal changes [4]. Two isozymogens, pepsinogen I (PgI) and pepsinogen II (PgII), are produced in different parts of the stomach [5]. PgI production is exclusively limited to acid-secreting glands of the gastric corpus (proximal stomach) whereas PgII production is widespread in diverse types of glands throughout the stomach as well as Brunner glands of the duodenum [6]. Thus, mucosal atrophy affecting the gastric corpus leads to decreased levels of PgI while PgII levels tend to be relatively stable. In particular, the presence of mucosal inflammation, also related to *H. pylori* infection, may increase levels of both PgI and PgII [7] and in some cases result in normal PgI values when both atrophy and inflammation are present [8]. To account for this draw-back, a decreased ratio of PgI to PgII (PgI/PgII) is considered to be the best serologic marker of gastric atrophy [2] as used for cancer screening in Japan [9] and increasingly in Europe [6].

Different methods for pepsinogen assessment are used in Eastern and Western countries. Miki and Fujishiro have expressed concerns about translating results between the assays [10]. Therefore, we aimed to compare the widely used pepsinogen assay in Japan with the

most popular assay in Europe. We also evaluated another assay manufactured in Russia since this country as well as its neighbours, sharing high burden of gastric cancer, usually favor domestic products. We correlated pepsinogen measurements by these different test-systems on the same patient samples, determined sensitivity and specificity to detect gastric atrophy and explored alternative cutoff values to improve performance.

MATERIAL AND METHODS

Study population

805 consecutive patients with dyspeptic complaints referred for upper endoscopy at Digestive Diseases Centre GASTRO and Riga East University hospital, Latvia were enrolled for this study (29% males; median age 51 years, age range 18-88 years). Patients with gastric cancer were excluded. Mucosal biopsies were collected according to the Sydney protocol. Pathology evaluation was performed by consensus among three expert pathologists (SI, ILK, DJ). Grade II (moderate) or III (severe) atrophy according to the updated Sydney classification were considered atrophy for the purposes of this analysis. Since pepsinogen levels primarily indicate atrophy status of the corpus, for histological assessment we used the higher grade of either of the two biopsies from this anatomic subsite. The study was approved by the Central Medical Ethics Committee of Latvia, protocol No.01-29.1/20 on September 22, 2011. All the patients provided informed consent upon enrolment.

Laboratory methods and manufacturers' pre-defined cutoff values

EDTA-anticoagulated plasma samples were obtained following an overnight fast, processed within 30 minutes after blood collection, and stored at -80°C until testing. Plasma samples were analysed by commercially available assays from three manufacturers: 1) a latex-agglutination test-system (Pgl kit cat. no. V-IZ51, PgiI kit cat. no. V-IZ53) made by Eiken Chemical Co., Tokyo, Japan [4], a widely used assay in Japan; 2) an Enzyme-Linked Immunosorbent Assay (ELISA) test made by Biohit, Plc., Helsinki, Finland (Pgl ELISA kit cat. no. 601 010.01, PgiI ELISA kit cat. no. 601 020.02) [6], the only test-system used in clinical practice in Europe; and 3) an ELISA test-system recently developed by Vector Best, Novosibirsk, Russia (Pgl ELISA kit cat. no. D-3762, PgiI ELISA kit cat. no. D-3764). Tests were conducted according to the respective manufacturers' instructions. Briefly, measurements of sample turbidity were performed on a CS-T240 auto-chemistry analyzer (Changchun, Jilin, P.R. China). Sample absorbance was measured on a Personal Laboratory automated ELISA processor (Adaltys, S.r.l., Milano, Italy). Standard curves for Pgl and PgiI were used to interpolate the concentrations of unknown samples. Based on the Japanese experience and manufacturer's recommendations, two cutoff specifications were used for the latex-agglutination assay: Pgl 70 ng/ml and Pgl/PgiI 3 for "any" atrophy, and Pgl 30 ng/ml and Pgl/PgiI 2 for "advanced" atrophy [4]. For the ELISA tests, the manufacturers' determined cutoff of $\text{Pgl/PgiI} < 3$ was used.

Statistical analysis

Pairwise correlations among the three different assays were assessed by Spearman statistics. Assay performance was evaluated by receiver operating curve (ROC) analysis with calculations of area under the curve (AUC) and compared by a non-parametric test [11].

Sensitivity, specificity and overall accuracy were assessed using histological diagnosis as the 'gold standard.' Three alternative analytical approaches were considered based on: 1) manufacturer-defined cutoffs, 2) ROC-defined cutoffs using minimal Euclidean distance, and 3) optimized cutoffs from the C4.5 algorithm decision tree method [12] with 10-fold cross-validation. In addition, sex-specific cutoffs were considered as a sensitivity analysis.

Statistical analyses were conducted with SPSS Statistics for Windows software, (version 20.0, IBM Corp., Armonk, NY). The decision tree algorithm was implemented in Weka software [13]. $p < 0.05$ was considered statistically significant for all comparisons.

RESULTS

Fifty patients were diagnosed with atrophy in the corpus (35 grade II and 15 grade III), and 755 were classified as not having corpus atrophy. Pair-wise scatter plots of their pepsinogen measurements by the three manufacturers' test-systems are shown in Figure 1. The measurements were highly correlated, with Spearman correlation coefficients ranging from 0.84 to 0.89 for PgI and from 0.87 to 0.90 for PgII, (all p -values < 0.01). Notably, absolute values of PgI and PgII differed substantially among the test-systems as illustrated by the deviation of the correlation line from the diagonal.

Means and standard deviations of PgI, PgII and PgI/PgII by atrophy status are presented in Table 1. For all 3 test-systems, PgI and PgI/PgII ratios were consistently lower in the patients with corpus atrophy.

Using the manufacturers' cutoffs of $\text{PgI/PgII} < 3$ for Biohit and Vector Best and $\text{PgI/PgII} < 3$ with $\text{PgI} < 70$ ng/ml for Eiken, 22 (44%) of the 50 patients with atrophy were correctly classified by all three test-systems while 12 cases (24%) were missed by all (Figure 2). The diagnostic performance of the three tests to detect atrophy is presented in Table 2. Under various alternative approaches, sensitivities ranged from 44-76.0%, specificities from 62.6-93.1%, and overall accuracy from 63.1-90.3%. These parameters did not systematically vary based on manufacturers', ROC-defined or decision tree-optimized cutoffs. Similarly, sex-specific cutoffs did not substantially modify diagnostic performance (Supplemental Table).

ROC analyses of PgI/PgII are presented in Figure 3. The AUCs were 0.70 (95% CI, 0.60-0.79) for Biohit, 0.76 (95% CI, 0.69-0.84) for Vector Best and 0.77 (95% CI, 0.69-0.85) for Eiken. Comparing among the three AUCs, differences were statistically significant for Biohit vs. Vector Best ($p = 0.024$) and Biohit vs. Eiken ($p = 0.003$) but not for Vector Best vs. Eiken ($p = 0.575$).

DISCUSSION

The first pepsinogen radioimmunoassay was developed by Samloff *et al.* in the early 1980s [14] and soon suggested as a potential biomarker of gastric cancer [15]. Pepsinogen testing has been utilized for screening purposes in Japan for decades [9,16] and more recently in Europe [6,17]. Current test-systems are based on latex-enhanced turbidimetric immunoassay or ELISA technologies. Decreases in either PgI levels or PgI/PgII ratio by latex-

agglutination test are commonly used for diagnosis of atrophy in Japan, while a decrease in PgI/PgII ratio by ELISA is the accepted criterion in Europe.

Our study has compared diagnostic performance of three widely used pepsinogen test-systems and, to the best of our knowledge, represents their first head-to-head comparison on symptomatic individuals with histological assessment of corpus atrophy.

Notable differences in absolute values for either PgI or PgII were found, however there was good relative agreement among the three test-systems. Our findings are similar to the comparison between Eiken and Biohit assays among asymptomatic individuals reported by Miki and Fujishiro [10]. One of the explanations for these differences could be the different laboratory principles (i.e. ELISA and latex-agglutination). At the same time, also the agreement between the two ELISA methods was imperfect, in particular with high pepsinogen values.

Previous evaluations of pepsinogens' diagnostic performance have been based on various assays, cutoff values, population characteristics and definition of atrophy, with inconsistent findings. As early as 2004, a meta-analysis by Dinis-Ribeiro *et al.* [4] included studies with sensitivity ranging from 6% to 99% and specificity ranging from 64% to 100%. A 2015 meta-analysis by Huang *et al.* [18] calculated a pooled 69% (95% CI, 52-83%) sensitivity, 84% (95% CI, 68-93%) specificity and ROC AUC of 0.83 (95% CI, 0.80-0.86) for PgI/PgII. Our results are generally comparable.

Diagnostic performance of pepsinogen testing is particularly dependent upon cutoff values [19]. Most of the studies performed in Asia addressing the potential to predict gastric cancer have used uniform cutoff values of PgI 70 and PgI/PgII 3.0 [20]. We tried several approaches to identify better cutoffs, including ROC-based selection and data mining decision tree methods, without substantial effects on test performance. Adjustments for age, sex, height, body weight, body surface area, smoking and drinking habit have been explored without meaningful improvement [21]. Further advances in blood-based diagnosis of atrophy may require application of additional tests, and circulating levels of trefoil factors [22] and ghrelin [23] have each been proposed as alternative markers for atrophy. In addition, cancer-specific biomarkers, including panels of micro-RNAs [24] and cancer-specific autoantibodies [25] have been suggested. Combining pepsinogens with one or more of these (or other) markers, as previously evaluated for trefoil factor 3 [22], could potentially improve the performance of non-invasive testing for gastric cancer risk.

In summary, our data demonstrated good correlations of PgI, PgII and PgI/PgII measurements among three test-systems, with all methods performing equivalently. Importantly, there were substantial differences in absolute values, which must be properly considered in comparisons between methods and populations. Adjusting the cutoff values does not substantially improve sensitivity and specificity as compared to definitive histopathological diagnosis. The clinical performance of pepsinogen blood levels to detect gastric corpus atrophy is not sufficient to recommend this approach for wide implementation in cancer risk-screening.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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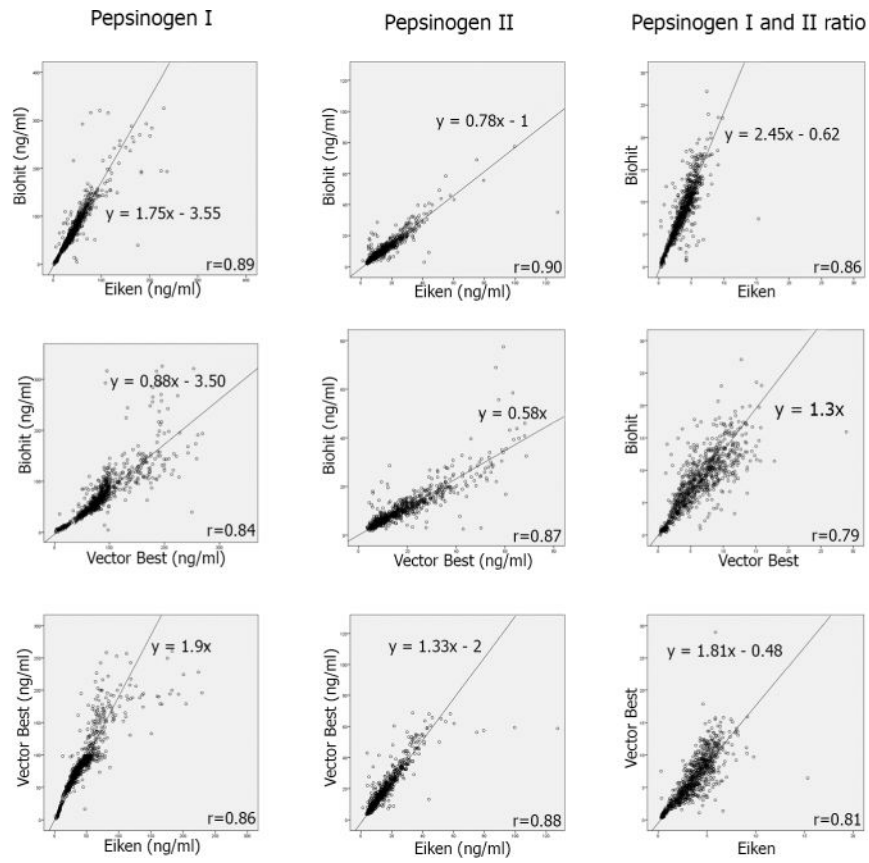


Figure 1. Pair-wise correlations of pepsinogen measurements by the three manufacturers' test-systems

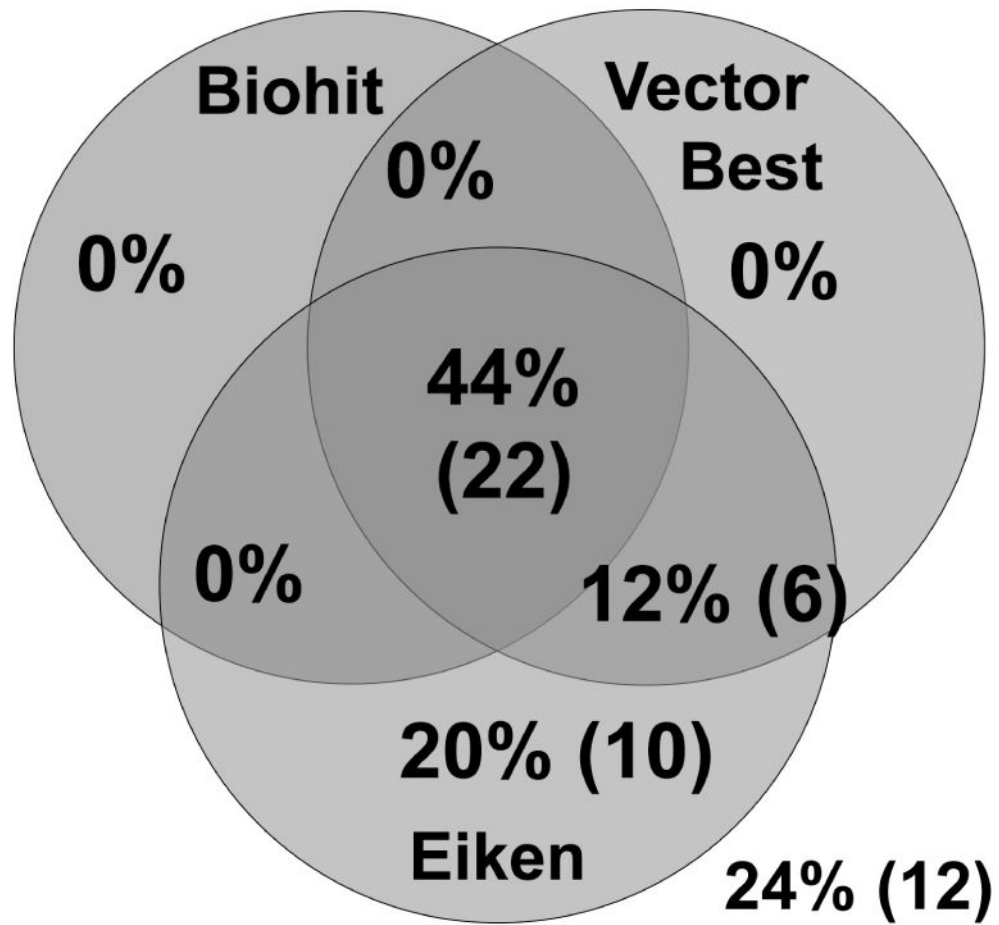


Figure 2. Diagnostic agreement among three pepsinogen test-systems in 50 patients with histologic diagnosis of corpus atrophy. The following cutoff values were used: Pgl 70 ng/ml and Pgl/PgII < 3 for Eiken, and Pgl/PgII < 3 for Biohit and Vector Best

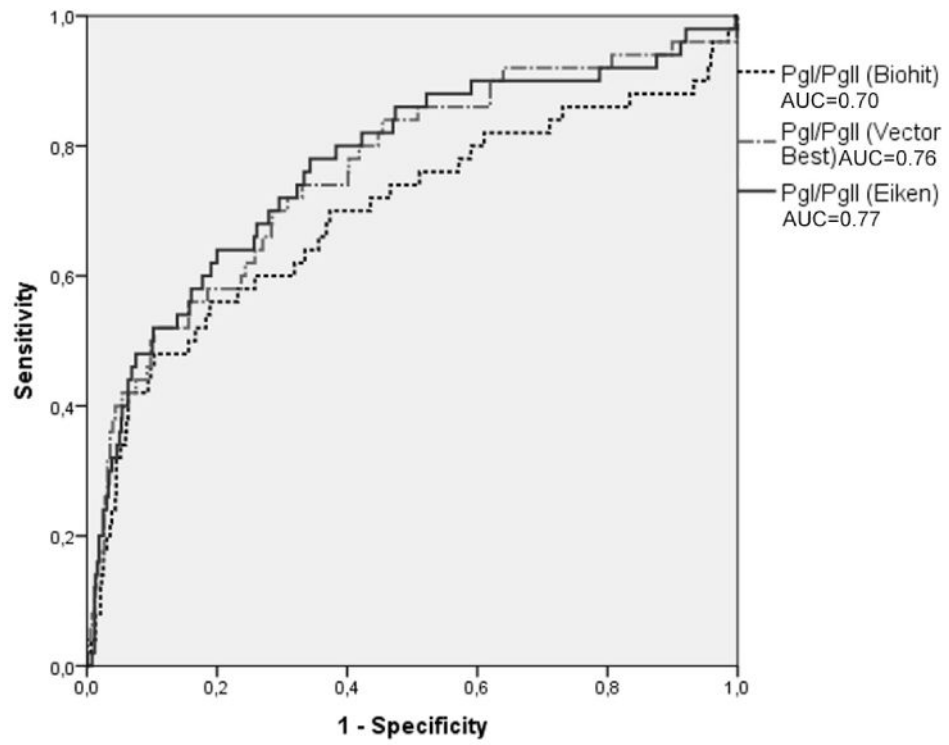


Figure 3.
Receiver operating characteristics of PgI/PgII to detect corpus atrophy

Mean pepsinogen levels by presence or absence of histologically defined atrophy of the gastric corpus

Table 1

Test-system	Pepsinogen I (SD) [ng/ml]			Pepsinogen II (SD) [ng/ml]			Pg I/II Ratio (SD)		
	Overall N=805	Atrophy N=50	No atrophy N=755	Overall N=805	Atrophy N=50	No atrophy N=755	Overall N=805	Atrophy N=50	No atrophy N=755
Biokit	73.9 (48.8)	60.5 (74.0)	74.7 (46.6)	10.7 (8.1)	10.3 (6.7)	10.7 (8.2)	8.3 (4.3)	5.7 (5.5)	8.4 (4.2)
Vector Best	87.9 (46.3)	59.4 (60.0)	89.8 (44.7)	17.8 (12.8)	17.8 (12.4)	17.8 (12.4)	6.3 (3.5)	3.9 (4.8)	6.5 (3.4)
Elken	44.9 (30.3)	29.7 (31.9)	45.9 (29.9)	14.3 (10.7)	13.8 (8.4)	14.4 (10.9)	3.6 (1.8)	2.1 (1.9)	3.7 (1.7)

Pg I/II – ratio between pepsinogen I and pepsinogen II

Atrophy is defined as grade II-III atrophy in the corpus

SD – Standard Deviation

Table 2

Performance indicators of the different pepsinogen test-systems based on varying cutoffs

Test-system (method)	Analytical Approach	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Overall accuracy ^a , % (95% CI)
Biohit (ELISA)	Manufacturer's (Pgl/PgII<3)	44.0 (30.2-57.8)	90.6 (88.5-92.7)	87.7 (85.4-90.0)
	ROC-defined cutoff (Pgl/PgII<6.9)	70.0 (57.3-82.7)	62.6 (59.2-66.1)	63.1 (59.8-66.4)
	DTC-optimized (Pgl<23 and Pgl/PgII<6)	48.0 (34.2-61.9)	93.1 (91.3-94.9)	90.3 (88.3-92.4)
Vector Best (ELISA)	Manufacturer's (Pgl/PgII<3)	56.0 (42.2-69.8)	83.7 (81.1-86.3)	82.0 (79.3-84.6)
	ROC-defined cutoff (Pgl/PgII<4.1)	70.0 (57.3-82.7)	71.5 (68.3-74.7)	71.4 (68.3-74.6)
	DTC-optimized (Pgl<42.9)	52.0 (38.2-65.9)	91.8 (89.8-93.8)	89.3 (87.2-91.5)
Eiken (Latex-agglutination)	Manufacturer's (Pgl 70 ng/ml and Pgl/PgII 3)	76.0 (64.2-87.8)	68.9 (65.6-72.2)	69.3 (66.1-72.5)
	Manufacturer's for strong positivity (Pgl 30 ng/ml and Pgl/PgII 2)	52.0 (38.2-65.9)	89.8 (87.6-92.0)	87.5 (85.2-89.7)
	ROC-defined cutoff (Pgl/PgII<2.7)	70.0 (57.3-82.7)	71.9 (68.7-75.1)	71.8 (68.7-74.9)
	DTC-optimized (Pgl<50 and Pgl/PgII<2.3)	58.0 (44.3-71.7)	83.7 (81.1-86.3)	82.1 (79.5-84.7)

^aSensitivity x Prevalence + Specificity (1 - Prevalence); Prevalence of atrophy=6.2% (50/805)

Abbreviations: CI – Confidence Interval; DTC – Decision tree classifier; ELISA -enzyme linked immunosorbent assay; ROC – Receiver Operating Characteristic; Pgl – Pepsinogen I; PgII – Pepsinogen II