Validity of Serum Pepsinogen I/II Ratio for the Diagnosis of Gastric Epithelial Dysplasia and Intestinal Metaplasia during the Follow-Up of Patients at Risk for Intestinal-Type Gastric Adenocarcinoma[1]

Mário Dinis-Ribeiro[*,†], Altamiro da Costa-Pereira[†], Carlos Lopes[‡], Joana Barbosa[‡], Mateus Guilherme[‡], Luís Moreira-Dias*, Helena Lomba-Viana*, Rui Silva*, Nuno Abreu* and Rafael Lomba-Viana**

*Department of Gastroenterology, Oncology Portuguese Institute, Oporto, Portugal; [†]Porto Faculty of Medicine, Department of Biostatistics and Medical Informatics, Oporto, Portugal; [‡]Department of Pathology, Oncology Portuguese Institute, Oporto, Portugal

Abstract

A cohort of individuals (*n* = 136) with lesions as severe as atrophic chronic gastritis (ACG) was cross-sectionally evaluated for the validity assessment of pepsinogen I (PGI) and pepsinogen II (PGII) serum levels for the diagnosis of intestinal metaplasia (IM) and gastric dysplasia. PGI/PGII ratio [median (range)] was 4 (0.5–7.5) in patients with ACG (*n* = 35); 4.6 (1.9–6.8) in type I IM (*n* = 18); 4.2 (1.4–5.9) in type II or type III IM limited to the antrum and incisura (*n* = 20); 2.4 (0.4–5.6) in extensive incomplete IM (*n* = 38); and 1.3 (0.4–6.4) in low-grade dysplasia (*n* = 23) (*P* = .002). Using histopathologic data as a reference test, the area under the receiver operating characteristic curves (CI 95%) was 0.73 (0.64–0.82) for extensive IM, 0.72 (0.58–0.85) for the diagnosis of dysplasia, and 0.81 (0.66–0.95) for the diagnosis of high-grade dysplasia. Using a PGI/PGII ratio of ≤3 as the cutoff for dysplasia diagnosis, the sensitivity was 70% (62–78%), the specificity was 65% (57–73%), and the negative predictive value estimates were over 90%. No differences in PG levels according to age or gender were observed. *Helicobacter pylori* did not significantly influence validity measurement estimates. PGI/PGII serum level ratio can be used even in the management of patients with a high *a priori* probability for a positive test. It may be useful for the exclusion of more advanced lesions (extensive IM and neoplastic lesions).

Neoplasia (2004) **6**, 449–456

Keywords: Pepsinogens, stomach neoplasms, receiver operating characteristic curve, disease management, precancerous conditions.

Introduction

At least for the intestinal type of gastric adenocarcinoma, a cascade of histopathologic lesions has been defined: chronic gastritis, atrophic chronic gastritis (ACG), intestinal metaplasia (IM), and dysplasia [1]. Although the diagnosis of these lesions and the cost-effectiveness of their management are still to be clearly defined, the follow-up of patients with such lesions may lead to the diagnosis of gastric cancer at an early stage, thus improving patients' survival rate [2,3]. The identification of such lesions depends on invasive tests such as upper gastrointestinal endoscopy, and it represents a challenge because they are scattered and multifocal.

Human pepsinogens I (PGI) and II (PGII) are proenzymes of pepsin—an endoproteinase of gastric juice. PGI is secreted mainly by chief cells in the fundic mucosa [4], whereas PGII is also secreted by the pyloric glands and the proximal duodenal mucosa [5]. *Helicobacter pylori*–related gastric atrophy and further changes tend to start at the antrum and antrum–corpus junction and the antral lesser curvature and incisura, and progress proximally in the distal corpus and, finally, the proximal corpus. In this way, serum PGI and PGII concentrations and the ratio between PGI and PGII may be related to the histologic and functional status of the gastric mucosa [6–16].

In fact, the "pepsinogen test" has been used [11,12,15,16] as a noninvasive "serologic biopsy" to select and improve patient compliance to generalized cancer screening programs in Japan.

Because such mass screening programs may not be feasible in Western countries, we aimed to determine the validity of the pepsinogen test for gastric epithelial dysplasia and IM diagnosis in a set of individuals with known gastric lesions as severe as ACG, hypothesizing its consistency even with a

Address all correspondence to: Mário Dinis-Ribeiro, MD, Gastroenterology Department, Oncology Portuguese Institute "Francisco Gentil," Rua Dr. António Bernardino de Almeida, Oporto 4200-072, Portugal. E-mail: mario@med.up.pt

[1]This work was supported by grants from the Portuguese Health Ministry (CFICS/MS 204/01), the Portuguese Gastrointestinal Endoscopy Society (2001), and the AstraZeneca, Jaba, MediBial, and Tecnimede research foundations.
Received 15 December 2003; Revised 3 March 2004; Accepted 3 March 2004.

DOI 10.1593/neo.03505

high *a priori* probability for a positive test. If it is valid, it may circumvent the problem of invasiveness of endoscopy and may add useful information to an as-yet-undefined schedule to be used even in Western countries for patients at high risk for gastric cancer.

Methods

Participants and Type of Study

A cross-sectional evaluation was performed on a cohort of 136 individuals [37% males, with a median age of 61 years old (minimum 26–maximum 75)] under follow-up at our institution after the diagnosis of gastric epithelial lesions associated with gastric cancer at least as severe as ACG (ACG, IM, or gastric epithelial dysplasia). No differences in age, gender, and time of follow-up were found according to histopathologic data.

After informed consent was given, the patients' data were analyzed and each patient submitted to 1) histopathologic assessment of endoscopic biopsies ("gold standard"); 2) blood collection for serum pepsinogen I (PGI) and pepsinogen II (PGII) determination; and 3) *H. pylori* infection status assessment, which was also considered because it is a recognized risk factor for gastric mucosal inflammation and atrophy and thus a possible confounder to consider in PG validity [17].

This study was fully approved by the ethical committee of the Instituto Português de Oncologia, Centro do Porto (Porto, Portugal).

Histopathologic Assessment of Endoscopic Biopsy Specimens (Reference Test or Gold Standard)

All participants were submitted to magnification chromoendoscopy using methylene blue (1%) as vital staining for IM mucosa and an Olympus Q240Z magnification endoscope (Olympus Corp., Tokyo, Japan) [18]. From each adult, a minimum of five endoscopic gastric biopsies was obtained from areas with differences in color or homogeneity, or randomly in the antrum, incisura, and corpus if no lesion was observed. Specimens were evaluated independently by two pathologists.

Atrophy was defined as the disappearance of normal glands in a determinate area of the stomach [19]. IM was classified as complete (type I) and incomplete (type II or III), according to sulpho staining or syalomucin staining [20]. IM extension [21,22] was estimated as a proportion of specimens with IM. As gastric mucosa specimens collected from the antrum, incisura, and corpus were available, an index for IM extension estimate was devised as follows: 1+/3 (only one of the three biopsies performed showed IM in histopathologic evaluation); 2+/3 (two of three specimens with IM); and 3+/3 (histopathologic evaluation of all specimens collected demonstrated IM, therefore representing multifocal extensive IM from the antrum to the corpus). According to the Vienna classification [23], low-grade dysplasia (LGD) was considered as low-grade noninvasive neoplasia. High-grade dysplasia (HGD) was considered as noninvasive carcinoma and was analyzed as high-grade neoplasia–invasive carcinoma.

Each subject was assigned a global histologic diagnosis to be used as the gold standard or reference test for the pepsinogen test of validity measurements. Seven groups were defined based on the presence of IM and its extension or dysplasia:

- ACG with no IM or dysplasia in any of the specimens collected (n = 35)
- type I IM limited to the antrum and/or the incisura (1+ or 2+/3) (n = 4)
- extensive multifocal type I IM (n = 14)
- 1+ or 2+/3 grade type II/III IM (n = 22)
- extensive 3+/3 grade type II/III IM (n = 38)
- patients with lesions as severe as LGD (n = 23)
- individuals with lesions as severe as high-grade noninvasive neoplasia or invasive carcinoma (n = 11).

No differences in statistical significance were found in age or gender distribution or time during follow-up across the groups defined above.

Pepsinogen Serum Level Determination

Approximately 40 ml of blood was collected from each fasting subject. The blood was centrifuged at 3000 rpm for 5 minutes and the serum aliquot was stored immediately at −20°C.

Using a solid-phase two-step sandwich enzyme immunoassay method (E PLATE Eiken PEPSINOGEN; Eiken Chemical Co., Ltd., Tokyo, Japan), all sera were analyzed for the determination of PGI and PGII concentrations. First, PGI or PGII standards or the sample (20 μl) and buffer (100 μl) were placed on antipepsinogen–coated microplates and incubated at 15°C to 30°C for 110 minutes. After cleaning the reaction mixture by washing, enzyme-labeled antipepsinogen was added (for 5 minutes) to the microplate and incubated at 15°C to 30°C for 55 minutes. After the reaction was completed, the plate was washed and the enzyme reaction was allowed to proceed with the addition of a substrate. The absorbance was then measured by using a microplate spectrophotometer [492 nm (480–500 nm)]. Pepsinogen (I or II) levels in the sample were obtained after a standard curve was drawn. Each sample was evaluated twice for each patient; a coefficient of variation of <15% was considered acceptable.

Possible ranges according to the supplier's PGI and PGII assay results can range from 2 to 200 ng/ml, and from 1 to 100 ng/ml, respectively.

H. pylori Infection Status

After gastric biopsies were obtained, they were evaluated for *H. pylori* infection using Giemsa, immunohistochemistry, and culture. In a previous report, we locally validated specific serum anti–*H. pylori* antibody levels and validity measures [the best cutoff is defined as serum-specific anti–*H. pylori* immunoglobulin G (IgG) antibodies ≥41 μg/ml] [24]. As IM

probably may not allow *H. pylori* to survive [24–27], in this study, patients were considered infected by *H. pylori* if at least one culture medium was positive or both bacterial culture returns were positive, or if serum IgG *H. pylori* antibody levels were considered positive. Thirty-eight percent of patients included were considered infected by *H. pylori*. Personal clinical files of all participants were reviewed and previous *H. pylori* eradication therapy was excluded.

Mucosal specimens were collected and then conserved in a transport medium (Portagerm Pylori; bioMérieux, Marcy l'etoile, France) until homogenization under aseptic conditions in 1.5 ml of sterile medium broth (Brain Heart Infusion; BioGerm, Maia, Portugal). Both nonselective (Columbia agar, with 5% sheep blood; bioMérieux) and selective (Pylori agar; bioMérieux) media were used and placed under microaerobic conditions (10% CO_2 and 10% O_2) in a gas-regulated incubator at 37°C and incubated for 5 to 10 days. All small, grey, and translucent colonies obtained were studied. Biochemical analyses were performed for catalase, oxidase, and urease activity, and Gram stain.

All patients' sera were tested for IgG with Serion ELISA Classic *H. pylori* kits (ELISA *Classic Helicobacter pylori*; Virion/Serion GmbH, Würzburg, Germany). The assays were performed in accordance with the manufacturer's instructions and without knowledge of the adults' *H. pylori* status. Immunoglobulin titer was interpreted from a graph obtained from the semilogarithmic axes analysis of the manufacturer's standard curve.

Statistical Analysis

Statistical Package for Social Sciences software (SPSS 9.0 Package Facility) was used for data support and analysis. Nonparametric tests were used to assess differences across groups of patients [see Histopathologic Assessment of Endoscopic Specimens (Reference Test or Gold Standard) section], namely, PGI, PGII, PGI/PGII ratio, and *H. pylori* IgG antibody levels. Receiver operating characteristic curves were plotted to determine the best cutoff as the tangent point to the curve parallel to the diagonal of the graphic. Sensitivity, specificity, predictive values, and likelihood ratio estimates were calculated by considering histopathology as a reference test (gold standard).

Results

Serum Levels of PGI and PGII and PGI/PGII Ratio According to Histopathologic Lesions

The median levels of PGI and of PGII were 50.6 ng/ml (ranging from 0 to 200) and 13 ng/ml (2.8–100), respectively.

Table 1 shows PGI and PGII serum level distribution, and PGI/ PGII ratio according to histopathologic lesions. No differences with statistical significance were observed in PGI or PGII distribution. Instead, the PGI/PGII ratio was significantly (P = .002) lower toward IM, in relation to its extension, LGD, and more severe lesions. No differences were observed between patients with ACG and those with complete IM or 1+ or 2+/3 incomplete IM. Patients with extensive incomplete IM and those with lesions as severe as LGD had significantly lower PGI/PGII ratios (P = .005).

Serum levels of PGI and PGII and PGI/PGII ratio did not vary with age or gender (Table 2). Patients infected with *H. pylori* did show higher levels of PGI (median, 52.4 ng/ml) and PGII (15.3) and lower ratios of PGI/PGII (2.3) (Table 2).

Although *H. pylori* infection contributes to the decrease in values of PGI/PGII ratio, more severe lesions such as dysplasia have significantly lower levels with (P = .050) or without *H. pylori* (P = .042) infection (Figure 1). In patients with no *H. pylori* infection, PGI/PGII ratio median was 2.7 (0.4–5.6) in those with extensive IM, and was 1.8 (1.3–6.4) in those with lesions as severe as dysplasia. Similar results were found in those infected: 2.4 (1.2–2.4) and 0.7 (0.4–4.9), respectively.

Best Cutoff for PGI/PGII Serum Level Ratio for the Diagnosis of Extensive IM and Lesions as Severe as LGD

For the diagnosis of HGD or cancer, the area under the curve (AUC) was 0.81 (0.66–0.95, 95% CI); for the diagnosis of lesions as severe as LGD, AUC = 0.72 (0.58–0.85, 95% CI); and for the diagnosis of lesions more severe than extensive incomplete IM or LGD, AUC = 0.73 (0.64–0.82, 95% CI) (Figure 2).

Drawn from the curves, the best cutoff was defined (see Methods section) for validity measurement estimates: PGI/PGII serum level ratio of ≤3.05 for the diagnosis of extensive IM or dysplastic lesions and PGI/PGII ratio of ≤3.1 for the diagnosis of HGD and invasive cancer.

Table 1. PGI and PGII Levels According to Histopathologic Lesions in a Set of Patients at High Risk for Gastric Cancer.

	n	PGI [ng/ml Median (Range); Mean (SE)]	PGII [ng/ml Median (Range); Mean (SE)]	PGI/PGII Ratio [Median (Range); Mean (SE)]
ACG without IM or dysplasia	35	50.0 (16.7–200); 57.6 (8.4)	12.9 (5.2–94); 19.8 (4.3)	4.0 (0.5–7.5); 3.9 (0.4)
IM as the most severe lesion	78	42.1 (2–151); 47.2 (3.9)	11.2 (4.3–63.4); 14.3 (1.2)	3.5 (0.4–6.8); 3.7 (0.2)
Type I IM as the most severe lesion	18	33.8 (19.7–93); 42.2 (6.9)	9.3 (4.3–63.4); 10.7 (1.7)	4.6 (1.9–6.8); 4.5 (0.5)
Extension: 1+ or 2+/3	4	32.3 (19.7–93); 48.3 (22.6)	5.8 (4.3–13.7); 7.9 (2.9)	5.7 (4.6–6.8); 5.6 (1.6)
Extension: 3+/3	14	35.2 (27.5–59.6); 39.6 (5.0)	10.4 (5.4–63.4); 11.0 (2.1)	4.5 (1.9–5.2); 4.0 (0.4)
Type II/III IM as the most severe lesion	60	39.6 (2–151); 42.4 (4.1)	13.6 (4.3–22); 14.4 (1.2)	3.0 (0.4–5.9); 3.1 (0.2)
Extension: 1+or 2+/3	22	43.5 (26.3–151); 53.8 (10.5)	13.2 (5.9–22); 14.4 (2.8)	4.2 (1.4–5.9); 4.1 (0.4)
Extension: 3+/3	38	35.1 (2–77.1); 37.7 (3.8)	14.0 (4.9–22); 14.4 (1.4)	*2.4 (0.4–5.6)*; 2.7 (0.2)*
At least LGD	23	24.6 (2.4–60.6); 20.8 (8.0)	11.3 (4.9–15.2); 10.1 (6.3)	*1.3 (0.4–6.4)*; 2.3 (0.9)*
HGD or invasive carcinoma	11	27.9 (0–192.9); 48.3 (16.8)	14.4 (2.8–100.0); 35.9 (9.1)	*1.6 (0.5–5.0)*; 1.7 (0.5)*
*P**		.208	.210	*.002**

*Comparison of levels of PGI, PGII, and PGI/PGII ratio according to histopathologic lesions [median (minimum and maximum)], using Kruskal-Wallis test.

Table 2. Serum Concentrations of PGI, PGII [median (min–max); ng/ml], and PGI/PGII Ratio According to Age, Gender, and *H. pylori* Infection Status.

	PGI [ng/ml Median (Range); Mean (SE)]	PGII [ng/ml Median (Range); Mean (SE)]	PGI/PGII Ratio [Median (Range); Mean (SE)]
Age (r^2)	0.001	0.002	0.021
Gender			
Male	42.9 (2–193); 49.4 (4.3)	14.5 (2.8–100); 16.3 (1.3)	3.4 (0.2–7.6); 3.5 (0.2)
Female	42.3 (2–200); 51.3 (4.4)	12.6 (4.3–63.4); 19.1 (2.5)	3.6 (0.4–8.1); 3.5 (0.3)
*P**	.417	.289	.825
H. pylori–positive (culture or IgG antibodies)			
Negative	31.6 (2–200); 38.5 (5.4)	7.5 (4.3–41.3); 10.8 (1.4)	4.3 (0.4–7.9); 4.0 (0.3)
Positive	53.4 (2.4–151); 50.5 (4.4)	15.3 (6.1–94.0); 18.8 (2.3)	2.3 (0.4–8.1); 3.0 (0.2)
*P**	*.013*	<.001	*.004*

*Mann-Whitney test for differences in PG distribution according to gender and *H. pylori* status.

Validity Measures of PGI/PGII Ratio for the Diagnosis of Gastric Histologic Lesions

Estimates of validity measurements were calculated by considering the PGI/PGII serum level ratio of ≤3.0 as a positive test for the presence of lesions as severe as extensive incomplete IM (including dysplasia) and for the presence of gastric mucosal lesions as severe as LGD and HGD or invasive carcinoma. Two cutoffs other than cutoff levels were considered for the comparison of PGI/PGII ratios of ≤2 and ≤4. Measures were determined by considering *H. pylori* infection status. No differences were observed (Table 3).

With a PGI/PGII ratio of ≤3 as a positive test, sensitivity estimates for the diagnosis of lesions as severe as IM and HGD varied between 66% and 82%, respectively. All cutoff levels showed very high negative predictive value estimates. If we considered *H. pylori* infection as an alternative positive test, no significant increase in accuracy is apparent. A slight increase in sensitivity with a marked decrease in specificity and negative predictive value occurs (Table 3).

Discussion

Gastric cancer prognosis clearly depends on stage at diagnosis [2,28]. Either general population screening or high-risk individual group follow-up can lead to the diagnosis of (early gastric) cancer and an improvement in patient survival [3,29–36]. Mass screening may not be feasible in all countries, but the follow-up of patients at high risk for gastric cancer may be useful even in Western countries.

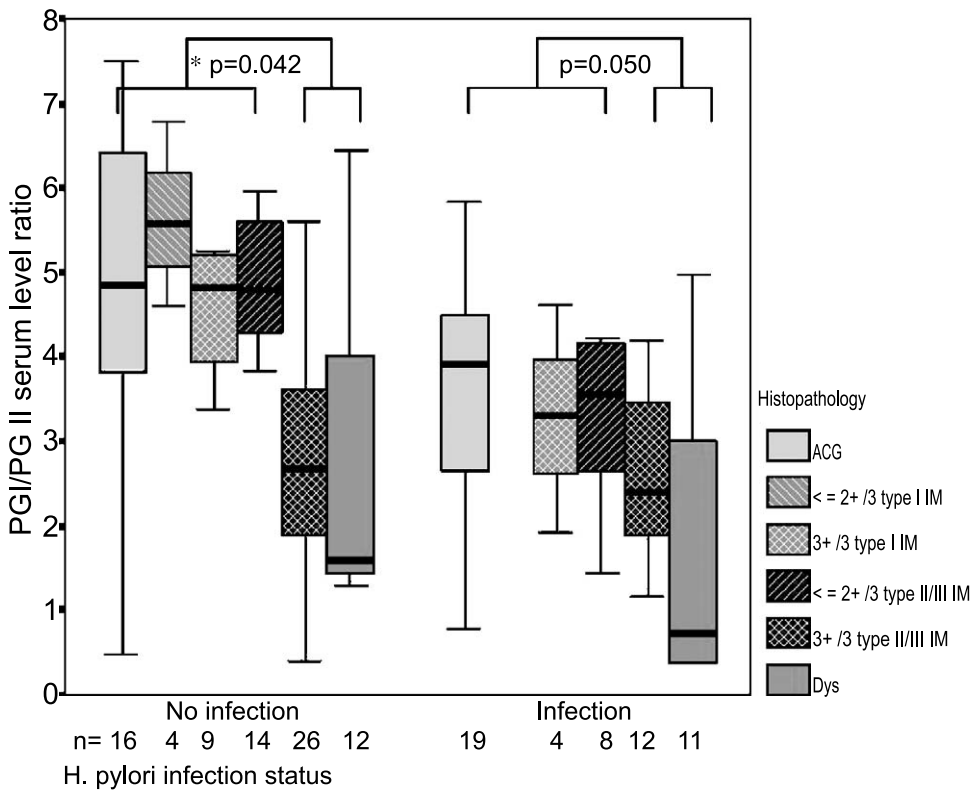

Figure 1. *PGI/PGII ratio according to histopathologic lesion and H. pylori status. Although H. pylori infection contributes to lower values of PGI/PGII, more severe lesions such as dysplasia have significantly lower levels with (P = .050) or without H. pylori (P = .042) infection.*

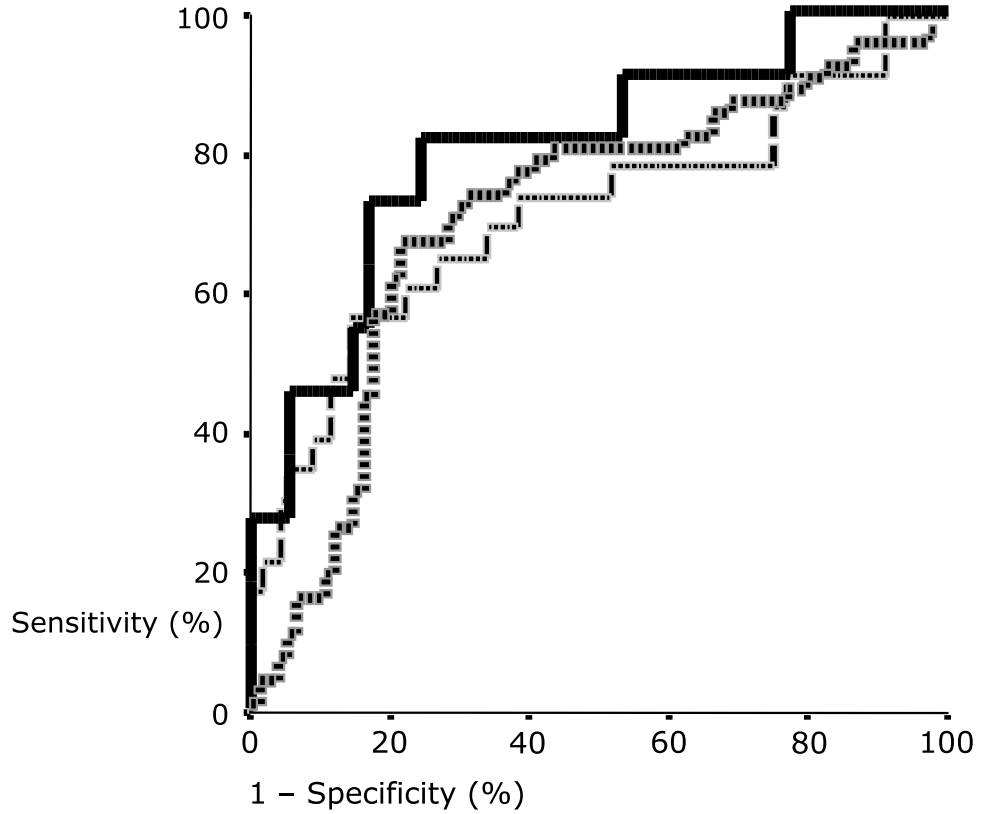

Figure 2. *Receiver operator characteristics curves for the diagnosis of histologic lesions: for the diagnosis of HGD or cancer (grey), AUC = 0.81 (0.66–0.95, 95% CI); for the diagnosis of lesions as severe as LGD (large dashes), AUC = 0.72 (0.58–0.85, 95% CI); and for the diagnosis of lesions more severe than extensive incomplete intestinal metaplasia or LGD (small dashes), AUC = 0.73 (0.64–0.82, 95% CI).*

At least for intestinal-type gastric adenocarcinoma, patients with ACG, namely, those with extensive areas of IM changes [21,22], seem to be at risk for gastric cancer. In Vienna, dysplasia was considered as a truly premalignant lesion, but to our knowledge, no recommendation on the management of patients with ACG or extensive IM exists, mostly because studies demonstrating the cost-effectiveness of such strategy are lacking.

This may be due to the fact that most studies tried to assess this issue after standardized endoscopic examinations and random biopsies. These procedures, even if performed in different and numerous sites, may still fail to diagnose dysplasia and cancer, both because those lesions may be scattered and multifocal and because endoscopy, at least for this purpose, shows a very high interobserver variability. In our study, we decided to use an improved endoscopic observation [18] that is reproducible and valid, leading to a more accurate assessment of the histopathologic status of gastric mucosa. However, even if an endoscopic method is improved [37–39], it will still be dependent on morphologic observations of limited areas and will clearly be badly accepted by patients.

Serum levels of PGI and the PGI/PGII ratio were used in several studies as markers of gastric mucosal changes (as ''serologic biopsy''). The focus was mainly on the selection of individuals with atrophic changes from the general population, with itself as an end point of the study [12–14], or as an associated lesion with gastric cancer to improve patients' compliance with the gastric cancer screening program in Japan.

In our study, we decided to evaluate this test's consistency by estimating its usefulness even in a selected set of patients at high risk for gastric cancer.

In fact, we observed that as changes in gastric mucosa become more severe in terms of carcinogenesis (type II or type III IM or low-grade neoplasia) or more extensive IM, PGI/PGII ratio decreases significantly. In the absence of a surface area estimate, an index of the extent of IM was conceived as a proportion of collected specimens with IM, based on the fact that IM evolves from the antrum–corpus junction and the antral lesser curvature to the antral greater curvature and distal corpus and, finally, to the greater curvature of the proximal corpus. A significant decrease of the PGI/PGII ratio seems to occur with extensive incomplete IM and LGD. In fact, this should be very important, as these are probably the more aggressive lesions leading to gastric cancer [35].

Age, gender, time of follow-up, and *H. pylori* infection status were considered in our analysis. No agreement exists [14,16] on the influence of age and gender on PG levels, and whenever assessment of changes in cutoff value according to those factors was tried, either no changes were obtained or significant losses in validity measures were noticeable. In our sample, we did not observe any differences with statistical significance on PG levels according to gender. Age does not explain more than 2% of the pepsinogen serum

Table 3. Validity Estimates of PGI/PGII Serum Level Ratio [% (CI 95%)] Either Alone or Considering *H. pylori* Infection as Risk Factor for the Diagnosis of Lesions as Severe as Extensive Incomplete IM, Dysplasia, or HGD and Its Variation After (n = 136).

Diagnosis	Positive Test Defined as PGI/PGII Serum Level Ratio			Positive Test Defined as Either *H. pylori* Infection – Detected or PGI/PGII Serum Level Ratio		
	≤4	≤3	≤2	≤4	≤3	≤2
Extensive IM (n = 61)						
Sensitivity	80% (73–87) 49/61	66% (57–74) 40/61	36% (28–44) 22/61	84% (78–90) 51/61	74% (66–81) 46/61	59% (51–67) 36/51
Specificity	60% (51–68) 44/74	78% (71–85) 58/74	82% (75–89) 61/74	39% (31–47) 29/74	46% (37–55) 34/74	46% (37–55) 34/74
Positive predictive value	62% (54–70) 49/79	66% (58–74) 40/56	63% (55–71) 22/35	53% (44–61) 51/96	54% (45–63) 46/86	47% (38–56) 36/76
Negative predictive value	78% (71–85) 44/56	73% (65–81) 58/79	61% (53–69) 61/100	74% (66–82) 29/39	69% (61–77) 34/49	58% (49–66) 34/59
Likelihood ratio of a positive test	1.98 (1.74–2.21)	3.00 (2.56–3.42)	2.00 (1.76–2.24)	1.38 (1.25–1.50)	1.37 (1.25–1.49)	1.09 (1.04–1.14)
Likelihood ratio of a negative test	0.34 (0.26–0.42)	0.44 (0.35–0.52)	0.78 (0.71–0.85)	0.41 (0.32–0.49)	0.56 (0.48–0.65)	0.89 (0.84–0.94)
Dysplasia (n = 23)						
Sensitivity	78% (71–85) 18/23	70% (62–78) 16/23	57% (49–65) 13/23	83% (77–89) 19/23	74% (66–82) 17/23	65% (57–73) 15/23
Specificity	46% (37–54) 51/112	65% (57–73) 72/112	81% (74–88) 90/112	31% (23–39) 35/112	38% (30–46) 43/112	46% (37–55) 51/112
Positive predictive value	23% (16–30) 18/79	29% (21–36) 16/56	37% (29–45) 13/35	20% (13–27) 19/96	20% (13–27) 17/86	20% (13–27) 15/76
Negative predictive value	91% (86–96) 51/56	91% (86–96) 72/79	90% (85–95) 90/100	90% (85–95) 35/39	88% (82–94) 43/49	86% (80–92) 51/59
Likelihood ratio of a positive test	1.44 (1.31–1.58)	2.00 (1.76–2.24)	3.00 (2.58–3.42)	1.20 (1.12–1.29)	1.19 (1.11–1.28)	1.20 (1.12–1.29)
Likelihood ratio of a negative test	0.48 (0.39–0.56)	0.46 (0.38–0.55)	0.53 (0.45–0.62)	0.55 (0.46–0.63)	0.68 (0.60–0.76)	0.76 (0.69–0.84)
HGD (n = 11)						
Sensitivity	91% (86–96) 10/11	82% (75–89) 9/11	73% (65–81) 8/11	90% (85–95) 10/11	82% (75–89) 9/11	73% (65–81) 8/11
Specificity	45% (36–54) 55/124	63% (55–71) 77/124	79% (72–86) 97/124	31% (23–39) 38/124	38% (30–46) 47/124	45% (36–54) 56/124
Positive predictive value	13% (7–19) 10/79	16% (10–22) 9/56	22% (15–29) 8/35	10% (5–15) 10/96	11% (6–16) 9/86	11% (6–16) 8/76
Negative predictive value	98% (96–100) 55/56	98% (96–100) 77/79	97% (94–99) 97/100	97% (94–99) 38/39	96% (93–99) 47/49	95% (91–99) 56/59
Likelihood ratio of a positive test	1.65 (1.48–1.83)	2.22 (1.93–2.50)	3.48 (2.97–3.98)	1.30 (1.20–1.41)	1.32 (1.21–1.43)	1.33 (1.21–1.44)
Likelihood ratio of a negative test	0.20 (0.13–0.27)	0.28 (0.21–0.36)	0.34 (0.26–0.42)	0.32 (0.24–0.40)	0.47 (0.39–0.56)	0.60 (0.52–0.68)

values. In fact, age seems to be related to an increase in acid secretion in humans [40], and the decrease of PGI level and PGI/PGII ratio found in most studies may be related not to age but to atrophic changes diagnosed that way.

H. pylori infection, a risk factor for gastric cancer, seems to be related to an increase of both PGI and PGII, which is probably associated with inflammation and cytokine release [41,42]. In our sample, we decided to define *H. pylori* infection status based on culture results and serum levels of IgG antibody to circumvent the possible "loss" of *H. pylori* from areas with IM. In fact, as others did [43] in a previous report, we observed [24] a negative correlation between the extent of metaplasia and the anti–*H. pylori* titer. In that report, Barbosa et al. [24] argue that as in other sets (such as evaluation after *H. pylori* eradication therapy for peptic ulcer disease) [1], noninvasive tests (namely, specific IgG serum levels), after proper assessment of cutoff values and their validation, may be used in the follow-up of patients with ACG and IM. A decrease to levels considered negative may be used to select patients for further evaluation, for instance, through endoscopic examination. Therefore, we decided to consider *H. pylori* status both to assess confounding effects for PG levels and to evaluate any modifications made to validity measurements according to the status of infection. We did find a variation of PG levels related to *H. pylori* infection, but as Fokuda et al. [44] did, we observed that although *H. pylori* justifies some decreases in the PGI/PGII ratio, it does not *per se* justify the decrease related to the severity of lesions. Furthermore, in this study, no differences were found in *H. pylori* infection status according to histopathologic lesions and no significant changes occurred in validity measurement estimates.

Several authors [11,13,16,17] reported on the relationship between the serum levels of PGI and PGII as related to mucosal changes in the stomach, mostly in asymptomatic and/or unknown gastric mucosal lesions. In the present

study, the pepsinogen test was used for the first time in our country in a set of patients with very high *a priori* probability of a positive test.

Although a systematic analysis of published reports would probably address this test's consistency in different population sets more accurately, we defined the same discriminative point (PGI/PGII ratio of ≤ 3) as the best cutoff, as in other studies with very similar results.

Considering validity measurement estimates, we found sensitivity values that may not be cost-effective for screening purposes. However, if we consider negative predictive values even in a select high-risk sample as we did, we may argue that in a clinical background where no clear recommendations had been made until now, PGI/PGII serum level ratio may be a useful tool [35].

We conclude that the evaluation in serum of both forms of pepsinogens and the calculation of the PGI/PGII ratio seem to be valid for the diagnosis of neoplastic forms, even in individuals with a high pretest probability of atrophy. Although its effectiveness needs evaluation, a management strategy based on this test may exclude patients from endoscopy and it may be able to noninvasively follow individuals with indolent lesions, such as ACG. Furthermore, when used together with endoscopic examination, it may give helpful information on the assurance and exclusion of more advanced lesions.

Acknowledgements

We acknowledge Carlos Torres for technical support and laboratory work, René Lambert (International Agency for Research in Cancer, Lyon, France) for suggestions and support, and Maria do Céu Barros for proofreading.

References

[1] Carneiro F, Machado JC, David L, Reis C, Nogueira AMMF, and Sobrinho-Simões M (2001). Current thoughts on the histopathogenesis of gastric cancer. *Eur J Cancer Prev* **10,** 101–102.

[2] Everett SM and Axon ATR (1997). Early gastric cancer in Europe. *Gut* **41,** 142–150.

[3] Di Gregorio C, Morandi P, Fante R, and De Caetani C (1993). Gastric dysplasia. A follow-up study. *Am J Gastroenterol* **88,** 1714–1719.

[4] Samloff IM (1971). Cellular localization of group I pepsinogens in human gastric mucosa by immunofluorescence. *Gastroenterology* **61,** 185–188.

[5] Samloff IM and Liebman WM (1973). Cellular localization of the group II pepsinogens in human stomach and duodenum by immunofluorescence. *Gastroenterology* **65,** 36–42.

[6] Basson M and Modlin I (1988). Pepsinogen: biological and pathophysiologic significance. *J Surg Res* **44,** 82.

[7] Fukunaga H, Hovita K, and Otsuka N (1989). Fundamental and clinical study for pepsinogen I and II RIABEAD Kit. *Igaku-To-Yakugaku Med Pharm Sci* **21,** 905–914.

[8] Huang S, Miki K, Furihata C, Ichinote M, Shimizu S, and Oka H (1988). Enzyme-like immunosorbent assays for serum pepsinogens I and II using monoclonal antibodies—with data on peptic ulcer and gastric cancer. *Clin Chem Acta* **175,** 37.

[9] Ley C, Mohar A, Guarner J, Herrera-Goepfert R, Figueroa LS, Halperin D, Johnstone I, and Parsonnet J (2001). Screening markers for chronic atrophic gastritis in Chiapas, Mexico. *Cancer Epidemiol Biomark Prev* **10,** 107–112.

[10] Nietsch H, Nametaka T, Rubin CE, Miki K, and Kimmey MB (2001). Gastric cancer screening in asymptomatic Japanese Americans using serum pepsinogen levels and endoscopy. *Endoscopy* **53,** AB209.

[11] Miki K, Ichinose M, Kawamura N, Matsushima M, Ahmad HB, Kimura M, Sano J, Tashiro T, Kakei N, Oka H, Furihata C, and Takahashi K (1989). The significance of low serum pepsinogen levels to detect stomach cancer associated with extensive chronica gastritis in Japanese subjects. *Jpn J Cancer Res* **1,** 45–50.

[12] Miki K, Ichinose M, Simizu A, Huang SC, Oka H, Furihata C, Matsushima T, and Takahashi K (1987). Serum pepsinogens as a screening test of extensive chronic gastritis. *Gastroenterol Jpn* **22,** 133–140.

[13] Varis K, Sipponen P, Laxen F, Samloff IM, Huttunen JK, Taylor PR, Heinonen OP, Albanes D, Sande N, Virtamo J, and Harkonen M (2000). Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia. Helsinki Gastritis Study Group. *Scand J Gastroenterol* **35,** 950–956.

[14] Webb PM, Hengels KJ, Moller H, Newell DG, Palli D, Elder JB, Coleman MP, De Backer G, and Forman D (1994). The epidemiology of low serum pepsinogen A levels and an international association with gastric cancer rates. *Gastroenterology* **107,** 1335–1344.

[15] Fahey MT, Hamada GS, Nishimoto IN, Kowalski LP, Iriya K, Gama-Rodrigues JJ, and Tsugane S (2000). Ethnic differences in serum pepsinogen levels among Japanese and non-Japanese Brazilian gastric cancer patients and controls. *Cancer Detect Prev* **24,** 564–571.

[16] Kato I, Miki K, Munoz N, Vivas JH, Lopez G, Peraza S, Carillo E, Castro D, Andrade O, and Sanchez V (1995). Determinants of plasma pepsinogen levels in a population at high risk for stomach cancer in Venezuela. *Int J Cancer* **62,** 512–518.

[17] Zhang L, Blot WJ, You WC, Chang YS, Kneller RW, Jin ML, Li JY, Zhao L, Liu WD, Zhang JS, Ma JL, Samloff IM, Correa P, Blaser MJ, Xu GW, and Fraumeni JF Jr (1996). *Helicobacter pylori* antibodies in relation to precancerous gastric lesions in a high-risk Chinese population. *Cancer Epidemiol Biomark Prev* **5,** 627–630.

[18] Dinis-Ribeiro M, Costa-Pereira A, Lopes C, Lara-Santos L, Guilherme M, Moreira-Dias L, Lomba-Viana H, Ribeiro A, Santos C, Soares J, Mesquita N, Silva R, and Lomba-Viana R (2003). Magnification chromoendoscopy for the diagnosis of gastric intestinal metaplasia and dysplasia. *Gastrointest Endosc* **57,** 498–504.

[19] Guarner J, Herrera-Goepfert R, Mohar A, Sanchez L, Halperin D, Ley C, and Parsonnet J (1999). Interobserver variability in application of the revised Sydney classification of gastritis. *Hum Pathol* **30,** 1431–1434.

[20] Filipe MI, Barbatis C, Sandey A, and Metz DC (1998). Expression of intestinal mucin antigens in the gastric epithelium and its relationship with malignancy. *Hum Pathol* **19,** 19–26.

[21] Stemmerman GN and Fenoglio-Preiser C (2002). Gastric carcinoma distal to the cardia: a review of the epidemiological pathology of the precursors to a preventable cancer. *Pathology* **34,** 494–503.

[22] Cassaro M, Rugge M, Gutierrez O, Leandro G, Graham DY, and Genta RM (2000). Topographic patterns of intestinal metaplasia and gastric cancer. *Am J Gastroenterol* **95,** 1431–1438.

[23] Schlemper RJ, Riddell RH, Kato Y, Borchard F, Cooper HS, Dawsey SM, Dixon MF, Fenoglio-Preiser CM, Flejou JF, Geboes K, Hattori T, Hirota T, Itabashi M, Iwafuchi M, Iwashita A, Kim YI, Kirchner T, Klimpfinger M, Koike M, Lauwers GY, Lewin KJ, Oberhuber G, Offner F, Price AB, Rubio CA, Shimizu M, Shimoda T, Sipponen P, Solcia E, Stolte M, Watanabe H, and Yamabe H (2000). The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* **47,** 251–255.

[24] Barbosa J, Dinis-Ribeiro M, Guilherme M, Filipe T, Lomba-Viana R, Guimaræs MA, and Lopes C (2003). Use of *Helicobacter pylori*–specific antibodies in the diagnosis of intestinal metaplasia and dysplasia. *Br J Biomed Sci* **60,** 175–179.

[25] Dixon M (1997). *Helicobacter pylori* gastritis: pathology and progression. *Int Med Publ*, 110–118.

[26] El-Zimaity HM, Ramchatesingh J, Saeed M, and Graham D (2001). Gastric intestinal metaplasia: subtypes and natural history. *J Clin Pathol* **54,** 679–683.

[27] Genta RM, Gurer IE, Graham DY, Krishnan B, Segura AM, Gutierrez O, Kim JG, and Burchette JL Jr (1996). Adherence of *Helicobacter pylori* to areas of incomplete intestinal metaplasia in the gastric mucosa. *Gastroenterology* **111,** 1206–1211.

[28] Ferlay J, Bray F, Sankila R, and Parkin DM (1999). Cancer Incidence, Mortality and Prevalence in the European Union 1997. IARC Cancer Base No. 4 IARC Press, Lyon.

[29] Conchillo JM, Houben G, deBruine A, and Stockbrugger RW (2001). Is type III intestinal metaplasia an obligatory precancerous lesion in intestinal-type gastric carcinoma? *Eur J Cancer Prev* **10,** 307–312.

[30] Whiting JL, Signedsson A, Rowlands DC, Hallissey MT, and Fielding JW (2002). The long term results of endoscopic surveillance of premalignant gastric lesions. *Gut* **50,** 378–381.

[31] Lahner E, Caruana P, D'Ambra G, Ferraro G, Di Giulio E, Delle Fave G, Bordi C, and Annibale B (2001). First endoscopic follow-up in patients with predominant atrophic gastritis: when should it be done? *Gastrointest Endosc* **53,** 443–448.

[32] Corral MJC, Pardo-Mindan FJ, Razquin S, and Ojeda C (1990). Risk of cancer in patients with gastric dysplasia. Follow-up study of 67 patients. *Cancer* **65,** 2078–2085.

[33] Correa P, Haenszel W, Cuello C, Zavala D, Fontham E, Zarama G, Tannenbaum S, Collazos T, and Ruiz B (1990). Gastric precancerous process in a high risk population: cohort follow-up. *Cancer Res* **50,** 4737–4740.

[34] Correa P (1992). Human gastric carcinogenesis: a multistep and multifactorial process—first American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* **52,** 6735–6740.

[35] Dinis-Ribeiro M, Lopes C, da Costa-Pereira A, Guilherme M, Barbosa J, Lomba-Viana H, Silva R, and Moreira-Diaz L (2004). A follow-up model for patients with atrophic chronic gastritis and intestinal metaplasia. *J Clin Pathol* **57,** 177–182.

[36] You WC, Li JY, Blot WJ, Chang YS, Jin ML, Gail MH, Zhang L, Liu WD, Ma JL, Hu YR, Mark SD, Correa P, Fraumeni JF Jr, and Xu GW (1999). Evolution of precancerous lesions in a rural Chinese population at high-risk of cancer. *Int J Cancer* **83,** 615–619.

[37] Bouma BE, Tearney GJ, Compton CC, and Nishioka NS (2000). High-resolution imaging of the human esophagus and stomach *in vivo* using optical coherence tomography. *Gastrointest Endosc* **51,** 467–473.

[38] Sivak MV Jr, Kobayashi K, Izatt JA, Rollins AM, Ung-Runyawee R, Chak A, Wong RC, Isenberg GA, and Willis J (2000). High-resolution endoscopic imaging of the GI tract using optical coherence tomography. *Gastrointest Endosc* **51,** 474–479.

[39] Tajiri H, Doi T, Endo H, Nishina T, Terao T, Hyodo I, Matsuda K, and Yagi K (2001). The application of magnifying endoscopy to gastric cancer diagnosis: a prospective study. *Endoscopy* **53,** AB123.

[40] Goldschmiedt M, Barnett CC, Schwarz BE, Karnes WE, Redfern JS, and Feldman M (1991). Effect of age on gastric acid secretion and serum gastrin concentrations in healthy men and women. *Gastroenterology* **101,** 977–990.

[41] Serrano MT, Lanas AI, Lorent S, and Sainz R (1997). Cytokine effects on pepsinogen secretion from human cells. *Gut* **40,** 42–48.

[42] Lorente S, Doiz O, Trinidad Serrano M, Castillo J, and Lanas A (2002). *Helicobacter pylori* stimulates pepsin secretion from isolated human peptic cells. *Gut* **50,** 13–18.

[43] Osawa H, Inoue F, and Yoshida Y (1996). Inverse relation of serum *Helicobacter pylori* antibody titres and extent of intestinal metaplasia. *J Clin Pathol* **49,** 112–115.

[44] Fukuda H, Saito D, Hayashi S, Hisai H, Ono H, Yoshida S, Oguro Y, Noda T, Sato T, and Katoh M (1995). *Helicobacter* infection, serum pepsinogens levels and gastric cancer: a case–control study in Japan. *Jpn J Cancer Res* **86,** 64–71.