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Pepsinogens to Distinguish Patients With Gastric Intestinal Metaplasia and *Helicobacter pylori* Infection Among Populations at Risk for Gastric Cancer

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OBJECTIVES: The objectives of this study were to investigate the serum pepsinogen test for the prediction of OLGIM (Operative Link on Gastric Intestinal Metaplasia Assessment) stages in first-degree relatives (FDR-GC) of patients with gastric cancer (GC) and autoimmune chronic atrophic gastritis (ACAG).

METHODS: In 67 consecutive patients with ACAG, 82 FDR-GC, and 53 controls (CTRL) without gastric disease (confirmed by biopsy), serum levels of pepsinogen 1 (PG1), pepsinogen 2 (PG2), G17, and the PG1/2 ratio were assessed by enzyme-linked immunosorbent assay kit. All ACAG patients had positive antiparietal cell antibody levels, estimated by indirect immunofluorescence. Biopsies taken in duplicate from the antrum, corpus, and fundus were stained with Giemsa for *Helicobacter pylori* detection. Endoscopic detection of metaplasia was confirmed by histological diagnosis. Histological classification of OLGIM stages was applied by using the criteria of severity and topography of intestinal metaplasia (IM).

RESULTS: The highest discrimination capacity for distinguishing ACAG from other groups of patients was the gastrin G17 test. The lowest mean for PG1 and PG2 serum levels was found in ACAG. In multivariate analysis by age, PG1 and PG1/PG2 were independent prognostic factors for metaplasia, and PG2 also for the presence of a histological *H. pylori* infection. The serum PG1 level was significantly lower in individuals with IM at OLGIM stage >2 than in those with IM at OLGIM stage <2, resulting in a useful method for the prediction of OLGIM stage. With the inclusion of patient age at diagnosis in the prediction of ≥ 2 vs. 0–1 OLGIM stages, the receiver operating characteristic (ROC) curve at 47.9 ng/ml PG1 level reached a significant area under the curve (AUC) value (0.978, $P<0.001$). We also observed a slight difference in PG2 serum levels between histological *H. pylori*-positive and *H. pylori*-negative subjects (ROC AUC: 0.599).

CONCLUSIONS: This study demonstrated an important increase in gastrin G17 serum level in autoimmune gastritis. PG1 serum level corrected by patient age can be used in the management of patients at risk for GC with a high predicted probability of having an OLGIM stage ≥ 2. Using a cutoff of 47.9 ng/ml, PG1 testing in FDR-GC and ACAG patients had a sensitivity of 95.83% and a specificity of 93.37. Although these results could be validated in a prospective study, the known importance of higher OLGIM stages in increasing the risk of GC development supports the rationale of proposing PG1 algorithm as a diagnostic tool for the selection of high-risk FDR-GC and ACAG patients at high-risk stages for subsequent detailed endoscopic examination to detect dysplasia and asymptomatic GC. In addition, serum PG1 and PG2 levels could stratify patients based on both *H. pylori* infection and OLGIM risk in consideration of the increased acknowledge regarding the role of *H. pylori* in the progression of gastritis to GC.

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INTRODUCTION

Gastric cancer (GC) incidence in Friuli Venezia Giulia (Italy) is medium/low rate at ~150 to 170 per 100,000 person-years.[1,2] Infection with *Helicobacter pylori* is the main risk factor for GC.[3] GC of the intestinal type occurs via a sequence of precancerous conditions known as the Correa's cascade (gastric atrophy,[4] intestinal metaplasia (IM), and gastric dysplasia), although not all patients with a precancerous lesion develop a GC. Moreover, patients with IM in autoimmune chronic athrophic gastritis (ACAG), an inherited autoimmune disease that destroys gastric parietal cells, have a threefold increased relative risk of developing a GC,[5,6] and the risk of familial aggregation of GC is increased 1–4- to

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7.0-fold in relatives of patients with intestinal and diffuse types of GC, respectively.[7–11]

The mortality rate of GC remains high, with a 5-year survival rate of <35% that is largely dependent on stage.[12] Thus, an early diagnosis and early treatment of GC patients is the key to improving prognosis. Endoscopy has a primary role in the surveillance of premalignant gastric conditions[4] and for the earliest detection of GC.[13,14] Endoscopic population-based screening in individuals with positive serum pepsinogen measurements has been performed in Korea, Taiwan, and Japan.[15–18] In the meantime, more of the world's attention is turning to primary prevention through the eradication of *H. pylori*. However, endoscopic screening is not suitable for use in areas that are medium/low risk for GC because endoscopy is costly, invasive, and time consuming, but it could be cost effective if limited to a subset of patients with a high absolute risk for GC development.[11,19,20] High gastric intestinal metaplasia assessment is known to be a precancerous condition carrying considerable GC risk that increases with older age, and mainly results from long-standing *H. pylori* infection.[4,21,22] *H. pylori* eradication alone in patients with nonatrophic gastritis has been demonstrated to prevent subsequent development of GC.[23] A concentration of pepsinogen 1 (PG1) of <70 ng/ml and a PG1/2 ratio of <3.0 are widely accepted as the cutoff points for GC screening in Japan.[24] However, there has been controversy in the literature with respect to the validity of this test for the identification of patients at risk for GC with low/moderate incidence rate. Moreover, several studies employing different analytical technologies exhibited different sensitivities and specificities (with different cutoff values) and tested populations with heterogenous clinical characteristics.

We proposed that measurement of gastrin G17 and PG1 serum levels including age bias targeted at population at risk for GC (i.e., first-degree relatives of GC patients (FDR-GC) and ACAG subjects) is likely to be an effective strategy for predicting individuals with OLGIM (Operative Link on Gastric Intestinal Metaplasia Assessment) stages 2 and 3 who would be referred for endoscopic surveillance. This model could offer the advantage of selecting from the general population patients who have ACAG, and in FDR-GC and ACAG populations at risk for GC, individuals with a high probability of having IM who would then be submitted to gastroscopy. The subsequent OLGIM stage evaluation of the biopsies by pathologists would be used to choose the follow-up according to the risk of GC development.

METHODS

Patient recruitment, ethical guidelines, and diagnosis. From January 2009 to March 2014, 202 patients were consecutively recruited at the Unit of Oncological Gastroenterology, Centro di Riferimento Oncologico, National Cancer Institute, Aviano, Italy. Patients recruited included 67 patients affected by ACAG, 82 FDR-GC, and 53 individuals without gastric disease as confirmed by biopsy (CTRL). None of the patients tested for the study were treated with proton pump inhibitors. Blood was processed only one time for the study. Biopsies were taken in duplicate in the antrum, corpus, and fundus regions. *H. pylori* infection was detected on tissue sections using hematoxylin and eosin and by Giemsa stains. Each patient underwent esophagogastroduodenoscopy performed using an Olympus 180 series gastroscope (Tokyo, Japan). ACAG was ascertained histologically and serologically.[25,26] Antiparietal cell antibody, levels were estimated by means of indirect immunofluorescence with a cutoff of ≥1/80 (EUROIMMUN, Lübeck, Germany).

The study was approved by the Institutional Review Board (no. 14) of National Cancer Institute, IRCCS, Centro di Riferimento Oncologico. All study participants, or their legal guardian, provided informed written consent before study enrollment. Ethical guidelines for research involving human subjects were respected.

Grading metaplasia with the OLGIM staging system. Every IM was confirmed by histology and staged using the OLGIM classification. On the basis of the standardized sites of biopsy, the gastritis stage was assessed according to the OLGA (Operative Link for Gastritis Assessment) staging system.[26] According to the Sydney protocol,[27] at least three biopsy samples were taken from the mucosecreting area (two antral samples plus one from the *incisura angularis*) and two from the oxyntic mucosa. In each biopsy, atrophy was scored as a percentage of atrophic glands. Ideally, atrophy is assessed on perpendicular (full thickness) mucosal sections. For each biopsy sample (from all areas), atrophy was scored on a four-tiered scale (no atrophy = 0%, score = 0; mild atrophy = 1–30%, score = 1; moderate atrophy = 31–60%, score = 2; and severe atrophy ≥60%, score = 3). In each of the two mucosal compartments (mucosecreting and oxyntic), an overall atrophy score was calculated, expressing the percentage of compartmental atrophic changes (i.e., taking into account all of the biopsies obtained from the same functional compartment). The OLGA stage was determined from the combination of the overall "antrum score" with the overall "corpus score." For the development of the IM staging system OLGIM, atrophic gastritis in the OLGA was replaced by IM, and a combination of the IM scores resulted in the OLGIM staging score, as reported elsewhere.[28]

Risk factor measurement. Peripheral blood (7.5 ml) was collected for each participant; serum was obtained by centrifugation at 2,608 × *g* for 10 min and stored at − 80 °C until assay for PGs.

The measurement of serum PG1, PG2, and G17 concentrations was carried out using an enzyme-linked immunosorbent assay kit (Biohit Oyj, Helsinki, Finland) according to the manufacturer's instructions. A blinded quality control was used and the normal ranges for serum PG1, PG2, PG1/2 ratio, and G17 were determined by the ISO9001-08 certified Clinical Pathology Laboratory as 30–166 ng/ml, 3–15 ng/ml, 3–20, and 1–7 pmol/l, respectively.

To isolate the effect of serum PG levels on GC occurrence, several baseline factors that were potential confounders were examined: age, gender sex, *H. pylori* infection confirmed by histological diagnosis, presence of IM and OLGIM stage of the biopsy samples. Individuals were divided into three groups: ACAG and FDR-GC, both at risk for GC development, and CTRL who were individuals with mild dyspepsia who had

Table 1 Baseline characteristics of the patient groups

Variable		CTRL ($N=53$)	FDR-GC ($N=82$)	ACAG ($N=67$)	*P*c
Age	Mean ± s.d.	54.7 ± 11.8	46.9 ± 12.3	53.5 ± 10.8	<0.001
Gender					
Female	No. (%)	24 (45.3)	46 (56.1)	51 (76.1)	0.01
Male	No. (%)	29 (54.7)	36 (43.9)	16 (23.9)	
HP[a]					
Positive	No. (%)	7 (13.2)	29 (36.7)	10 (16.7)	0.002
Negative	No. (%)	46 (86.8)	50 (63.3)	50 (85.3)	
IM					
Positive	No. (%)	—	7 (8.5)	42 (62.7)	<0.001
Negative	No. (%)	53 (100.0)	75 (91.5)	25 (37.3)	

ACAG, autoimmune chronic atrophic gastritis; CTRL, controls, general population; FDR-GC: first-degree relatives of patient with gastric cancer; HP, *Helicobacter pylori*; IM, intestinal metaplasia.
*P*c: Bonferroni corrected value of analysis of variance (ANOVA) for gender, *Helicobacter pylori*, and IM covariates.
[a]The sum does not add up to the total because of missing values.

undergone gastroscopy but had no endoscopic and histopathological esophagogastric lesions.

Statistical analysis. The Shapiro–Wilk test was used to assess the normal distribution of the data. Where values were normally distributed, the *t*-test for independent samples or the one-way analysis of variance were performed for comparisons of quantitative data among the groups. The Mann–Whitney *U*-test and the Kruskall–Wallis test were applied when the assumption of normality was not confirmed. Fisher's exact test was used to analyze group differences in qualitative data. The effects of logistic factors and PGs or G17 on the risk of IM were expressed as odds ratio (OR) and 95% confidence intervals (CIs). Receiver operating characteristic (ROC) curves were constructed to extract the corresponding cutoff values for the respective groups. The cutoff values from each evaluation were use to determine sensitivity and specificity. Multivariate logistic regression analysis was used to examine the relationship between variables and patient characteristics. All tests were two sided, and a significance level of 0.05 was selected. Linear regression line was used to determine the impact of age on PG1 serum level.

RESULTS

General characteristics of the patient groups. Table 1 shows the means and number of variables for the CTRL, FDR-GC, and ACAG. Analysis of variance revealed significant differences in age (mean years 54, 47, and 54 in CTRL, FDR-GC, and ACAG, respectively), gender (male: 55%, 44%, and 24%, respectively), *H. pylori* infection (13%, 37%, and 17%, respectively), and the presence of metaplasia (0%, 9%, and 63%) among the patient groups. Female gender and metaplasia were most frequent in the ACAG group, whereas *H. pylori* infection was more frequent in the FDR-GC group.

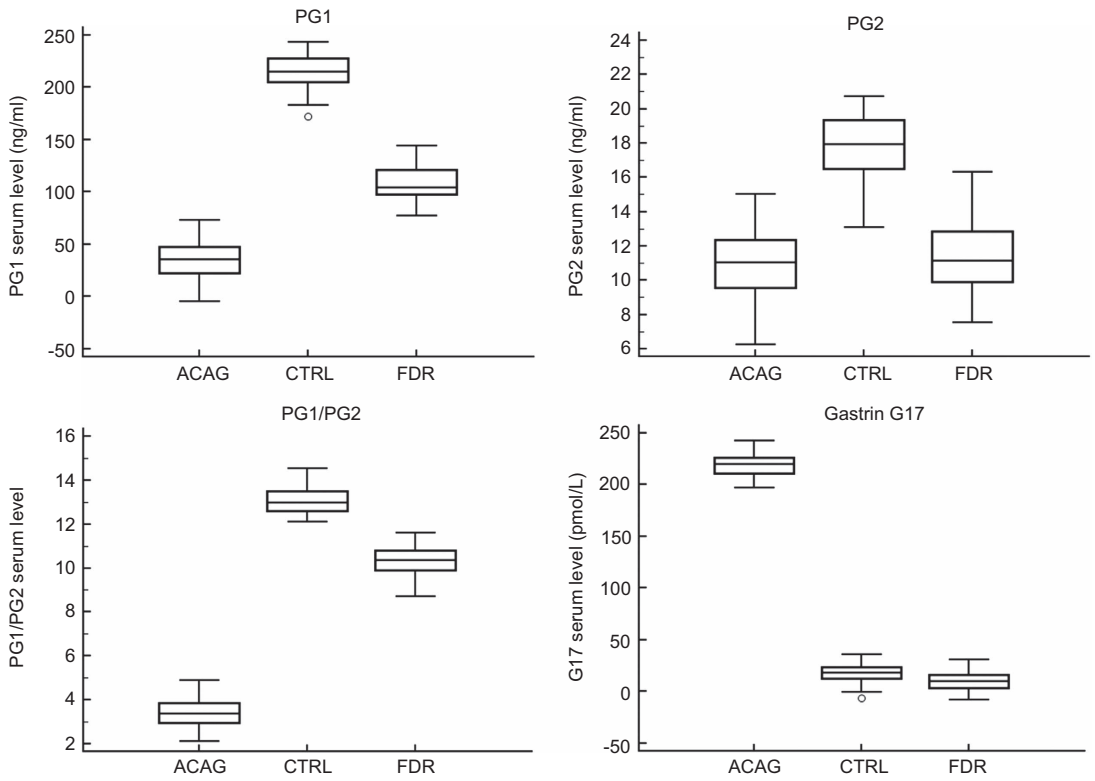
Variables and mean concentrations of serum PGs and gastrin G17 among CTRL, ACAG, and FDR-GC individuals (mean ± s.e.m.). The overall means of PG levels and gastrin G17 distribution were statistically different among the groups: the mean levels of PG1 and PG2 and the PG1/PG2 ratio were higher in CTRLs, whereas the level of gastrin G17 was markedly higher in ACAG (Figure 1).

Results of multiple comparison graphs showed that the serum level of PG1 and the PG1/PG2 ratio were the lowest in patients with ACAG, whereas the mean of PG2 levels were similar in ACAG and FDR-GC. The mean serum G17 level was found to be the best marker for discriminating ACAG in all groups tested (Figure 1).

In the multivariate analysis, we tested patient age, male gender, PG1, PG2, gastrin G17 levels, and PG1/PG2 ratio, and the variables were retained in the model if $P<0.05$ (Table 2). Age (OR 1.05, 95% CI 1.00–1.09), PG1 level (OR 0.98, 95% CI 0.97–0.99), and PG1/PG2 ratio (OR 0.82, 95% CI 0.71–0.95) were identified as independent predictive factors of metaplasia, whereas the presence of a histological *H. pylori* infection was related to PG2 (OR 1.08, 95% CI 1.02–1.14) serum level. The level of PG1 approached a borderline statistical significance ($P=0.054$).

Of note, we found that the mean values of PG1 serum level decreased with the presence of IM. The summary sensitivity and summary specificity for IM diagnosis were 85.71 and 85.00, respectively, with a cutoff value of PG1 $\leq$58 ng/ml, as determined by ROC curve analysis performed in 202 individuals (53 CTRLs, 82 FDR-GC, and 67 ACAG; data not shown). Considering that generally the PG1 value was found lower in ACAG than in FDR-GC (Figure 1), and that being female is a significant risk factor for several autoimmune diseases, it is plausible that a genetic mechanism determines the association of IM and female gender in ACAG. Box plot analysis of gastritis stage by using intestinal IM (OLGIM stages) with PG1 serum level demonstrated a significant decrease in the PG1 serum level in association with an increase in OLGIM stage (Figure 2a, $P<0.001$). By incorporating age as a covariable into the ROC curve analysis, we identified a PG1 cutoff of $\leq$47.9 ng/ml to be most discriminatory for an OLGIM stage $\geq$2 (Figure 2b). The area under the curve (AUC) value was 0.978 (95% CI 0.948–0.994), with a sensitivity of 95.83 and a specificity of 93.37.

The median PG2 serum level increased with the presence of *H. pylori* infection; using ROC curve analysis the summary sensitivity and summary specificity for an histological *H. pylori* infection were 69.6 and 50.1, respectively, with a cutoff value of PG2 $\leq$12.4 (Figure 3). The accuracy of the AUC value increased if we considered individuals without IM (AUC = 0.599 compared with AUC = 0.624 for individuals without IM; data not shown).



Variable	n	PG1 Mean ng/ ml (Std)	PG2 Mean ng/ ml (Std)	PG1/PG2 ratio	G17 Mean pmol/L (Std)
CTRL	53	215.7 (15)	18.3 (2)	13.2 (0.6)	19.3 (16)
FDR-GC	82	110.2 (13)	11.2 (2)	10.0 (0.5)	14.1 (14)
ACAG	67	40.7 (13)	10.8 (2)	3.5 (0.5)	221 (15)
p[c]		***<0.001***	***0.001***	***<0.001***	***<0.001***

Figure 1 Box-and-whisker plots of age- and gender-adjusted means of pepsinogens 1 and 2 (PG1 and PG2), PG1/PG2 ratio, and gastrin G17 for comparison of patients and control groups. Mean and s.e. are reported in more detail in the graph below. Median PG1 level and PG1/PG2 ratio were found significantly decreased in individuals at risk for GC (i.e., ACAG and FDR-GC) compared with controls. Gastrin G17 showed the highest mean level associated with ACAG status. ACAG, autoimmune chronic atrophic gastritis; CTRL, general population; FDR-GC, first-degree relatives of patient with gastric cancer. *P*c: Bonferroni corrected value of analysis of variance (ANOVA) for age and gender.

Attempts to improve the performance of the PG test to identify patients with high-risk OLGIM stages. In order to discriminate among FDR-GC and ACAG patients with a high-risk OLGIM stage according to the results reported and based on OLGIM stage ≥ 3 as a risk factor for GC development, we proposed to use a combined gastrin G17-PG1 score (illustrated in Figure 4) for the assessment and management of patients with an OLGIM stage ≥ 2 to take into consideration for gastroscopy. Premalignant OLGIM stage 3, identified from pathologist examination of the biopsies, would be considered for strict endoscopic surveillance. Several PG cutoff points have been used to evaluate gastric atrophy and GC risk in clinical practice, cutoff of PG1 ≤ 70 ng/ml and PG1/2 ratio ≤ 3.0 were the most common,[29,30] with lower cutoff point indicating a more severe atrophy and therefore a greater cancer risk. By using PG1 ≤ 70 ng/ml and PG1/2 ratio ≤ 3.0 as markers in our series, we discriminated 49 individuals at risk for preneoplastic lesions, of whom 17 cases showed an OLGIM stage 2 and 4 showed an OLGIM stage 3; by using our model in the same series of individuals we predicted an OLGIM stage ≥ 2 in 61 cases, of whom 16 cases had an OLGIM stage 2 and 6 had an OLGIM stage 3.

DISCUSSION

Serum PG measurements have significant value in screening for atrophic gastritis and in predicting the risk for GC. The test is inexpensive and noninvasive; the cutoff value commonly used in Japan is PG1 <70 ng/ml and PG1/PG2 ratio <3,[29,30]

Table 2 Prognostic factors for presence of metaplasia and histological HP infection by multivariate regression model

Multivariate analysis[a]	OR	Cases ($n=202$)	
		95% CI	*P*
Metaplasia predictors			
Age	1.05	(1.0017–1.0893)	0.04
PG1	0.98	(0.9657–0.9947)	<0.01
PG1/PG2	0.82	(0.7065–0.9510)	<0.01
HP infection predictors			
PG1	0.99	(0.9921–0.9999)	0.054
PG2	1.08	(1.0212–1.1410)	<0.01

CI, confidence interval; HP, *Helicobacter pylori*; OR, odds ratio; PG1, pepsinogen 1; PG2, pepsinogen 2.
Variables tested: age, gender, presence of HP (for metaplasia predictors) or intestinal metaplasia (IM, for HP infection predictors), PG1, PG2, PG1/PG2, and G17 levels.
$P<0.05$ values indicate statistical significance.
[a]Stepwise logistic regression with dependent variable retained if $P<0.05$, excluded if $P>1$.

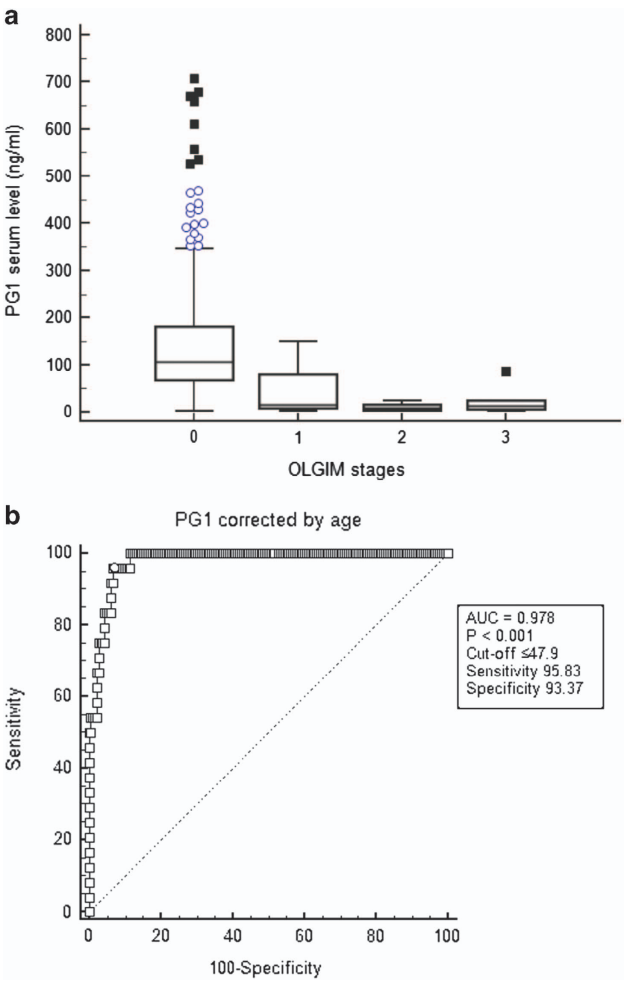


Figure 2 Box plot data of PG1 serum level based on OLGIM stages (**a**). Area under the PG1 serum value ROC curve (AUC) for the diagnosis of OLGIM stage >2 (**b**). Box plot analysis of gastritis by using the OLGIM stage system with PG1 level indicated a decrease in the PG1 level that was associated with worsening of the OLGIM stage. ROC curve analysis of PG1 level corrected for age of patients at diagnosis predicts the presence of an OLGIM stage ≥2. AUC, area under the ROC curve; OLGIM, Operative Link on Gastric Intestinal Metaplasia Assessment; PG1, pepsinogen 1; ROC, receiver operating characteristics.

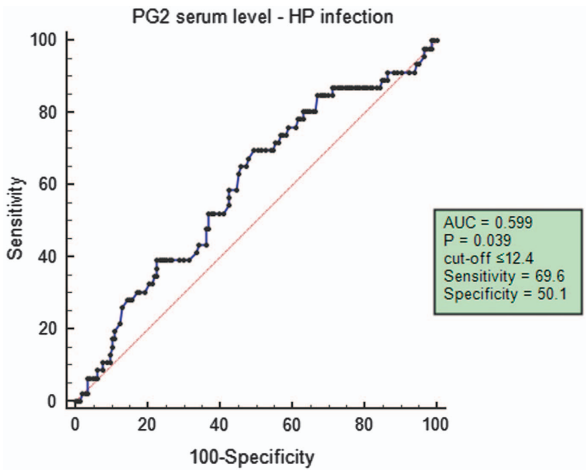


Figure 3 ROC curve analysis of PG2 levels for discriminating individuals with *Helicobacter pylori* (HP) infection. ROC curve analysis of PG2 levels discriminates individuals with *H. pylori* infection from those without a proven histological presence of the bacteria. PG2 values were corrected for age and gender covariables. AUC, area under the ROC curve; PG2, pepsinogen 2; ROC, receiver operating characteristic.

but this showed a low specificity (<75%) and high levels of heterogeneity among studies.[4,30–35] Geographic and ethnic variations in the population, as well as different clinical and histopathological entities, may cause the differences in the optimal cutoff values for screening tests. Follow-up endoscopy in patients with chronic atrophic gastritis in order to find GC at an early stage is not cost effective in a region with medium/low incidence of GC, but this may be improved by the identification of patients with high-risk OLGIM stages (>2) in selected populations at risk for GC (i.e., FDR-GC and ACAG).

In our population, the best marker for discriminating ACAG was gastrin G17 (Figure 1). This hormone is almost exclusively produced by the antrum G-cells and its production is regulated by gastric acid secretion. Thus, as occurs in the ACAG cases, G17 results at high level as an indicator of the inhibition of acid secretion because of the destruction of the parietal cells and loss of the feedback inhibition of gastrin secretion.[4,34,36–39] In our ACAG population the age- and gender-corrected median for gastrin G17 concentration was 219 (s.d. 7.5). Identification of ACAG cases with higher gastrin G17 concentration could be important as gastrin is involved in cell proliferation, migration, invasion, angiogenesis, apoptosis, maintenance of gastric stem cells, and hypertrophy of enterochromaffin-like cells.[40–42] This last effect is hypothesized to be one mechanism to explain the malignant transformation of enterochromaffin-like cells into carcinoid tumors that are more frequently found in patients affected by ACAG and pernicious anemia than in the general population (a 13-fold higher risk than in the general population).[42] However, hypergastrinemia may also be caused by regular proton pump inhibitor therapy, and consequently these data could be extrapolated during gastrin G17 test. In this study, none of the patients tested were treated with proton pump inhibitors.[43,44]

We also found a significant decrease in the level of PG1 in ACAG and FDR-GC compared with the CTRLs (Figure 1). There are two main causes of athrophic gastritis resulting in

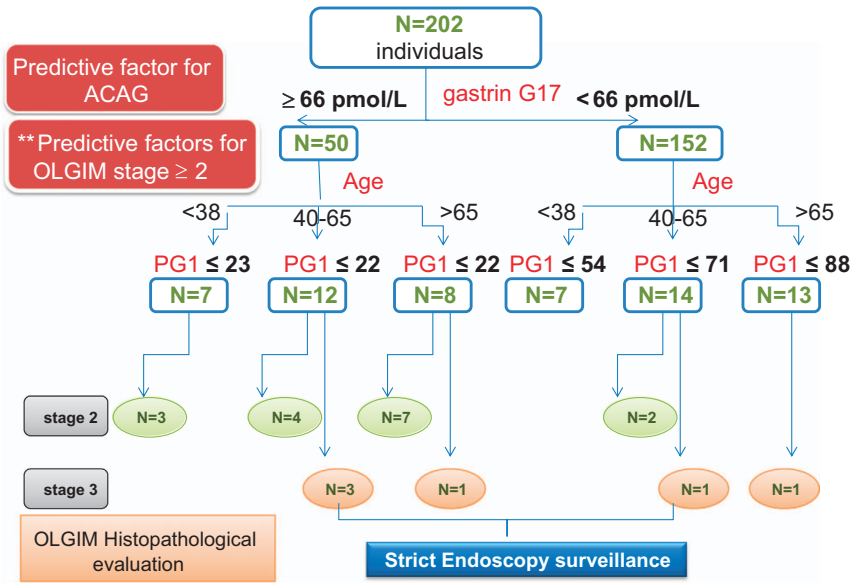


Figure 4 Predictive risk stratification model for advanced OLGIM stages. A combination of gastrin G17 and PG1 serum levels was used to define a model for predicting OLGIM stage ≥ 2. The number of patients resulting in each category is indicated (*N*). **Linear logistic regression was used to assess the impact of age on PG1 serum level. To this aim we select the optimal G17 cutoff value obtained by ROC curves; the obtained equation was PG1 = 23 − 0.01006× for G17 value > 66 pmol/l and PG1 = −12 + 1.6636× for G17 < 66 pmol/l, respectively. Risk stratification based on these factors stratified patients with a good performance: 24 of the 202 cases who were tested by pathologists showed an OLGIM stage ≥ 2 (18 with stage 2 and 6 with stage 3); 22 cases (16 cases with stage 2 and all of the 6 cases with stage 3) were correctly predicted using the proposed model. ACAG, autoimmune chronic atrophic gastritis; OLGIM, Operative Link on Gastric Intestinal Metaplasia Assessment; PG1, pepsinogen 1.

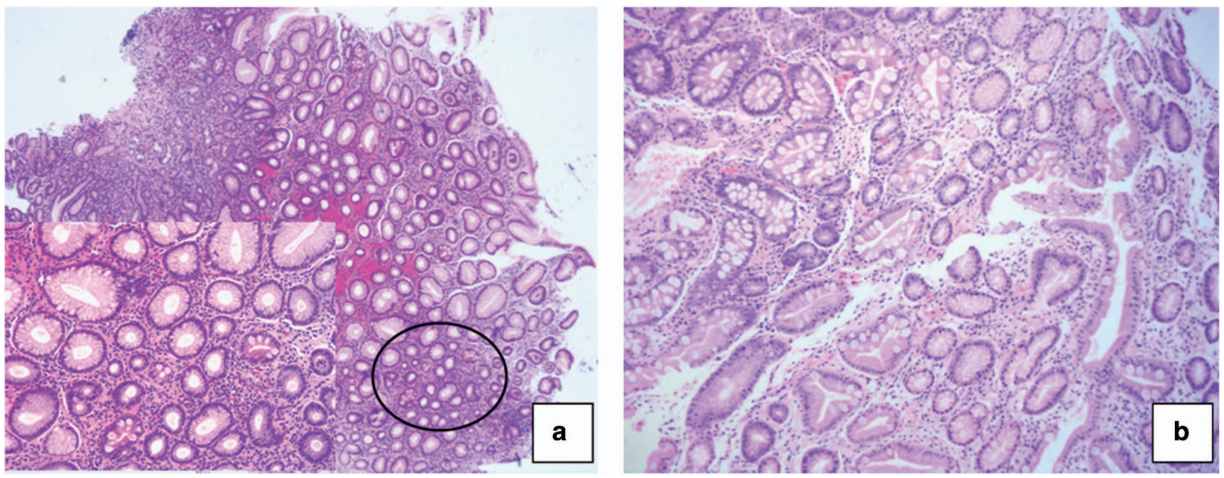


Figure 5 Images of representative OLGIM score 1 and score 3 diagnostic histological biopsies. In the gastric mucosa, atrophy is defined as the "loss of appropriate glands." Intestinal metaplasia (IM) is defined as replacement of gastric glands by glands with a different (intestinal) commitment. (**a**) Scattered glands showing that IM changes (OLGIM score 1) are evident in this antrum biopsy (active chronic gastritis is also present throughout the biopsy) (insert: higher magnification image of metaplastic glands showing goblet cells). (**b**) Diffuse metaplastic changes involving nearly 80% of all gastric glands could be identified in this antrum biopsy (OLGIM score 3). OLGIM, Operative Link on Gastric Intestinal Metaplasia Assessment.

distinct topographic types of gastritis: *H. pylori*-associated atrophic gastritis, which is usually a multifocal process involving antrum, corpus, and fundus of the stomach, as well as autoimmune gastritis, which is essentially restricted to the gastric corpus and fundus.[34,42,45,46] Data from our series were in agreement with this model, as in the presence of corpus atrophy, such as that present in ACAG, the PG1 serum level markedly decreases, whereas PG2 slightly diminishes compared with that found in FDR-GC. Moreover, we noted a decrease in PG1 levels with the presence of IM in both ACAG and FDR-GC endoscopic biopsies. Low serum levels of PG1 (< 28 ng/ml) found 2 years before IM was diagnosed in 4 patients affected by ACAG even more support this association (data not shown). However, probably because ACAG, like other autoimmune diseases,[47] has shown highest incidence in females, PG1 level better discriminated ACAG patients of the female gender, whereas it better discriminated FDR-GC patients of the male gender (data not shown).

By combining results obtained in this study, we propose an algorithm to discriminate patients at risk for OLGIM staging (≥2) for gastroscopic evaluation and histopathological evaluation in ACAG and FDR-GC population at risk for GC (Figures 4 and 5). Cases with a higher GC risk (OLGIM stage 3) would be included in a strict endoscopic follow-up. Furthermore, as individuals with a high PG2 serum levels (≥12.4 ng/ml) showed an association with a histological *H. Pylori* infection (Figure 3), and this association was more evident in patients without IM (data not shown), it is probable that in the more advanced stage of *H. pylori* infection, when atrophy is extensive and metaplasia is established, the environment becomes unfavorable for *H. pylori* persistence.[48–50] Recently it was suggested that as these individuals are at the last stage of atrophy, they are probably at the highest risk for progression to GC.[45]

In conclusion, considering the elevated cost and invasive nature of endoscopy, gastrin G17 and PG1 serum testing may be a relatively inexpensive method for identifying, among selected ACAG and FDR-GC patients at risk for GC, those with an additional important risk factor for GC who should be referred for endoscopic surveillance. This model could offer the advantage of selecting from the general population patients with autoimmune athrophic gastritis, and in FDR-GC and ACAG populations at risk for GC, individuals with a high probability of having OLGIM stage ≥2 who would then be subjected to gastroscopy. Among selected individuals with high-risk OLGIM stage 3 confirmed by histopathology, endoscopic surveillance could be used to potentially screen for detecting early dysplastic and cancerous lesions. A primary limitation to this study is that data will need to be validated in further cohorts and in a prospective study; nonetheless, the results could be useful for improving the clinical value of a combined gastrin G17/PG1 test for GC screening in regions with low GC incidence. In addition, although waiting for consistent data regarding the effect of *H. pylori* eradication in infected individuals (The European *H. pylori* Study Group, presented in the Maastricht IV Consensus Report[51]), we expect that patients with high-risk OLGIM stage >3 and especially those with a PG2 <12 ng/ml (*H. pylori* negative) have the highest risk for GC.

CONFLICT OF INTEREST

Guarantor of the article: Renato Cannizzaro, MD.
Specific author contributions: Valli De Re analyzed and interpreted data and wrote the paper; Enrico Orzes analyzed and interpreted data; Vincenzo Canzonieri and Paolo De Paoli planned the study; Stefania Maiero, Mara Fornasarig, Silvia Cervo, Agostino Steffan, Giorgio Zanette, and Cinzia Mazzon conducted the study; Lara Alessandrini conducted the study and analyzed and interpreted data; Renato Cannizzaro planned and conducted the study, and analyzed and interpreted data. All authors have approved the final draft submitted.
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Potential competing interests: None.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Gastric cancer (GC) remains a significant medical and social problem.
- ✓ Atrophy, intestinal metaplasia, and *Helicobacter pylori* infection are the most important risk factors for nonhereditary gastric adenocarcinoma development.
- ✓ Endoscopic follow-up of patients at high risk for GC and *H. pylori* eradication are effective approaches to reducing GC incidence.
- ✓ Pepsinogen and gastrin G17 testing provides important clinical data useful in predicting atrophy in gastric mucosa; however, its utility in the detection of patients with premalignant and malignant lesions is not sufficiently cost effective, especially in low- and medium-risk areas.

WHAT IS NEW HERE

- ✓ We found that gastrin G17 was the best marker for discriminating patients affected by autoimmune atrophic gastritis.
- ✓ Patients with Operative Link on Gastric Intestinal Metaplasia Assessment (OLGIM) stage ≥2 were found to be significantly associated with a mean reduction in the serum pepsinogen 1 (PG1) level and an older age compared with patients with OLGIM stage 0–1.
- ✓ PG2 level combined with high-risk OLGIM stage might be a marker to identify a subgroup of patients who had probably cleared the *H. pylori* infection.
- ✓ We proposed an algorithm based on gastrin G17-PG1 level and age of patients for initial assessment and management of patients with a high-risk OLGIM stage.

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