Autoantibodies Toward ATP4A and ATP4B Subunits of Gastric Proton Pump H+,K+-ATPase Are Reliable Serological Pre-endoscopic Markers of Corpus Atrophic Gastritis

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INTRODUCTION: **Noninvasive assessment of corpus atrophic gastritis (CAG), a condition at increased risk of gastric cancer, is based on the measurement of pepsinogens, gastrin, and *Helicobacter pylori* antibodies. Parietal cell autoantibodies (PCAs) against the gastric proton pump (ATP4) are potential serological biomarkers of CAG. The purpose of this study was to compare the diagnostic performance of PCA and pepsinogen I tests in patients with clinical suspicion of CAG with the histopathological evaluation of gastric biopsies as reference standard.**

METHODS: **A prospective case-finding study was performed on 218 naive adult patients (131 women, median age 65 years) who underwent gastric biopsies to confirm/exclude CAG. Patients with histopathological CAG were defined as cases, conversely as controls. Autoantibodies against the individual alpha (ATP4A) and beta (ATP4B) subunits of ATP4 were measured by luciferase immunoprecipitation, and global PCA and pepsinogen I by enzyme-linked immunosorbent assay.**

RESULTS: **Histopathology classified 107 subjects (49%) as cases (CAG+, autoimmune 81.2%, and multifocal extensive 18.8%) and 111 subjects (51%) as controls (CAG−). In cases, ATP4A, ATP4B, and PCA titers were increased compared with controls, whereas pepsinogen I was reduced ($P < 0.0001$ for all). ATP4B, ATP4A, and pepsinogen I tests showed sensitivities of 77%, 75%, and 73% and specificities of 88%, 88%, and 80%, respectively. The receiver operating characteristic (ROC) area under the ROC curve (AUC) of these serological biomarkers confirmed their ability to discriminate cases from controls (ATP4B = 0.838, ATP4A = 0.826, pepsinogen I = 0.775, and PCA = 0.805), whereas the partial ROC-pAUC$_{90}$ analysis showed that the ATP4B test had the best diagnostic performance ($P = 0.008$ vs ATP4; $P = 0.0002$ vs pepsinogen I). The presence of autoimmune or extensive gastritis was not significantly different between ATP4B positive or negative cases ($P = 0.217$).**

DISCUSSION: **PCAs are promising serological biomarkers for the identification of CAG in high-risk individuals, particularly in an autoimmune pattern but also in an extensive-multifocal atrophy pattern.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A396, http://links.lww.com/CTG/A397, http://links.lww.com/CTG/A398, http://links.lww.com/CTG/A399

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INTRODUCTION

Atrophic gastritis (AG) is a precancerous condition in which gastric dysplasia and cancer may occur (1,2). Extensive AG, affecting both the antral and corpus mucosa, is considered an advanced stage with an increased gastric cancer risk (3,4). AG is also linked to the development of gastric type 1 neuroendocrine

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tumors (5). The timely diagnosis of this precancerous condition is important because high-risk patients may benefit from regular endoscopic follow-up that could provide an early identification of neoplastic conditions, as recommended by the European guidelines (4). Importantly, serological assessments estimate the worldwide AG prevalence to be 23.9% and 27.0% in the general population and in the selected cohorts, respectively (6). Similarly, biopsy-based histological analyses suggest that the worldwide AG prevalence is approximately 33.4% and 31.6% in the same groups (6). Thus, AG may potentially affect 1 of 3 adults globally.

The histopathologic evaluation of gastric biopsies is the gold standard for diagnosing AG (7). Potential noninvasive alternatives include serological tests for pepsinogens, gastrin-17, and antibodies against *Helicobacter pylori* (*Hp*) (8–10). These "virtual" or "serological" biopsies may be useful for screening subjects at high risk of AG to identify those to refer for histological confirmation (6). Indeed, low pepsinogen I serum levels and/or a low pepsinogen I/II ratio can be used to identify patients with advanced stages of AG (11–16) in whom endoscopy is recommended, particularly if *H. pylori* serology is negative (5).

Autoantibodies against parietal cells (PCAs) are primarily directed against the gastric proton pump (ATP4) and are considered diagnostic markers of autoimmune gastritis and pernicious anemia, conditions characterized by the presence of corpus atrophic gastritis (CAG) (17). PCAs are currently used to screen patients with other autoimmune disorders such as autoimmune thyroid disease, type 1 diabetes, LES, and vitiligo for autoimmune gastritis (17–21). Circulating serum PCAs can be identified by indirect immunofluorescence, a semiquantitative operator-dependent method, or enzyme-linked immunosorbent assay (ELISA), a quantitative and generally more sensitive method (17,22,23). Recently, we developed luciferase immunoprecipitation system (LIPS) assays against the alpha (ATP4A) and beta (ATP4B) subunits of ATP4 (24) that showed a good diagnostic performance in patients with proven CAG (25).

Previous studies indicated that PCAs are prevalent in patients with CAG (5,25,26). Therefore, these autoantibodies may be suitable candidates as serological biomarkers of atrophic damage of the oxyntic mucosa and could be useful for noninvasive, pre-endoscopic assessment of CAG. Data on the effectiveness of PCA in a serological case-finding strategy for CAG are lacking. This study aimed to assess the diagnostic performance of PCA and pepsinogen I in a cohort of adult patients with clinical suspicion for CAG in comparison with a histopathological evaluation of gastric biopsies as reference standard.

METHODS

This study was drafted according to STARD 2015 guidelines to ensure the quality of reporting (27).

Study subjects

We performed a prospective case-finding study on 218 consecutive adult patients (131 women, 87 men, median age 65 years, interquartile range 53–77 years) presenting at our center between May 2017 and April 2018 with clinical suspicion of CAG. The inclusion criteria were the presence of at least one of the following conditions: anemia (iron or cobalamin deficiency), autoimmune disease, family history for CAG, or history of long-standing noninvestigated dyspepsia (for at least 12 months). The exclusion criteria were age younger than 18 years, a previous diagnosis of CAG, and a previous inclusion in an endoscopic surveillance program for gastric malignant conditions.

All patients underwent gastroscopy with standard biopsies for histopathology to confirm or to exclude CAG. Patients with a histopathological diagnosis of CAG were defined as cases; patients without a histopathological diagnosis of CAG were defined as controls.

For PCA, pepsinogen I, and *Hp* antibody tests, serum samples from each patient were drawn and preserved at −20°C.

Serological assays

Parietal cell autoantibodies. PCAs were detected by (i) LIPS assays targeting the ATP4A and ATP4B subunits individually and (ii) ELISA to detect global PCAs without differentiating between subunits. LIPS assays were performed as previously described (24,25,28) (threshold for negativity: <52 units for the ATP4A assay and <28 AU for the ATP4B assay). Global PCAs were measured by ELISA (Quanta LiteTM GPA; Inova Diagnostics, San Diego, CA) according to the manufacturer's instructions (threshold for negativity: <20 relative units/mL).

Pepsinogen I. Serological levels of pepsinogen I were assessed by ELISA (Biohit Oyi, Helsinki, Finland) (threshold for negativity: <30 ng/mL).

All serological assays were performed by operators blinded to the patients' identity and histopathological diagnosis. The LIPS assays were performed at the San Raffaele Diabetes Research Institute of the IRCCS Ospedale San Raffaele, Milan, Italy. The other serological analyses were performed at Sant'Andrea Hospital, University Sapienza, Rome, Italy.

***Antibodies against* Hp.** *Hp* antibodies were determined by ELISA (GAP test IgG, Bio-Rad, Milan, Italy) (threshold for negativity: <15 ng/mL).

All subjects (n = 218) were tested for ATP4A, ATP4B, and pepsinogen I according to the original study protocol. At a later point and based on sera availability, subsets were analyzed for global PCAs (n = 128) and *Hp* antibodies (n = 127) to provide additional data on comparison between the LIPS assays and the commonly used traditional ELISA.

Histopathology of gastric biopsies

The presence of CAG was defined by the histological confirmation of gastric corpus mucosal atrophy (26,29). All patients underwent gastroscopy with standardized biopsy sampling from the antrum (n = 3) and body (n = 3) mucosa for conventional histopathological examination. The degree of gastritis was assessed according to the updated Sydney System (7). Atrophy of the gastric corpus mucosa was defined as focal or complete oxyntic gland loss and/or their replacement by metaplastic pyloric or intestinal glands (26,29). In all patients, the gastric biopsy specimens were evaluated by an expert gastrointestinal pathologist (E.P.).

Patients with CAG were defined as those who had an active *Hp* infection when the bacterium was retrieved at the histopathological evaluation of gastric biopsies (26,29).

For the purpose of this study, the presence of dyspepsia was defined as the presence of recurrent, burdensome, upper gastrointestinal symptoms such as epigastric pain and/or burning, postprandial fullness, and/or early satiety for at least once a week in the past 3 months (30). The presence of anemia was defined as the presence of a hemoglobin concentration <14 g/dL for men and <12 g/dL for women (26,31). Autoimmune gastritis was defined on histopathological grounds, following the updated

Table 1. Main features of the 218 patients investigated for clinical suspicion of corpus atrophic gastritis

	Cases (n = 107 [49.1])	Controls (n = 111 [50.9])	*P*
Female sex	71 (66.4)	60 (54.1)	ns
Age, yr, median (interquartile range)	64 (18–88)	67.5 (23–90)	ns
Clinical suspicion of corpus atrophic gastritis			
Anemia	60 (56.1)	73 (65.8)	ns
Dyspepsia	36 (33.6)	30 (27.0)	ns
Miscellaneous (autoimmune diseases, family history)	11 (10.3)	8 (7.2)	ns
Helicobacter pylori infection			
Positive at histology	6 (5.6)	18 (16.2)	<0.01
Positive at serology	36/68 (52.9)[a]	35/59 (59.3)[a]	ns

When not otherwise indicated, data are expressed al number (percentage) or number (interquartile range).
ns, not significant.
[a]Of the 127 subjects tested for *H. pylori* antibodies.

Sydney criteria and including the presence of corporal mucosa atrophy with a spared antrum (7).

Informed consent was provided by all participants, and approval of local ethical committee was achieved (No. 5390/2019).

Statistics

An intended sample size of 101 cases and 124 controls was calculated by considering an estimated sensitivity of current serological tests of 70% (13,16) and hypothesizing an increase by about 20% by the use of LIPS PCAs (25) at type I (alpha, significance) and type II (beta, 1-power) errors of 0.05.

Data are expressed as median (range or interquartile range) and/or number/total (percentage). Differences between groups were analyzed by the Fisher exact test or the Mann-Whitney test, as appropriate. The diagnostic performance of serological assays was evaluated by receiver operating characteristic (ROC) curve analysis and expressed in sensitivity, specificity, and positive and negative predictive values. Both the full area under the ROC curve (ROC-AUC) and the partial ROC-AUC requiring a specificity greater than 90% ($pAUC_{90}$) of the different assays were compared (32,33). The correlation of serological biomarker positivity with age was tested by binomial logistic regression, whereas the correlation of titers with age was tested with the Spearman rank correlation test. For all statistical analyses, 2-tailed *P* values <0.05 were considered significant. Statistical analyses were performed by MedCalc Statistical Software version 19.0.4 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2019).

RESULTS

Patient demographics

After the histopathological evaluation of the gastric biopsies in all 218 patients, we classified 107 (49%) of the study subjects as cases (CAG present) and 111 (51%) as controls (CAG absent). The median age did not differ between cases and controls (cases: 64 years, range 18–88, vs controls: 67.5 years, range 23–90; Mann-Whitney test $P = 0.46$), although female sex showed a trend toward increased frequency in cases (66.4% vs 54.1%, $P = 0.073$).

Anemia and dyspepsia, the main reasons for the clinical suspicion of CAG, did not differ between cases and controls (anemia: 56.1% vs 65.8%; dyspepsia: 33.6% vs 27%, $P = 0.165$ and $P = 0.305$, respectively).

The presence of *Hp* was ascertained histologically in all patients, showing a higher prevalence of active infection in controls (cases: 5.6% vs controls: 16.2%, $P = 0.009$). The prevalence of antibodies to *Hp* was tested in 127 subjects and did not differ between the 2 groups (cases: 52.9% vs control: 59.3%, $P = 0.480$).

CAG was associated with a histological corpus-restrictive atrophy pattern suggestive of autoimmune gastritis in 81.2% of the cases and with a multifocal (extensive) atrophy pattern in the remaining 18.8% of the cases. The patients' main characteristics are summarized in Table 1.

Diagnostic performance of serological markers

Titers of ATP4A, ATP4B, and global PCAs were increased, whereas the pepsinogen I levels were reduced in cases compared with controls ($P < 0.0001$ for all, Mann-Whitney test) (Figure 1). Sensitivity was the highest for autoantibodies to ATP4B (77%), ATP4A (75%), and then pepsinogen I (73%). Similarly, specificity was the highest for ATP4B (88%), ATP4A (88%), and pepsinogen I (80%) (Table 2).

Sensitivity and specificity were also higher for ATP4B autoantibodies (74% and 94%, respectively) compared with global PCAs (69% and 91%, respectively) in the subset of subjects tested by ELISA (n = 128).

Hp antibodies were tested in 127 subjects and did not differ between cases and controls ($P = 0.392$, Mann-Whitney test; sensitivity and specificity of 47% and 44%, respectively).

The ability of the ATP4A and ATP4B and pepsinogen I tests to discriminate cases from controls was confirmed by the ROC curve analysis (ROC-AUC) ($P < 0.05$ for all) (Figure 2). The ROC-AUC includes regions of very low specificity irrelevant to most practical diagnostic applications. Therefore, as a more relevant proxy of diagnostic performance, we also calculated the partial ROC-AUC after imposing a specificity ≥90% ($ROC\text{-}pAUC_{90}$) (34). The ATP4B test had a significantly enhanced performance compared with both the ATP4A ($P = 0.008$) and pepsinogen I tests ($P = 0.0002$) (Figure 2). In addition, the ATP4A test performed better than pepsinogen I alone ($ROC\text{-}pAUC_{90}$: 0.028 vs 0.00, $P = 0.0245$). In the subgroups tested by ELISA for PCA or *Hp* antibodies, the ROC-AUC confirmed that only the PCA test discriminated cases from controls ($P < 0.05$) (see Figure S1, Supplementary Digital Content 1, http://links.lww.com/CTG/A396).

Diagnostic performance of serological marker combinations

Combined scores classifying the study subjects as positive in the presence of either reduced pepsinogen I levels and/or elevated autoantibodies (reduced pepsinogen I and/or ATP4A+; reduced pepsinogen I and/or ATP4B+; reduced pepsinogen I and/or PCA+) showed increased sensitivity but reduced specificity (Table 3). The ROC-AUC analysis confirmed the ability of selected combinations to discriminate cases from controls (ROC-AUC: pepsinogen I and ATP4A = 0.788; pepsinogen I and ATP4B = 0.875; pepsinogen I and PCA = 0.904, $P < 0.05$ for all) (see Figure S2, Supplementary Digital Content 2, http://links.lww.com/CTG/A397). None of the biomarker combinations

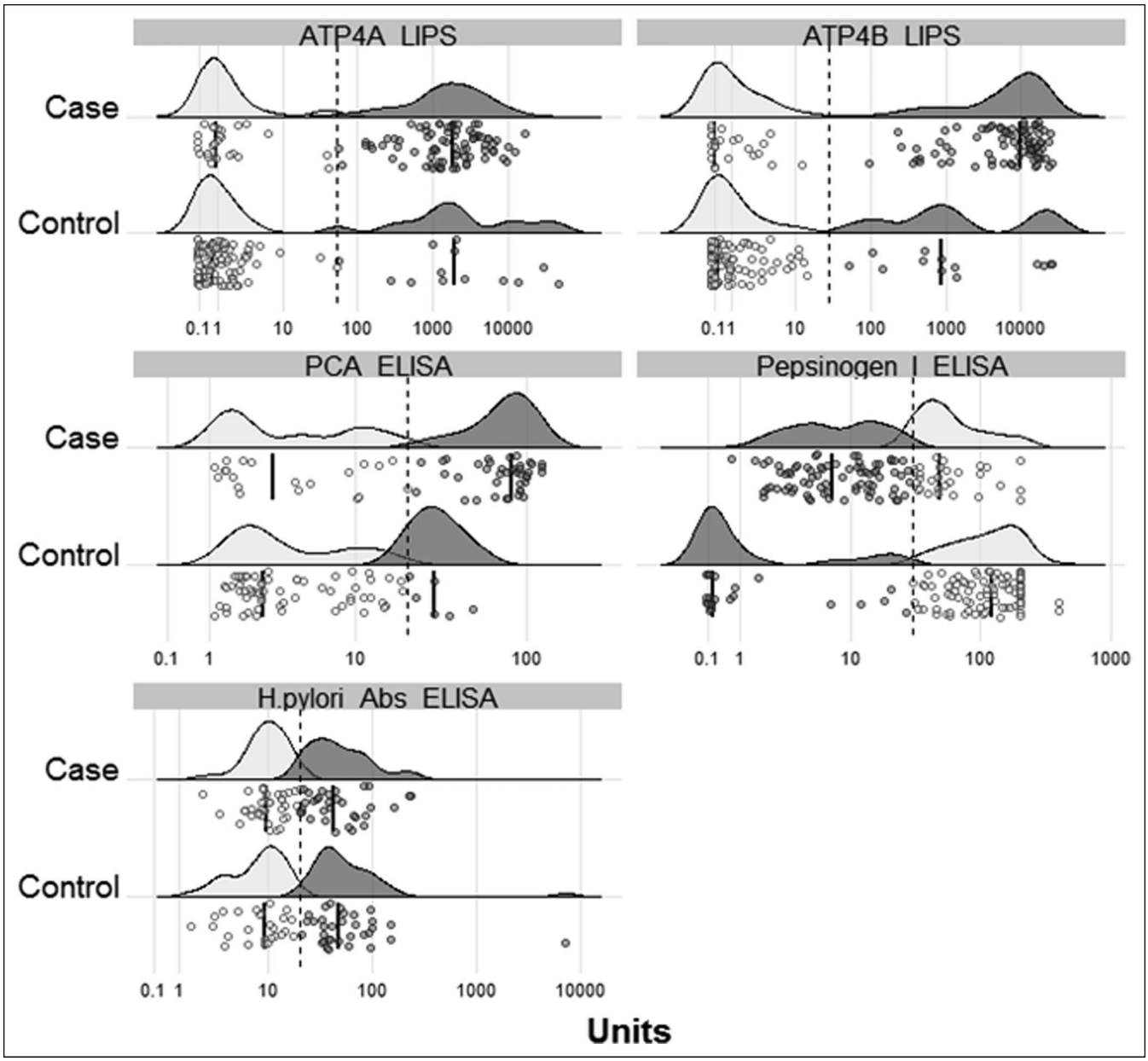

Figure 1. Biomarker distributions in corpus atrophic gastritis (CAG). The values measured in each subject with (case) or without (control) CAG are shown as circles. In addition, the overall probability density estimates in biomarker positive (dark gray fill) or negative (light gray fill) subjects are shown. The dashed vertical lines mark the threshold of positivity in each assay. Median values in biomarker positive and negative subgroups are indicated by solid black lines. ELISA, enzyme-linked immunosorbent assay; LIPS, luciferase immunoprecipitation system; PCA, parietal cell autoantibody.

significantly improved either the ROC-AUC or the ROC-$pAUC_{90}$ over that of the ATP4B test (Table 3).

Correlation of ATP4B autoantibodies with the histopathology of gastric biopsies

Histopathological features of gastric biopsies were stratified according to the presence or absence of ATP4B autoantibodies, the serological biomarker with the best individual performance (Table 4). The presence of corpus-restricted atrophy (typically associated with autoimmune gastritis) was not significantly different between ATP4B positive or negative cases ($P = 0.217$). Severe corpus atrophy and intestinal metaplasia were more frequent in cases with ATP4B antibodies ($P < 0.0001$ and 0.0488, respectively), but no correlation was present between ATP4B titers and severity of corpus atrophy ($P = 0.241$). Active *H. pylori* infection was rare and apparently more frequent in ATP4B autoantibody–negative subjects ($P = 0.02362$ and 0.0077, respectively), with the very low numbers strongly limiting statistical significance.

Relationship between age and serological markers

No significant correlation was observed between age and CAG ($P = 0.484$), and in cases, no significant correlation of age and seropositivity for ATP4B autoantibodies ($P = 0.543$) or other

Table 2. Diagnostic performance of single serological markers in patients at clinical suspicion of corpus atrophic gastritis

Serological markers	n	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Subunit ATP4A antibodies	218	75% (65–83)	88% (81–94)	86% (77–92)	78% (70–85)
Subunit ATP4B antibodies	218	77% (67–84)	88% (81–94)	86% (78–93)	80% (71–86)
Parietal cell antibodies	128	69% (56–80)	91% (82–97)	88% (75–95)	76% (65–85)
Pepsinogen I	218	73% (63–81)	80% (72–87)	78% (69–86)	75% (67–83)
Helicobacter pylori antibodies	127	47% (35–60)	44% (31–58)	49% (37–62)	42% (30–55)

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

serological biomarkers was evident (see Figure S3, Supplementary Digital Content 3, http://links.lww.com/CTG/A398). However, the proportion of ATP4B autoantibody–negative cases in subjects younger than 70 years of age was significantly lower than that in older individuals (31.5% vs 59.35, $P = 0.009$), although the overall prevalence of ATP4B autoantibodies showed a trend toward a reduction with increasing age that did not reach statistical significance ($P = 0.0952$) (Figure 3).

In cases, a weak negative correlation between ATP4B autoantibody titer and age was observed (rho = −0.220, $P = 0.0227$) that was no longer significant when only the ATP4B autoantibody–positive subjects were analyzed (rho = −0.1998, $P = 0.0718$). Similar findings were observed for ATP4A and PCA. No correlation between pepsinogen I titer and age was found (rho = 0.068, $P = 0.3201$) (see Table S1, Supplementary Digital Content 4, http://links.lww.com/CTG/A399).

Diagnostic performance of serological markers regarding anemia or dyspepsia

A subanalysis was performed to assess whether the diagnostic performance of serological markers was different regarding anemia and/or dyspepsia. None of the serological biomarkers differed in prevalence between anemic or dyspeptic cases (P = ns for all) (see Table S2, Supplementary Digital Content 4, http://links.lww.com/CTG/A399). Combinations of autoantibody biomarkers with pepsinogen I levels increased sensitivity and reduced specificity in both anemic and dyspeptic cases, but no statistically significant differences were observed between the 2 subgroups (P = ns for all).

DISCUSSION

The main finding of this study was that PCA showed a good pre-endoscopic diagnostic performance in patients with clinical suspicion of CAG. CAG is a condition increasing risk for developing gastric neoplasias (1,2), and a diagnostic delay may potentially be

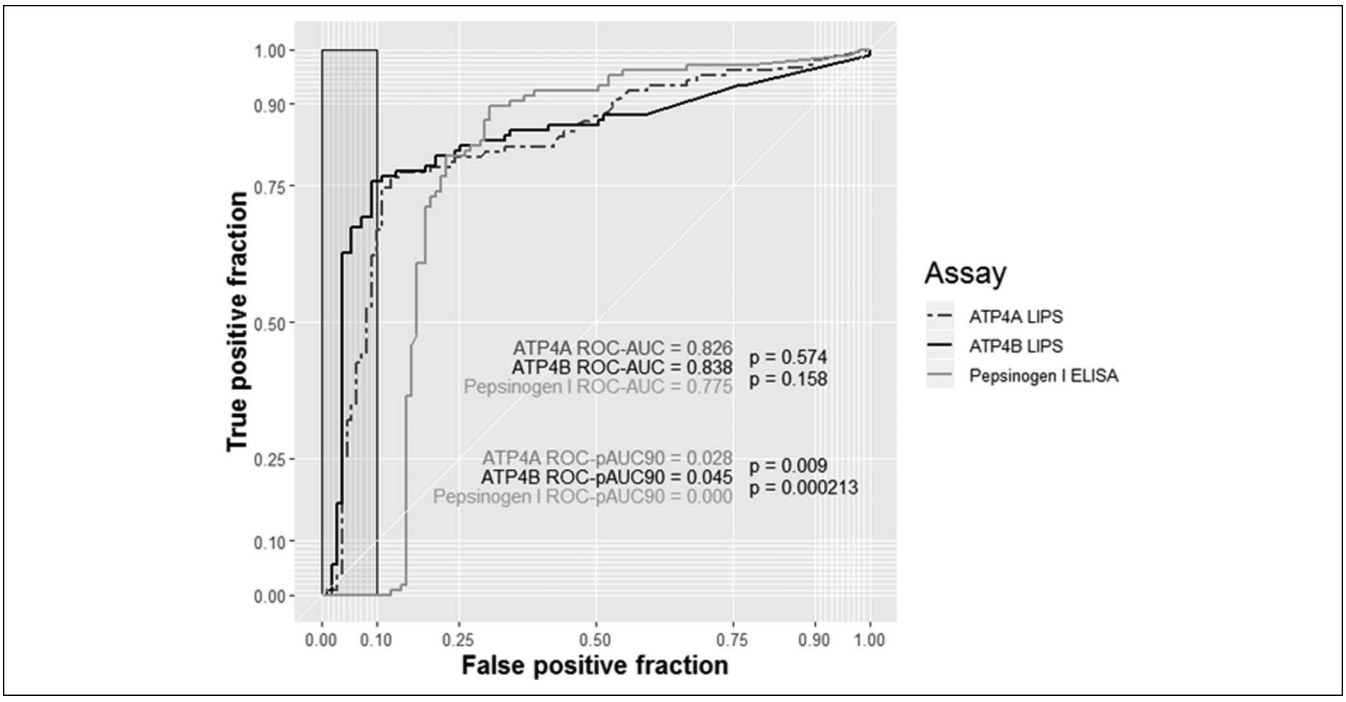

Figure 2. ROC curve analysis of selected CAG serological biomarker assays. The ROC-curves of the tests for ATP4A autoantibodies (dashed gray line), ATP4B autoantibodies (black line), and pepsinogen I levels (gray line) are shown. A rectangular background box highlights the partial area under the ROC curve after imposing a specificity ≥90% (ROC-pAUC$_{90}$). The P values shown are those of the ROC-AUC and ROC-pAUC$_{90}$ comparisons of the ATP4B vs ATP4A and of the ATP4B vs pepsinogen I tests. AUC, area under the ROC curve; CAG, corpus atrophic gastritis; ELISA, enzyme-linked immunosorbent assay; LIPS, luciferase immunoprecipitation system; ROC, receiver operating characteristic.

Table 3. Diagnostic performance of serological marker combinations compared with that of the single ATP4B test[a,b]

Serological markers	Tested subjects	ROC-AUC	*P* vs single ATP4B	ROC-$pAUC_{90}$	*P* vs single ATP4B	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ATP4A and/or pepsinogen I	218	0.788	0.2687	0.0461	0.0021[c]	84% (76–90)	72% (63–80)	74% (66–82)	82% (73–89)
ATP4B and/or pepsinogen I	218	0.875	0.1557	0.0074	0.8886	85% (77–91)	72% (63–80)	75% (66–82)	83% (74–90)
PCA and/or pepsinogen I	128	0.904	0.3576	0.0665	0.2792	87% (76–94)	78% (66–87)	78% (66–87)	87% (76–94)

AUC, area under the ROC curve; CI, confidence interval; NPV, negative predictive value; PCA, parietal cell autoantibody; PPV, positive predictive value; ROC, receiver operating characteristic.
[a]Single ATP4B test ROC-AUC = 0.8383 and ROC-AUC_{90} = 0.0455.
[b]In the subset of 128 patients tested for PCA the single ATP4B test showed ROC-AUC = 0.8718 and ROC-AUC_{90} = 0.0717.
[c]The single ATP4B test had better performance.

linked to irreversible long-term complications (35,36). The clinical spectrum of CAG is often not clearly defined (37), with symptoms including more frequent long-standing dyspepsia (38), mainly postprandial fullness (39), and deficiencies of erythropoietic micronutrients potentially leading to anemia (6,40,41). Currently, the serum PCAs are generally used for the noninvasive diagnostics of autoimmune gastritis, especially in patients with other autoimmune disorders (9,17–20). Neither current guidelines (4,6) nor physicians in clinical practice routinely use PCA as a serological screening tool to identify patients with CAG.

In the current study, all the patients underwent gastric biopsies after showing evidence of long-standing dyspepsia or anemia, irrespective of the clinical suspicion of gastric autoimmunity. Our results show that the measurement of PCAs by LIPS assay showed a sensitivity up to 77% and a specificity of 88%, with a ROC-AUC of 0.83. To the best of our knowledge, this study is the first to provide evidence that PCAs may be a potential screening tool in patients with clinical suspicion of CAG.

Combined serological tests assessing the functional or morphological status of the gastric mucosa, a strategy often referred to as "virtual" or "serological" biopsy (9–16), show close correlation with gastric precancerous conditions and have been proposed as a screening tool in gastric disease (8,42). According to recommendations of the European guidelines on the management of precancerous conditions of the stomach (MAPS II) (4), low pepsinogen I serum levels or/and low pepsinogen I/II ratio, already exhaustively validated as serological markers of AG, may identify patients with advanced stages of the disease, in whom gastroscopy is recommended, especially if *Hp* serology is negative (4,6). A meta-analysis reported the usefulness of pepsinogens for the noninvasive diagnosis of AG with a summary sensitivity and specificity of 69% and 88% despite substantial heterogeneity between studies (13). Similarly, a further meta-analysis of a panel test (combining pepsinogen, gastrin 17, and anti-*Hp* assays) for the diagnosis of AG reported a summary sensitivity and specificity of 74.7% and 95.6%, respectively (16).

In our study, pepsinogen I levels alone showed a reasonable diagnostic performance (ROC-AUC 0.77) for CAG but with a lower sensitivity and specificity compared with the autoantibody biomarkers. Our data provide evidence that the use of a PCA test might improve the differential diagnosis of subjects with strong clinical suspicion of CAG and that the combination of PCA and pepsinogen levels might further increase sensitivity with a reasonable penalty in specificity.

PCAs are routinely assessed for the noninvasive diagnosis of autoimmune gastritis, especially in patients with other autoimmune disorders (9,17–20), but the potential utility of the pepsinogen I test is not usually considered (9,42). In the current study, the diagnostic performance of all tested serological biomarkers was similar in subjects clinically presenting with anemia or dyspepsia, and the biomarker combinations led to an increased sensitivity (albeit slightly reduced specificity) in both anemic and dyspeptic subjects. These findings show that a serological panel composed of PCA and pepsinogen I may be used in subjects with the most common clinical presentations causing suspicion of autoimmune but also *Hp*-related extensive CAG.

The combination of functional serological tests of the gastric mucosa (e.g., pepsinogen I) with PCAs should allow identification of a larger spectrum of patients with oxyntic mucosa damage requiring gastroscopic/histological assessment for a definite diagnosis than using serological biomarkers for gastric autoimmunity or those for gastric precancerous conditions individually. Furthermore, our results suggest a potential inverse correlation between PCA and age, unlike for pepsinogen I levels. A potential loss of PCA positivity in elderly patients with CAG has been previously reported by us and others (43–45) and attributed to antigen loss because of the advancing disease. Therefore, we believe that a combination of serum pepsinogen I with PCA may provide a more accurate detection of CAG in elderly patients in whom long-term complications of undiagnosed CAG, such as gastric neoplasms, are more frequent (35).

Table 4. Stratification of gastric histological features according to positivity for ATP4B autoantibodies

Cases	ATP4B Ab positive n (%)	ATP4B Ab negative n (%)	*P*
Corpus restricted atrophy	67 (83.7)	15 (71.4)	0.2174
Severe corpus atrophy	47 (58.0)	4 (16.0)	<0.0001
Corpus intestinal metaplasia	60 (74.0)	13 (52.0)	0.0488
Antral atrophy	12 (15.0)	4 (19.0)	0.7379
Positivity to *Helicobacter pylori* corpus	2 (2.5)	4 (16.7)	0.0236
Positivity to *H. pylori* antrum	0 (0)	3 (14.3)	0.0077

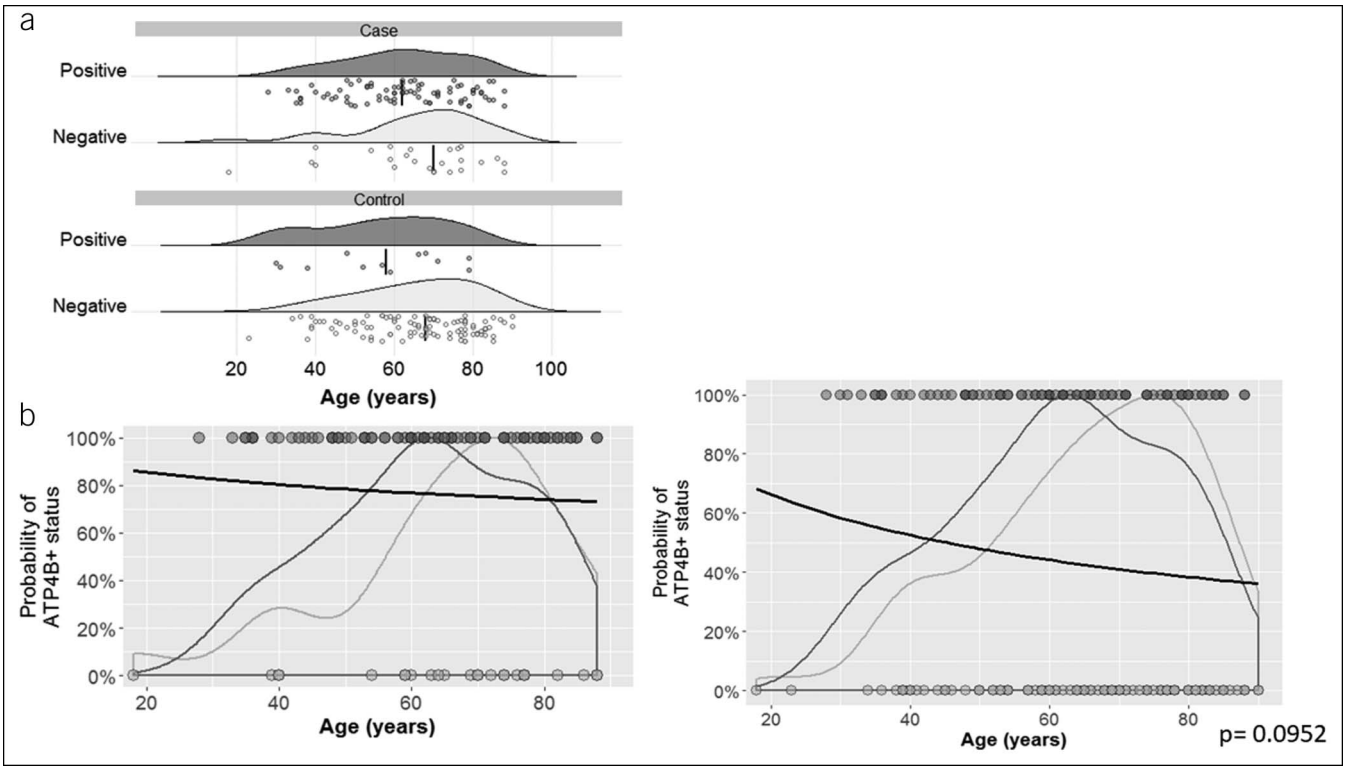

Figure 3. ATP4B autoantibody status according to age and presence of CAG. The age of each subject with (case) or without (control) CAG is shown as circles in ATP4B autoantibody positive (dark gray fill) or negative (light gray fill) subjects. (**a**) In cases, younger subjects are more likely to be ATP4B positive than negative. Shown are the corresponding age probability density estimates in ATP4B autoantibody positive (dark gray ridges) or negative (light gray ridges) subjects. Solid black lines stand for the median value. (**b**) Trend toward an increased prevalence of ATP4B autoantibody positive subjects with increasing age. The black line shows the probability of ATP4B autoantibody positive status according to age estimated by logistic regression. The corresponding age probability density estimates in ATP4B autoantibody positive (dark gray line) or negative (light gray line) subjects are super imposed after upscaling for visibility. CAG, corpus atrophic gastritis.

The mechanisms of the production of PCAs are not fully elucidated. The gastric proton pump H+/K+ ATPase located on parietal cells is the major target autoantigen recognized by PCAs (46). PCAs target both the alpha and beta subunits of the proton pump, albeit the alpha subunit is considered the major antigen (47). These autoantibodies are often associated with AIG but are found also in individuals with other autoimmune diseases, *Hp*-related CAG, or *Hp* infection without AG (17–20,24). Positivity to PCAs is found in up to 20.7% of *Hp*-infected patients (44). Older studies showed that the production of these antibodies seem to result from antigen mimicry between *Hp*-lipopolysaccharides and blood group antigens Lewis Y and X present on the beta subunit of the proton pump of the parietal cells (48) and that the cross-reacting antigen seem to be one of the surface proteins of *Hp* with strong immunogenic features similar to the human heat shock protein (49). It might be reasonably speculated that the gastric proton pump H+/K+ ATPase becomes an immunogenic antigen leading to PCA production when its epitopes are altered by inflammation and/or destruction. This might be possible as a consequence of autoreactive cytotoxic CD4+ T cells or of the *Hp*-induced inflammatory cascade. Basic studies are needed to provide data on this.

We are aware of some limits of the study, including the higher frequency of active *Hp* infection in controls than in cases. This unbalanced distribution between cases and controls may be explained by the prospective case-finding strategy study design as patients were consecutively recruited based on clinical suspicion of CAG, mainly presenting with persistent dyspepsia or anemia, both clinical manifestations of *Hp* infection (50,51). This higher prevalence of *Hp* infection among controls might have accounted for a lower specificity; *Hp* positivity was slightly more frequent in ATP4B and PCA negative patients than in positive patients, but statistical significance was missed, and our results should not have been seriously affected by this potential bias.

Among patients diagnosed with CAG, there was a high percentage (80%) of patients with corpus-limited atrophy and a spared antrum, possibly increasing the *a priori* probability of PCA positivity. However, the histological pattern of corpus-restricted atrophy was not different between autoantibody-positive and -negative patients so that this uneven distribution should not have seriously biased the results obtained.

A strength of our study is the use of both a commercial ELISA (17,22) and subunit-specific LIPS assay to monitor PCAs. Individually, both analytical approaches showed a good diagnostic performance, although LIPS assays, particularly the ATP4B test, performed better than the ELISA. Technical differences that may explain the LIPS' improved performance include the use of human recombinant antigens tagged with a highly active luciferase reporter and antigen autoantibody binding in liquid phase, with the likely detection of both conformational and linear epitopes. By contrast, solid phase assays such as direct ELISA often show a narrow dynamic range for measuring autoantibodies and a sub optimal detection of conformational epitopes, particularly after optimization for background noise (24,52,53).

In conclusion, our study strongly supports the concept that PCAs, detected by LIPS assay or ELISA, represent a promising serological biomarker of autoimmune but also extensive-multifocal CAG that might be used singly, or in combination with tests for pepsinogen, for

identifying those high-risk individuals most in need of histological diagnosis to rule out neoplastic complications.

CONFLICTS OF INTEREST

Guarantor of the article: Edith Lahner, MD, PhD.

Specific author contributions: Edith Lahner, MD, PhD, and Ilaria Marzinotto, PhD, contributed equally to this work. E.L., B.A., and V.L.: designed the study. E.L., I.M., V.L., L.D., C.L., and B.E.: contributed by conducting the study and collecting data. V.L., B.E., C.B., M.S., L.P., H.W.D., and J.M.W.: contributed by developing and/or performing the serological assays. E.L., I.M., and V.L.: analyzed and interpreted the results and drafted the manuscript. E.P.: performed histopathological evaluation of gastric biopsies. B.A. and H.W.D.: supervised the study and contributed to the completion of the draft. All authors approved the final draft submitted.

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Study Highlights

WHAT IS KNOWN

- ✓ Noninvasive assessment of corpus atrophic gastritis (CAG), known as "virtual" or "serological" biopsy," includes serological tests for pepsinogens, gastrin, and anti-*Hp* antibodies considered reliable tools to identify subjects at high risk of CAG to refer for gastroscopy with gastric biopsy for histological confirmation.
- ✓ Autoantibodies against parietal cells (PCA) are class G immunoglobulins (IgGs) directed against the gastric proton pump (ATP4) and considered diagnostic markers of autoimmune gastritis and pernicious anemia (conditions characterized by the presence of CAG). They are currently used as a serological marker to screen for autoimmune gastritis in patients with other autoimmune disorders such as autoimmune thyroid disease, type 1 diabetes, LES, and vitiligo.
- ✓ In addition to the global PCAs, ELISA and LIPS assays against the 2 subunits of ATP4 can detect PCAs with high specificity and sensitivity in patients with proven CAG.
- ✓ Data on the effectiveness of PCA as a serological case-finding strategy for CAG are lacking.

WHAT IS NEW HERE

- ✓ Assays of serum PCAs showed a good pre-endoscopic diagnostic performance in patients with clinical suspicion of CAG.
- ✓ PCAs may represent an effective screening tool in patients with clinical suspicion of CAG.
- ✓ Pepsinogen I levels showed a reasonable diagnostic performance for CAG but with a lower specificity compared with the autoantibody biomarkers.
- ✓ In subjects with high clinical suspicion of CAG, the diagnostic performance of the pepsinogen test alone can be increased by adding PCA assays to the serological panel.
- ✓ PCAs, detected by LIPS or ELISA, are a promising serological biomarker of CAG that might be used for stratifying high-risk individuals for further histological follow-up.

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