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Biomarkers, Autoantibodies, and Micronutrient Deficiencies in Gastric Precancerous Lesions

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I dedicate this work to the "Polki w Medycynie" Foundation Team. I dream of an increased presence of women researchers in Polish and international scientific communities.

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1. List of abbreviations

AIG	Autoimmune Gastritis
AIFA	Anti-Intrinsic Factor Antibody
AG	Atrophic gastritis
ANA	Anti-Nuclear Antibodies
APCA	Anti-Parietal Cell Antibody
AUC	Area Under Curve
CAG	Chronic Atrophic Gastritis
CLEIA	Chemiluminescent Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
G-17	Gastrin 17
GC	Gastric Cancer
GPL	Gastric Precancerous Lesions
H. pylori	*Helicobacter pylori*
IL-6	Interleukin-6
HE-4	Human epididymal protein 4
KL-6	Krebs von den Lungen 6
NAIG	Non-autoimmune Gastritis
NETs	Neuroendocrine tumors
PG	Pepsinogen
ROC	Receiver-Operating Curve
Se	Sensitivity
Sp	Specificity

2. Summary in English

Gastric cancer (GC), ranked as the fifth most prevalent cancer in the world, results in almost 800.000 deaths annually; early diagnosis is imperative to improve survival rates for patients with this cancer. Gastric precancerous lesions (GPL) precede the appearance of GC as a consequence of chronic infection with *H. pylori,* inducing non-atrophic gastritis, which may progress into chronic atrophic gastritis (CAG), intestinal metaplasia, dysplasia, and ultimately to GC. Another type of gastritis is autoimmune gastritis (AIG), which may also precede GC due to an autoimmune reaction. In this doctoral dissertation, various aspects of patients with GPL were examined, including non-invasive biomarkers, autoantibodies, and micronutrient deficiencies.

Article 1 assessed the diagnostic performance of serum pepsinogen I and II, and ratio (PGI, PGII, PG I/II ratio) measured by chemiluminescent enzyme immunoassay (CLEIA), as well as other biomarkers: interleukin-6 (IL-6), human epididymal protein 4 (HE-4), adiponectin, ferritin and Krebs von den Lungen (KL-6), for the detection of atrophic gastritis. Overall, the PG I/II ratio demonstrated 75.0% sensitivity and 92.6% specificity for the detection of moderate to severe corpus atrophic gastritis. While pepsinogens alone have limitations as biomarkers for the detection of antrum atrophic gastritis, IL-6 showed a promising sensitivity of 72.2% for this location. Combining the PG I/II ratio with HE-4 increased the sensitivity to 85.2% for detecting moderate to severe atrophic gastritis at any location. The study highlights the accuracy of pepsinogen testing for corpus atrophic gastritis. It suggests that IL-6 and HE-4 might be potential markers for antrum atrophic gastritis, offering insights into the early identification of individuals at risk for GC through serum biomarkers assessment.

Article 2 aimed to analyze the diagnostic value of pepsinogen testing for the diagnosis of atrophic gastritis by comparing two different diagnostic methods, CLEIA, and enzyme-linked immunosorbent assay (ELISA). Additionally, the article assessed the results according to the type (autoimmune *vs.* non-autoimmune) and location of atrophic gastritis. The study showed excellent diagnostic performances of PG I testing for detecting corpus CAG, with sensitivity and specificity of 92.7% and 99.1% for ELISA and 90.5% and 98.2% for CLEIA, respectively. For AIG, the corresponding values were 97.7% and 97.4% for ELISA and 95.6% and 97.1% for CLEIA. In conclusion, pepsinogens appear highly efficient for the detection of corpus-

limited CAG, especially for AIG. Subsequently, it allows to discriminate between autoimmune and non-autoimmune gastritis.

Article 3 aimed to search for the presence of autoantibodies in patients with GPL. Indeed, GC incidence has been shown to increase recently, especially in young female patients, with the underlying mechanism for this phenomenon remaining unknown but with the suggested role of autoimmunity. Since GPL precedes the development of GC, we aimed to test the possible existence of the stigmas of autoimmunity in patients with GPL. The study analyzed the prevalence of several autoantibodies in patients with GPL (AIG and *H. pylori*-related gastritis, NAIG) compared to control patients. Patients were tested for 19 autoantibodies (anti-nuclear antibodies, ANA, anti-parietal cell antibody, APCA, anti-intrinsic factor antibody, AIFA, and 16 myositis-associated antibodies). The frequency of ANA positivity was significantly higher in AIG than in NAIG or control patients (46.7%, 29%, and 27%, respectively, $p = 0.04$). Female gender was positively associated with ANA positivity (OR 0.51 (0.31–0.81), $p = 0.005$), while age and *H. pylori* infection were not. Myositis-associated antibodies were found in 8.9% of AIG, 5.5% of NAIG, and 4.4% of control patients, without significant differences among the groups ($p = 0.8$). Higher APCA and AIFA positivity was confirmed in AIG and was not associated with *H. pylori* infection, age, or gender in the multivariate analysis. Overall, the results of this study do not support an overrepresentation of common autoantibodies in patients with GPL, except ANA, which are significantly more frequent in AIG, but the clinical significance of this finding remains to be established.

Article 4 investigated micronutrient concentrations in patients with AIG, NAIG, and control patients to assess the prevalence of iron and vitamin B12 deficiencies and studied the associated factors. AIG exhibited significantly lower median vitamin B12 and ferritin concentrations than NAIG and controls. Vitamin B12 deficiency rates were 13.3%, 1.5%, and 2.8% in AIG, NAIG, and controls, respectively. Similarly, the median ferritin concentration was significantly lower in AIG than in NAIG and control patients, with iron deficiency presented in 28.9% of AIG, 12.8% of NAIG, and 12.9% of controls, respectively. Multivariate analysis demonstrated that AIG patients had a higher risk of developing vitamin B12 (OR 11.52 (2.85-57.64) $p=0.001$) and iron (OR 2.92 (1.32-6.30) $p=0.007$) deficiencies as compared to controls. Factors like age, sex, and *H. pylori* status did not affect the occurrence of micronutrient deficiencies. The study highlights the importance of screening for micronutrient deficiencies, particularly iron, in AIG patients and incorporating their management into treating patients with GPL.

In conclusion, these studies collectively contribute to understanding the diagnostic landscape of GPL, emphasizing the potential of serum markers like pepsinogens and shedding light on the associated factors, such as autoimmunity and micronutrient deficiencies.

3. Summary in Polish

Rak żołądka (GC), będący piątym pod względem częstości występowania nowotworem na świecie, prowadzi do około 800.000 zgonów rocznie na całym świecie. Wczesna diagnoza jest niezbędna, aby poprawić przeżywalność pacjentów chorych na ten nowotwór. Zmiany przedrakowe żołądka (GPL) zwykle poprzedzają wystąpienie GC i są najczęściej związane z zakażeniem *H. pylori,* wywołującym przewlekle zapalenie żołądka, które może przejść w przewlekłe zanikowe zapalenie żołądka (CAG), metaplazję jelitową, dysplazję, aż do raka żołądka. Innym, rzadszym, typem zanikowego zapalenia żołądka jest zapalenie autoimmunologiczne (AIG) które również może predysponować do rozwoju raka żołądka. W tej rozprawie doktorskiej zbadano różne aspekty pacjentów z GPL, w tym nieinwazyjne biomarkery, autoprzeciwciała i niedobory mikroelementów.

W artykule 1 oceniono skuteczność diagnostyczną badania pepsynogenu I, II i wskaźnika (PGI, PGII, wskaźnik PGI/II) w surowicy przy użyciu metody chemiluminescencyjnej (CLEIA), jak również innych biomarkerów: interleukiny-6 (IL-6), ludzkiego białka najądrza 4 (HE-4), adiponektyny, ferrytyny i białka Krebs von den Lungen (KL-6) do wykrywania GPL. Wskaźnik PGI/II wykazał czułość 75% i swoistość 92.6% w przypadku umiarkowanego do ciężkiego CAG. Podczas gdy pepsynogeny wykazują ograniczenia diagnostyczne w przypadku CAG zlokalizowanego w antrum żołądka, IL-6 wykazała obiecującą czułość na poziomie 72.2% w tym rozpoznaniu. Łącząc wskaźnik PG I/II z HE-4 uzyskano czułość 85.2% w wykrywaniu umiarkowanego do ciężkiego CAG w każdej lokalizacji. Badanie to pokazuje skuteczność diagnostyczną nieinwazyjnych biomarkerów w diagnostyce CAG, w tym dobre wskaźniki swoistości i czułości pepsynogenów oraz potencjalną rolę IL-6 i HE-4 jako nowych markerów zanikowego zapalenia żołądka.

Artykuł 2 miał na celu analizę wartości diagnostycznej oznaczania PG dla wykrywania zanikowego zapalenia żołądka, przez porównanie dwóch metod diagnostycznych- CLEIA i immunoenzymatycznej (ELISA) oraz w zależności od typu zapalenia żołądka (autoimmunologiczne i nie autoimmunologiczne) i lokalizacji CAG. Badanie wykazało doskonałe zdolności diagnostyczne PG I do wykrywania CAG, z czułością i swoistością na poziomie odpowiednio 92.7% i 99.1% dla testu ELISA oraz 90.5% i 98.2% dla CLEIA. W

przypadku AIG, odpowiednie wartości wynosiły 97.7% i 97.4% dla metody ELISA oraz 95.6% i 97.1% dla CLEIA. Podsumowując, PG są wysoce skuteczne w diagnozowaniu CAG ograniczonego do trzonu żołądka, szczególnie AIG, oraz pomagają odróżnić AIG od CAG wywołanych przez *H. pylori.*

Artykuł 3 miał na celu zbadanie obecności autoprzeciwciał u pacjentów z GPL. Częstość występowania GC wzrasta w ostatnich latach u pacjentów <50 roku życia, szczególnie u kobiet i chociaż mechanizm leżący u podstaw tego zjawiska pozostaje nieznany, sugeruje się rolę reakcji autoimmunologicznej w procesie kancerogenezy. Ponieważ GPL poprzedza rozwój GC, naszym celem było sprawdzenie obecności cech autoimmunizacji u pacjentów z GPL, poprzez zbadanie autoprzeciwciał u tych chorych. W badaniu analizowano częstość występowania autoprzeciwciał u pacjentów z GPL (AIG oraz zapalenie żołądka wywołane przez *H. pylori,* NAIG) w porównaniu z pacjentami kontrolnymi. Pacjentów badano na obecność 19 autoprzeciwciał (przeciwciała przeciwjądrowe, ANA, przeciwciała przeciw komórkom okładzinowym, APCA, przeciwciała przeciwko czynnikowi wewnętrznemu, AIFA i 16 przeciwciał związanych z zapaleniem skórno-mięśniowym). Wynik pozytywny ANA był istotnie wyższy u pacjentów z AIG niż u pacjentów z NAIG lub grupy kontrolnej (odpowiednio 46.7%, 29% i 27%, p = 0.04). U płci żeńskiej występował znamiennie wyższy odsetek dodatnich wyników ANA (OR 0.51 (0.31–0.81), p = 0.005), podczas gdy wiek pacjentów i zakażenie *H. pylori* nie wykazały takiego związku. Przeciwciała związane z zapaleniem skórno-mięśniowym stwierdzono u 8,9% pacjentów z AIG, 5.5% z NAIG i 4.4% pacjentów z grupy kontrolnej, bez istotnych różnic między grupami (p = 0.8). W grupie AIG, potwierdzono wyższy odsetek dodatnich przeciwciał APCA i AIFA, która w analizie wieloczynnikowej nie była powiązana z infekcją *H. pylori,* wiekiem ani płcią. Podsumowując, wyniki badania nie potwierdzają wyższej obecności autoprzeciwciał u pacjentów z GPL, poza wyższym odsetkiem dodatnich wyników ANA w grupie AIG, jednak znaczenie kliniczne tego faktu wymaga dalszych badań.

W artykule 4 zbadano stężenie mikroelementów (żelaza i witaminy B12) u pacjentów z AIG, NAIG i w grupy kontrolnej, aby ocenić częstość występowania tych niedoborów i czynników na nie wpływających. Pacjenci z rozpoznaniem AIG wykazali znacząco niższą medianę stężenia witaminy B12 i ferrytyny niż pacjenci z NAIG i grupy kontrolnej. Odsetek pacjentów z niedoborem witaminy B12 wynosił, odpowiednio, 13.3%, 1.5% i 2.8% w grupie AIG, NAIG i w grupie kontrolnej. Podobnie niedobór żelaza występował u 28.9% pacjentów z AIG, 12.8% NAIG i u 12.9% pacjentów z grupy kontrolnej. Analiza wieloczynnikowa wykazała, że u pacjentów z AIG ryzyko wystąpienia niedoborów witaminy B12 (OR 11,52 (2,85-57,64)

p=0,001) i żelaza (OR 2,92 (1,32-6,30) p=0,007) było wyższe w porównaniu z grupą kontrolną. Czynniki takie jak wiek, płeć i status *H. pylori* nie miały wpływu na występowanie niedoborów mikroelementów. Wyniki tego badania podkreślają znaczenie badań pod kątem niedoborów mikroelementów, szczególnie żelaza, u pacjentów z AIG, aby skuteczniej leczyć pacjentów z GPL.

Podsumowując, badania te wspólnie przyczyniają się do lepszej diagnostyki stanów przedrakowych żołądka, pokazują potencjał diagnostyczny biomarkerów z surowicy takich jak pepsynogen, jednocześnie rzucając światło na czynniki związane z GPL, takie jak autoimmunizacja i niedobory mikroelementów.

4. Introduction

4.1 Gastric cancer

4.1.1 Epidemiology

With more than one million new cases yearly, gastric cancer (GC) is the fifth most frequently diagnosed cancer, with almost 800.000 deaths annually, ranking the fourth cause of cancer-related death in the world [1]. Gastric cancer displays substantial global variation in incidence; the highest rates are observed in Eastern Asia (annual incidence rates up to 60/100,000 inhabitants), South America, and Eastern Europe (17/ 100,000). A gradual decline in the incidence of GC has been observed in Western Europe and North America (annual incidence rates varying from 5/100,000 to 10/100,000) [1]. Gastric cancer rates are two-fold higher in men than in women [1]. France, whose population was included in the studies of this doctoral dissertation, is classified as a low-risk GC area, with incidence rates around 7/100,000 in males and 2.6/100,000 in females [2]. The incidence rates in Poland are 2.5-fold higher than in France: 18.8/100,000 in males and 7.8/100,000 in females [3].

Gastric cancer was the leading cause of cancer death worldwide until the 1980s. Since then, GC incidence has been decreasing in parallel to the decreasing prevalence of its primary carcinogen, *Helicobacter pylori (H. pylori)* infection.

However, there is a worrying recent epidemiological trend in GC with a rising incidence in low-incidence countries such as the UK and the US among younger individuals (below 50 years), especially women [4,5]. The causal mechanism for this "new" type of GC has not been identified; however, an increase in autoimmune disorders in this age group and dysbiosis of the

gastric microbiome associated with modern lifestyles have been evoked as a causative factor [5–7].

Gastric cancer is a heterogeneous disease; different types of GC are distinguished according to their location: distal (non-cardia) GC and proximal (cardia) GC. These entities differ in terms of risk factors and epidemiologic patterns. Another heterogeneity can be seen in the histological subtypes. Historically, we distinguish 3 subtypes according to the Laurén classification: intestinal, diffuse, or mixed type [8]. According to the newer WHO classification of gastric cancer, we distinguish papillary, tubular, mucinous, signet-ring cell, poorly cohesive, mixed carcinoma, and other less common subtypes [9]. Gastric cancer classification systems are presented in Table 1. Additionally, there has been a recently developed molecular atlas of GC (TCGA), dividing gastric cancer into 4 molecular subtypes: Eppstein-Barr Virus positive (EBV-positive) GC (present in 9% of cases), microsatellite instable GC (22%), genomically stable GC (20%), and with chromosomal instability (50%).[10]

Table 1 Gastric cancer classification systems: WHO classification and Laurén classification

WHO classification (2019) [9]	Laurén classification (1965) [8]
Papillary carcinoma	
Tubular carcinoma	Intestinal type
Mucinous carcinoma	
Poorly cohesive carcinoma (including Signet-ring cell carcinoma)	Diffuse type
Mixed carcinoma	Mixed type
Other subtypes	-

WHO, World Health Organization

The intestinal non-cardia type is the most common (~80% of global cases), where almost all cases are attributed to chronic *H. pylori* infection. In contrast, cardia GC has a different etiology, with only a small proportion of cases linked to *H. pylori* infection [1]. Regarding the epidemiological pattern, cardia GC is more common in Western Europe and North America [1].

Up to now, GC screening programs have been only implemented in the countries with a high incidence of GC (e.g., Japan, South Korea, and China), enabling the diagnosis at the earlier

stage and improving survival. So far, there are no established screening programs for GC in Europe. However, there are currently ongoing European programs (EUROHELICAN, TOGAS, GISTAR) aiming at the evaluation of feasibility and the most appropriate modalities of screening programs in Europe [11].

4.1.2 Risk factors and genetic predispositions for gastric cancer

4.1.2.1 Risk factors for gastric cancer

The established carcinogens for non-cardia GC are infectious factors, mainly *H. pylori*, which is roughly responsible for over 80% of all GC cases. Dietary factors related to GC include alcohol use, high intake of salty and smoked food, and low consumption of fruit and vegetables [12]. Besides, older age, cigarette smoking, previous gastric surgery, and living in a population at high risk might be additional risk factors [13]. Gastric cancer demonstrates familial aggregation in ~10% of cases [14]. Although a family history of GC is a risk factor for gastric cancer, it is not clear whether it is caused by shared environmental factors, a genetic predisposition, or rather a multifactorial cause that may include these factors together. Additionally, according to TCGA, EBV-positive GC is more prevalent in the gastric corpus and fundus [10].

In contrast to distal GC, the most common risk factors for proximal (cardia) cancer are obesity and gastro-esophageal reflux [1,12,13]. The risk factors for the development of GC are presented in Table 2.

Table 2 Environmental, dietary, and lifestyle factors associated with gastric cancer.

	Cardia GC	Non-cardia GC
Infectious factors	*H. pylori* (part of cases)	*H. pylori, EBV*
Tobacco	Smoking	
Dietary factors	Low fruit and vegetable intake, high alcohol intake, high intake of processed food	
	Intake of hot beverages	Intake of salt and salty foods, pickled foods
	Obesity	
Family history	Positive family history of gastric cancer	
Other conditions	Gastro-esophageal reflux disease, Barret's esophagus	
Protective factors	High fruit and vegetable intake, physical activity	

EBV, Epstein-Barr virus; GC, gastric cancer; *H. pylori, Helicobacter pylori,*

4.1.2.2 Genetic predispositions for gastric cancer

Genetic mutations are responsible for around 3% of GC cases. Germline mutations include CDH1 gene mutation that encodes E-cadherin, responsible for cell-to-cell adhesion in epithelial tissues. Less common is in the CTNNA1 gene mutation (encoding alpha-E-catenin). Mutations in those genes predispose to hereditary diffuse gastric cancer, characterized by the presence of poorly cohesive gastric cancer and highly aggressive disease [14–16].

Another genetic syndrome associated with predisposition to GC is Lynch syndrome (germline mutation in one of the genes: MLH1, MSH2, MSH6, PMS2, EPCAM, leading to DNA mismatch repair deficiency and microsatellite instability, MSI, within the tumor). Lynch syndrome carriers have up to a 10% lifetime risk of GC [14].

Apart from that, patients with familial adenomatous polyposis, FAP (germline mutation in the adenomatous polyposis coli, APC gene), an autosomal dominant hereditary polyposis syndrome have an increased risk of GC. We distinguish two forms of FAP syndrome. The classic form of FAP is clinically defined by the presence of 100 or more synchronous colorectal adenomas, often associated with gastric and small intestine adenomas. Attenuated FAP is a less severe entity, defined as the presence of fewer than 100 adenomatous polyps [14]. Loss of function in both APC alleles is highly penetrant and causes polyp development in childhood,

leading to cancer in young adults. Patients with FAP have a 100% lifetime risk of cancer development unless prevented. Prevention includes endoscopic clearing of polyps or surgical resection of affected organs. Less commonly, the development of GC is associated with Li-Fraumeni syndrome (mutation in TP53 gene), Peutz-Jeghers syndrome, and Juvenile polyposis syndrome (mutation in STK11 and SMAD4, BMPR1A genes, respectively) [14].

4.1.3 Treatment modalities and outcomes in gastric cancer

The overall survival rates in GC are closely related to the stage. The overall survival rate, all stages included, is around 30% and has not been improved considerably during the last three decades [12,13]. In Poland, the 5-year survival rate in patients at all stages of GC is ~20% [3]. Whereas in patients with stage I disease, the 5-year survival rate is around 65% [17].

Locally advanced unresectable or metastatic GC has a poor prognosis; survival in clinical trials assessing the value of chemotherapy did not exceed one year [13]. In the field of medical treatment of advanced/metastatic GC, tremendous improvement has been observed over the last 2 decades. Advancement in the knowledge of GC molecular biology [10], notably in tumors with microsatellite instability, led to a change in the standard of care in this subgroup of patients. Other advancements in the treatment of patients with GC include the combination of different chemotherapeutical agents (fluoropyrimidines, platinum salts, and taxanes) versus single-agent chemotherapy [12]. Currently, the established predictors for the systemic treatment in locally advanced/metastatic GC are human epidermal growth factor receptor 2 (HER2) expression, Programmed Death Ligand 1 (PD-L1) according to combined positive score (CPS), and MSI-H/dMMR status [12,13]. The emerging predictors are claudin-18.2 and factor 2 isoform IIb receptor (FGFR2b) overexpression [18].

4.3.1.1. Treatment of localized gastric cancer

In the locally advanced, resectable tumors, in stages IB-III, adding perioperative chemotherapy based on the FLOT regimen (consisting of docetaxel, oxaliplatin, 5-fluorouracil, and leucovorin) helps to improve patient outcomes, with almost 50% of patients living more than 5 years based on the results of the phase II/III trial FLOT4 [19]. The future perspectives in the management of patients with localized GC with microsatellite instability (MSI-H/dMMR) include the usage of immunotherapy, based on the results of GERCOR NEONIPIGA phase II

study where perioperative immunotherapy helped to achieve a histological complete response in 58.6% of 29 included patients [20]. New approaches in the treatment of localized GC include adding immunotherapy to the FLOT chemotherapy as a part of the perioperative regimen. The preliminary results of the phase III MATTERHORN trial show statistically significant improvement in complete pathological response with the addition of immunotherapy (durvalumab, immune checkpoint inhibitor) to FLOT versus placebo (19% vs 7%; p<0.00001) [21].

The quality of the surgery plays a crucial role in the treatment of patients with GC. Data show that patients with localized GC, with stage Ib-III according to the AJCC/UICC TNM 8th edition, undergoing radical gastrectomy with D2 lymphadenectomy have superior outcomes than gastrectomy with D1 lymphadenectomy [12]. Also, patients should undergo operations in high-volume centers with appropriate surgical expertise and post-operative care. A German study shows that low-volume centers for GC surgery have post-operative mortality of 7.9%. In contrast, in centers with 30 gastric resections per year, mortality is below 4% [22]. Therefore, patients with GC should undergo surgery in dedicated, high-volume centers.

4.3.1.2. Treatment of locally advanced and metastatic gastric cancer

Recent improvement in the medical treatment of GC includes the development of targeted therapies. The addition of targeted therapy of anti-HER2 (trastuzumab) to chemotherapy in HER-2 positive metastatic GC (present in around 20% of GC, primarily intestinal type), based on the results of the ToGA trial [23], results in better survival of patients (overall survival, OS 13.8 months for trastuzumab and chemotherapy, and 11.1 months in patients with chemotherapy alone (hazard ratio, HR 0.74; 95% confidence interval, CI 0.60–0.91, p 0,005)). Future treatment modalities in HER-2-positive GC include the usage of an antibody-drug conjugate, trastuzumab-deruxtecan, based on the results of the phase II trial, DESTINY- Gastric 01, that evaluates trastuzumab-deruxtecan, compared with chemotherapy in HER2-positive pre-treated GC in the third line of chemotherapy. Trastuzumab-deruxtecan treatment leads to significant improvement in objective response rate (51% vs. 14%; p < 0.001) and OS (median 12.5 vs. 8.4 months; HR 0.59; 95% CI 0.39 to 0.88; p 0.01), in the Asian population [24]. The results are similar in the Western population; the results of the phase II study DESTINY-Gastric 02 show confirmed objective response to the treatment in 42 % (95% CI 30.8-53.4) of included

patients [25]. Currently, the phase III global study DESTINY-Gastric 04 is recruiting patients to evaluate the effectiveness of trastuzumab-deruxtecan with chemotherapy in patients who progressed after trastuzumab in the first line [26].

High hopes in the medical oncology field are linked with immunotherapy's efficacy in the treatment of metastatic and locally advanced/unresectable GC. The efficacy of the addition of immunotherapy to chemotherapy in patients with GC with a combined positive score (CPS) >=5 is shown in the phase III CheckMate 649 trial, which evaluates the addition of nivolumab (anti-PD-1 immune checkpoint inhibitor) to the first-line chemotherapy (capecitabine or 5-fluorouracil and oxaliplatin). Nivolumab plus chemotherapy significantly improves overall survival, with HR of 0.71 (98.4% CI 0.59–0.86), $p < 0.0001$. [27]. In the phase III KEYNOTE-062 trial, pembrolizumab (anti-PD-1 immune checkpoint inhibitor) monotherapy was non-inferior to cisplatin and fluoropyrimidine chemotherapy for overall survival in patients with CPS score greater than 1. Additionally, Pembrolizumab prolongs OS in comparison with chemotherapy in patients with a CPS score of 10 or greater (median OS, 17.4 months *vs.* 10.8 months; HR, 0.69; 95% CI, 0.49-0.97), but this difference was not statistically tested [28]. The search for predictive factors for the response to immune checkpoint inhibitors is still necessary, which would allow better selection of the patients susceptible to benefit from this treatment.

Advances in the treatment of GC are also observed with the emerging treatment targets. The phase III SPOTLIGHT trial investigates the effect of targeting claudin-18.2 (expressed by ~ 40% of metastatic GC), using targeted therapy with the monoclonal antibody zolbetuximab plus modified FOLFOX regimen (consisting of folinic acid, 5-fluorouracil, and oxaliplatin), in patients with claudin-18.2 positive, untreated, locally advanced or metastatic GC. The study shows an improvement in progression-free survival (10.61 months in the zolbetuximab group vs. 8.67 months in the placebo group; HR 0.75, 95% CI 0.60–0.94; p 0.007) [29]. In the same way, the GLOW trial showed an improvement in OS in patients with claudin-18.2 positive GC treated with zolbetuximab in combination with CAPOX regimen (consisting of capecitabine and oxaliplatin) versus CAPOX in the first-line setting. Median OS was 14.4 months for the experimental arm versus 12.2 months for the chemotherapy arm (HR 0.77, p 0.012), respectively[30]. A phase II FIGHT study investigates the efficacy of a fucosylated, humanized IgG1 anti-fibroblast growth factor 2 isoform IIb receptor (FGFR2b) monoclonal antibody bemarituzumab with modified mFOLFOX regimen in patients with FGFR2b-selected GC. Despite no statistically significant improvement in progression-free survival in

this exploratory phase II study, treatment with bemarituzumab showed promising clinical efficacy [31]. A phase III trial of bemarituzumab in patients with GC is currently under investigation.

4.3.1.3 Personalized medicine in the treatment of gastric cancer

Personalized medicine is an emerging practice of oncology that uses patients' genetic profiles to guide decisions made regarding the prevention, diagnosis, and treatment of disease. This approach is an opportunity to turn “one size fits all” therapy into an individualized treatment. Taking personalized medicine into account, some rare genetic alterations, also in patients with GC, might be treated with actionable treatment. Promising targets include neurotrophic tyrosine receptor kinase (NTRK) fusion or that occur in a broad spectrum of tumors (including breast, cholangiocarcinoma, colorectal, gynecological, neuroendocrine, non-small cell lung, salivary gland, pancreatic, sarcoma and thyroid cancers). NTRK fusion is a predictive factor for the response to TRK inhibitors, like larotrectinib and entrectinib [32]. Although extremely rare (the exact prevalence in patients with GC has not been assessed), there are reported cases of GC with NTRK fusion [33]. The VIKTORY umbrella trial (a type of study that evaluates multiple targeted therapies in a single disease setting) was designed to classify patients with metastatic GC based on clinical sequencing. It included 8 different biomarker groups (RAS aberration, TP53 mutation, PIK3CA mutation/amplification, MET amplification, MET overexpression, TSC2 deficient, or RICTOR amplification, all negative) to assign patients to a targeted therapy in second-line treatment. 14.7% of patients received biomarker-assigned treatment. The results of the biomarker-assigned treatment cohort show encouraging response rates and survival comparable with conventional second-line chemotherapy [34].

Despite the growing efficacy of the above treatments and the increase in the availability of the treatment, the global efficacy of GC treatment still needs improvement since long-term responses or complete remissions in this setting are rare. Therefore, preventive measures should be undertaken to improve outcomes in patients with GC.

4.2 Gastric carcinogenesis: gastric precancerous lesions

The development of non-cardia intestinal-type GC follows a pattern of stepwise progression from gastric precancerous lesions (GPL). According to the model of gastric carcinogenesis known as "Correa's cascade" [35], GC is preceded by a progression from a normal mucosa through non-atrophic gastritis, usually following chronic infection with *H. pylori*, and precancerous lesions, successively, chronic atrophic gastritis (CAG), intestinal metaplasia (IM), dysplasia (low-grade dysplasia, and high-grade dysplasia), and finally cancer [35–37]. Less frequently, atrophic gastritis can result from an autoimmune reaction and then is called autoimmune gastritis (AIG).

In *H. pylori*-related gastritis, non-autoimmune gastritis (NAIG), the lesions first appear in the antrum and eventually spread to the corpus, causing pangastritis. In contrast, in AIG, the lesions are limited to the gastric corpus and fundus, sparing the antrum, Figure 1 [38,39].

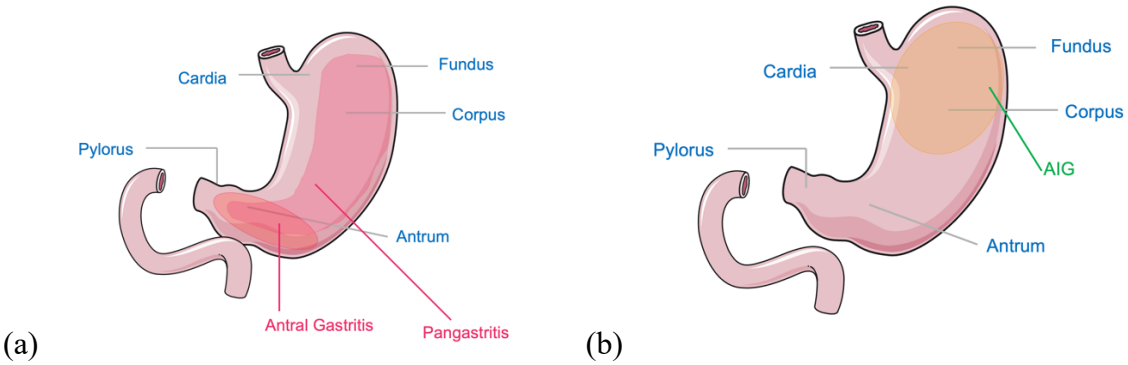

Figure 1 Distribution of different types of atrophic gastritis in the stomach.

(a) *H. pylori*-related gastritis affects the gastric antrum and eventually spreads to the corpus, causing pangastritis.
(b) Autoimmune gastritis (AIG) affects the gastric corpus and fundus, causing mucosal atrophy that spares the antrum.
The figure was developed in Microsoft ® PowerPoint version 16.82 2024 based on the image from the SMART website.

Gastric precancerous lesions, whose intensity is evaluated according to histologic classification OLGA and OLGIM, are associated with an increased risk of GC [40]. The annual incidence of GC in patients with GPL, according to a PALGA study conducted on the Dutch population, was 0.1% for atrophic gastritis, 0.25% for intestinal metaplasia, 0.6% for mild-to-moderate dysplasia, and 6% for severe dysplasia (for the latter, HR 40.14, 95% CI; 32,2-50,1) [41]. Studies have demonstrated that the most common location of gastric atrophy is the antrum, but patients with pangastritis have a major risk of progression to GC [42]. To sum up, patients with atrophic gastritis have an increased risk of GC; thus, they would benefit from close surveillance.

Since most GC cases progress from gastric precancerous lesions, several actions have been made to reinforce the oncological surveillance in patients with GPL. It includes open-access endoscopy services in patients with high-risk GPL lesions [43]. Also, combined colonoscopy and esophagogastroduodenoscopy screening have been proposed as concomitant colon and gastric cancer screening [44]. Nevertheless, endoscopic evaluation of pre-malignant conditions in the stomach is imperfect as a screening measure. Despite the low rate of adverse events, esophagogastroduodenoscopy is an invasive diagnostic procedure with reported complications [45]. The estimated number of procedures for one cancer avoided by detecting a premalignant condition exceeds 230, even in countries with an intermediate prevalence of GC [46].

Moreover, the endoscopic diagnosis of GPL - atrophic gastritis and intestinal metaplasia - is questionable. The real-world data shows that the sensitivity of the detection of AG does not exceed 70% and the detection of IM 20% [47,48]. The diagnostic performance depends on the operator's expertise and may vary significantly between centers [48,49]. Because of low detection by optical judgment, the diagnosis of AG and IM still relies on "mapping" biopsies. It can be missed by biopsy due to a "patchy" distribution of GPL. The current diagnostic standard for GPL proposed by MAPS II guidelines consists of high-definition chromoendoscopy and systematic biopsies of at least two topographic sites (from both the antrum and corpus) [50]. Therefore, the development of non-invasive markers is required to "support" or replace endoscopy in searching for pre-malignant conditions. It would apply, especially in countries with low to moderate GC incidence, where nationwide screening programs concerning cost-effectiveness and patient burden seem inappropriate.

4.2.1 *H. pylori*-related gastritis

4.2.1.1 Physiopathology of H. pylori-related gastritis

H. pylori is a Gram-negative bacterium that colonizes half of the human population but only causes overt gastric disease in a subset of infected hosts. Colonization and persistence in such an inhospitable place as the stomach lumen, with its low pH, requires the presence of exquisite adaptive mechanisms that *H. pylori* has mastered. After *H. pylori* enters the host's stomach, four steps are necessary for bacteria to establish successful colonization and persistent infection that leads to the development of atrophic gastritis: (i) production of the urease by the bacterium to raise the gastric pH and dissolve gastric mucins; (ii) movement through the mucins toward the epithelium by flagella-mediated motility; (iii) attachment to host cells by adhesins, that enables binding to the gastric epithelium adhesins; (iv) tissue damage by toxins (vacuolating cytotoxin, Vac; cytotoxin associated gene, CagA, CagL, CagY) released by the bacterium, (v) the ability of the evasion and subversion of the host's immune system, through modification of own pathogen-associated molecular patterns (PAMP's), and avoidance of recognition by Toll-like receptors of immune cells [51]. Most *H. pylori*-infected individuals are asymptomatic; only a small proportion will develop chronic atrophic gastritis, gastric or duodenal ulcer, gastric mucosa-associated lymphoid tissue lymphoma, or gastric cancer during long-term infection.

4.2.1.2 Location of lesions and symptoms in H. pylori-related gastritis

When *H. pylori* colonization becomes persistent, acid secretion is crucial for the distribution of gastritis. Since acid has a limiting effect on bacterial growth, in subjects with intact acid secretion, *H. pylori* colonizes only the gastric antrum, with few acid-secretory parietal cells present. Subjects in whom acid secretion is impaired, including those chronically ingesting proton pump inhibitors (PPIs), have bacterial colonization in the gastric antrum and corpus, leading to pangastritis [52] (Figure 1). Patients with *H. pylori* infection may report the following symptoms: pain or discomfort (usually located in the upper abdomen), bloating, early satiety, loss of appetite, and nausea and vomiting. NAIG does not present with symptoms other than those mentioned above caused by *H. pylori*.

4.2.1.3 Prevalence of H. pylori-related gastritis

Chronic atrophic gastritis is more prevalent in the older population, although it varies in different regions worldwide. The assessment of the prevalence is difficult due to the lack of symptoms in most individuals. In a population-based cohort study in Western Europe (Germany), where the diagnosis of chronic atrophic gastritis was based on the serological assessment of pepsinogen I and II and *H. pylori* serology, the prevalence was 4.8% in the age group 50-54 years old and increases to 8.7% in the 70- 74 age group and tend to be more prevalent in men [53]. The prevalence is higher in East Asia. Studies performed in high-incidence areas such as Japan and China showed a prevalence of NAIG between 33- 84% [54].

4.2.1.4 Diagnosis and treatment of H. pylori-related gastritis

H. pylori infection can be diagnosed through invasive and non-invasive diagnostic methods. Noninvasive approaches involve detecting *H. pylori* antigens in stool and *H. pylori* IgG antibodies in serum or conducting a urea breath test based on a high urease activity of the bacterium. Invasive tests include upper endoscopy, which necessitates gastric tissue and encompasses methods such as rapid urease test, histopathology, polymerase chain reaction, and culture [55]. The current standard for the NAIG diagnosis is upper endoscopy, but serological and other non-invasive tests are emerging.

Based on current Maastricht VI/ Florence guidelines, the recommended treatment of *H. pylori* infection is quadruple therapy with antibiotics and bismuth or triple therapy with amoxicillin and clarithromycin, depending on the local antibiotic (especially clarithromycin) resistance

[55]. Eradication of *H. pylori* is recommended even in the absence of symptoms in infected individuals, with the primary objective of GC prevention. Data from the literature consistently confirm that eradication of *H. pylori* decreases the risk of developing GC, both in the subjects with a family history of GC and in the general population [56,57].

H. pylori eradication cures non-atrophic gastritis and may reduce or even cure chronic atrophic gastritis, but in patients with more advanced lesions such as intestinal metaplasia and dysplasia, its effect is less certain [50,55,58]. Reduction of the risk of developing metachronous GC after *H. pylori* eradication was also confirmed in patients who underwent endoscopic resection of early GC [59]. Therefore, *H. pylori* eradication is recommended in patients with early GC [12,55]. More disputable is the interest in *H. pylori* eradication in patients with locally advanced GC after gastrectomy and metastatic GC. One study confirms improved survival in patients who received *H. pylori* treatment after gastrectomy [60], but such treatment is not yet included in the guidelines. In the case of metastatic disease, no studies confirm the efficacy of *H. pylori* eradication on patients' survival. Additionally, such treatment from an ethical point of view – imposing antibiotics on patients with advanced disease, already receiving toxic treatment – is questionable. Notably, a recent study reports that patients with GC and positive serology for *H. pylori* have a negative impact on the efficacy of treatment with immune checkpoint inhibitors [61]. This phenomenon is explained by chronic *H. pylori* infection being associated with less responsive immune T-cells in the tumor microenvironment, and smaller infiltration of immune cells in the tumor microenvironment leads to lower response to immunotherapy [62]. However, more data are necessary to draw firm conclusions. To sum up, H. *pylori* eradication is recommended only in patients with early GC to prevent metachronous GC and not in patients with advanced and metastatic GC.

4.2.2 Autoimmune gastritis

4.2.2.1 Patomechanisms of autoimmune gastritis

AIG is characterized by immune-mediated destruction of gastric oxyntic glands, particularly parietal cells, in the gastric corpus. This immune response is related to the production of autoantibodies, specifically anti-parietal cell antibodies (APCA) and anti-intrinsic factor antibodies (AIFA) [39,63,64]. APCA targets the proton pump (H+/K+ ATPase) located on the surface of parietal cells. These cells secret hydrochloric acid (HCl) into the gastric lumen, which

is essential for activating pepsinogen, facilitating digestion and iron absorption. Immune-mediated destruction of parietal cells leads to decreased HCl secretion. AIFA interferes with the secretion of intrinsic factor, a glycoprotein secreted by parietal cells, which binds to vitamin B12, enabling its absorption in the ileum. The physiology of gastric oxyntic mucosa is presented in Figure 2.

Chronic inflammation and parietal cell destruction lead to gastric mucosal atrophy and metaplasia. Increased gastric pH leads to hypergastrinemia and hyperplasia of enterochromaffin cells, increasing the risk of developing gastric neuroendocrine type 1 tumors, frequently observed in this context [65]. The role of AIG in the development of GC is currently debated [66–68], but the GC risk appears lower than in pangastritis due to *H. pylori* infection. In AIG, underlying longstanding *H. pylori* infection is potentially responsible for the development of GC [65,68].

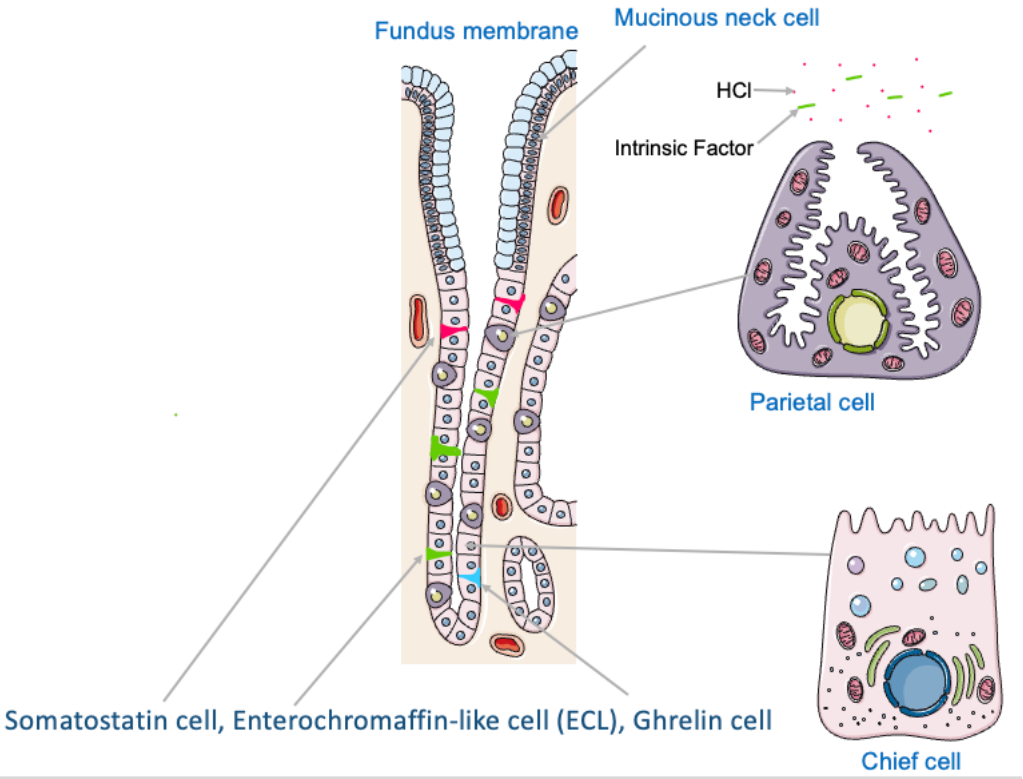

Figure 2 Physiology of gastric oxyntic mucosa in the gastric corpus

Parietal cells in the gastric oxyntic mucosa in the proximal stomach (gastric corpus and fundus) have two main functions: hydrochloric acid secretion and intrinsic factor (vitamin B12-binding glycoprotein) production. Parietal cells reside along with other cells, including chief cells (producing pepsinogen), mucinous neck cells (producing mucins), enterochromaffin-like cells (ECL), ghrelin cells, and somatostatin cells.

In autoimmune gastritis, parietal cells are the main target of autoimmune reactions. The destruction of parietal cells leads to the loss of intrinsic factor and reduced acid output. These alterations result in malabsorption of iron and vitamin B12. Besides, increased gastric pH leads to hypergastrinemia and hyperplasia of enterochromaffin cells, increasing the risk of developing gastric neuroendocrine type 1 tumors.

The figure was developed in Microsoft ® PowerPoint version 16.82 2024 based on the image from the SMART website.

4.2.2.2 Prevalence of autoimmune gastritis

AIG is rare and occurs in ~ 0.5–2 % of the general population [69]. The prevalence of AIG increases in the population > 60 years and affects women more, with an average female-to-male ratio of 2–3:1. Nevertheless, a recent study showed an increased prevalence of AIG among the younger 35–45-year-old patients [70]. In contrast, it rarely affects children [71]. Patients with other autoimmune diseases, notably diabetes mellitus type 1 and thyroiditis, are more susceptible to AIG [64,72].

4.2.2.3 Symptoms of autoimmune gastritis

AIG may be asymptomatic, but the main symptom leading to the diagnosis of AIG is anemia (micro- or macrocytic). Gastrointestinal symptoms may include epigastric pain, weight loss, heartburn, and nausea, exhibited by around 1/3 of patients. Less commonly, patients can present with bloating, diarrhea, abdominal pain, early satiety, and vomiting. Rare symptoms are constipation, dysphagia, and glossitis, which are present in <5% of patients [63,64].

4.2.2.4 Diagnosis and treatment of autoimmune gastritis

Diagnosis of AIG is based on histological analysis of the gastric biopsies obtained during the upper endoscopy. Macroscopic evaluation of the gastric mucosa during an endoscopic procedure, especially high-definition endoscopy with chromoendoscopy, to identify areas of the mucosa suspected of atrophy or intestinal metaplasia, but histological confirmation is still necessary [50]. The search for serum autoantibodies should be performed, with elevated titers of APCA and AIFA autoantibodies indicative of AIG, keeping in mind that their sensitivity is not perfect. APCA is detected in 85-90% of patients with AIG but may also be found in around 10% of the healthy population. AIFA is present in 35–60% of AIG cases and is highly specific to AIG [73]. In the late stage of AIG, seroconversion may occur; therefore, the clinical importance of AIFA and APCA antibodies is limited [55,63,74]. Of note, APCA and AIFA positivity levels do not correlate with the severity of the lesions in AIG. Some guidelines recommend assessing gastrin levels to diagnose AIG [55].

All patients with AIG should be screened for other autoimmune diseases due to the frequent coexistence of other autoimmune disorders in AIG [75]. Unfortunately, no curative treatment is currently available, and the management includes supplementation of micronutrient deficiencies and upper endoscopy for the screening of GC and neuroendocrine tumors.

4.3 Non-invasive Biomarkers of gastric precancerous lesions

4.3.1 Pepsinogens

Serum pepsinogens (PGs), the precursors of pepsin, are the most studied biomarkers of gastric atrophy. PGs include pepsinogen I and II (PGI and PG II), which are secreted to the stomach lumen and circulation. PGI is secreted by the chief cells present only in the gastric corpus, while PGII is secreted throughout the stomach and proximal duodenum.

Therefore, in the case of CAG affecting the corpus, the level of PGI drops significantly. In contrast, the level of PGII remains unchanged, hence allowing the use of the decreased levels of PGI and PGI/PGII ratio as potential biomarkers of corpus atrophy. One of the weaknesses of the non-invasive diagnosis of CAG using PG testing is its low level of performance for the detection of antrum atrophy.

The diagnostic value of PG testing has been assessed in several studies using different methods (enzyme-linked immunosorbent assay, ELISA, chemiluminescent enzyme immunoassay, CLEIA) and in different populations (Asian, Caucasian). Although discordant results have been obtained concerning its sensitivity (ranging from 32 to 98%) [76], assessment of PG serology in atrophic gastritis is recommended by international guidelines: MAPS I and II consensus stated that serum pepsinogen levels could predict extensive atrophic gastritis. Also, Low PGI serum levels or/and low PGI/II ratio identify patients with advanced stages of atrophic gastritis, and endoscopy is recommended for these patients, mainly if *H. pylori* serology is negative [50,77]. Maastricht VI/Florence consensus also confirmed the role of PG: the available data consistently recognize PG serology as the most useful non-invasive test to explore the gastric mucosa status (non-atrophic vs. atrophic) [55]. Nevertheless, the PGI/PGII ratio can never be assumed to be a biomarker of gastric neoplasia [78]. The summary of the diagnostic performance of pepsinogens across different populations and with different techniques is summarized in Table 3.

Table 3. The comparison of the diagnostic performance of pepsinogens.

Study author (year)	Study type, country/region	Targeted condition	Cut-off values	No. of patients included	Age of patients included	Sensitivity (95%CI)	Specificity (95%CI)	AUC ROC (95%CI)
Lin [79] (2023)	Single-center, China	AG	PG I ≤70 ng/ml and PGI/PGII ratio ≤3	965 (275 AG)	n/a	8.73%	94.49%	n/a
			PG II >11.05 ng/ml and PGI/PGII ratio <3.75)			21.82%	86.09%	n/a
Nguyen [80] (2022)	Single center, Vietnam	AG moderate to severe	PGI ≤63.5 ng/ml	273 (77 moderate to severe AG)	56.3± 9.7	79.2%	41.3%	0.612
			PGI/PGII ratio ≤5.2			61%	68.9%	0.689
			PGI ≤63.5 ng/ml and PGI/PGII ratio ≤5.2			49.4%	82.1%	Na/
			PGI ≤63.5 ng/ml or PGI/PGII ratio ≤5.2			90.9%	28.1%	n/a
Miftahussurur [81] (2022)	Cross-sectional, Multicenter Indonesia	AG, GC, gastroesophageal reflux	PG I ≤70 ng/ml and PGI/PGII ratio ≤3	646 (171 AG)	44.93 ± 12.98	7.6% (4.5–9.2)	99.2% (98.2–99.8)	n/a
			PGII ≥12.45 ng/mL	646 (27 AG)		59.3 (38.8-77.6)	77.1 (73.0-80.8)	0.755 (0.702-0.811)
			PGI/II ratio ≤4.75			81.5 (61.9-93.7)	78.7 (74.3-82.3)	0.821 (0.763-0.855)
Koc [82] (2022)	Single center, Turkey	AG	PGI/II ratio ≤11.9 for AG and autoimmune AG	147 (79 AG, 16 AIG)	57.7±12	45.6%	84.4%	0.644
			PGI/II ratio ≤9.2 for AG			47.5%	90.6%	0.711
			PGI/II ratio ≤1.9 for autoimmune AG			100%	100%	1
			PGI ≤13.5 ng/ml for autoimmune AG			100%	100%	1
Cai [83] (2021)	Multicenter, China	AG	PGI ≤73.14 ng/mL	1922 (1590	52.3 ± 9.8	62.1%	53.8%	0.585
			OLGA 0 vs I/II	OLGA 0, 273				

			PGI/PGII ratio ≤ 11.54 ng/mL OLGA 0 vs I/II	OLGA I/II, 49 OLGA III/IV)		43.2%	77.7%	0.611
			PGI ≤64.0 ng/mL OLGA 0/I/II vs III/IV			67.2%	61.2%	0.631
			PGI/PGII ratio ≤ 9.11 ng/mL OLGA 0/I/II vs III/IV			53.0%	91.8%	0.740
Whary [84] (2020)	Single center, Colombia	AG, GC	PGI/PGII ratio n/a value for AG/GC	153	n/a	44.7%	83%	n/a
			PGI/PGII ratio and interleukine-5 n/a values for AG/GC			63.8%	67.9%	0.66
Miftahussurur [85] (2020)	Multicenter, Southeast Asia	AG, *H.pylori*	PG I ≤70 ng/mL, PGI/PGII ratio ≤3	1206	44 years (range 13–88)	15.9%	96.9%	n/a
			PGII ≥10.35 ng/mL			72.6%	56.9%	0.664
			PGI/PGII ratio ≤4.95			66.2%	67.5%	0.718
Zeng [86] 2020	Single-center, China	AG, GC	PG I < 71.56 μg/l	197 (86 GC, 61AG)	n/a	77.1%	66.0%	0.719
			PG I/II ratio < 5.6			60.1%	82.0%	0.755
			PG I <71.56 μg/l; PG I/II ratio < 5.6			67.2%	84.0%	0.807
Bang [87] (2019)	Metaanalysis, 14 studies for AG, 43 for GC	AG, GC	PG I ≤70 ng/mL; PGI/PGII ratio ≤3	AG 130	n/a	AG: 0,59 (0.38–0.78)	AG: 0,89 (0,70–0,97)	0,81 (0,77–0,84
Mezmale [88] (2019)	Multicenter, Kazakhstan	AG	PG I ≤70 ng/mL; PGI/PGII ratio ≤3	157	51±6.98	50.0% (1.2 - 98.7)	50.0% (1.2 - 98.7)	n/a
			PG I ≤30 ng/mL and PGI/PGII ratio ≤2			73.5% (65.8 - 80.3)	90.9% (85.3 - 94.9)	n/a

Loong [89] (2017)	Single-center, Malaysia	AG	PGI ≤87,2 µg/L) PG I/II ratio ≤10 G-17 <5.6	71 (36/35)	56.2± 16.2	PGI: 66.7% PGI/II ratio: 83.3% G17:68.8%	PGI:85.3% PGI/II ratio:77.9% G17:44.8%	PGI:0.659 PGI/PGII ratio:0.902 G17<0.5
Zagari [76] (2017)	Metaanalysis, 20 studies	AG	PGI; PGI/PGII ratio; G17b; HpAb; different cut-offs	4241	n/a	74,7% (62,0-84,3)	95,6% (92,6-97,4)	n/a
Leja [90] (2017)	Case-control Multicenter, Latvia	AG	L-AA PgI≤70 ng/ml; PgI/PgII≤3 for "any" atrophy; PgI≤30ng/ml; PgI/PgII≤2 for advanced atrophy, ELISA: PgI/PgII<3	805 (50/755)	51 (range 18-88)	44%	91%	n/a
Huang [91] (2015)	Metaanalysis, 14 studies AG, 17 GC	AG, GC	PG I ≤70 ng/mL and/or PG I/PG II ratio ≤3	AG: 2220	n/a	0.69 (0,55- 0,8)	0,88 (0,77-0.94)	0.83 (0,8-0,86)
McNicholl [92] (2014)	Multicenter, Spain	AG	PGI < 25lg/L G-17b < 0,1 HpAb < 30	85	44 ±14	50% (39–61%),	80% (71–88%),	n/a

AG, atrophic gastritis; AUC, area under curve; CLEIA, chemiluminescent enzyme immunoassay; ELISA enzyme-linked immunosorbent assay; GC gastric cancer; HpAb, *H. pylori* antibodies [EIU]; EIU, enzyme immune units; PGI, pepsinogen I; PGII, pepsinogen II; G-17b, Gastrin-17, basal; L-AA, latex-agglutination assay; ROC, receiver operating characteristic curve; n/a, not available; values are presented as mean ± standard deviation or percentage unless stated otherwise

4.3.2 Gastrin

Gastrin is produced by gastric G cells located in the gastric antrum. Gastrin initiates the release of gastric acid in the stomach after food intake. Its secretion is regulated by a feedback system involving (i) the presence of peptides in the stomach, (ii) high pH in the stomach, and (iii) the release of somatostatin, which stimulates G cells to gastrin release. Gastrin has few active isoforms, but only gastrin 17 (G17) is used in clinical practice [93]. G-17 production increases after food intake; evaluating G17 following a protein-rich meal is more accurate than fasting gastrin [94].

In autoimmune gastritis, reduction in gastric acid secretion triggers a compensatory response, resulting in an increase in gastrin levels that stimulates the release of gastric acid from parietal cells. Therefore, increased G-17 is a good serological marker of AIG [95]. Gastrin levels are also higher (~1.5-fold) in patients with *H. pylori* infection than in uninfected patients and long-term proton pump inhibitor users [55,96].

In atrophic gastritis of the antrum, the loss of antral glands results in a decreased number of G cells, which leads to a low output of G-17. Therefore, a low G17 level could be a marker of gastric antral atrophy. Some previous studies evaluated the diagnostic value of gastrin in this indication; the test's sensitivity was 36.8%, specificity was 86.5%, and the overall accuracy was 82.6% after protein-meal stimulation. To sum up, the low sensitivity of the G-17 test made it less useful for diagnosing antral atrophy in clinical practice.

4.3.3 Other potential biomarkers

Due to the high frequency of gastric cancer, the search for new biomarkers of GPL are under investigation to improve the diagnostic performance of pepsinogen.

4.3.3.1 Human epididymal protein 4

Increased serum level of human epididymal protein 4 (HE-4) is an ovarian cancer biomarker established in the clinical guidelines. HE-4 is upregulated in GPL in the metaplastic transition following acute parietal cell loss in mice and humans and has been suggested as a surrogate marker of preneoplastic lesions in the stomach [97].

GC can also express HE-4 – the expression in immunohistochemistry is present in 25% of intestinal type and around 60% of diffuse type GC of stages I and II; its expression correlates

with tumor size, stage, and survival [98,99]. HE-4 expression is also present in other gastrointestinal cancers, like pancreatic and esophageal cancer [98]. Nevertheless, up to now, the serum HE-4 levels have not been measured in patients with GPL.

4.3.3.1 Interleukin-6

Interleukin-6 (IL-6) is a pleiotropic cytokine that plays a role in inflammation and tumor progression. Recent studies have shown that *H. pylori* induces signal transducer and activator of transcription 3 (STAT3) that plays a vital role in gastric carcinogenesis. STAT3 activation is mediated through reactive oxygen species (ROS)-induced upregulation of IL-6 expression in human GC cells [100]. These findings provide a novel molecular mechanism responsible for *H. pylori*-induced gastritis and gastric carcinogenesis and a possibility to use serum IL-6 as a GPL biomarker. Besides, Higher IL-6 serum levels were detected in *H. pylori*-infected individuals [101]. Increased levels of IL-6 and other chemokines have been associated with GC growth, and IL-6 serum levels increase during tumor progression and correlate with patient survival. Several studies have investigated the IL-6 value as a diagnostic marker of GC, with a range of sensitivity and specificity of 0.39–0.85 and 0.50–0.97 [102–104]. Of note, IL-6 values may be influenced by other factors, including autoimmune diseases, inflammation, and physical exercise, and thus, this parameter is susceptible to giving false-positive results. Nevertheless, the serum assessment of IL-6 in patients with different types and severity of GPL has not been performed before.

4.3.3.2 Adiponectin

Adiponectin is a hormone adipocytes produce and plays a vital role in energy metabolism and insulin sensitivity. Adiponectin serum levels correlate inversely with the volume of visceral abdominal fat tissue. Several cancers have been associated with low levels of adiponectin and altered levels of adiponectin receptors; therefore, it can potentially be a marker for those cancers [105]. In patients with *H. pylori* infection, adiponectin was used to identify the patients at risk of developing metabolic syndrome [106,107]. Adiponectin may enhance carcinogenesis through its well-recognized effects on insulin resistance and its direct impact on tumor cells [108].

The literature shows contradictory data on serum adiponectin levels in patients with GC. A study by Ishikawa et al. suggested that serum adiponectin concentrations are lower in patients with GC than healthy controls [109]. However, in a study by Seker et al., there was no statistical

significance between the groups [110]. Nevertheless, serum adiponectin levels may vary due to multiple factors (sex, body fat distribution, renal and cardiac function, smoking, dietary factors, and physical exercise) [108], making the implementation in clinical practice more challenging. Nevertheless, the serum assessment of adiponectin as a biomarker of different types and severity of GPL has not been performed before.

4.3.3.3 Krebs von den Lungen 6

Krebs von den Lungen 6 (KL-6) is a subtype of membrane-associated mucins (MUC), and its extracellular domain is widely expressed in gastrointestinal tissues. Its expression is higher in various cancer tissues and is associated with a worse prognosis and more invasive disease [111]. Historically, in the 90', the KL-6 serum marker served as a biomarker of gastrointestinal cancers, but in clinical practice, it was replaced by a more specific carcinoembryonic antigen (CEA). Currently, KL-6 is used as a serum marker of interstitial lung disease in clinical practice [112]. The serum assessment of KL-6 as a biomarker of different types of GPL has not been studied before.

4.3.4 Combinations of different biomarkers

4.3.4.1 Gastropanel®

Gastropanel® is a combination of serological assays, including serum PGs (PGI and PGII), G-17, and anti-*H. pylori* antibodies (HpAb) and has been proposed as a 'serological biopsy' for diagnosing atrophic gastritis [113]. The interplay of interdependent biomarkers measured in serum samples can help to assess the presence of AG and the activity of inflammation in the gastric mucosa. Serum PGI levels and the PGI/PGII ratio are lower in patients with corpus atrophic gastritis. In contrast, a low G-17 serum level, in combination with positive HpAb, would indicate the presence of antrum atrophic gastritis. Thus, combining the results of HpAb, PGI or PGI/PGII ratio, and G-17 tests would allow us to detect the presence and site of inflammation [114]. Gastropanel® has shown promising results for the diagnosis of GPL, although wide variations of its diagnostic accuracy among different populations have been observed [76]. In Europe, in a study by Chapelle et al., sensitivity and specificity for detecting AG by Gastropanel® were 39.9% and 93.4%, respectively. The sensitivity was significantly higher for the detection of severe AG [60,8% (95% CI 46,1-74,6) P = .015] and corpus AG

[61% (49,2-72), *P* = .004]. Diagnostic performances of Gastropanel® were not statistically different from the assessment of PG I alone (*P* = .068)[115].

Metanalysis performed by Zagari et al. included 20 studies assessing the accuracy of a combination of serological assays (PGI, PGI/PGII ratio, G17, *H. pylori* serology) for the diagnosis of AG, compared to histology. Pooling data from these studies yielded a summary sensitivity of 74,7% (95% CI; 62-84,3). and the specificity 95,6% (95%CI; 92.6-97.4). Based on the median prevalence of atrophic gastritis across the studies of 27%, the negative predictive value of the panel test was 91%, and the positive predictive value was 86% [76]. In summary, Gastropanel® can be an interesting diagnostic tool for diagnosing GPL, but its sensitivity is too low to implement in clinical practice.

4.3.4.2 Other combinations of markers

Since a single biomarker is imperfect in distinguishing the origin and severity of gastritis, the current Maastricht VI guidelines recommend a combination of different serological markers for the non-invasive assessment of gastric mucosa and distinguishing between the two main etiologies: AIG and NAIG. The recommended combination is PG I, II, and PGI/PGII ratio, gastrin 17, and APCA [55].

4.4 Autoimmunity in gastric precancerous lesions and gastric cancer

As mentioned above, despite a global decrease in GC, there is a rise in the incidence in young, predominantly female patients [4,5]. The causal mechanisms for this "new" type of GC have not been identified. However, a role for autoimmunity or changes in the microbiota has been proposed [5–7]. This is supported by studies suggesting an association between autoimmune conditions, such as dermatomyositis, pernicious anemia, Addison disease, and herpetiform dermatitis, and an increased risk of GC [116–118]. In the recent meta-analysis by Song et al., an autoimmune condition is associated with GC pooled relative risk (RR) of 1.37 (95% CI, 1.24 to 1.52). Among the 24 autoimmune conditions, two autoimmune diseases were mainly associated with increased risk of GC: dermatomyositis (RR, 3.69; 95% CI, 1.74 to 7.79) and pernicious anemia (RR, 2.84; 95% CI, 2.30 to 3.50) [116]. If autoimmunity is associated with the development of GC, we could expect the presence of a biological stigma of autoimmunity in patients with GPL, which precedes the appearance of cancer. To date, this aspect has never been studied. In the case of NAIG, the association of *H. pylori* with the development of many

autoimmune diseases (organ-specific and systemic) is evoked [119]. Conversely, autoimmune thrombocytopenia is the only autoimmune disease in which the role of H. pylori as a causative factor has been confirmed [120]. Patients with AIG are at higher risk of developing an autoimmune disease, present in around 20% of patients at diagnosis [63,64].

4.5 Micronutrient deficiencies in gastric precancerous lesions

Iron and vitamin B12 deficiencies represent a significant health problem affecting a patient's quality of life. They often manifest as a range of clinical symptoms, such as anemia (iron deficiency anemia and pernicious anemia in vitamin B12 deficiency), persistent fatigue, dizziness, chest pain, and neuropsychiatric disorders in the case of vitamin B12 deficiency [121,122]. While iron and vitamin B12 deficiencies can arise from various causes, it is essential to highlight that GPL, including AIG and *H. pylori* gastritis, are recognized as distinct underlying factors frequently associated with these deficiencies.

4.5.1 Micronutrient deficiencies in AIG

Around half of patients with AIG are anemic, and even more present iron and vitamin B12 deficiencies [123]. Iron and vitamin B12 deficiencies in AIG vary across sexes and age groups.

In autoimmune gastritis (AIG), a cascade of pathophysiological events unfolds due to the destruction of parietal cells in the gastric corpus. This process results in an elevated stomach pH, referred to as achlorhydria, and a concomitant loss of intrinsic factor. These changes collectively culminate in impaired absorption of iron and vitamin B12, ultimately leading to anemia [124].

Vitamin B12 stores in the liver can suffice for several years, meaning that vitamin B12 deficiency tends to manifest later in the disease course than iron deficiency. Vitamin B12 deficiency in the context of AIG presents a unique clinical challenge. Its symptoms can manifest independently of anemia and often require prompt treatment to reverse symptoms. The clinical presentation varies and encompasses neurological symptoms driven by demyelination, spinal cord atrophy, and potential axonal loss. These manifestations include spastic paraparesis, an unsteady gait, altered nerve reflexes, and visual disturbances [125,126]. Another notable symptom is sensory polyneuropathy, characterized by symmetrical numbness in the extremities and pins-and-needles sensations [126]. Vitamin B12 deficiency can

contribute to cognitive deficits and memory loss, mimicking dementia, particularly among elderly patients [126,127]. Additionally, psychiatric disorders such as manic and depressive episodes, psychosis, and chronic fatigue often manifest in cases of severe vitamin B12 deficiency [128]. These diverse clinical presentations emphasize the importance of early detection and timely vitamin B12 supplementation to mitigate its potential implications.

Impaired iron absorption in AIG stems from achlorhydria, which interferes with the favorable conversion of ferric iron to ferrous iron in the stomach, making iron absorption impossible. In contrast to vitamin B12, iron stores in the liver last only a few months. Consequently, iron deficiency anemia manifests earlier than pernicious anemia in AIG.

Surprisingly, clinicians often overlook iron deficiency in AIG despite evidence from the literature indicating its prevalence, particularly among women under 50 years old [123,129]. This information implies that iron deficiency emerges earlier than vitamin B12 deficiency in the pathogenesis of AIG and can serve as an initial disease symptom.

Iron deficiency can cause symptoms both in the presence and absence of anemia, and it also can be asymptomatic. The clinical manifestation of iron deficiency includes fatigue, reduced concentration, dizziness, headache, and restless leg syndrome [122]. Skin presentation includes dry hair or skin, hair loss, koilonychia, and skin pallor. ID and anemia can also exacerbate symptoms of cardiovascular diseases, including heart failure and ischemic heart disease. It worsens performance status and quality of life in oncological patients [130]. Iron is transported in the bloodstream via transferrin. In healthy individuals, transferrin is saturated in approximately 30% with iron. Excess iron is bound and stored by ferritin, an intracellular protein found mainly in the liver and macrophages. Different indices and thresholds are proposed to assess iron deficiency. The most common is the assessment of serum ferritin concentration, with thresholds below 25 ng/mL for women and 30 ng/mL for men [122]. Ferritin protein synthesis also increases during inflammation, behaving as an acute phase protein independently of iron stores. Some data shows that ferritin as a marker of iron deficiency should be adjusted to c-reactive protein (CRP), which is a marker of existing inflammation [131], with the threshold for CRP > 5 mg/dL and ferritin < 70 ng/mL.

In summary, AIG leads to significant micronutrient deficiencies, primarily affecting iron and vitamin B12 absorption. Understanding the distinct clinical presentations and the timing of these deficiencies is vital for accurate diagnosis and timely intervention.

4.5.2 Micronutrient deficiencies in *H. pylori*-related gastritis

In NAIG, *H. pylori* damages the gastric mucosa and raises gastric juice pH levels, which can hinder the effective absorption of iron [38,39,132]. *H. pylori* actively absorbs iron, *which is* vital for the bacteria's survival and movement. *H. pylori* uses ferric iron through the Fur receptor to activate its flagella, which enables bacteria's motility and colonization [133]. Additionally, *H. pylori* infection leads to peptic ulcers, and the associated gastrointestinal bleeding exacerbates iron loss, ultimately leading to anemia.

Recent meta-analyses have demonstrated the positive impacts of eradicating *H. pylori* infection on the amelioration of iron deficiency anemia [134]. Specifically, eradicating *H. pylori* has been shown to elevate hemoglobin levels, particularly in patients with moderate to severe anemia [134,135].

Additionally, evidence suggests that vitamin B12 levels tend to be lower in *H. pylori*-positive individuals compared to those without the infection, and the eradication of *H. pylori* can lead to improvements in serum vitamin B12 levels, particularly among children [136]. It is important to note that data on the connection between vitamin B12 deficiency and *H. pylori* gastritis is relatively scarce and is derived from a single Arabic country.

The precise mechanism behind vitamin B12 deficiency in *H. pylori* infection remains elusive. Still, several potential mechanisms have been proposed, including (i) dysfunction in the secretion of the intrinsic factor, (ii) concurrent decreased levels of ascorbic acid, leading to impaired vitamin B12 absorption, (iii) diminished acid secretion (achlorhydria) leading to a failure of splitting of vitamin B12 from food binders, (iv) concurrent autoimmune gastritis [136,137]. Current guidelines for the management of *H. pylori* infection recommend *H. pylori* eradication for patients with vitamin B12 deficiency [55].

4.5.3 Treatment of micronutrient deficiencies in gastric precancerous lesions

Iron supplementation in case of deficiency in AIG or *H. pylori* gastritis does not differ from iron supplementation in other medical conditions. Oral iron is comparable in efficacy to parenteral iron in treating iron deficiency anemia in absolute iron deficiency (low ferritin levels). Oral iron supplementation has its limitations. An upregulation of iron regulator hepcidin limits the absorption efficiency of high-dose oral iron supplementation and iron absorption from the gastrointestinal tract during inflammation, respectively. In the latter, iron deficiency is usually functional (elevated ferritin levels but low iron availability). Patients who fail to

respond to oral supplementation, defined as hemoglobin increases of <1 g/dl at 2-8 weeks following oral iron supplementation, or have functional iron deficiency require parenteral iron therapy [63,138]. A retrospective study showed the efficacy of parenteral iron therapy in patients with AIG. It shows a significant hemoglobin (around 3 g/dL) and increases ferritin levels. Nevertheless, iron deficiency anemia relapsed in almost half of patients with AIG after two years of observation [139]. It is important to note, that iron-replacement therapy improves quality of life and reduces fatigue in patients under supplementation [140].

5. The rationale for combining the works into a series of publications

This series of articles focuses on various aspects of patients with GPL. The study design in all works is multicenter and prospective and involves the same patient cohort (n=344-356), encompassing those with NAIG, AIG, and a control group.

Publication No. 1 examined the diagnostic accuracy of non-invasive biomarkers in the detection of GPL. Serum biomarkers included pepsinogen assessment with the CLEIA technique, which has not been used on the Caucasian population before. Additionally, it explores other non-invasive biomarkers not studied before in GPL, like IL-6, HE-4, adiponectin, ferritin, and KL-6.

Publication No. 2 compared the diagnostic performance of pepsinogen testing for GPL of different origins, severity, and location using ELISA and CLEIA techniques.

Publication No. 3 looked for the possible presence of autoimmunity in patients with GPL compared to the control group, which could be a potential factor for the development of gastric cancer. This aspect of GPL is described for the first time in the literature.

Publication No. 4 searched for micronutrient deficiencies in patients with GPL. It evaluated the prevalence of iron and vitamin B12 deficiency in patients with NAIG and AIG and control patients. Additionally, it searched for the factors influencing those deficiencies, like age, gender, *H. pylori* infection, and type of gastritis, since data about the vitamin B12 deficiency in NAIG or iron deficiency in AIG are scarce.

6. The aim of the studies

GPL precedes the development of gastric cancer. Therefore, this group of patients should be strictly monitored to prevent the development of this deadly cancer. The presented studies focused on non-invasive biomarkers, autoantibodies, and micronutrient deficiencies and compared them between NAIG, AIG, and the control group that could help to manage patients with GPL in clinical practice.

The prospective studies that were performed for this doctoral dissertation aimed to:

1. Analyze the performance of non-invasive biomarkers and pepsinogens in the diagnosis of GPL with different techniques: CLEIA and ELISA.

2. Explore the role of other non-invasive biomarkers not previously studied in GPL, including IL-6, HE-4, adiponectin, ferritin, and KL-6.

3. Look for the possible presence of autoimmunity in patients with GPL compared to the control group, with the assessment of 19 autoantibodies (ANA, APCA, AIFA, and 16 myositis-associated antibodies).

4. Explore the prevalence of micronutrient deficiencies, including vitamin B12 and iron deficiency, and associated factors like age, sex, *H. pylori* infection, and the origin of gastritis.

7. Articles for the doctoral dissertation

7.1 Serum Pepsinogens Combined with New Biomarkers Testing Using Chemiluminescent Enzyme Immunoassay for Non-Invasive Diagnosis of Atrophic Gastritis: A Prospective, Multicenter Study.

Chapelle Nicolas, Osmola Małgorzata, Martin Jérôme, Blin Justine, Leroy Maxime, Jirka Iva, Moussata Driffa, Lamarque Dominique, Olivier Raphael, Tougeron David, Hay-Lombardie Anne, Bigot-Corbel Edith, Masson Damien, Mosnier Jean-François, Matysiak-Budnik Tamara.
Diagnostics. 2022; 12(3): 1-17

7.2 Serum pepsinogens can help to discriminate between *H. pylori*-induced and auto-immune atrophic gastritis: Results from a prospective multicenter study.

Chapelle Nicolas, Martin Jérôme, Osmola Małgorzata, Hémont Caroline, Leroy Maxime, Vibet Marie-Anne, Tougeron David, Moussata Driffa, Lamarque Dominique, Bigot-Corbel Edith, Masson Damien, Blin Justine, Josien Regis, Mosnier Jean-François, Matysiak-Budnik Tamara.
Digestive and Liver Disease. 2023; 55 (10):1345-1351

7.3 Atrophic Gastritis and Autoimmunity: Results from a Prospective, Multicenter Study.

Osmola Małgorzata, Hémont Caroline, Chapelle Nicolas, Vibet Marie-Anne, Tougeron David, Moussata Driffa, Lamarque Dominique, Bigot-Corbel Edith, Masson Damien, Blin Justine, Leroy Maxime, Josien Regis, Mosnier Jean-François, Matysiak-Budnik Tamara
Diagnostics. 2023; 13(9): 1-10

7.4 Iron and Vitamin B12 Deficiency in Patients with Autoimmune Gastritis and *Helicobacter pylori* Gastritis: Results from a Prospective Multicenter Study

Osmola Małgorzata, Chapelle Nicolas, Vibet Marie-Anne, Bigot-Corbel Edith, Masson Damien, Hemont Caroline, Jirka Adam, Blin Justine; Tougeron David, Moussata Driffa, Lamarque Dominique, Josien Regis, Mosnier Jean-François, Martin Jérôme, Matysiak-Budnik Tamara.
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Serum Pepsinogens Combined with New Biomarkers Testing Using Chemiluminescent Enzyme Immunoassay for Non-Invasive Diagnosis of Atrophic Gastritis: A Prospective, Multicenter Study

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Abstract: Background: Analysis of serum biomarkers for the assessment of atrophic gastritis (AG), a gastric precancerous lesion, is of growing interest for identification of patients at increased risk of gastric cancer. The aim was to analyze the diagnostic performance of serum pepsinogen testing using another method, chemiluminescent enzyme immunoassay (CLEIA), as well as of other new potential biomarkers. Material and Methods: The sera of patients considered at increased risk of gastric cancer and undergoing upper endoscopy collected in our previous prospective, multicenter study were tested for pepsinogen I (PGI) and II (PGII), interleukin-6 (IL-6), human epididymal protein 4 (HE-4), adiponectin, ferritin and Krebs von den Lungen (KL-6) using the CLEIA. The diagnostic performance for the detection of AG was calculated by taking histology as the reference. Results: In total, 356 patients (162 men (46%); mean age 58.6 (±14.2) years), including 152 with AG, were included. For the detection of moderate to severe corpus AG, sensitivity and specificity of the pepsinogen I/II ratio were of 75.0% (95%CI 57.8–87.9) and 92.6% (88.2–95.8), respectively. For the detection of moderate to severe antrum AG, sensitivity of IL-6 was of 72.2% (95%CI 46.5–90.3). Combination of pepsinogen I/II ratio or HE-4 showed a sensitivity of 85.2% (95%CI 72.9–93.4) for the detection of moderate to severe AG at any location. Conclusion: This study shows that PG testing by CLEIA represents an accurate assay for the detection of corpus AG. Additionally, IL-6 and HE-4 may be of interest for the detection of antrum AG. Mini-abstract: Pepsinogens testing by chemiluminescent enzyme immunoassay is accurate for the detection of corpus atrophic gastritis. IL-6 and HE-4 maybe of interest for the detection of antrum atrophic gastritis.

Keywords: atrophic gastritis; non-invasive markers; pepsinogens; diagnostic performance

1. Introduction

Gastric cancer (GC) incidence has been decreasing over the past five decades in parallel to the decreasing prevalence of *H. pylori* infection [1]. However, it still represents the fifth most common cancer and the third leading cause of cancer-related death in the world. GC incidence varies considerably among different countries, being particularly high in the "Eastern world" (annual incidence rates up to 60/100,000 in East Asia) as compared with the "Western world" (annual incidence rates varying from 5/100,000 to 10/100,000 in Western Europe or USA) [2]. France is classically described as a low-risk GC area, with incidence rates around 7/100,000 in males and 2.6/100,000 in females [3].

Although important progress has been made in the field of cancer treatment, the overall survival in GC remains poor and is closely related to the stage of the disease at diagnosis [4]. Thus, as in other cancers, making early diagnosis is the best way to improve prognosis in GC. For decades, the Correa cascade of gastric precancerous lesions (GPL)—i.e., atrophic gastritis (AG), intestinal metaplasia (IM), low grade dysplasia (LGD), and high grade dysplasia (HGD), appearing successively following chronic infection with *H. pylori*—has been described and considered as the main pathway of gastric carcinogenesis [5,6]. Large population-based studies demonstrated increasing risk of GC parallel to the increasing severity of the lesions [7,8], and most of the studies on GPL focused on AG and IM, which are the most commonly observed [9–11]. In Asia, the knowledge of gastric physiology and carcinogenesis has led to the development of blood tests, and especially pepsinogen testing, which have shown their usefulness for the stratification of the patients according to their GC risk ("ABC method") [12]. In Western countries, the standard method of assessing the status of the gastric mucosa remains histological analysis of gastric biopsies obtained during an upper endoscopy, which is an invasive, costly, and often not well-accepted procedure. Moreover, the correlation between endoscopic evaluation of the mucosa and histologic findings is very poor [9], and there is a risk of false diagnosis due to the sampling error since the distribution of the GPL may be patchy.

However, the recent European guidelines recognize the usefulness of pepsinogen testing for identifying the most at-risk patients in whom endoscopic evaluation would be required [13]. Pepsinogen I (PGI) is secreted by the chief cells present only in the corpus mucosa, while pepsinogen II (PGII), is secreted by both antrum and corpus cells. The decrease in PGI level and in the PGI/PGII ratio is considered a marker of gastric, and especially corpus, atrophy. Combination of biomarkers, as proposed in the Gastropanel® (PGI, PG II, Gastrin 17: G-17, and *H. pylori* serology), based on enzyme linked immunosorbent assay (ELISA), has shown promising results for the diagnosis of AG [14], although wide variations in its diagnostic accuracy among the different populations studied have been observed [15]. We have previously reported the results of Gastropanel® in France [16], which has shown good diagnostic performance for the detection of corpus AG and of severe atrophy, but which has been insufficient for the detection of antral or mild atrophy. In the present study, we wanted to evaluate in the same setting another method for pepsinogen testing, ChemiLuminescent Enzyme ImmunoAssay (CLEIA), which has never been used for the detection of gastric atrophy in a European population. Our second aim was to test other potential biomarkers, i.e., adiponectin, human epididymal protein 4 (HE-4), interleukin-6 (IL-6), Krebs von den Lungen 6 (KL-6) and ferritin, which according to some published data could be involved in gastric carcinogenesis [17–20]. We hypothesized that blood level of these markers could be increased in GPL, and in consequence, they could increase our ability to detect gastric atrophy, and in particular antrum atrophy, for which no validated markers exist.

2. Patients and Methods

2.1. Design of the Study

This study was based on the analysis of the sera collected during our previous prospective, multicenter study, including all the consecutive patients considered at increased risk for GC, presented between 2016 and 2019 in four French University Hospitals for an upper endoscopy with gastric biopsies. The sera collected during that study were kept frozen, until being retrieved for the present analysis. The details on patients' selection, endoscopy protocol used, blood sample collection, and histological evaluation of gastric biopsies are described in our previous article [16]. Briefly, all the consecutive patients considered at risk for GC were proposed for inclusion. An upper endoscopy with at least 4 gastric biopsies (2 from the antrum and 2 from the corpus) was performed and a fasting blood sample was obtained. The presence, intensity, and distribution of GPL (AG and IM) were evaluated using the updated Sydney system [21]. According to the results of histopathological analysis, the patients were classified into 5 groups: normal gastric mucosa (N), non-atrophic gastritis (NAG), AG restricted to the antrum (AGA), AG restricted to the corpus (AGC), and AG extended to the antrum and to the corpus (AGAC). Additionally, patients with moderate to severe AG were distinguished from the patients with mild AG.

2.2. Measurement of Serum Biomarkers

Serum biomarkers (HE4, IL6, KL6, Adiponectin, Pepsinogen I and II) were analyzed using the CLEIA (ChemiLuminescent Enzyme ImmunoAssay) on the fully automated LUMIPULSE G instrument (Fujirebio® France SARL, Courtaboeuf, France). The system uses a unique mono test cartridge concept for the quantitative determination of each parameter. Ferritin was analyzed by immunoturbidimetric method (Cobas 8000, Roche®, Basel, Switzerland).

2.3. Statistical Analysis

The diagnostic accuracy of the markers was assessed by receiver operating characteristic (ROC) curve analysis, with evaluation of sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV). Because of the selection of the patients for this study, to better explore the performance of the test independent of the prevalence of GPL in the studied population, in addition to PPV and NPV, the positive likelihood ratio (PLR) and the negative likelihood ratio (NLR) were calculated. Statistical analysis was performed separately for AG of the antrum, of the corpus, and of the whole stomach, as well as according to the severity of AG (graded as mild or moderate/severe).

For pepsinogens, the cut-off levels commonly recommended in the Western populations (PGI: <30 µg/L; PGII: <3 µg/L, PGI/PGII ratio: <3.0) were used, and the values below these cut-off levels were considered as indicators of atrophy. Additionally, the ROC curves were developed to establish the best cut-off values for the study population using CLEIA technique (Youden's index). For other markers, since no recommended cut-off values are available, the evaluation was based on the best cut-off values identified by the ROC curves analysis for each parameter.

The ANOVA and post hoc Tukey test analysis were used to compare the values obtained for different biomarkers, considered alone or in combination, by taking histology as the reference. Statistical analysis was performed using the R version 3.6.0. software.

3. Results

3.1. Patients—Serum Samples

From the 397 serum samples initially collected, 7 were excluded from the initial study (5 because of synchronous adenocarcinoma and 2 for not fulfilling the inclusion criteria), 29 were not analyzed because of an incomplete biopsy protocol, and 5 others were not available. Finally, 356 patients (162 men (46%); mean age 58.6 (±14.2) years) were included in the study. Mean age in N, NAG, AGA, AGC, and AGAC groups were 56.1 (±14.3), 56.9 (±14.1), 61.9 (±12.2), 62.6(±14.2), respectively. The mean delay between endoscopy

and blood sample intake was 5.4 days (Q1:0.0; Q3: 0.0), and 79% of blood samples were collected the day of endoscopy.

3.2. Histology

According to the results of histopathological analysis, the patients were categorized into three groups: those with a normal gastric mucosa (N) (n = 113, 48 males, mean age 56.1 (±14.3) years), those with a non-atrophic gastritis (NAG) (n = 91, 37 males, mean age 56.9 (±14.1) years), and those with AG (n = 152, 77 males, mean age 61.4 (±13.8) years). Furthermore, within the group of the patients with AG, three groups were distinguished: patients with antrum-limited AG (AGA) (n = 72), corpus-limited AG (AGC) (n = 42), and pangastric (involving antrum and corpus) AG (AGAC) (n = 38).

In 129 out of 152 patients with AG (84.0%), IM was also present. *H. pylori* infection was found in 47 out of 356 patients (13.2%) by histology and in 61 patients (17%) by serology. Advanced gastric atrophy or IM (graded as moderate or severe) according to the Sydney classification was found in 54 out of 152 patients (35.5%).

3.3. Serum Biomarkers Testing Results

The results of the tests are presented according to the clinical situations of interest encountered by the clinicians—i.e., AG restricted to the antrum (AGA), to the corpus (AGC) or extensive, pangastric AG (AGAC). Additionally, the results for the patients with the most severe lesions (moderate or severe atrophy) are presented since the patients harboring these lesions are considered at the highest risk of progression to cancer. Because the patients with non-atrophic gastritis (NAG) are not considered at increased risk for GC, in some analyses they were categorized together with the patients with normal gastric mucosa (N) as controls. However, separate results for these two categories of patients, for each marker, and for each clinical situation are available upon request. Since PPI-therapy may influence the results of certain markers (particularly pepsinogens), the results for long-term PPI users were analyzed separately.

The values of all biomarkers studied, PG I, PGII, PG/PGII ratio, adiponectin, ferritin, HE-4, IL-6, and KL-6, according to different histological groups, are presented in Table 1. Post hoc analysis (Tukey's) for 2 by 2 comparison is available in Supplementary Table S1.

Table 1. Serum levels of all the biomarkers in different patient groups according to histology results.

	N	NAG	AGA	AGC	AGAC	*p*-Value
***n* =**	**113**	**91**	**72**	**42**	**38**	
PG I	70.93 (66.52)	59.81 (44.40)	70.70 (64.52)	14.03 (33.25)	48.45 (51.56)	<0.001
PG II	14.10 (11.52)	13.63 (8.78)	16.56 (16.09)	10.36 (6.08)	13.77 (8.92)	0.027
PGI/PGII	4.86 (1.37)	4.61 (1.75)	4.54 (1.82)	1.07 (1.54)	3.30 (2.68)	<0.001
Adiponectin	5.07 (2.91)	4.31 (2.81)	4.92 (4.10)	5.29 (3.47)	5.31 (3.32)	0.204
Ferritin	91.81 (88.67)	81.22 (61.15)	115.01 (121.68)	68.58 (67.45)	99.95 (98.58)	0.105
HE-4	75.70 (57.59)	73.94 (42.49)	86.42 (49.67)	93.38 (83.34)	115.34 (136.04)	0.012
IL-6	5.28 (11.44)	4.80 (3.83)	4.56 (2.83)	6.86 (11.77)	4.98 (4.62)	0.249
KL-6	291.63 (123.05)	326.02 (181.11)	328.81 (136.57)	353.64 (157.71)	337.21 (197.75)	0.182

N: normal gastric mucosa, NAG: non-atrophic gastritis, AGA: atrophic gastritis of the antrum, AGC: atrophic gastritis of the corpus, AGAC: atrophic gastritis of the antrum and corpus. HE-4: human epididymal protein 4, IL-6: interleukin-6, KL-6: Krebs von den Lungen 6. PGI: pepsinogen I, PGII: pepsinogen II. Results are presented in ng/mL for PGI, PGII, and ferritin; in pg/mL for IL-6; in pmol/l for HE-4; in µg/mL for adiponectin; and in International Units/mL for KL-6.

Pepsinogens

Patients with AGC had significantly decreased PGI levels as compared with N ($p < 0.001$), NAG ($p < 0.001$), and AGA ($p < 0.001$) patients, and borderline as compared with AGAC patients ($p = 0.051$). For PGII, the difference was statistically significant only between AGC and AGA patients ($p = 0.039$). PGI/PGII ratio was significantly lower in patients with AGC than in patients with N ($p < 0.001$), NAG ($p < 0.001$), AGA ($p < 0.001$), and AGAC ($p < 0.001$).

Similarly, the PGI/PGII ratio was significantly lower in patients with extensive AG (AGAC) as compared with N ($p < 0.001$), NAG ($p = 0.001$), and AGA ($p = 0.004$) patients. There was no significant difference in PG levels between the AGA patients and N ($p = 0.756$) or NAG ($p = 0.999$) patients (Supplementary Table S1).

HE-4

A significantly higher level of HE-4 was found in patients with AGAC as compared with N ($p = 0.020$) and NAG ($p = 0.011$) patients.

Other markers

No significant difference was found among the different groups for adiponectin, ferritin, IL-6, or KL-6 (Table 1).

(1) Diagnostic performance of biomarkers for the detection of any atrophy (AGA, or AGC, or AGAC)

For the detection of any gastric atrophy, PGI/PGII ratio showed the best performance, with Se and Sp of 44.7% (95%CI 36.7; 53.0) and 92.6% (95%CI 88.2; 95.8), respectively, using a standard cut-off <3.0 (AUC 0.685). The corresponding values for the best cut-off (<3.03) were of 46.7% (95%CI 38.6; 55.0) and 92.6% (95%CI 88.2; 95.8), respectively. This performance was improved in the case of moderate to severe atrophy, with Se of 57.4% and Sp of 92.6% for the best cut-off (Table 2).

Among other markers, the best diagnostic performance was observed with HE4, in particular in combination with PGI/PGII: Se of 69.7% and Sp 67.6% with AUC of 0.687 for any atrophy and Se of 85.2% and Sp of 52.0% with AUC 0.686 for moderate to severe atrophy (Table 2, Figure 1).

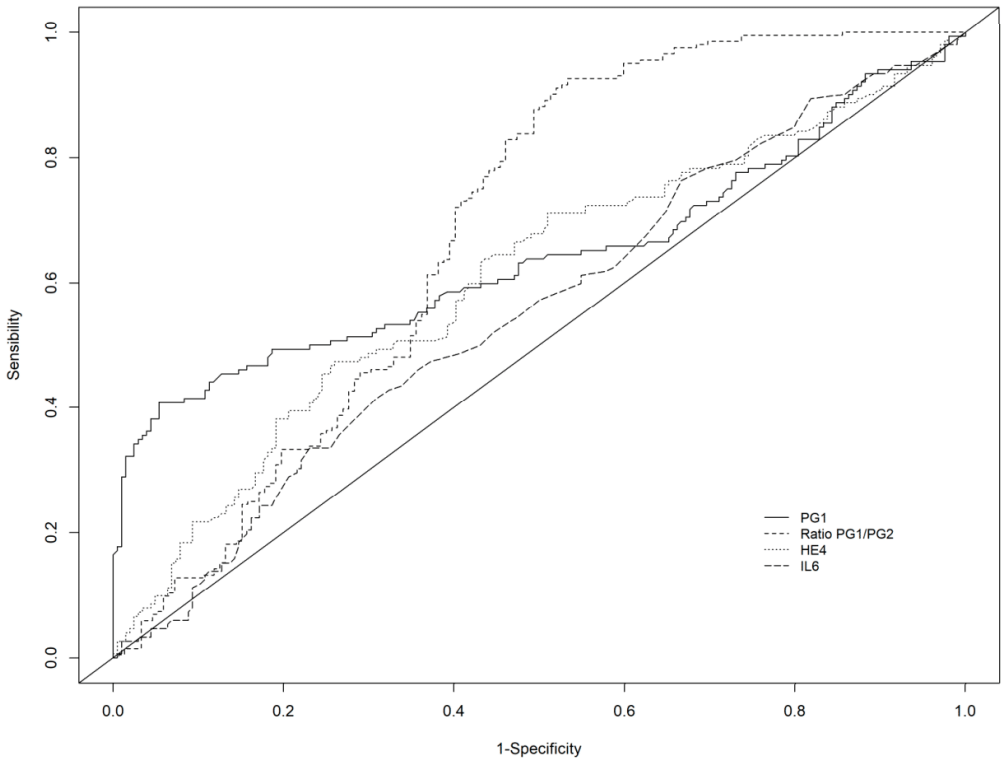

Figure 1. Receiver operating characteristic curve of PGI, PGI/PGII ratio, HE-4, and IL-6 for the detection of any atrophy (AGA or AGC or AGAC).

Table 2. Diagnostic performance of different markers for the detection of AG: comparison between all patients with AG (AGA or AGC or AGAC, *n* = 152) and all control patients (N + NAG, *n* = 204), presented for all patients (white space, *n* = 152) and patients with moderate to severe atrophy (grey space, *n* = 54).

	n =	AUC	Cut-Off	Se (95%CI)	Sp (95%CI)	PPV (95%CI)	NPV (95%CI)	PLR (95%CI)	NLR (95%CI)
PGI	356	0.642	≤30 *	46.7% (38.6; 55.0)	83.8% (78.0; 88.6)	68.3% (58.4; 77.1)	67.9% (61.7; 73.6)	2.89 (2.02; 4.12)	0.64 (0.54; 0.75)
	356	0.642	≤21.1 #	40.8% (32.9; 49.0)	94.6% (90.6; 97.3)	84.9% (74.6; 92.2)	68.2% (62.4; 73.6)	7.56 (4.13; 13.86)	0.63 (0.55; 0.72)
PGI/PGII	356	0.685	≤3 *	44.7% (36.7; 53.0)	92.6% (88.2; 95.8)	81.9% (72; 89.5)	69.2% (63.4; 74.7)	6.08 (3.62; 10.21)	0.6 (0.51; 0.69)
	356	0.685	≤3.03 #	46.7% (38.6; 55.0)	92.6% (88.2; 95.8)	82.6% (72.9; 89.9)	70.0 % (64.2; 75.4)	6.35 (3.79; 10.64)	0.58 (0.49; 0.67)
Adiponectin	356	0.512	≥6.6	30.3% (23.1; 38.2)	79.4% (73.2; 84.7)	52.3% (41.4; 63.0)	60.4% (54.3; 66.3)	1.47 (1.02; 2.11)	0.88 (0.77; 1.0)
Ferritin	356	0.510	≥150	19.1% (13.2; 26.2)	83.3% (77.5; 88.2)	46.0 % (33.4; 59.1)	58.0 % (52.1; 63.7)	1.14 (0.73; 1.79)	0.97 (0.88; 1.07)
HE4	356	0.606	≥75.8	47.4% (39.2; 55.6)	74.0 % (67.4; 79.9)	57.6% (48.4; 66.4)	65.4% (58.8; 71.5)	1.82 (1.37; 2.43)	0.71 (0.6; 0.84)
IL6	356	0.555	≥4.5	41.4% (33.5; 49.7)	69.1% (62.3; 75.4)	50.0 % (41.0; 59.0)	61.3% (54.7; 67.6)	1.34 (1.02; 1.77)	0.85 (0.72; 1.0)
KL6	356	0.564	≥322	50.7% (42.4; 58.9)	62.3% (55.2; 68.9)	50.0 % (41.8; 58.2)	62.9% (55.8; 69.5)	1.34 (1.06; 1.7)	0.79 (0.65; 0.96)
PGI/PGII +/− HE-4	356	0.687	PGI/PGII ≤ 3.03 OR HE4 ≥ 75.8	69.7% (61.8; 76.9)	67.6% (60.8; 74.0)	61.6% (53.9; 68.9)	75.0 % (68.1; 81.1)	2.16 (1.72; 2.7)	0.45 (0.35; 0.58)
	356	0.614	PGI/PGII ≤ 3.03 AND HE4 ≥ 75.8	23.7% (17.2; 31.3)	99.0% (96.5; 99.9)	94.7% (82.3; 99.4)	63.5% (58.0; 68.8)	24.16 (5.91; 98.78)	0.77 (0.7; 0.84)
PGI	258	0.740	≤30 *	55.6% (41.4; 69.1)	83.8% (78.0; 88.6)	47.6% (34.9; 60.6)	87.7% (82.2; 92.0)	3.43 (2.32; 5.09)	0.53 (0.39; 0.72)
	258	0.740	≤20.2 #	53.7% (39.6; 67.4)	95.6% (91.8; 98.0)	76.3% (59.8; 88.6)	88.6% (83.7; 92.5)	12.17 (6.14; 24.15)	0.48 (0.36; 0.65)
PGI/PGII	258	0.758	≤3 *	55.6% (41.4; 69.1)	92.6% (88.2; 95.8)	66.7% (51.0; 80.0)	88.7% (83.7; 92.6)	7.56 (4.39; 13.0)	0.48 (0.36; 0.65)
	258	0.758	≤3.03	57.4% (43.2; 70.8)	92.6% (88.2; 95.8)	67.4% (52.0; 80.5)	89.2% (84.2; 93.0)	7.81 (4.56; 13.38)	0.46 (0.34; 0.63)
HE-4	258	0.637	≥63.2	70.4% (56.4–82.0)	55.4% (48.3–62.3)	29.5% (21.8–38.1)	87.6% (80.6–92.7)	1.58 (1.25–1.99)	0.53 (0.35–0.82)
PGI/PGII +/− HE-4	258	0.686	PGI/PGII ≤ 3.03 OR HE4 ≥ 63.2	85.2% (72.9; 93.4)	52.0 % (44.9; 59.0)	31.9% (24.4; 40.2)	93.0 % (86.6; 96.9)	1.77 (1.48; 2.12)	0.29 (0.15; 0.55)
		0.684	PGI/PGII ≤3.03 AND HE4 ≥ 63.2	40.7% (27.6; 55.0)	96.1% (92.4; 98.3)	73.3% (54.1; 87.7)	86.0 % (80.8; 90.2)	10.39 (4.9; 22.03)	0.62 (0.49; 0.77)

* Commonly used cut-off, # best cut-off; AUC: area under curve, Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative likelihood ratio. N: normal gastric mucosa, NAG: non-atrophic gastritis, AG: atrophic gastritis, AGA: atrophic gastritis of the antrum, AGC: atrophic gastritis of the corpus, AGAC: atrophic gastritis of the antrum and corpus. PGI: pepsinogen I, PGII: pepsinogen II, HE-4: human epididymal protein 4, IL-6: interleukin-6, KL-6: Krebs von den Lungen 6. Results are presented in ng/mL for PGI, PGII and ferritin; in pg/mL for IL-6; in pmol/L for HE-4; in μg/mL for adiponectin; and in International Units/mL for KL-6.

Associations of biomarkers allowed an increase in Sp or Se, whether they were used together (marker 1 AND marker 2) or independently (marker 1 OR marker 2). To maximize Se, the most interesting combination for the detection of any AG was PGI/PGII OR HE4, with Se of 69.7% (95%CI 61.8–76.9) and Sp of 67.6% (95%CI 60.8–74.0) (cut-off: PGI/PGII <3.03, HE4 >75.8 µg/mL) for the detection of any AG. To maximize Sp, the best combination of biomarkers for the detection of any AG was the association of PGI/PGII and HE4, giving a Sp of 99.0% (95%CI 96.5–99.9) but a Se of only 23.7% (95%CI 17.2–31.3).

(2) Diagnostic performance for the detection of corpus atrophy

With the commonly used cut-off (<30 µg/L), PG I showed a Se of 71.2% and Sp of 83.8% for the detection of corpus AG, with corresponding PLR and NLR values of 4.4 and 0.34, respectively. Results were comparable for PGI/PGII ratio, with Se of 67.5% and Sp of 92.6% (PLR and NLR of 9.18 and 0.35, respectively). The results were improved in the case of moderate to severe corpus AG (PGI: Se 77.8%, Sp 83.8%; PGI/PGII: Se 75.0%, Sp 92.6%). PGI and PGI/II were superior to all other markers for the detection of AGC. (Table 3, Figure 2).

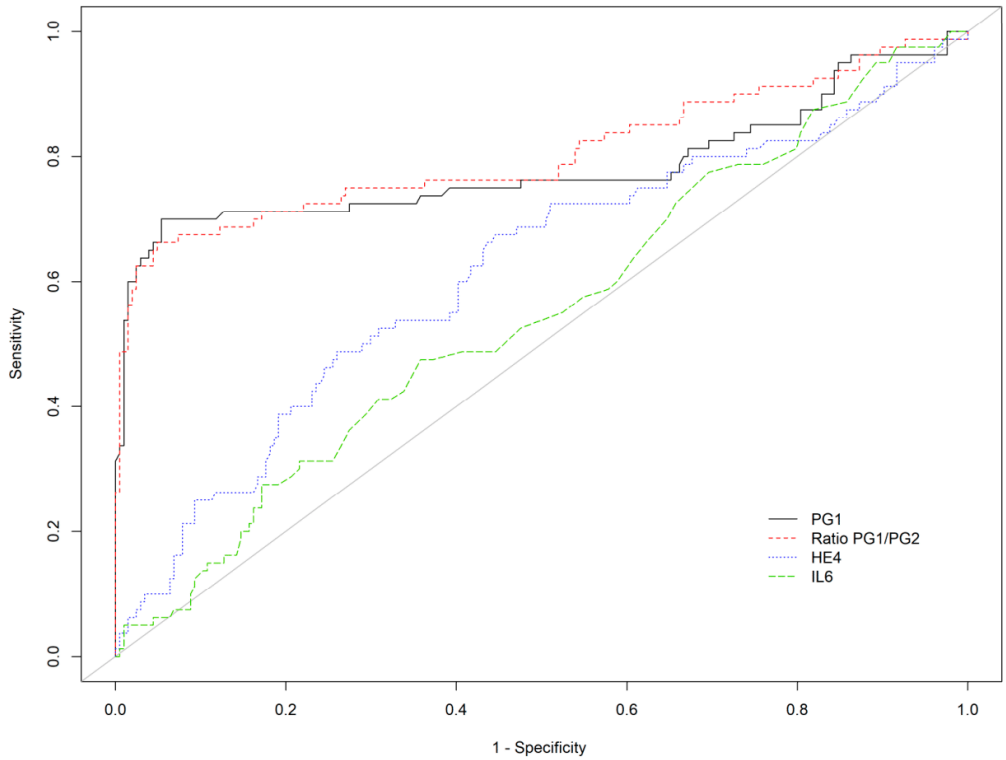

Figure 2. Receiver operating characteristic curve of PGI, PGI/PGII ratio, HE-4, and IL-6 for the detection of corpus AG (AGC + AGAC).

(3) Diagnostic performance for the detection of antrum atrophy

As expected, pepsinogens were not efficient for the detection of AGA, and the results are not provided in Table 4 (but can be available upon request) since the PGI levels of the patients with AGA were even slightly above the level of control patients (N + NAG). Among the other markers, HE4 and IL-6 yielded the bests results, with Se of 66.7% (95%CI 41.0–86.7) and 72.2% (95%CI 46.5–90.3), respectively, for the detection of moderate to severe antrum atrophy. Surprisingly, adiponectin showed a Se of 58.3% for the detection of any antrum AG but only of 22.2% for the detection of moderate to severe AG. KL6 showed a

very good Se (77.8%) for the detection of antrum AG, especially severe AG (94.4%), but with a very poor Sp (Table 4, Figure 3).

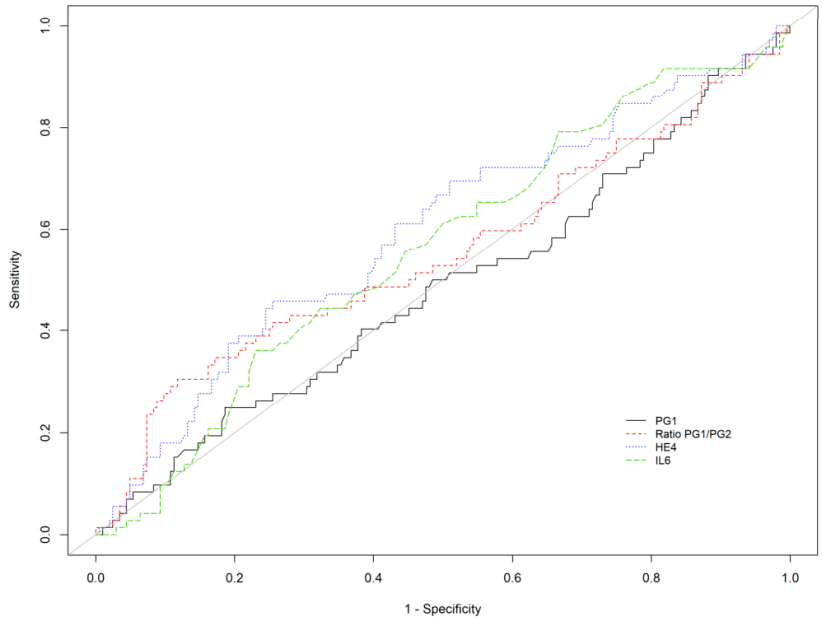

Figure 3. Receiver operating characteristic curve of PGI, PGI/PGII ratio, HE-4, and IL-6 for the detection of antrum AG (AGA).

(4) Diagnostic performance for the detection of the pangastric (antrum and corpus) atrophy

Among all the biomarkers tested, PGI/PGII ratio (cut-off <3) and HE4 (cut-off >75.8 µg/mL) showed the best performance for the detection of pangastric atrophy, with an AUC of 0.664 and 0.638 and Se of 44.7% (95%CI 28.6–61.7) and 52.6% (95%CI 35.8–69), respectively (Table 5, Figure 4).

(5) Diagnostic performance for the detection of moderate to severe atrophy

The diagnostic performance of PG and HE-4 increased in the case of moderate to severe atrophy as compared with any atrophy: Se and Sp for PGI/PGII ratio (cut off <3.03) were of 57.4% and 92.6%, for PGI/PGII ratio, respectively, and for HE4 (cut off >63.2 µg/mL) of 70.4% and 55.4%, respectively (Table 2). Corresponding AUCs for PGI/PGII ratio and HE4 were 0.740 and 0.637, respectively (Figure 2). A combination of markers allowed a further increase in Se up to 85.2% (95%CI 72.9–93.4). Consequently, the most interesting NLR for the detection of moderate to severe atrophy was obtained with a combination of PGI/PGII (<3.03) or HE4 (>63.2 µg/mL): 0.29 (95%CI 0.15–0.55). The best PLR was obtained with PGI/PGII ratio (7.56 (95%CI 4.39; 13)) (Table 2).

Table 3. Diagnostic performance of different markers for the detection of corpus atrophic gastritis: comparison between the patients with AGC + AGAC (n = 80) and control patients (N + NAG, n = 204), presented for all patients (white space, n = 80) and patients with moderate to severe atrophy (grey space, n = 36).

	n =	AUC	Cut-Off	Se (95%CI)	Sp (95%CI)	PPV (95%CI)	NPV (95%CI)	PLR (95%CI)	NLR (95%CI)
PGI	284	0.782	≤30 *	71.2% (60.0; 80.8)	83.8% (78.0; 88.6)	63.3% (52.5; 73.2)	88.1% (82.7; 92.3)	4.4 (3.13; 6.2)	0.34 (0.24; 0.49)
PGI	284	0.782	≤21.1 #	70.0% (58.7; 79.7)	94.6% (90.6; 97.3)	83.6% (72.5; 91.5)	88.9% (84.0; 92.8)	12.98 (7.18; 23.48)	0.32 (0.23; 0.44)
PGI/PGII	284	0.805	≤3 *	67.5% (56.1; 77.6)	92.6% (88.2; 95.8)	78.3% (66.7; 87.3)	87.9% (82.8; 91.9)	9.18 (5.51; 15.29)	0.35 (0.26; 0.48)
PGI/PGII	284	0.805	≤2.59 #	66.2% (54.8; 76.4)	95.1% (91.2; 97.6)	84.1% (72.7; 92.1)	87.8% (82.7; 91.8)	13.51 (7.24; 25.23)	0.35 (0.26; 0.48)
Adiponectin	284	0.540	≥6.66	37.5% (26.9; 49.0)	79.4% (73.2; 84.7)	41.7% (30.2; 53.9)	76.4% (70.1; 82.0)	1.82 (1.23; 2.69)	0.79 (0.66; 0.95)
Ferritin	284	0.463	≥150	15.0% (8.0; 24.7)	83.3% (77.5; 88.2)	26.1% (14.3; 41.1)	71.4% (65.2; 77.1)	0.9 (0.49; 1.65)	1.02 (0.91; 1.14)
HE-4	284	0.616	≥63.2	67.5% (56.1; 77.6)	55.4% (48.3; 62.3)	37.2% (29.4; 45.7)	81.3% (73.8; 87.4)	1.51 (1.22; 1.88)	0.59 (0.42; 0.82)
IL-6	284	0.549	≥4.2	47.5% (36.2; 59.0)	64.2% (57.2; 70.8)	34.2% (25.5; 43.8)	75.7% (68.6; 81.9)	1.33 (0.99; 1.78)	0.82 (0.65; 1.03)
KL-6	284	0.564	≥421	35.0 % (24.7; 46.5)	85.3% (79.7; 89.9)	48.3% (35.0; 61.8)	77.0 % (70.9; 82.3)	2.38 (1.52; 3.72)	0.76 (0.64; 0.9)
PGI	240	0.856	≤30 *	77.8% (60.8; 89.9)	83.8% (78.0; 88.6)	45.9% (33.1; 59.2)	95.5% (91.4; 98.1)	4.81 (3.36; 6.88)	0.27 (0.14; 0.49)
PGI	240	0.856	≤20.2 #	77.8% (60.8; 89.9)	95.6% (91.8; 98.0)	75.7% (58.8; 88.2)	96.1% (92.4; 98.3)	17.63 (9.09; 34.18)	0.23 (0.13; 0.43)
PGI/PGII	240	0.859	≤3 *	75.0 % (57.8; 87.9)	92.6% (88.2; 95.8)	64.3% (48.0; 78.4)	95.5% (91.5; 97.9)	10.2 (6.05; 17.2)	0.27 (0.15; 0.48)
PGI/PGII	240	0.859	≤0.96 #	72.2% (54.8; 85.8)	98.0 % (95.1; 99.5)	86.7% (69.3; 96.2)	95.2% (91.4; 97.7)	36.83 (13.67; 99.25)	0.28 (0.17; 0.48)

* Commonly used cut-off, # best cut-off; AUC: area under curve, Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative likelihood ratio. N: normal gastric mucosa, NAG: non-atrophic gastritis, AGC: atrophic gastritis of the corpus. PGI: pepsinogen I, PGII: pepsinogen II, HE-4: human epididymal protein 4, IL-6: interleukin-6, KL-6: Krebs von den Lungen 6. Results are presented in ng/mL for PGI, PGII and ferritin; in pg/mL for IL-6; in pmol/L for HE-4; in µg/mL for adiponectin; and in International Units/mL for KL-6.

Table 4. Diagnostic performance of different markers for the detection of antrum atrophic gastritis: comparison between the patients with AGA (n = 72) and control patients (N + NAG, n = 204), presented for all patients (white space, n = 72) and patients with moderate to severe atrophy (grey space, n = 18).

	n =	AUC	Cut-off	Se (95%CI)	Sp (95%CI)	PPV (95%CI)	NPV (95%CI)	PLR (95%CI)	NLR (95%CI)
Adiponectin	276	0.520	≤4.22	58.3% (46.1; 69.8)	50.5% (43.4; 57.5)	29.4% (22.1; 37.6)	77.4% (69.4; 84.2)	1.18 (0.93; 1.5)	0.83 (0.61; 1.12)
Ferritin	276	0.563	≥150	23.6% (14.4; 35.1)	83.3% (77.5; 88.2)	33.3% (20.8; 47.9)	75.6% (69.4; 81.0)	1.42 (0.85; 2.37)	0.92 (0.8; 1.06)
HE-4	276	0.595	≥77.6	45.8% (34.0; 58.0)	74.5% (68.0; 80.3)	38.8% (28.4; 50.0)	79.6% (73.2; 85.1)	1.8 (1.28; 2.54)	0.73 (0.58; 0.91)
IL-6	276	0.561	≥5.1	36.1% (25.1; 48.3)	77.0 % (70.6; 82.6)	35.6% (24.7; 47.7)	77.3% (71.0; 82.9)	1.57 (1.05; 2.33)	0.83 (0.69; 1.0)
KL-6	276	0.564	≥226	77.8% (66.4; 86.7)	33.8% (27.4; 40.8)	29.3% (23.0; 36.3)	81.2% (71.2; 88.8)	1.18 (1.0; 1.38)	0.66 (0.41; 1.05)
Adiponecti	258	0.501	≥8.47	22.2% (6.4; 47.6)	88.2% (83.0; 92.3)	14.3% (4.0; 32.7)	92.8% (88.2; 96.0)	1.89 (0.74; 4.85)	0.88 (0.69; 1.13)
Ferritin	258	0.550	≥150	16.7% (3.6; 41.4)	83.3% (77.5; 88.2)	8.1% (1.7; 21.9)	91.9% (87.0; 95.4)	1.0 (0.34; 2.94)	1.0 (0.81; 1.24)
HE-4	258	0.600	≥64.8	66.7% (41.0; 86.7)	56.9% (49.8; 63.8)	12.0 % (6.4; 20.0)	95.1% (89.6; 98.2)	1.55 (1.08; 2.22)	0.59 (0.3; 1.14)
IL-6	258	0.588	≥3.1	72.2% (46.5; 90.3)	41.2% (34.4; 48.3)	9.8% (5.3; 16.1)	94.4% (87.4; 98.2)	1.23 (0.9; 1.67)	0.67 (0.31; 1.45)
KL6	258	0.565	≥192	94.4% (72.7; 99.9)	22.5% (17.0; 28.9)	9.7% (5.8; 15.1)	97.9% (88.7; 99.9)	1.22 (1.07; 1.39)	0.25 (0.04; 1.68)

AUC: area under curve, Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative likelihood ratio. N: normal gastric mucosa, NAG: non-atrophic gastritis, AGA: atrophic gastritis of the antrum. HE-4: human epididymal protein 4, IL-6: interleukin-6, KL-6: Krebs von den Lungen 6. Results are presented in ng/mL for ferritin, in pg/mL for IL-6, in pmol/l for HE-4, in µg/mL for adiponectin, and in International Units/mL for KL-6.

Table 5. Diagnostic performance of different markers for the detection of pangastric (antrum and corpus) atrophic gastritis: comparison between the patients with AGAC (n = 38) and control patients (N + NAG, n = 204).

	n	AUC	Cut-Off	Se (95%CI)	Sp (95%CI)	PPV (95%CI)	NPV (95%CI)	PLR (95%CI)	NLR (95%CI)
PGI	242	0.613	≤30 *	47.4% (31.0; 64.2)	83.8% (78.0; 88.6)	35.3% (22.4; 49.9)	89.5% (84.3; 93.5)	2.93 (1.85; 4.63)	0.63 (0.46; 0.85)
PGI	242	0.613	≤21.1 #	47.4% (31.0; 64.2)	94.6% (90.6; 97.3)	62.1% (42.3; 79.3)	90.6% (85.9; 94.2)	8.78 (4.52; 17.09)	0.56 (0.41; 0.75)
PGI/PGII	242	0.664	≤3 *	44.7% (28.6; 61.7)	92.6% (88.2; 95.8)	53.1% (34.7; 70.9)	90.0% (85.1; 93.7)	6.08 (3.33; 11.11)	0.6 (0.45; 0.8)
PGI/PGII	242	0.664	≤2.86 #	44.7% (28.6; 61.7)	92.6% (88.2; 95.8)	53.1% (34.7; 70.9)	90.0% (85.1; 93.7)	6.08 (3.33; 11.11)	0.6 (0.45; 0.8)
Adiponectin	242	0.542	≥6.79	44.7% (28.6; 61.7)	79.9% (73.7; 85.2)	29.3% (18.1; 42.7)	88.6% (83.1; 92.8)	2.23 (1.42; 3.48)	0.69 (0.52; 0.93)
Ferritin	242	0.527	≥150	21.1% (9.6; 37.3)	83.3% (77.5; 88.2)	19.0% (8.6; 34.1)	85.0% (79.3; 89.6)	1.26 (0.63; 2.51)	0.95 (0.8; 1.13)
HE-4	242	0.638	≥75.8	52.6% (35.8; 69.0)	74.0% (67.4; 79.9)	27.4% (17.6; 39.1)	89.3% (83.7; 93.6)	2.03 (1.38; 2.96)	0.64 (0.45; 0.9)
IL-6	242	0.529	≥6.4	31.6% (17.5–48.7)	83.8% (78.0; 88.6)	26.7% (14.6–41.9)	86.8% (81.3–91.2)	1.95 (1.11–3.43)	0.82 (0.65–1.02)
KL-6	242	0.525	≥400	36.8% (21.8; 54.0)	80.4% (74.3; 85.6)	25.9% (15.0; 39.7)	87.2% (81.6; 91.6)	1.88 (1.14; 3.1)	0.79 (0.61; 1.01)

* Commonly used cut-off, # best cut-off; AUC: area under curve, Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative likelihood ratio. N: normal gastric mucosa, NAG: non-atrophic gastritis, AGAC: atrophic gastritis of the antrum and corpus. HE-4: human epididymal protein 4, IL-6: interleukin-6, KL-6: Krebs von den Lungen 6. Results are presented in ng/mL for ferritin, in pg/mL for IL-6, in pmol/L for HE-4, in µg/mL for adiponectin, and in International Units/mL for KL-6.

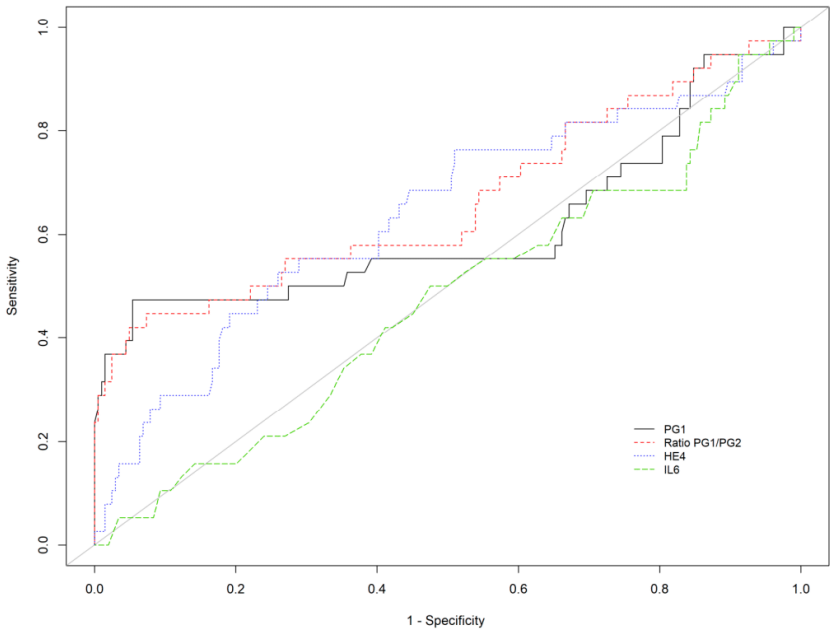

Figure 4. Receiver operating characteristic curve of PGI, PGI/PGII ratio, HE-4, and IL-6 for the detection of extensive AG (AGAC).

3.4. Diagnostic Performance in Patients without PPI Therapy

There was no significant change when analyzing the performance of the markers in this subgroup of patients (Supplementary Table S2).

3.5. Comparison between H. pylori-Positive and H. pylori-Negative Patients

There was no significant difference in PGI level (Mean ± SD) between *H. pylori*-positive patients (56.44 ± 42.91 ng/mL) and *H. pylori*-negative patients (59.99 ± 62.09 ng/mL, $p = 0.594$). However, *H. pylori*-positive patients, presented a lower PGI/PGII ratio (3.40 ± 1.58) than *H. pylori*-negative patients (4.30 ± 2.19, $p < 0.001$), and this difference was particularly observed in the group of control patients (3.69 ± 1.27 vs. 4.92 ± 1.55, respectively, $p < 0.001$), while it was not statistically significant in the group of the patients with AG (3.18 ± 1.78 vs. 3.37 ± 2.65, respectively, $p = 0.619$). Consequently, the PGII level was significantly higher in *H. pylori*-positive patients (17.34 ± 10.01) than in *H. pylori*-negative patients (13.34 ± 11.64, $p = 0.007$).

3.6. Comparison between the Results of the Previous Study (Gastropanel®) and the Current Study (CLEIA Fujirebio®)

There was not a significant difference in the diagnostic performance for the detection of any atrophy or corpus atrophy between the two tests, either for PGI or for PGI/PGII ratio (Supplementary Table S3).

4. Discussion

Our study is to our knowledge the first report of pepsinogen testing for the detection of AG using CLEIA in Europe. Only two studies have tested this technique so far, both of them performed in Japan, with one showing the normalization of PG levels after eradication of *H. pylori* [22] and the other showing that PG testing may be useful in classifying GC risk according to ABCD classification [23].

The PG I and PGII, whose levels reflect the functional state of the gastric mucosa, are the most validated markers. We report here a good performance of PGI and PGI/PGII

ratio measured by CLEIA for the detection of corpus AG, with a Se of 70% and Sp of over 94%. The sensitivity of this test further increases in the case of severe AG (about 78%), indicating that the more the atrophic lesions are pronounced, the more sensitive is the test. This observation is important from clinical point of view since the patients with more severe lesions are considered at most at risk of gastric cancer. These results are comparable with those achieved in most of the studies reported in the literature [24] and similar to those obtained in the same population in our previous study using ELISA assay [16]. Thus, this study shows that CLEIA is not only technically easy (results available in 20 min) but also efficient for the detection of corpus AG. Indeed, this technique is of growing interest in biology laboratories due to its easy use in a routine practice [25]. In a previous publication by Leja and colleagues, the comparison of three assays (two of them using ELISA and one using a latex agglutination test) did not show any significant changes in the diagnostic performance of pepsinogens among the different techniques used [26].

One of the weaknesses of non-invasive diagnosis of AG using PG testing is its relative low level of performance for the diagnosis of antrum atrophy. Although current evidence suggests that corpus atrophy is a major marker of risk of progression to GC, several studies have demonstrated that the most common location of gastric atrophy is the antrum [9–11,27] and that not only the location but also other parameters, such as severity of atrophy or incomplete type of intestinal metaplasia, are important factors associated with an increased risk of GC [28–30]. There is no currently established marker for the detection of antral atrophy. Some previous studies evaluated the diagnostic value of gastrin in this indication, but the results were discordant, and there were important methodological issues that made this marker less useful in clinical practice [31]. Therefore, we tried to investigate other potential markers of impaired gastric function in addition to pepsinogens—namely, those that have been reported to be involved in gastric carcinogenesis, particularly in the development of IM, and those whose value in the detection of GPL has not been investigated yet.

Adiponectin is a hormone whose blood concentration is inversely correlated with the level of visceral abdominal fat, and which has been associated with various human diseases [32]. It is believed to play a role in several malignancies through various mechanisms, among which are the regulation of cytokines and hormone release, insulin-resistance, and tumor cell proliferation [17]. A low adiponectin level has been associated with an increased risk of GC and has been correlated with clinical stage [33]. In our study, with a Se of 58%, serum adiponectin did not appear as a marker with performance sufficiently high to be considered as a potential candidate marker for the detection of AG. Krebs von Lungen 6, which is a subtype of mucin 1 (MUC1), has been mostly investigated in biliary or pancreatic cancers [18]. However, several studies have also shown aberrant expression of MUC1 in GC, which could be associated with deeper invasion and lymph node metastasis [18,34]. Although in our study, KL6 showed a very good Se for the detection of antral atrophy, and especially severe atrophy (>90%), due to a very low Se (22.5%), this marker does not appear reliable as a detection marker.

It has been suggested that consecutively to *H. pylori* infection and inflammation, the IL-6/STAT3 signaling pathway is activated, promoting epithelial to mesenchymal transition [19]. Increased levels of IL-6 and other chemokines have been associated with GC growth, and IL-6 serum level has been shown to increase in parallel to tumor progression and to be correlated with survival. Several studies have investigated the IL-6 value as a diagnostic marker of established GC, with a wide range of Se and Sp reported (0.39–0.85 and 0.50–0.97, respectively) and a wide variation in the cut-off values used [35–37]. In our study, IL-6 showed promising Se for the detection of marked antrum AG (72%) but with rather poor Sp (41%). Of note, IL-6 values may be influenced by several other conditions (autoimmune diseases, inflammation, physical exercise), and thus this parameter is susceptible to give false-positive results.

In addition to IL-6, HE-4 turned out to be one of the most promising markers in our study. HE-4 has been mostly investigated in ovarian and endometrial cancer, but several

studies have shown that HE-4 expression is increased in GC, particularly of diffuse-type, and its expression correlated with tumor size, stage, and survival [38,39]. More interestingly, HE-4 was upregulated in the metaplastic transition following acute parietal loss cell in mouse and in humans and has been suggested as a surrogate marker of preneoplastic lesions in the stomach [20], such as spasmolytic polypeptide-expressing metaplasia (SPEM) [40]. In the present study, HE-4 appeared of particular interest in combination with PGI/PGII ratio.

The combination of "functional" (PGI and II) and "morphological" (HE-4) markers could be an interesting approach for studying gastric precancerous lesions in the future.

We confirmed that patients with *H. pylori* infection have increased levels of PGII, probably related to chronic gastric inflammation, and in consequence, they present a lower PGI/PGII ratio, as already reported before [22].

Several points should be taken into account while interpreting the performance of diagnostic markers. First, the performance and usefulness may vary according to the population studied [41,42], the method used [26], the cut-off value set for each parameter [26,43], and the severity of AG [16]. Indeed, in highly selected patients such as in the present study, the prevalence does not reflect the distribution of the disease in the general population. The prevalence of AG varies largely between the Western and the Eastern populations, from 0–8% to more than 80%, respectively [9,44]. Moreover, its distribution varies according to age and ethnicity of the individuals within the same country [44–46]. Regarding the tools used to judge the diagnostic performance of a diagnostic test, Se and Sp are the most commonly used. PPV and NPV are also of interest but are influenced by the prevalence of the disease in the studied population, thus limiting the comparison from one study to another. To surmount this limitation, positive and negative likelihood ratios are used. They are expressed as the ratio between the probability of obtaining a positive (or negative) test in sick patients and the probability of obtaining a positive (or negative) test in controls. Usually, a PLR >10 (or NLR <0.1) is considered a sufficient value for assessing the diagnostic, whereas a PLR between 1 and 2 (or NLR 0.5–1) is considered useless.

For the assessment of a biomarker, the cut-off may be adjusted to maximize either Se or Sp. Increasing Se is privileged to exclude the disease (when the test is negative with a high Se) and when a false positive result does not have serious consequences. In the case of AG, this approach could be used in a screening strategy, allowing identification of the patients with positive test and thus those susceptible to bearing GPL. The second approach consists in increasing Sp and could be privileged in the follow up of patients with known GPL, allowing a reduction in the number (and frequency) of follow-up endoscopies. Indeed, systematic endoscopic follow-up of all the patients with GPL is costly, time consuming, not well-accepted, and consequently not well-applied [9]. In several studies, it has been shown that only a small proportion of patients with GPL will develop a GC or progress to more severe lesion [11,27,28,42,47–49]. Among these studies, several have shown that *H. pylori* eradication leads to a decreased score of GPL, and even its regression. Thus, one application of non-invasive markers would be to use them regularly to avoid systematic, repeated endoscopies in patients with stable non-invasive marker results.

Our study has several strengths. The prospective design and the rigorous methodology ensured reliable data. The study was performed under "real-life" conditions, including the data from four different centers, thus allowing generalization of the data for the French population considered as a low GPL prevalence area. This is the first study investigating the new, selected markers suspected to be involved in gastric carcinogenesis, never studied before in this setting. We report here for the first time that IL-6 and HE-4 may be useful for the assessment of antrum AG, and we demonstrate that pepsinogens testing using CLEIA shows good performance for the diagnosis of severe and corpus AG.

Our study also has some limitations. Only a third of the patients had advanced atrophy, and we did not have enough patients with pangastric advanced atrophy to reliably test the markers in this group. However, the proportion of patients with advanced atrophy was in line with data previously reported in Europe [50–52]. A high definition chromoendoscopy, which is known to be superior to white-light endoscopy for the diagnosis of GPL and is

currently recommended by the guidelines [13], was not required in the present study. Several studies reported that other factors than the extent of severity of AG could be associated with an increased risk of GC, such as the presence of incomplete type IM [28–30,53,54]; however, we were not able to provide these data for our population due to the absence of systematic IM subtyping. We did not perform a cost-efficiency analysis for this study in particular, but a recent and nice review summarized the results of studies conducted in this setting, and addressed the pros and cons in the different situations [55].

In conclusion, this is the first study evaluating PGI and PGII tested by CLEIA, which shows the good diagnostic performance of these markers for the diagnosis of AG in a European population, comparable with previously reported data and comparable with our previous results obtained in the same population with another technique. Additionally, we demonstrate here a potential interest in some new markers, such as HE4 and IL-6 in particular, for the assessment of antrum AG.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/diagnostics12030695/s1, Table S1: Post hoc analysis (Tukey's test) of the comparison between the different histological subgroups (only the markers for which the significant differences were found are presented), Table S2: Diagnostic performance of different biomarkers for the detection of atrophic gastritis: comparison between the control patients (N+NAG, n = 164) and patients with atrophic gastritis (AGA + AGC + AGAC, n = 119) without PPI treatment, Table S3: Comparison of diagnostic performance of PG I (A) and PGI/PGII (B) testing for the detection of any atrophic gastritis (AG) and corpus atrophic gastritis (AGC+ AGAC) between the current study (Fujirebio®test) and previous study (Gastropanel). Comparison of the ROC curves using the DeLong test.

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Institutional Review Board Statement: The study was approved by the local ethics committee (Comité de Protection des Personnes Ouest IV, 8 November 2011). The study that was allowed to conduct the biocollection was registered on clinicaltrials.gov under the number NCT02624271. The bio-collection was registered under the number DC-2011-1399.

Informed Consent Statement: A written, informed consent was obtained from all the patients before inclusion.

Data Availability Statement: All raw data are available upon request.

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Alimentary Tract

Serum pepsinogens can help to discriminate between *H. pylori*-induced and auto-immune atrophic gastritis: Results from a prospective multicenter study

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ABSTRACT

Background: Serum pepsinogen (PG) testing is recommended by the European guidelines for diagnosis of chronic atrophic gastritis (CAG). However, wide variations in diagnostic performances are observed, due to the differences in the extent of gastric atrophy, and possibly in its origin (*Helicobacter pylori*-, autoimmune (AIG)). Aim. To analyze the diagnostic performances of PGs testing according to these different parameters, using enzyme-linked-immunosorbent serologic assay (ELISA) and chemiluminescent immunoassay (CLEIA).

Methods: Serum samples from patients having undergone gastroscopy with biopsies in five French centers were collected prospectively. Sensitivity (Se), specificity (Sp), and Area Under Curve were analyzed according to the extent and origin of CAG.

Results: Overall, 344 patients (156 males [45%]; mean age 58.8 [±14.2] years) were included, among whom 44 had AIG. Diagnostic performances of PG I for the detection of corpus CAG were excellent, with Se and Sp of 92.7% and 99.1% for ELISA and 90.5% and 98.2% for CLEIA, respectively. For AIG, corresponding values were 97.7% and 97.4% for ELISA, and 95.6% and 97.1% for CLEIA. In multivariate analysis, PG levels were associated with the auto-immune origin ($p<0.001$) but not with the extent of the atrophic gastritis.

Conclusions: Pepsinogens are highly efficient for the diagnosis of corpus-limited CAG and allow to discriminate AIG from *H. pylori*-induced gastritis.

1. Introduction

With over one million new cases each year, responsible for almost 800 000 deaths, gastric cancer (GC) represents one of the deadliest cancers worldwide [1]. Gastric carcinogenesis is a multistep process usually induced by chronic infection with *Helicobacter pylori* (*H. pylori*) [2,3]. Screening and surveillance of patients with gastric precancerous lesions (chronic atrophic gastritis (CAG)

Abbreviations: AIG, Auto-immune Gastritis; AG, Atrophic gastritis; AUC, Area Under Curve; CAG, Chronic Atrophic Gastritis; CLEIA, Chemiluminescent Immunoassay; ELISA, Enzyme linked immunoabsorbent assay; NAI, Non-auto-immune; NLR, Negative likelihood ratio; NPV, Negative Predictive Value; PG, Pepsinogen; PLR, positive Likelihood ratio; PPV, Positive Predictive Value; ROC, Receiver-operating curve; Se, Sensitivity; Sp, Specificity.

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and/or intestinal metaplasia) and eradication of *H. pylori* appear as the best strategy to decrease the incidence of advanced GC and GC-related mortality [4].

Besides *H. pylori* infection, an alternative pathway promoting CAG is triggered by autoimmune gastritis (AIG) classically located in the corpus, and usually characterized by the presence of autoantibodies, including and anti-parietal cell- and anti-intrinsic factor antibodies. AIG is characterized by a progressive destruction of gastric corpus and fundus glands, responsible for decreased gastric acid secretion leading to hypergastrinemia and gastric enterochromaffin cell hyperplasia, leading in some cases to altered vitamin B12 absorption which may be responsible for megaloblastic anemia and sometimes neurological damage [5].

Altogether, at least three types of CAG can thus be distinguished based on the origin and localization of the lesions in the stomach: atrophic gastritis confined to the antrum, probably corresponding to the early phase of *H. pylori*-induced gastritis, atrophic gastritis confined to the corpus, most probably corresponding to AIG (upon clinical and biological confirmation of this diagnosis), and atrophic gastritis in both the antrum and the corpus (extensive gastritis), which may correspond to either the late stage *H. pylori*–induced gastritis, or mixed form of AIG and *H. pylori* gastritis [6,7].

Whatever the mechanism pathophysiological is, CAG may ultimately lead to the destruction of gastric glands and perturbations of gastric mucosal physiology. This has served as a basis for the development of blood tests proposed for a non-invasive strategy to detect gastric atrophy, more specifically by assessing serum levels of pepsinogen (PG) I and II. While PGI is secreted by chief cells and mucus neck cells of the corpus mucosa, PGII is secreted throughout the stomach and proximal duodenum. Therefore, in case of CAG affecting the corpus, the level of PGI drops significantly, while the level of PGII remains usually unchanged, hence allowing to use the decreased levels of PGI and/or PGI/PGII ratios as potential biomarkers of corpus CAG.

The diagnostic value of PG testing has been assessed in several studies, in different populations and using different methods. Although discordant results have been obtained with respect to its sensitivity (Se) [8] (ranging from 32 to 98%), this marker is the only one currently recommended by international guidelines for the screening of patients with gastric precancerous lesions, infected with *H. pylori*, or more generally at increased risk of gastric cancer [4,9]. Discrepancies existing among these results may be related to heterogeneous patient populations included in these studies, and especially to the proportions of the three types of atrophic gastritis mentioned above. Indeed, it has been suggested that, as compared to *H. pylori*-induced gastritis, AIG could lead to deeper destruction of gastric glands and more severe perturbations of gastric physiology [10,11] although these results need to be confirmed.

In our previous prospective multicenter studies, we reported the value of pepsinogen testing using ELISA (Enzyme linked immunoabsorbent assay) [12] or CLEIA- (Chemiluminescent Immunoassay) [13] for the detection of corpus gastric atrophy in the French population, with more than 70% Se and 90% specificity (Sp) for both methods.

In the present study, we aimed to analyze the PG I and II levels and the diagnostic performance of pepsinogen testing by ELISA and CLIA according to the histology-based subtypes of CAG, that means, according to its origin (autoimmune or non-autoimmune) and extent in the stomach.

2. Methods

The data obtained in our two previously published studies [12,13] were re-analyzed for the subgroups of patients with different types of CAG. The histological, clinical and biological data of the patients have been reviewed and on the basis of these data, the subgroup of patients with autoimmune gastritis has been identified. The diagnosis of AIG was based on the presence of typical histological lesions, namely atrophic gastritis and/or intestinal metaplasia confined to the corpus with linear or nodular hyperplasia of enterochromaffin-like cells. Serological anti-parietal cell antibodies and/or anti-intrinsic factor antibodies were also tested, but since they may be absent in AIG (sensitivity around 80% for APCA, 20% for anti-intrinsic factor), the only mandatory criterion was histology. Accordingly, two subgroups of patients with CAG have been identified: patients with autoimmune gastritis (AIG) and patients with non-autoimmune atrophic gastritis (NAIG, *H. pylori*-related atrophic gastritis).

2.1. Statistical analysis

The blood levels of PGI, PGII and PGI/PGII ratio were calculated for all groups of patients and were compared among each other's. The diagnostic performances for the detection of gastric atrophy were evaluated and compared among the groups (Auto-immune, non-auto-immune, and patients without atrophic gastritis).

Variables were described using mean, standard deviation, minimum and maximum value for continuous variables, median [first quartile, third quartile, minimum and maximum] for discontinuous variables, and frequencies for qualitative variables. Comparisons among the groups were realized using Student's test for continuous variables, and chi-squared tests (or Fisher's exact test if required) for qualitative variables. For any biomarker statistically significantly different among the groups ($p<0.05$), pairwise comparisons were performed by post hoc Tukey test analysis with Bonferroni correction. The diagnostic accuracy of the different biomarkers was evaluated for ELISA and CLEIA by Receiver Operating Characteristic (ROC) curve analysis, with evaluation of Se, Sp, Positive predictive value (PPV), Negative Predictive Value (NPV), and positive and negative likelihood ratios. The best cutoff value was defined for each biomarker by maximizing the Youden index. Multivariate analysis was conducted using logistic regression modeling. Kolmogorov and Smirnov test was used to analyze the distributions of the populations.

Statistical analysis was done at a two-tailed a level of 0.05. Statistical analysis was performed using the R version 4.0.2. software.

2.2. Research ethics and patient consent

The study was approved by the ethical review board "Protection des Personnes Ouest IV", November 8, 2011. The bio-collection was registered under the number DC-2011–1399. A written, informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's human research committee.

3. Results

3.1. Description of the population

From the initial cohort, patients with synchronous gastric cancer ($n = 5$), incomplete biopsy protocol (i.e. no histology available for antrum and/or corpus, $n = 29$), dosage failure ($n = 7$), or not fulfilling the inclusion criteria ($n = 2$) were excluded. Altogether, 344 patients (156 males [45%]; mean age 58.8 [±14.2] years) were included in our first study, in which PG I, PGII, and *H. pylori* serology were assessed by ELISA [12], and 356 patients (162 males (46%); mean age 58.6 (±14.2) years) were included in our second study, in which the PGI and II levels were measured by CLEIA[13]. The distribution of patients according to the extent of the CAG

Table 1
Diagnostic performances of Pepsinogens testing in patients with atrophic gastritis limited to the corpus, measured using ELISA and CLEIA methods.

Biomarker (ELISA)	AUC	Cut-off	Sensitivity [95% CI]	Specificity [95% CI]	Positive predictive value [95% CI]	Negative predictive value [95% CI]	Positive likelihood ratio [95% CI]	Negative likelihood ratio [95% CI]
PGI	0,971	≤ 39.5*	92.7% [80.1 - 98.5]	99.1% [94.9 - 100]	97.4% [86.5 - 99.9]	97.2% [92.2 - 99.4]	99.17 [14.07 - 698.91]	0.07 [0.02 - 0.22]
PGI	0,971	≤ 30	85.4% [70.8 - 94.4]	100% [96.6 - 100]	100% [90 - 100]	94.7% [88.8 - 98]	NA	0.15 [0.07 - 0.31]
PGI/PGII	0,946	≤ 3.1*	87.5% [73.2 - 95.8]	99.1% [94.9 - 100]	97.2% [85.5 - 99.9]	95.5% [89.8 - 98.5]	93.62 [13.26 - 660.89]	0.13 [0.06 - 0.29]
PGI/PGII	0,946	≤ 3	85% [70.2 - 94.3]	99.1% [94.9 - 100]	97.1% [85.1 - 99.9]	94.6% [88.7 - 98]	90.95 [12.87 - 642.54]	0.15 [0.07 - 0.32]
Biomarker (CLEIA)	AUC	Cut-off	Sensitivity [95% CI]	Specificity [95% CI]	Positive predictive value [95% CI]	Negative predictive value [95% CI]	Positive likelihood ratio [95% CI]	Negative likelihood ratio [95% CI]
PGI	0,942	≤ 19.2*	90.5% [77.4 - 97.3]	98.2% [93.8 - 99.8]	95.0% [83.1 - 99.4]	96.5% [91.3 - 99]	51.12 [12.9 - 202.6]	0.10 [0.04 - 0.25]
PGI	0,942	≤ 30	92.9% [80.5 - 98.5]	86.7% [79.1 - 92.4]	72.2% [58.4 - 83.5]	97% [91.6 - 99.4]	7.0 [4.33 - 11.29]	0.08 [0.03 - 0.25]
PGI/PGII	0,941	≤ 2.33*	88.1% [74.4 - 96]	99.1% [95.2 - 100]	97.4% [86.2 - 99.9]	95.7% [90.3 - 98.6]	99.55 [14.1 - 702.8]	0.12 [0.05 - 0.27]
PGI/PGII	0,941	≤ 3	88.1% [74.4 - 96]	98.2% [93.8 - 99.8]	94.9% [82.7 - 99.4]	95.7% [90.2 - 98.6]	49.77 [12.55 - 197.47]	0.12 [0.05 - 0.28]

AUC: Area Under Curve; CI: Confidence Interval.
ELISA: Enzyme-linked immunosorbent assay CLEIA: Chemiluminescent Enzyme immunoassay, PG I: Pepsinogen I, PGII: Pepsinogen II, PGI/PGII: Pepsinogen I/Pepsiongen II ratio; AUC: Area Under Curve, CI: Confidence interval, *: Best Cut-off.

(Antrum-limited, corpus-limited or extensive) in these two populations, has been already described previously [12,13]. In brief, the histological analysis found normal mucosa or non-atrophic gastritis, antrum limited, corpus limited or extensive gastritis in 57%, 20,2%, 11,8% and 11% of patients, respectively. The mean delay between the blood sample intake and the upper GI endoscopy was 5.4 days, but in almost 80% of the cases, the blood sampling and the endoscopy were performed the same day. In total, a mean of 6.8 (SD ±3.2) biopsies per patients were retrieved, and was 3.6 (SD±1.8) and 3.1 (SD±1.8) in the antrum and corpus, respectively. After reviewing the medical files, 44 patients with auto-immune gastritis (AIG) were identified, among whom 37 had an atrophic gastritis limited to the corpus, and 7 an extensive, antrum and corpus- involving gastritis. *H. pylori* infection (past or present, diagnosed either by serology or histology) was found in 14.9% of patients with AIG, and 30.3% of patients with NAIG.

3.2. Diagnostic performances of pepsinogens testing for the detection of corpus-limited atrophic gastritis

In our previous studies, we found that in case of corpus involvement (that is, corpus-limited or extensive CAG) PG levels were significantly decreased. In the present study, we wanted to analyze the performances according to the extent of atrophy, and in particular in cases of exclusive corpus-limited atrophic gastritis. In these cases, PG testing yielded excellent diagnostic performances, with AUC of 0.971 and 0.942 for ELISA and CLEIA methods, respectively. Similarly, diagnostic performances of PGI/PGII in these patients showed AUC of 0.946 and 0.941 for ELISA and CLEIA, respectively. The comparison of both methods did not show significant differences. The detailed results of Se, Sp, PPV, NPV, and positive and negative likelihood ratios are provided in Table 1.

3.3. Comparison of serum pepsinogens levels between the patients with similar localization of CAG according to the origin (autoimmune or not)

Considering that pepsinogens are not reliable markers for antrum-limited gastritis, and that none of the patients with AIG had antrum-limited gastritis, we further focused on extensive and corpus-limited atrophic gastritis. We wondered whether the origin of corpus limited or extensive lesions (AIG or NAIG) may impact the depth of atrophy reflected by a decreased level of PG. To this aim, we compared the PG values in patients with AI-corpus limited and AI-extensive to those with NAIG-corpus limited and NAIG-extensive atrophic gastritis.

Overall, patients with AIG (AI-corpus limited and AI-extensive) had significantly lower PGI level and PGI/PGII ratio than their NAIG (NAIG-corpus limited and NAIG-extensive) counterparts (Table 2). When focusing on corpus limited or extensive lesions separately, similar results were found with clearly lower PGI levels and PGI/PGII ratio in patients with AIG CAG (Table 2).

Fig. 1 summarizes the distribution of the population according to PGI and PGI/PGII ratio, depending on the presence or not of auto-immune gastritis**.**

3.4. Diagnostic performances of pepsinogen testing for the detection of AIG

As previously mentioned, PG testing showed very good diagnostic performances in patients with AGC. When focusing on patients with AIG, diagnostic performances still increased, with areas under curve up to 0.991 and 0.985, and 0.969 and 0.970 for PGI and PGI/PGII ratio, for ELISA and CLEIA methods, respectively (Table 3). These diagnostic performances were superior to those reported for AGC of any origin (AUC 0.963 and 0.935 for ELISA and CLEIA, respectively). Fig. 2 shows the ROC curves for PG testing in patients with AGC and AI-AGC. Finally, we conducted a multivariate regression analysis to investigate if PGI was a parameter independently associated with the origin of CAG. In univariate analysis, both location ($p<0.001$) and origin ($p<0.001$) were parameters associated with PG values, however in multivariate analysis, PGI was only significantly associated with the origin of the CAG ($p<0.001$), and no longer to the extent of CAG ($p = 0.433$). In the other words, PGI variations are more likely related to the origin of CAG, rather than to the location/extent of atrophy. The Kolmogorov and Smirnov test showed clear separation of the patients with AIG and NAIG, with a significant difference in the distribution of the patients according to PG values (Fig. 3).

Table 2
Comparison of serum pepsinogen I (PGI) levels (mean +/- SD) and PGI/PGII ratio (mean+/-SD), between the patients with auto-immune gastritis (AIG) and Non-auto-immune gastritis (NAIG) in ELISA and CLEIA study.

	AIG (corpus limited and extensive with AIG component, $n = 44$)	NAIG (antrum limited or extensive without AIG, $n = 33$)	p-value
ELISA			
PGI	12.5 (11.3)	118.8 (91.3)	<0.001
PGI/PGII	1.3 (1.4)	8.1 (4.8)	<0.001
CLEIA			
PGI	10.0 (29.4)	56.6 (50.5)	<0.001
PGI/PGII	0.7 (1.0)	3.9 (2.5)	<0.001
	AI-corpus limited ($n = 37$)	NAIG-corpus limited ($n = 4$)	p-value
ELISA			
PGI (pg/ml)	13.114 (11.883)	81.350 (70.414)	0.002
PGI/PGII	1.353 (1.437)	12.500 (7.339)	0.010
CLEIA			
PGI (pg/ml)	11.295 (32.344)	42.325 (36.651)	0.005
PGI/PGII	0.797 (1.136)	3.740 (2.480)	0.016
	AI-extensive ($n = 7$)	NAIG-extensive ($n = 29$)	p-value
ELISA			
PGI (pg/ml)	9.043 (7.115)	124.021 (93.664)	<0.001
PGI/PGII	0.957 (0.842)	8.152 (4.879)	<0.001
CLIA			
PGI (pg/ml)	4.286 (3.046)	62.231 (51.777)	0.002
PGI/PGII	0.481 (0.413)	4.187 (2.453)	<0.001

AIG: Auto-immune gastritis, ELISA: Enzyme-linked immunosorbent assay CLEIA : Chemiluminescent Enzyme immunoassay, PGI: Pepsinogen I, PGI/PGII : Pepsinogen I/ Pepsinogen II ratio, NAIG: Non-auto-immune atrophic gastritis,.

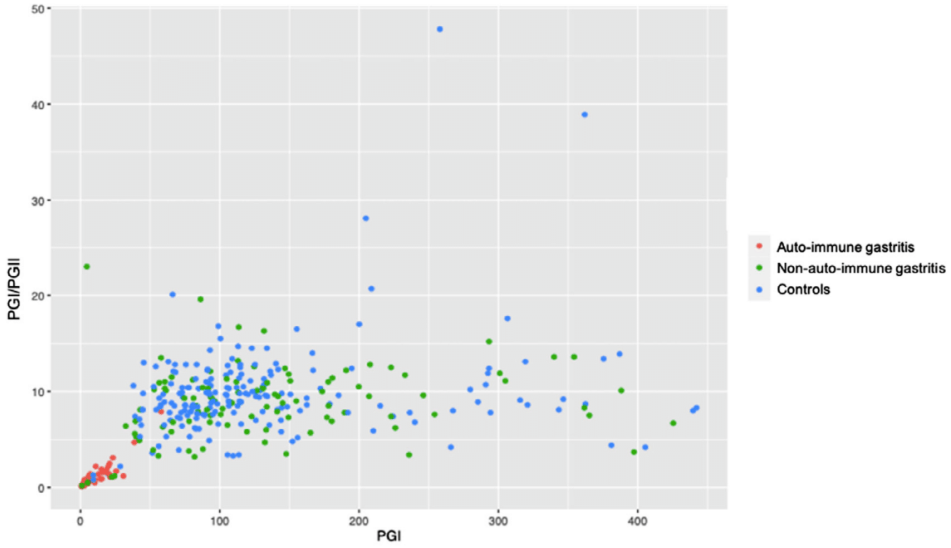

Fig. 1. Distribution of patients according to PGI and PGI/PGII ratio depending on the presence of atrophic gastritis.

In all the situations, there was no difference with one or the other technics of measurement of pepsinogens (CLEIA or ELISA), but the best cut-off varied as shown in Tables 2 or 3.

4. Discussion

This study represents a more detailed analysis of the results obtained in our two previously published studies, in which we tried to describe in more details the gastric physiology changes depending on the origin (autoimmune and non-autoimmune) of atrophic gastritis, and localization of atrophy. Our results suggest that AIG, as compared to NAIG ("environmental" gastritis, usually induced by chronic *H. pylori* infection), is associated with the most profound changes of gastric physiology as reflected by the lowest PG I levels and the lowest PG/I/PGII ratio. Since the severity of atrophy has been associated with the increased risk of evolution toward gastric adenocarcinoma, we may postulate that PG testing is the reliable method for identifying the patients at highest risk. However, in the

Table 3
Diagnostic performances of pepsinogen testing in patients with AIG, tested with ELISA and CLEIA.

Biomarker (ELISA)	AUC	Cut-off	Sensitivity [95% CI]	Specificity [95% CI]	Positive predictive value [95% CI]	Negative predictive value [95% CI]	Positive likelihood ratio [95% CI]	Negative likelihood ratio [95% CI]
PGI	0.991	≤ 38.7*	97.7% [88 - 99.9]	97.4% [94.1 - 99.2]	89.6% [77.3 - 96.5]	99.5% [97.1 - 100]	38.31 [16.11 - 91.12]	0.02 [0 - 0.16]
PGI	0.991	≤ 30	93.2% [81.3 - 98.6]	98% [94.9 - 99.4]	91.1% [78.8 - 97.5]	98.5% [95.6 - 99.7]	45.66 [17.25 - 120.83]	0.07 [0.02 - 0.21]
PGI/PGII	0.985	≤ 3.1*	95.3% [84.2 - 99.4]	97.4% [94.1 - 99.2]	89.1% [76.4 - 96.4]	99% [96.3 - 99.9]	37.38 [15.69 - 89.02]	0.05 [0.01 - 0.18]
PGI/PGII	0.985	≤ 3	93% [80.9 - 98.5]	97.4% [94.1 - 99.2]	88.9% [75.9 - 96.3]	98.5% [95.5 - 99.7]	36.47 [15.29 - 86.96]	0.07 [0.02 - 0.21]
Biomarker (CLEIA)	AUC	Cut-off	Sensitivity [95% CI]	Specificity [95% CI]	Positive predictive value [95% CI]	Negative predictive value [95% CI]	Positive likelihood ratio [95% CI]	Negative likelihood ratio [95% CI]
PGI	0.969	≤ 16.3*	95.6% [84.9 - 99.5]	97.1% [93.7 - 98.9]	87.8% [75.2 - 95.4]	99% [96.4 - 99.9]	32.49 [14.73 - 71.64]	0.05 [0.01 - 0.18]
PGI	0.969	≤ 30	97.8% [88.2 - 99.9]	83.8% [78 - 88.6]	57.1% [45.4 - 68.4]	99.4% [96.8 - 100]	6.04 [4.41 - 8.29]	0.03 [0 - 0.18]
PGI/PGII	0.970	≤ 2.33*	95.6% [84.9 - 99.5]	95.6% [91.8 - 98]	82.7% [69.7 - 91.8]	99% [96.4 - 99.9]	21.66 [11.4 - 41.15]	0.05 [0.01 - 0.18]
PGI/PGII	0.970	≤ 3	95.6% [84.9 - 99.5]	92.6% [88.2 - 95.8]	74.1% [61 - 84.7]	99% [96.3 - 99.9]	13 [7.95 - 21.24]	0.05 [0.01 - 0.19]

AUC: Area Under Curve; CI: Confidence Interval, AIG: Auto-immune gastritis, ELISA: Enzyme-linked immunosorbent assay CLEIA: Chemiluminescent Enzyme immunoassay, PG I: Pepsinogen I, PGII: Pepsinogen II, PGI/PGII: Pepsinogen I/Pepsiongen II ratio, Units: ug/l *:Best Cut-off.

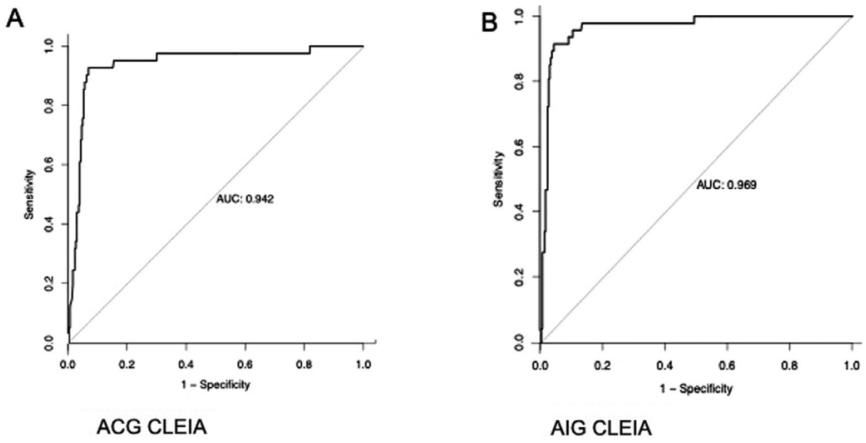

Fig. 2. ROC curves for PG testing in corpus limited gastric atrophy (A) and AIG (B). CLEIA: Chemiluminescent Enzyme ImmunoAssay.

previously published studies, wide discrepancies existed concerning the population studied, the geographic area (and related *H. pylori* prevalence), and the types of atrophic gastritis included in the studies (location and origin of CAG). These discrepancies are probably responsible for very heterogeneous results obtained within the different studies, as summarized in a recent meta-analysis [8]. Therefore, in the present study, we aimed to refine our previous analysis and provide precise data not only according to the extent, but also to the origin of gastritis.

Our results show that in the patients with both AIG or NAIG, the lesions localized exclusively in the corpus are associated with more profound PG abnormalities than when the lesions are localized only in the antrum or both in the antrum and the corpus. Although the patients with exclusive antrum gastritis or pangastritis (most probably of environmental origin) are also at risk for gastric cancer, pepsinogen testing may be less informative in this situation [14]. Therefore, additional non-invasive biomarkers should be investigated to identify more accurately the patients at highest risk of GC in this group

Very interesting results came from the comparison of PG levels between the patients not only according to the extent of the CAG, but also according to its origin. Although clearly limited by a small sample size, the comparison between the patients with and without AIG, showed striking differences in PG values being significantly lower in AIG. One may argue that the expected overrepresentation of AGC within the patients with AIG is responsible for this result. However, while grouping all the patients with CAG involving the corpus (corpus limited and extensive lesions), the difference in PG level remained highly significant, suggesting that glands atrophy is deeper and leads to more profound alteration of PGI secretion in patients with AIG[5]. Secondly, in our multivariate analysis, the serum PG levels were significantly related to the origin, but not to the location of the gastritis. This result is in line with a previous histopathological study that showed a more advanced atrophy (in the corpus in particular) in patients with auto-immune gastritis as compared to patients with *H. pylori*-related gastritis [10]. The underlying mechanisms leading to the atrophic gastritis in AIG and NAIG seem very different, as suggested by distinct inflammatory infiltrates, and deserve further investigation [15]. Our results suggest that PG could be a precise marker of AIG. However, several other markers were tested in this setting. Notably, a case-control study showed interesting diagnostic perfor-

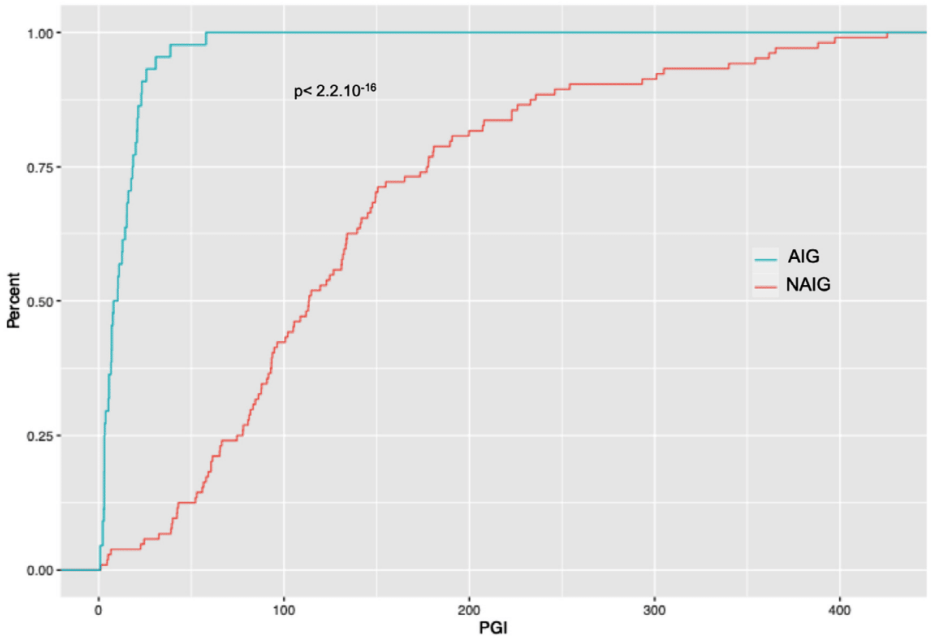

Fig. 3. Cumulative distribution of the patients according to the PGI levels.

mance of a score combining hemoglobin, mean corpuscular volume and gastrin, with Se and Sp of 85.7% and 83.7%, respectively [16]. This score has, however not been validated outside a referral center and thus is not currently used in general practice.

Our study has several strengths. Firstly, its prospective design and the validation of the results with two distinct methods of PG testing supports the conclusions. Secondly, the precise identification of patients with AIG, not only based on serology as in similar studies [17] (which is known to be sometimes responsible of false positive and false negative diagnostics) but on the mandatory typical pathology findings to ensure an accurate classification of the patients. Finally, we considered the environmental origin of CAG not only based on histology, but also on serology, as well as on the past history of *H. pylori* infection. Recent studies also focused on PG levels in patients with AIG, but with smaller sample-size, and no direct comparison with non-auto-immune gastritis [18,19].

This study has, however some limitations. Firstly, in the initial protocol, there was no requirement for the assessment of the depth and severity of atrophy according to OLGA staging, although it has been validated for both *H. pylori* and auto-immune gastritis [6,7]. A study investigating OLGA/OLGIM scores in patients with corpus gastritis from the two origins (AIG and NAIG) would provide a direct comparison at histology level. Some comparisons performed in the present study were made based on small sample size groups, limiting their robustness. However, when grouping extensive and corpus-limited lesions altogether, we found consistent results. Larger studies are needed, such as GISTAR study[20], involving thousands of patients, and will probably give the opportunity to confirm these results. Similarly, although we attempt to classify as accurately as possible all the patients, we cannot exclude that some single patients were misclassified, due to non-typical histology findings (or sampling errors) and/or seronegativity (with respect to *H. pylori*-induced CAG since may become seronegative after a long disease course). Moreover, the separation of *H. pylori* and AIG maybe artificial, and several studies, showed interactions between these two entities [5,21]. Besides, a recent study suggested that PG and Gastrin 17 could be interesting markers for the detection of gastric neuro-endocrine tumors in patients with auto-immune gastritis. We were not able to provide such information due to the relative small sample size of AIG group, and the diagnostic of gastric neuro-endocrine tumor was not systematically recorded in our study [22].

5. Conclusion

The present study shows that patients with AIG present lower levels of PGI than those with *H. pylori* -induced atrophic gastritis, suggesting a deeper gastric atrophy in AIG. Accordingly, PGs testing is very accurate in predicting the presence of corpus-limited CAG and especially AIG. These results need to be confirmed in larger studies, and additional non-invasive markers are still to be identified for the detection of antral- or extensive *H. pylori*-related gastritis.

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Financial disclosure

None.

Conflict of Interest

The authors listed above declare no conflict of interest for this article.

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Article

Atrophic Gastritis and Autoimmunity: Results from a Prospective, Multicenter Study

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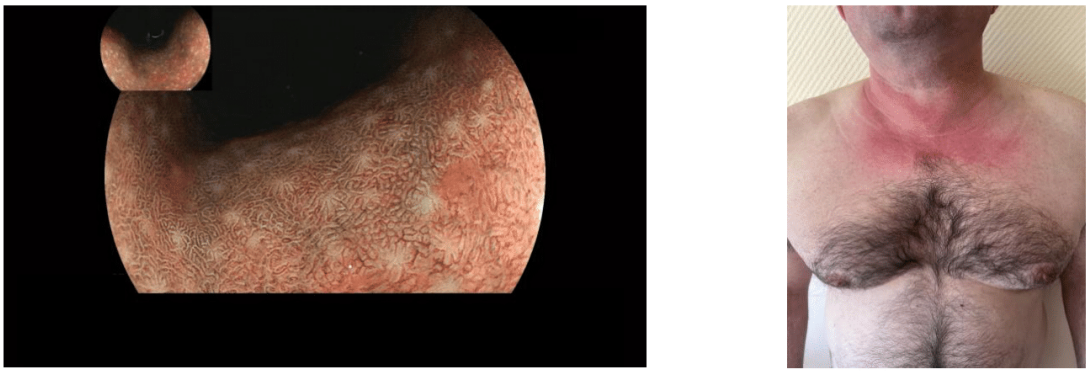
Abstract: Despite a global decrease, gastric cancer (GC) incidence appears to be increasing recently in young, particularly female, patients. The causal mechanism for this "new" type of GC is unknown, but a role for autoimmunity is suggested. A cascade of gastric precancerous lesions, beginning with chronic atrophic gastritis (CAG), precedes GC. To test the possible existence of autoimmunity in patients with CAG, we aimed to analyze the prevalence of several autoantibodies in patients with CAG as compared to control patients. Sera of 355 patients included in our previous prospective, multicenter study were tested for 19 autoantibodies (anti-nuclear antibodies, ANA, anti-parietal cell antibody, APCA, anti-intrinsic factor antibody, AIFA, and 16 myositis-associated antibodies). The results were compared between CAG patients (n = 154), including autoimmune gastritis patients (AIG, n = 45), non-autoimmune gastritis patients (NAIG, n = 109), and control patients (n = 201). ANA positivity was significantly higher in AIG than in NAIG or control patients (46.7%, 29%, and 27%, respectively, p = 0.04). Female gender was positively associated with ANA positivity (OR 0.51 (0.31–0.81), p = 0.005), while age and *H. pylori* infection status were not. Myositis-associated antibodies were found in 8.9% of AIG, 5.5% of NAIG, and 4.4% of control patients, without significant differences among the groups (p = 0.8). Higher APCA and AIFA positivity was confirmed in AIG, and was not associated with *H. pylori* infection, age, or gender in the multivariate analysis. ANA antibodies are significantly more prevalent in AIG than in control patients, but the clinical significance of this finding remains to be established. *H. pylori* infection does not affect autoantibody seropositivity (ANA, APCA, AIFA). The positivity of myositis-associated antibodies is not increased in patients with CAG as compared to control patients. Overall, our results do not support an overrepresentation of common autoantibodies in patients with CAG.

Keywords: autoimmune gastritis; chronic atrophic gastritis; autoimmunity; gastric cancer; *H. pylori*

1. Introduction

With almost one million new cases every year, gastric cancer (GC) is the fifth most frequently diagnosed cancer and the third cause of cancer-related death worldwide [1]. According to the model of gastric carcinogenesis known as "Correa's cascade" [2], GC is preceded by the sequential development of gastric precancerous lesions (GPL) (i.e., chronic atrophic gastritis (CAG), intestinal metaplasia (IM), and dysplasia), usually following a chronic infection with *Helicobacter pylori* (*H. pylori*) [2–4]. Less frequently, atrophic gastritis can result from an autoimmune reaction (autoimmune gastritis, AIG), which destroys gastric glands in the fundus [5–7]. In *H. pylori*-related gastritis, the lesions first appear in the antrum and eventually spread to the corpus [5,6,8,9]; in contrast, in AIG, the lesions are typically limited to the corpus (Figure 1a).

Despite a global decrease in GC incidence over the last decades, recent epidemiological studies have shown a rising incidence in young, especially female, patients [10,11]. The causal mechanisms for this "new" type of GC have not been identified. However, a role for autoimmunity or changes in the microbiota has been proposed [11–13]. This is supported by recent studies suggesting an association between autoimmune conditions, such as dermatomyositis, Addison disease, and herpetiform dermatitis, and an increased risk of GC [14–16].



(**a**) (**b**)

Figure 1. (**a**) Upper gastrointestinal endoscopy with virtual chromoendoscopy (BLI) showing intestinal metaplasia and gastric atrophy in the corpus in a patient with autoimmune gastritis. Photo from the private archive of Dr. Nicolas Chapelle. (**b**) A 45-year-old male patient with dermatomyositis presented with a skin rash and pruritus. Clinical examination revealed macular erythema over the sun-exposed parts of the anterior neck and upper chest, known as "V-sign", a skin manifestation of dermatomyositis. Data from the literature indicate a strong association between dermatomyositis and GC [14,15]. Patient informed consent for the photo publication was obtained.

To test whether a possible overrepresentation of autoimmunity-associated autoantibodies in patients with CAG could exist, this study aimed to analyze the prevalence of routinely assessed autoantibodies in patients with CAG as compared to control patients. We tested 19 different autoantibodies, including anti-nuclear antibodies (ANA), anti-parietal cell antibody (APCA), anti-intrinsic factor antibody (AIFA), and 16 different myositis-associated antibodies. APCA and AIFA were included as "classical" AIG-associated antibodies [14], and ANA were included because of their presence in multiple autoimmune diseases [17]. The panel of myositis antibodies was selected according to the data from the literature indicating a strong association between dermatomyositis and GC [14,15], while its possible

association with GPL has yet to be studied. The clinical picture of dermatomyositis is presented in Figure 1b.

2. Materials and Methods

2.1. Design of the Study

Serum samples collected from patients during our previous prospective, multicenter, cross-sectional study were analyzed. Out of 394 patients initially included in the study, 33 were excluded due to the absence of biopsies from two sites (corpus and antrum), 4 due to gastric adenocarcinoma at the initial examination, and 2 due to the lack of serum samples. Finally, 355 patients were included in the current study. Detailed descriptions of the study population, criteria for patient selection, endoscopy protocol used, blood sample collection, and histopathological evaluation of gastric biopsies were reported previously [18,19]. In brief, patients presented for upper endoscopy with gastric biopsies in four French University Hospitals between 2016 and 2019, and considered at increased risk of GC, were candidates for inclusion. Upper endoscopy with at least four gastric biopsies (two from the antrum and two from the corpus) was performed, and a fasting blood sample was obtained. The presence and intensity/distribution of GPL was evaluated with histopathological analysis of gastric biopsies according to the updated Sydney system [20]. The diagnosis of AIG was based on typical histology, including atrophic gastritis or intestinal metaplasia limited to the corpus with concomitant hyperplasia of enterochromaffin-like cells. Patients with CAG with typical histology were classified as NAIG. Other patients included in the study, with normal gastric mucosa or with non-atrophic gastritis on the histopathological examination, were classified as the control group. *H. pylori* status was assessed in all patients with histology and serology and was considered positive if at least one of the results was positive.

2.2. Antibodies

Nineteen autoantibodies, including ANA, APCA, AIFA, and 16 different myositis-associated antibodies were tested. APCA and AIFA were screened with fluorescence enzyme immunosorbent assay (FEIA) on an automated Phadia^{TM} 250 analyzer according to the supplier's recommendations (Thermo Fisher Scientific Inc., Waltham, MA, USA). The cut-off values the manufacturer recommended are presented in Table 1.

Table 1. Antibodies and the cut-off values.

Antibody	Negative	Equivocal	Positive
APCA, AIFA [U/mL]	<7	7–10	>10
ANA	<1:80	1:80	≥1:160
Myositis-associated antibodies	≤10	>10	>25

APCA, anti-parietal cell antibody; AIFA, anti-intrinsic factor antibody; ANA, anti-nuclear antibodies; myositis-associated antibodies including Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, SRP, Jo-1, PL-7, PL-12, EJ, OJ, Ku, PM-Scl100, PM-Scl-75, SSA-52 were assessed.

ANA were screened with indirect immunofluorescence assay on HEp-2 cells (screening dilution 1:80) according to the supplier's recommendations (Bio-Rad, Hercules, CA, USA). Positive sera were titrated with a 2-fold dilution up to a maximum of 1:2560. ANA results were classified as negative for dilution <1:80, equivocal for dilution 1:80, weakly positive for dilution 1:160, positive for dilution 1:320 or 1:640, and strongly positive for dilution ≥1:1280.

Myositis autoantibodies were analyzed with Immunoblot assay (EUROLINE Myositis Profile; Euroimmun, Lübeck, Germany) according to the supplier's recommendations. This immunoblot detected 12 myositis-specific autoantibodies (Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, SRP, Jo-1, PL-7, PL-12, EJ, OJ) and 4 myositis-associated autoantibodies (Ku, PM-Scl100, PM-Scl-75, SSA-52). Immunoblot bands were analyzed with the EUROLineScan

software (Euroimmun), allowing semi-quantitative determinations based on signal intensity (Table 1).

2.3. Statistical Analysis

Differences between the groups with CAG (origin or location) versus controls were tested using Pearson's chi-squared test for binary characteristics and the Student's t or Fisher's test for continuous characteristics. In order to identify characteristics that are more associated with ANA, AIFA, or APCA positivity, univariate and multivariate logistic regressions were carried out. Analyses were performed using R and R-studio. A significance level of $p < 0.05$ was adopted.

3. Results

3.1. Descriptive Analysis of the Study Population

A comparison of demographic characteristics, *H. pylori* status, and autoantibody positivity between CAG and control patients is presented in Table 2. The data, according to the type of CAG (AIG or NAIG), are presented in Table 3. Patients were categorized into two major groups: patients with CAG ($n = 154$), and control patients ($n = 201$) including those with normal gastric mucosa or non-atrophic gastritis. Subsequently, within the CAG group, patients were classified into two sub-groups: autoimmune gastritis (AIG, $n = 45$) and non-autoimmune gastritis (NAIG, $n = 109$). In our cohort, patients in the CAG group were older than the control patients (mean age 61.5 ± 13.8 years vs. 56.4 ± 14.2 years, respectively, $p < 0.001$). Within the CAG group, NAIG patients were significantly older than control patients (62.5 ± 12.8 vs. 56.4 ± 14.2 years, respectively, with significance in post hoc analysis $p < 0.001$). There was no significant age difference between the AIG and control patients (58.9 ± 15.8 vs. 56.4 ± 14.2 years, $p = 0.5$). *H. pylori* infection was more frequent in the CAG than in the control group (27.3% vs. 15.4%, respectively, $p = 0.006$) and in NAIG as compared to AIG patients (33.9% vs. 11.1%, $p = 0.02$).

Table 2. Comparison of patient characteristics, autoantibody seropositivity, and *H. pylori* status in chronic atrophic gastritis and control patients.

Parameter	CAG ($n = 154$)	Control ($n = 201$)	p-Value	Total ($n = 355$)
Age (year) mean (±SD)	61.5 (±13.8)	56.4 (±14.2)	<0.001	58.6 (±14.2)
Range (year)	22–89	18–82		18–89
Sex			0.09	
Female *n* (%)	76 (49.4)	117 (58.2)		193 (54.4)
Male *n* (%)	78 (50.6)	84 (41.8)		162 (45.6)
H. pylori status			0.006	
Histology positive *n* (%)	25 (16.2)	22 (10.9)		47 (13.2)
Serology positive *n* (%)	35 (22.7)	27 (13.4)		62 (17.5)
Any *H. pylori* positive *n* (%)	42 (27.3)	31 (15.4)		73 (20.6)
APCA *n* (%)	41 (27.0)	8 (4.0)	<0.001	49 (13.9)
AIFA *n* (%)	20 (13.5)	0	<0.001	20 (5.8)
ANA *n* (%)	52 (34.2)	54 (27.0)	0.1	106 (30.1)
Myositis-associated antibodies			0.6	
At least one antibody equivocal or positive *n* (%)	22 (14.5)	26 (12.9)		59 (13.8)
At least one positive antibody *n* (%)	9 (5.9)	9 (4.4)		19 (5.3)

CAG, chronic atrophic gastritis; APCA, anti-parietal cell antibody; AIFA, anti-intrinsic factor antibody. Cut-off values for APCA and AIFA, negative: <7 U/mL, equivocal: 7–10 U/mL, positive: >10 U/mL. Values qualified as positive for APCA and AIFA were with cut-off >10 U/mL. ANA, anti-nuclear antibodies; ANA results: negative dilution <1:80, equivocal 1:80, positive ≥1:160. Values qualified as positive for ANA were ≥1:160. Myositis-associated antibodies seropositivity, equivocal > 10; positive >25; myositis antibodies included Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, SRP, Jo-1, PL-7, PL-12, EJ, OJ, Ku, PM-Scl100, PM-Scl-75, SSA-52; *H. pylori*, *Helicobacter pylori*. Values are presented as n (%), mean (±SD). Pearson's chi-squared test or Linear Model ANOVA was used for statistical analysis, and a significance level of $p < 0.05$ was adopted.

Table 3. Comparison of patients' characteristics, *H. pylori* status, and antibody seropositivity among the patients with autoimmune gastritis, with non-autoimmune gastritis, and control patients.

Parameter	AIG (*n* = 45)	NAIG (*n* = 109)	Control (*n* = 201)	*p*-Value	Total (*n* = 355)
Age (year) mean (±SD)	58.9 (±15.7)	62.5 (±12.8)	56.4 (±14.2)	0.001	58.6 (±14.2)
Range (year)	23–89	22–87	18–82		18–89
Sex				0.059	
Female *n* (%)	27 (60.0)	49 (45.0)	117 (58.2)		193 (54.4)
Male *n* (%)	18 (40.0)	60 (55.0)	84 (41.8)		162 (45.6)
H. pylori status				<0.001	
Histology positive *n* (%)	0	25 (22.9)	22 (10.9)		47 (13.2)
Serology positive *n* (%)	5 (11.1)	30 (27.5)	27 (13.4)		62 (17.5)
Any *H. pylori* positive *n* (%)	5 (11.1)	37 (33.9)	31 (15.4)		73 (20.6)
APCA *n* (%)	33 (73.3)	8 (7.5)	8 (4.0)	<0.001	49 (13.9)
AIFA *n* (%)	17 (40.5)	3 (2.8)	0	<0.001	20 (5.8)
ANA *n* (%)	21 (46.7)	31 (29.0)	54 (27.0)	0.03	106 (30.1)
Myositis antibodies				0.8	
At least one antibody equivocal or positive *n* (%)	7 (14.3)	15 (15.6)	26 (12.9)		59 (13.8)
At least one positive antibody *n* (%)	4 (8.9)	6 (5.5)	9 (4.4)		19 (5.3)

AIG, autoimmune gastritis; NAIG, non-autoimmune gastritis; APCA, anti-parietal cell antibody; AIFA, anti-intrinsic factor antibody. Cut-off values for APCA and AIFA, negative: <7 U/mL, equivocal: 7–10 U/mL, positive: >10 U/mL. Values qualified as positive for APCA and AIFA with cut-off >10 U/mL. ANA, anti-nuclear antibodies; ANA results: negative dilution <1:80, equivocal 1:80, positive ≥1:160. Values qualified as positive for ANA were ≥1:160. Myositis-associated antibodies seropositivity, equivocal >10; positive >25; myositis antibodies included Mi-2α, Mi-2β, TIF1γ, MDA5, NXP2, SAE1, SRP, Jo-1, PL-7, PL-12, EJ, OJ, Ku, PM-Scl100, PM-Scl-75, SSA-52; *H. pylori*, *Helicobacter pylori*. Values are presented as n (%) or mean (± SD). Pearson's chi-squared test or Linear Model ANOVA was used for statistical analysis; a significance level of $p < 0.05$ was adopted.

3.2. Autoantibodies

APCA and AIFA antibody positivity was overall significantly higher in the CAG group than in the control group (APCA 27% vs. 4%; AIFA 13.5% vs. 0, respectively, $p < 0.001$). Within the subgroups of CAG, APCA, and AIFA, antibody positivity was significantly higher in the AIG than in the NAIG and control groups (APCA: 73.3% vs. 7.5% vs. 4%, respectively, $p < 0.001$; AIFA: 40.5% vs. 2.8% vs. 0, respectively, $p < 0.001$, significant differences were noted between AIG and NAIG and AIG and controls, $p < 0.001$ for both antibodies), while there was no significant difference in APCA and AIFA seropositivity between the NAIG and control patients (Table 3). Although ANA positivity was not significantly different between CAG and the control group ($p = 0.1$), it was significantly higher in AIG than in NAIG or control patients (46.7%, 29%, and 27%, respectively, $p = 0.03$, a significant difference was present between AIG and control groups $p = 0.04$, and not between AIG and NAIG, $p = 0.1$) (Table 3 and Table S2).

Overall, there was no difference between the CAG and the control group with respect to myositis-associated antibodies positivity (Table 2). Myositis antibodies were found in 8.9%, 5.5%, and 4.4% of patients with AIG, NAIG, and in the control group, respectively, ($p = 0.8$) (Table 3). The antibody with the highest percentage of at least an equivocal result was PM75 (4.5% in the whole cohort). Beyond PM75, other myositis antibodies with at least equivocal results were detected only in less than 2% of the cohort (Table S1).

3.3. Multivariate Analysis

To look for other factors that could potentially affect the ANA, APCA, and AIFA seropositivity, we performed a multivariate analysis for the following factors: age, gender, and *H. pylori* infection. We found that the only factor influencing ANA positivity was female gender (OR 0.51 (0.31–0.81, $p = 0.005$)). Neither age nor *H. pylori* infection affected ANA seropositivity (Table 4). Whereas for APCA and AIFA, we found no factor affecting their positivity (Table 5). Considering that positivity for myositis antibodies was rare, it was not included in the multivariate analysis.

Table 4. Multivariate analysis for ANA.

Parameter		ANA Negative	ANA Positive	OR (Univariate)	OR (Multivariate)
Age *n* (%)	≤50	70 (72.2)	27 (27.8)		
	>50	176 (69.0)	79 (31.0)	1.16 (0.70–1.97, $p = 0.5$)	1.23 (0.73–2.11, $p = 0.4$)
Sex *n* (%)	Female	122 (63.5)	70 (36.5)		
	Male	124 (77.5)	36 (22.5)	0.51 (0.31–0.81, $p = 0.005$)	0.50 (0.31–0.80, $p = 0.004$)
H. Pylori n (%)	Negative	199 (71.1)	81 (28.9)		
	Positive	47 (65.3)	25 (34.7)	1.31 (0.75–2.25, $p = 0.3$)	1.31 (0.74–2.27, $p = 0.3$)

ANA, anti-nuclear antibodies; ANA results: negative, dilution <1:80, positive, ≥1:16; *H. pylori, Helicobacter pylori*. OR, odds ratio (95% confidence interval). Values are presented as n (%). The chi-square test was used for statistical analysis.

Table 5. Multivariate analysis for APCA and AIFA.

Parameter		APCA Negative	APCA Positive	OR (Univariate)	OR (Multivariate)	AIFA Negative	AIFA Positive	OR (Univariate)	OR (Multivariate)
Age *n* (%)	≤50	80 (82.5)	17 (17.5)			87 (90.6)	9 (9.4)		
	>50	223 (87.5)	32 (12.5)	0.68 (0.36–1.31, $p = 0.2$)	0.69 (0.37–1.34, $p = 0.3$)	240 (95.6)	11 (4.4)	0.44 (0.18–1.13, $p = 0.08$)	0.46 (0.18–1.12, $p = 0.09$)
Sex *n* (%)	Female	163 (84.9)	29 (15.1)			176 (93.6)	12 (6.4)		
	Male	140 (87.5)	20 (12.5)	0.80 (0.43–1.47, $p = 0.5$)	0.83 (0.44–1.52, $p = 0.5$)	151 (95.0)	8 (5.0)	0.78 (0.30–1.93, $p = 0.6$)	0.85 (0.34–2.09, $p = 0.7$)
H. Pylori n (%)	Neg.	239 (85.4)	41 (14.6)			258 (92.8)	20 (7.2)		
	Pos.	64 (88.9)	8 (11.1)	0.73 (0.30–1.56, $p = 0.4$)	0.74 (0.31–1.58, $p = 0.5$)	69 (100.0)	0	-	0.09 (0.006–1.5, $p = 0.09$)

APCA, anti-parietal cell antibody; AIFA, anti-intrinsic factor antibody. Cut-off values for APCA and AIFA, negative: <7 U/mL, equivocal: 7–10 U/mL, positive: >10 U/mL.Values qualified as positive for APCA were with cut-off >10 U/mL; *H. pylori, Helicobacter pylori;* Neg., negative; Pos., positive. OR, odds ratio (95% confidence interval). Values are presented as n (%). The chi-square test was used for statistical analysis.

4. Discussion

It has been shown that different autoantibodies are more prevalent in patients with cancer, including GC [21,22], and that autoimmune diseases are associated with GC [14,15]. The aim of this study was thus to test the hypothesis that an increased prevalence of commonly assessed autoantibodies could be found already in patients with GPL, preceding the development of GC. Not surprisingly, APCA and AIFA positivity was more frequent in CAG than in control patients, explained by the high rate of seropositivity in patients with AIG [5]. No difference existed regarding ANA and myositis antibodies between CAG and controls, whereas ANA positivity was more frequent in AIG than in controls. To our knowledge, this is the first study investigating the ANA profile in a large group of patients with well-defined atrophic gastritis, particularly assessing the difference between the two types of chronic atrophic gastritis, autoimmune and *H. pylori*-induced.

ANA positivity is detected in several autoimmune conditions, including systemic lupus erythematosus, systemic sclerosis, and Sjogren's syndrome, but also in about 10% of the general population [23]. ANA are more prevalent in women and older individuals [24] and detected in around 30% of patients with malignancies [25]. In our study, seropositivity for ANA was detected in almost half of AIG patients (46.7%), which is a higher rate as compared to other studies, where seropositivity for ANA ranged between 17.4% in patients with AIG [26] to 19.1% in patients with *H. pylori*-negative CAG [27]. However, some of these studies were limited by a small sample size [26]. The higher ANA rate observed in our study may be related to the differences in methodology of ANA assessment, but also due to the high percentage of weakly positive results in our study (almost half of ANA positive results in AIG were weakly positive, Table S2). Another possible explanation of high ANA seropositivity in AIG patients is the presence of concomitant autoimmune thyroiditis in patients with AIG, which might be associated with ANA seropositivity. In the literature, the seropositivity of ANA in autoimmune thyroiditis was described in 20–35%

of patients [28,29]. We did not confirm the association between *H. pylori* infection and ANA positivity, as suggested by other studies [30]. The rate of ANA positivity in the control group in our study was also quite high (27%), but one third of the positive results were patients with weakly positive results (Table S2). Our study confirms that high ANA might be partially attributed to a higher percentage of women in the AIG group. This is consistent with the data from the literature [24].

Another original investigation of our research was the assessment of myositis antibodies in CAG. Although we observed an overall low prevalence of myositis antibodies (5.3%), this rate appears higher than expected when compared to the general population (close to 1%) [31]. Consequently, firm conclusions cannot be drawn, given the lack of direct comparisons and different techniques used to analyze myositis antibodies. Interestingly, there was no association with a particular myositis antibody. The highest seropositivity was noted for PM75, which, together with PM100, are the antibodies characteristic for polymyositis, systemic sclerosis, and overlap syndromes [32,33]. Seropositivity for the PM75 antibody has low specificity which increases, in the case of double seropositivity for both PM75 and PM100, which was rare in our study. Other antibodies, including the most specific for dermatomyositis, associated with malignancy (NXP2 and TIF1g), remained low in our study population (0.3–0.6%) [34]. Thus, our results may instead suggest that dermatomyositis develops together with GC as a paraneoplastic syndrome and is not a causative factor [35].

Not surprisingly, APCA and AIFA were more prevalent in CAG than in the control group, but seropositivity of these antibodies is the hallmark of AIG and pernicious anemia [36,37]. On the other hand, APCA and AIFA positivity did not differ between the NAIG group and control patients. APCA is usually detected in 85–90% of AIG patients but may also be found in around 10% of the healthy population. AIFA is present in 35–60% of AIG cases and is highly specific for AIG [5,38]. APCA and AIFA can also be found in patients with other autoimmune diseases, such as celiac disease and diabetes mellitus type I [36,39].

The role of AIG as a precancerous condition is currently debated [40,41]. Some studies reported an increased GC risk in patients with AIFA [13], but recent studies found no association [42,43]. According to recent data, the increased GC risk reported in patients with AIG would be mainly related to the concomitant *H. pylori* infection [42,43]. Indeed, another important aspect is the role of *H. pylori* infection and its relationship to AIG. Some data suggest that *H. pylori* infection triggers AIG [44,45] and that *H. pylori* eradication may even lead to the regression of AIG [46]. However, the exact role of *H. pylori* in AIG has yet to be elucidated [5,36]. In the present study, *H. pylori* infection did not affect APCA and AIFA seropositivity, which is consistent with the data from the literature [47]. The association of *H. pylori* with the development of many autoimmune diseases (organ-specific and systemic) is evoked [48]. Conversely, the only autoimmune disease in which the role of *H. pylori* as a causative factor has been admitted is autoimmune thrombocytopenia [49].

Overall, our results do not support the initial hypothesis of the autoimmune response in patients with GPL beyond the known association with ACPA and AIFA. Nevertheless, they do not preclude that an autoimmune response may appear later in the gastric carcinogenesis.

Our study has several strengths, including its multicentric and prospective design. It is the first prospective study investigating the presence of autoantibodies, with an emphasis on myositis antibodies, in patients with well-defined CAG. The patients were divided according to the origin of gastritis (AIG and NAIG) to better understand the differences in autoimmunity in CAG.

Our study also has some limitations. Firstly, the CAG group is relatively small. Even so, this condition is rare in regions with a low GC incidence, such as France (prevalence in Western Europe is around 3.2% [50], compared to >20% in Southeast Asia and South America [51]). Secondly, we did not adjust the antibody's level according to information from past medical history, such as the history of autoimmune diseases, which is a major drawback, but the initial study design did not imply the collection of these data from the patients. Moreover, the median age in our cohort is above 50 years. Therefore, the higher

level of antibodies may be related to age, even though multivariate analysis did not confirm the influence of age on antibody seropositivity.

5. Conclusions

Overall, our results do not support the association between the presence of common autoantibodies, particularly myositis-associated antibodies, and GPL, except for an expected overrepresentation of APCA and AIFA in AIG. Interestingly, ANA appear more prevalent in AIG than in control patients, and the significance of this finding, both on pathophysiological and diagnostic levels, deserves further investigation. Additionally, *H. pylori* infection does not appear to affect the autoantibody positivity (ANA, APCA, AIFA).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/diagnostics13091599/s1, Table S1: Seropositivity of myositis antibodies in patients with CAG and control patients; Table S2: ANA concentrations in patients with CAG and control patients.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to protection of patients' privacy.

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Abbreviations

GC	Gastric Cancer
GPL	Gastric Precancerous Lesions
H. pylori	*Helicobacter pylori*
CAG	Chronic Atrophic Gastritis
AIG	Autoimmune Gastritis
NAIG	Non-autoimmune Gastritis
ELISA	Enzyme-Linked Immunoabsorbent Assay
ANA	Anti-Nuclear Antibodies
APCA	Anti-Parietal Cell Antibody
AIFA	Anti-Intrinsic Factor Antibody

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Iron and Vitamin B12 Deficiency in Patients with Autoimmune Gastritis and *Helicobacter pylori* Gastritis: Results from a Prospective Multicenter Study

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Keywords
Autoimmune gastritis · Atrophic gastritis · *Helicobacter pylori* · Iron deficiency · Vitamin B12 deficiency

Abstract
Introduction: Iron and vitamin B12 deficiencies are common in patients with atrophic gastritis, but there are limited data on the prevalence of these deficiencies in different types of atrophic gastritis. ***Methods:*** This multicenter, prospective study assessed micronutrient concentrations in histologically confirmed autoimmune gastritis (AIG, n = 45), *Helicobacter pylori*-related non-autoimmune gastritis (NAIG, n = 109), and control patients (n = 201). A multivariate analysis was performed to determine factors influencing those deficiencies. ***Results:*** The median vitamin B12 concentration was significantly lower in AIG (367.5 pg/mL, Q1, Q3: 235.5, 524.5) than in NAIG (445.0 pg/mL, Q1, Q3: 355.0, 565.0, p = 0.001) and control patients (391.0 pg/mL, Q1, Q3: 323.5, 488.7, p = 0.001). Vitamin B12 deficiency was found in 13.3%, 1.5%, and 2.8% of AIG, NAIG, and control patients, respectively. Similarly, the median ferritin concentration was significantly lower in AIG (39.5 ng/mL, Q1, Q3: 15.4, 98.3 ng/mL) than in NAIG (80.5 ng/mL, Q1, Q3: 43.6, 133.9, p = 0.04) and control patients (66.5 ng/mL, Q1, Q3: 33.4, 119.8, p = 0.007). Iron deficiency and iron deficiency adjusted to CRP were present in 28.9% and 33.3% of AIG, 12.8% and 16.5% of NAIG, and 12.9% and 18.4% of controls, respectively.

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Multivariate analysis demonstrated that AIG patients had a higher risk of developing vitamin B12 deficiency (OR: 11.52 [2.85–57.64, p = 0.001]) and iron deficiency (OR: 2.92 [1.32–6.30, p = 0.007]) compared to control patients. Factors like age, sex, and *H. pylori* status did not affect the occurrence of vitamin B12 or iron deficiency. ***Conclusion:*** Iron and vitamin B12 deficiencies are more commonly observed in patients with AIG than in those with NAIG or control patients. Therefore, it is essential to screen for both iron and vitamin B12 deficiencies in AIG patients and include the treatment of micronutrient deficiencies in the management of atrophic gastritis patients.

Introduction

Iron and vitamin B12 deficiencies represent a significant health problem with wide-ranging implications for individuals' overall well-being. They contribute to a spectrum of clinical manifestations, including anemia (iron deficiency anemia and pernicious anemia in vitamin B12 deficiency), fatigue, dizziness, chest pain [1], and neuropsychiatric manifestation in case of vitamin B12 deficiency [2].

While iron and vitamin B12 deficiencies can arise from various causes, gastric precancerous lesions (GPLs), including autoimmune gastritis (AIG) and *Helicobacter pylori* (*H. pylori*)-related non-autoimmune gastritis (NAIG), are recognized as distinct etiologies commonly associated with those deficiencies. The appearance of GPL, i.e., chronic atrophic gastritis, intestinal metaplasia, and dysplasia, usually precedes gastric cancer [3]. Chronic infection with *H. pylori* is the most common cause of GPL. However, autoimmune reaction leading to AIG can also contribute [3–5]. In AIG, the destruction of parietal cells in the gastric corpus by autoantibodies results in achlorhydria and impaired intrinsic factor production and may lead to subsequent iron and vitamin B12 malabsorption [6]. Conversely, in NAIG, the lesions typically begin in the antrum and eventually spread to the corpus, damaging the gastric mucosa and increasing gastric juice pH, possibly leading to impaired iron absorption [5–9].

Understanding the prevalence and underlying mechanisms of iron and vitamin B12 deficiency in GPL is crucial for effective diagnosis and management. Therefore, in this prospective multicenter study, we aimed to evaluate the prevalence of iron and vitamin B12 deficiency in well-defined and histologically confirmed AIG, NAIG, and control patients without atrophic gastritis. Additionally, we evaluated C-reactive protein (CRP)-adjusted ferritin levels and the prevalence of iron deficiency in *H. pylori*-positive patients. We also performed a multivariate analysis including age, gender, *H. pylori* infection, and the state of the gastric mucosa to search for the factors influencing vitamin B12 and iron deficiencies.

Patients and Methods

The serum samples from the patients included in our previous prospective, multicenter, cross-sectional study were retrieved and analyzed for micronutrient concentrations. Out of 394 patients initially included in this study, 33 were excluded due to the absence of biopsies from two sites (corpus and antrum), 4 due to gastric adenocarcinoma at the initial examination, and 2 due to the lack of serum samples. Finally, 355 patients were included in the current study.

The study protocol has been described previously [10–13]. In brief, patients presented for upper endoscopy according to usual care from four university hospitals in France between 2016 and 2019 were candidates for inclusion. Additional inclusion criteria were patients with increased risk of gastric cancer (at least one of the following criteria): (1) age >50 years, (2) family history of gastric cancer, (3) known precancerous lesions, (4) pernicious anemia, (5) *H. pylori* infection, (6) genetic predisposition (Lynch syndrome, adenomatous familial polyposis), (7) history of gastric MALT lymphoma, (8) dyspepsia, (9) anemia of unknown origin, (10) personal history of GC resected endoscopically.

Exclusion criteria for the study were (1) subjects with known active cancer, (2) pregnancy, (3) active digestive bleeding, and (4) conditions that may interfere with the study objectives, according to the investigator. The upper endoscopy with gastric biopsies according to the Sydney protocol (non-targeted biopsies requiring at least four biopsies – two from the gastric antrum and two from the gastric body) was performed in all patients, and a fasting blood sample was obtained. The presence, severity, and extent of GPL were evaluated by histopathological analysis of gastric biopsies according to the updated Sydney system [14]. The diagnosis of AIG was based on typical histology, i.e., atrophic gastritis or intestinal metaplasia in the corpus with concomitant hyperplasia of enterochromaffin-like cells. Patients with chronic atrophic gastritis without clear AIG were classified as NAIG, whereas patients with normal gastric mucosa or non-atrophic gastritis were classified as the control group.

Additionally, the antibody characteristics for AIG were assessed, including anti-parietal cell antibodies and anti-intrinsic factor antibodies. According to the supplier's recommendations, anti-parietal cell antibodies and anti-intrinsic factor antibodies were screened by fluorescence enzyme immunosorbent assay on an automated Phadia™ 250 analyzer (Thermo Fisher Scientific Inc, Waltham, USA). The cut-off values the manufacturer recommended are presented in Table 1.

A serum vitamin B12 concentration threshold below 200 pg/mL was used to define vitamin B12 deficiency. Serum ferritin concentration was used as the indicator for iron deficiency, with thresholds below 25 ng/mL for women and 30 ng/mL for men [1]. Iron deficiency adjusted to CRP was assessed separately according to some data from the literature indicating the necessity to adapt ferritin level to existing inflammation [15], with the threshold for CRP >5 mg/dL and ferritin <70 ng/mL. Serum ferritin and vitamin

Table 1. Comparison of basic characteristics and micronutrient concentrations among AIG, NAIG, and control patients

Parameter	AIG (*N* = 45)	NAIG (*N* = 109)	Control (*N* = 201)	Total (*N* = 355)
Age, mean (±SD), years	58.9 (±15.7)	62.5 (±12.8)	56.4 (±14.2)	58.6 (±14.2)
Range, year	23–89	22–87	18–82	18–89
Sex, *n* (%)				
Female	27 (60.0)	49 (45.0)	117 (58.2)	193 (54.4)
Male	18 (40.0)	60 (55.0)	84 (41.8)	162 (45.6)
H. pylori status				
Histology positive, *n* (%)	0	25 (22.9)	22 (10.9)	47 (13.2)
Serology positive, *n* (%)	5 (11.1)	30 (27.5)	27 (13.4)	62 (17.5)
Any *H. pylori* positive, *n* (%)	5 (11.1)	37 (33.9)	31 (15.4)	73 (20.6)
APCA, *n* (%)	33 (73.3)	8 (7.5)	8 (4.0)	49 (13.9)
AIFA, *n* (%)	17 (40.5)	3 (2.8)	0	20 (5.8)
Vitamin B12, median (Q1, Q3), pg/mL	367.5 (235.5, 524.5)	445.0 (355.0, 565.0)	391.0 (323.5, 488.7)	403.0 (326.5, 517.5)
Vitamin B12 deficiency, *n* (%)	6 (13.3)	3 (1.5)	3 (2.8)	12 (3.4)
Ferritin, median (Q1, Q3), ng/mL	39.5 (15.4, 98.3)	80.5 (43.6, 133.9)	66.5 (33.4, 119.8)	69.5 (30.6, 120.3)
Iron deficiency, *n* (%)	13 (28.9)	14 (12.8)	26 (12.9)	53 (14.9)
Iron deficiency adjusted to CRP, *n* (%)	15 (33.3)	18 (16.5)	37 (18.4)	70 (19.7)
Concomitant iron and vitamin B12 deficiency, *n* (%)	1 (2.2)	1 (0.9)	1 (0.5)	3 (0.8)

AIG, autoimmune gastritis; APCA, anti-parietal cell antibody; AIFA, anti-intrinsic factor antibody, cut-off values for APCA and AIFA, negative: <7 U/mL, equivocal: 7–10 U/mL, positive: >10 U/mL, values qualified as positive for APCA and AIFA with cut-off >10 U/mL; CRP, C-reactive protein; *H. pylori*, *Helicobacter pylori*; NAIG, non-autoimmune gastritis; ferritin, normal range: 30–300 ng/mL for males, 25–300 ng/mL for females; iron deficiency, ferritin level below the lower threshold; iron deficiency adjusted to CRP, if CRP >5 mg/dL, ferritin lower threshold is < 70 ng/mL; vitamin B12, normal range 200–800 pg/mL. Values are presented as *n* (%), mean (±SD), or median (quartile 1, quartile 3, Q1, Q3).

B12 assays were performed by electrochemiluminescent assay on Cobas 8000 e 602® (Roche Diagnostics, Meylan, France) according to the manufacturer's instructions.

H. pylori status was assessed in all patients by histology and serology and was considered positive if at least one of the results was positive. *H. pylori* serology was assessed with IgG antibody by ELISA using GastroPanel®, Biohit Oyj; levels above 30 enzyme-immunoassay units were considered an indicator of *H. pylori* infection (ongoing or recent).

Differences among the groups (AIG, NAIG, and control patients) were tested using Pearson's χ^2 test for binary characteristics or Kruskal-Wallis's test for continuous variables. Post hoc comparisons were made using the Tukey test or χ^2 with adjustment for multiplicity. Univariate and multivariate logistic regressions were carried out to identify characteristics associated with iron deficiency and vitamin B12 deficiency. Odds ratios (ORs) were presented with their 95% confidence interval (CI). A significance level of $p < 0.05$ was adopted. Analyses were performed using R and RStudio.

Results

Demographic characteristics, micronutrient levels, and *H. pylori* status in AIG (n = 45), NAIG (n = 109), and control patients (n = 201) are presented in Table 1.

Micronutrient Deficiencies

The median B12 concentration was of 367.5 pg/mL (quartile 1, quartile 3, Q1, Q3: 235.5, 524.5 pg/mL) in AIG, 445.0 pg/mL (Q1, Q3: 355.0, 565.0 pg/mL) in NAIG, and 391.0 pg/mL (Q1, Q3: 323.5, 488.7 pg/mL) in control patients (shown in Table 1; Fig. 1a). The differences were statistically significant between AIG and NAIG (p = 0.001) and AIG and control groups (p = 0.05), but not between NAIG and control groups (p = 0.9). Vitamin B12 deficiency was found in 13.3%, 1.5%, and 2.8% of patients with AIG, NAIG, and control, respectively.

The median ferritin concentration was of 39.5 ng/mL (Q1, Q3: 15.4, 98.3 ng/mL) in AIG, 80.5 ng/mL (Q1, Q3: 43.6, 133.9 ng/mL) in NAIG, and 66.5 ng/mL (Q1, Q3: 33.4, 119.8 ng/mL) in the control groups (shown in Fig. 1b). The differences were statistically significant between AIG and NAIG (p = 0.04), and AIG and control groups (p = 0.007), but not between NAIG and control groups (p = 0.2). Iron deficiency was present in 28.9%, 12.8%, and 12.9% of AIG, NAIG, and control patients, respectively. Iron deficiency adjusted to CRP was found in 33.3%, 16.5%, and 18.4% of AIG, NAIG, and control patients, respectively (shown in Table 1). Concomitant iron and vitamin B12 deficiency was found in 1 patient in each group (shown in Fig. 2).

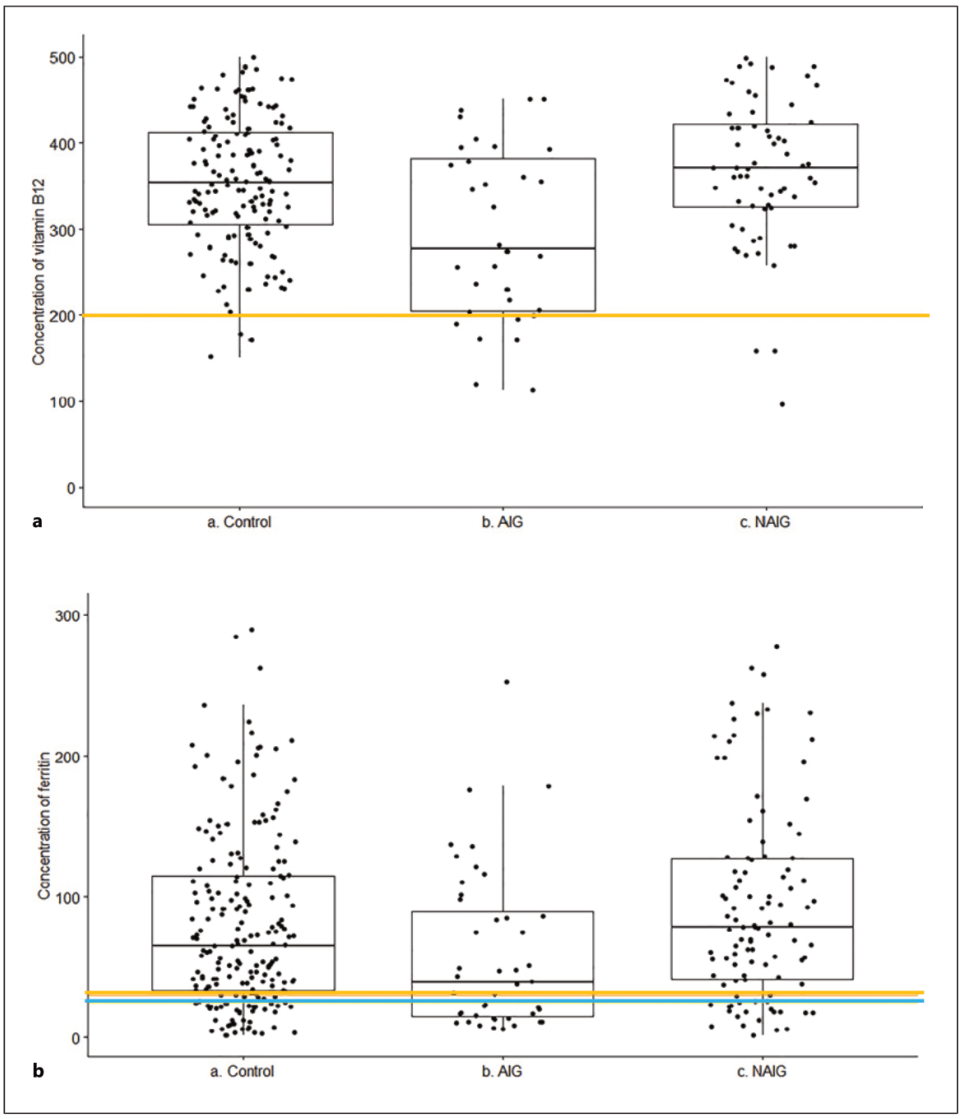

Fig. 1. Comparison of micronutrient concentrations among AIG, NAIG, and control patients. **a** Median vitamin B12 concentration; vitamin B12 deficiency is defined as <200 pg/mL (orange line). **b** Median ferritin concentration; iron deficiency is defined as ferritin <30 ng/mL for males and <25 ng/mL for females (orange and blue lines, respectively).

H. pylori *Infection and Micronutrient Deficiency*

Since some data from the literature indicate that *H. pylori* infection may lead to iron and vitamin B12 deficiency, we analyzed the data according to *H. pylori* status. In the NAIG group, 37 patients were *H. pylori* positive. Among them, 3 (8.1%) had iron deficiency, and 2 (5.4%) had vitamin B12 deficiency, whereas among *H. pylori* negative (n = 72), 15.3% were iron deficient, and 1.4% were vitamin B12 deficient. Only 5 out of 45 patients in the AIG group had confirmed *H. pylori* infection; among

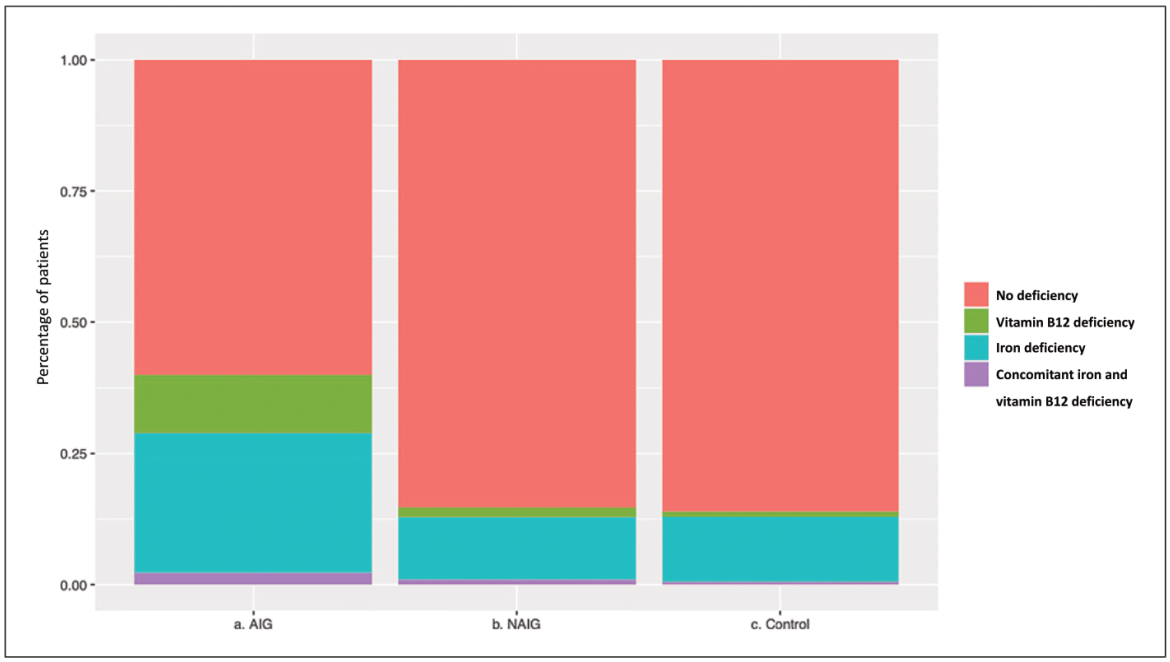

Fig. 2. Vitamin B12 deficiency, iron deficiency, and concomitant iron and vitamin B12 deficiency in AIG, NAIG, and control patients.

H. pylori-positive patients (n = 5), only 1 had iron deficiency, and none had vitamin B12 deficiency, whereas among *H. pylori*-negative patients (n = 40), 30% (n = 12) had iron deficiency and 15% (n = 6) had vitamin B12 deficiency (shown in Table 2).

Multivariate Analysis

To search for the factors that could potentially affect vitamin B12 and iron deficiencies, we performed a multivariate analysis using the following factors: age, gender, *H. pylori* infection, and the state of the gastric mucosa (AIG, NAIG, and control patients). Our multivariate modeling for vitamin B12 deficiency revealed that the autoimmune origin of gastritis influenced vitamin B12 deficiency; AIG has a significantly higher risk of developing vitamin B12 deficiency as compared with controls (OR multivariate 11.52 [95% CI: 2.85–57.64, p = 0.001]), whereas for NAIG group, the risk of developing vitamin B12 deficiency, as compared to control group, was not elevated {OR multivariate 0.09 (95% CI: 2.10 [0.36–12.08, p = 0.4])}. Other factors like age, sex, *and H. pylori* status did not affect vitamin B12 deficiency (shown in Table 3).

In multivariate modeling for iron deficiency, the autoimmune origin of gastritis influenced iron deficiency, AIG has a significantly higher risk of developing iron deficiency as compared to controls (OR multivariate 2.92 [1.32–6.30, p = 0.007]), whereas NAIG did not show an increased risk of developing iron deficiency compared to controls (OR multivariate 1.07 [0.50–2.19, p = 0.9]). Neither age, sex, *nor H. pylori* status did affect iron deficiency (Table 2).

Discussion

Our study tested micronutrient concentrations in patients with GPL, depending on the origin of this gastritis (AIG and NAIG), as compared to control patients. We found significant differences in micronutrient concentrations depending on the origin of gastritis: vitamin B12 deficiency was much more frequent in AIG (13.3%, n = 6) than in NAIG (1.5%, n = 3), whereas iron deficiency occurred two times more frequently in AIG (28.9%, n = 13) than in the NAIG and control patients (around 12% in each group, n = 14, n = 26, respectively). In the literature, AIG is often linked with vitamin B12 deficiency and pernicious anemia, while iron deficiency is

Table 2. *H. pylori* infection and micronutrient deficiency

	AIG (*N* = 45)		NAIG (*N* = 109)		Control (*N* = 201)		Total (*N* = 355)	
H. pylori status	pos	neg	pos	neg	pos	neg	pos	neg
	N = 5	*N* = 40	*N* = 37	*N* = 72	*N* = 31	*N* = 170	*N* = 73	*N* = 282
Ferritin median (Q1, Q3), ng/mL	83.9 (43.8, 86.1)	34.75 (15.0, 99.0)	87.9 (42.5, 169.8)	78.8 (51.65, 122.20)	54.5 (28.2, 92.7)	69.9 (33.9, 124.5)	74.3 (36.3, 127.3)	69.3 (30.6, 120.1)
Iron deficiency, *n* (%)	1 (20)	12 (30)	3 (8.1)	11 (15.3)	5 (16.1)	21 (12.4)	9 (12.3)	44 (15.6)
Vit. B12, median (Q1, Q3), pg/mL	421.5 (358.0, 487.0)	358.0 (227.7, 524.5)	445.0 (361.5, 592.0)	444.5 (347.2, 560.0)	402.5 (327.5, 514.0)	389.0 (322.7, 487.5)	417.0 (344.0, 559.5)	397.5 (324.3, 512.0)
Vit. B12 deficiency, *n* (%)	0	6 (15)	2 (5.4)	1 (1.4)	1 (3.2)	2 (1.2)	3 (4.1)	9 (3.2)

AIG, autoimmune gastritis; NAIG, non-autoimmune gastritis; Vit., Vitamin B12 N, normal range 200–800 pg/mL, deficiency <200 pg/mL; *H. pylori*, *Helicobacter pylori*; neg, negative; pos, positive (in histology and/or serology); AIG, autoimmune gastritis; NAIG, non-autoimmune gastritis; ferritin, normal range: 30–300 ng/mL for males, 25–300 ng/mL for females; iron deficiency, ferritin level below the lower threshold.

less well described in this setting [6, 16]. Our study shows that iron deficiency occurs twice more often than vitamin B12 deficiency (28.9%, $n = 13$ vs. 13.3%, $n = 6$, respectively) in patients with AIG. Hence, clinicians should carefully screen for iron deficiency in patients with AIG and supplement when necessary. Iron deficiency in our study was present in one-third of AIG patients, whereas in one previously published study, this rate was as high as 57% in AIG (with or without anemia) [17]. Iron deficiency anemia is the main presentation of AIG in children [18].

The rates of iron deficiency adjusted to inflammatory biomarkers (in our study to CRP level) were slightly higher than in the "classical" definitions of iron deficiency. Since data about adjusting ferritin levels to CRP are scarce in adults and patients with GPL, adjusting ferritin levels in clinical practice needs further studies [15, 19].

In a multivariate analysis, only the autoimmune origin of gastritis influenced vitamin B12 deficiency. Patients with AIG have around 12 times (OR: 11.52 [2.85–57.64, $p = 0.001$]) higher risk of developing vitamin B12 deficiency than the control patients. In contrast, patients with NAIG do not exhibit a higher risk of developing vitamin B12 deficiency. Additional factors, like *H. pylori* positivity (confirmed by serology and/or histology), sex, and age, did not affect the vitamin B12 deficiency. Other studies indicate that pernicious anemia is more prevalent in the elderly [17], occurs on average 20 years later than iron deficiency [20, 21], and is more prevalent in women [20]. However, data from our study did not show such correlations. One of the explanations might be the small sample size in our study. Some data indicate that *H. pylori* may cause vitamin B12 deficiency, and its eradication improves anemia and serum vitamin B12 levels, but the causal mechanism remains unknown [22]. Current Maastricht VI guidelines recommend *H. pylori* eradication for patients with vitamin B12 deficiency [23], but our data did not confirm the influence of *H. pylori* on vitamin B12 deficiency. However, this result must be interpreted cautiously, given a small number of *H. pylori*-positive patients in our study.

In multivariate modeling for iron deficiency, the autoimmune origin of gastritis influenced iron deficiency. AIG had around three times (OR: 2.92 [1.32–6.30, $p = 0.007$]) higher risk of developing iron deficiency than the controls, whereas NAIG patients' risk was not higher than the control group. Neither age nor sex or *H. pylori* status affected iron status.

Iron body stores are sufficient only for a few months; in consequence, iron deficiency anemia develops earlier in AIG, whereas vitamin B12 stores may be sufficient for a few years, and pernicious anemia manifests itself later in the course of the disease. *H. pylori* can lead to anemia due to increased gastric pH, but the prevalence of iron deficiency in NAIG patients was less important than in AIG.

Table 3. Multivariate modeling for vitamin B12 and iron deficiency in AIG, NAIG, and control patients

Parameter	Vit. B12 N	Vit. B12 deficiency	OR (univariate)	OR (multivariate)	No iron deficiency	Iron deficiency	OR (univariate)	OR (multivariate)
Age, *n* (%)								
≤60 years	166 (95.4)	8 (4.6)			144 (82.8)	30 (17.2)		
>60 years	177 (97.8)	4 (2.2)	0.47 (0.12–1.52, $p = 0.2$)	0.46 (0.12–1.52, $p = 0.2$)	158 (87.3)	23 (12.7)	0.70 (0.38–1.25, $p = 0.2$)	0.64 (0.34–1.18, $p = 0.1$)
Sex, *n* (%)								
Female	185 (95.9)	8 (4.1)			168 (87.0)	25 (13.0)		
Male	158 (97.5)	4 (2.5)	0.59 (0.15–1.90, $p = 0.4$)	0.66 (0.17–2.20, $p = 0.4$)	134 (82.7)	28 (17.3)	1.40 (0.78–2.53, $p = 0.3$)	1.63 (0.88–3.04, $p = 0.1$)
H. pylori, *n* (%)								
Neg	273 (96.8)	9 (3.2)			238 (84.4)	44 (15.6)		
Pos	70 (95.9)	3 (4.1)	1.30 (0.28–4.49, $p = 0.7$)	1.73 (0.36–6.53, $p = 0.4$)	64 (87.7)	9 (12.3)	0.76 (0.33–1.57, $p = 0.5$)	1.73 (0.36–6.53, $p = 0.4$)
State of the gastric mucosa								
Control	198 (98.5)	3 (1.5)			175 (87.1)	26 (12.9)		
AIG	39 (86.7)	6 (13.3)	10.15 (2.57–49.74, $p = 0.001$)	11.52 (2.85–57.64, $p = 0.001$)	32 (71.1)	13 (28.9)	2.73 (1.25–5.82, $p = 0.01$)	2.92 (1.32–6.30, $p = 0.007$)
NAIG	106 (97.2)	3 (2.8)	1.87 (0.34–10.24, $p = 0.5$)	2.10 (0.36–12.08, $p = 0.4$)	95 (87.2)	14 (12.8)	0.99 (0.48–1.96, $p = 0.9$)	1.07 (0.50–2.19, $p = 0.9$)

Vit., Vitamin B12 N, within normal range 200–800 pg/mL, deficiency <200 pg/mL; *H. pylori*, *Helicobacter pylori*; neg, negative; pos, positive (in histology and/or serology); AIG, autoimmune gastritis; NAIG, non-autoimmune gastritis; iron deficiency, ferritin concentration <25 ng/mL for women and 30 ng/mL for men; OR, odds ratio, presented as OR (95% CI, *p* value). Values are presented as *n* (%). The χ^2 test was used for statistical analysis.

In both AIG and NAIG groups, we did not find significant differences in micronutrient deficiencies between *H. pylori*-positive and *H. pylori*-negative patients (Table 3). On the contrary, we even found lower rates of iron deficiency in *H. pylori*-positive patients, which might be explained by more careful medical attention and prompt supplementation in *H. pylori*-positive patients. These results must also be interpreted with caution because of a small sample size, especially of *H. pylori*-positive patients with AIG. Data from the literature show an association between *H. pylori* infection and iron deficiency [23, 24], which might be caused by *H. pylori*-induced gastric and duodenal mucosa injury and associated bleeding or other, not well-understood mechanisms [25]. Indeed, 18% of *H. pylori*-associated gastritis leads to refractory iron deficiency in the absence of bleeding, especially in younger patients [26, 27].

The prevalence of iron deficiency in the control group was 12%, which is consistent with data from the literature, showing the prevalence of iron deficiency in the European population of 26.8% in patients over 70 years old [28] and 5–16% in adults 20–49 years old [29]. Interestingly, serum B12 and ferritin concentrations were lower in the control group than in the NAIG group (391.0 pg/mL, Q1, Q3: 323.5, 488.7 vs. 445.0 pg/mL, Q1, Q3: 355.0, 565.0 for vitamin B12, and 66.5 ng/mL, Q1, Q3: 33.4, 119.8 vs. 80.5 ng/mL, Q1, Q3: 43.6, 133.9 for ferritin, respectively). The possible explanation is that patients with atrophic gastritis were given more medical attention and were more likely to get vitamin B12 and iron supplementation than the patients from the control group.

Concomitant iron and vitamin B12 deficiency in our study was a rare event noted in 2.2% of AIG patients. In

contrast, another study reports a much higher incidence of dimorphic anemia (a result of iron and vitamin B12 deficiency, with clinical manifestation of normal mean corpuscular volume and anisocytosis) present in 30% of patients with AIG [17].

In patients with AIG, only 5 patients (11.1%) were *H. pylori* positive, and only by serology, while none was positive by histology. This is consistent with the current knowledge that in such an atrophic environment, the scarce bacteria may be absent on the biopsies despite rigorous adherence to the Sydney protocol in this study (at least two biopsies obtained from the antrum and two from the corpus). Besides, in this group, there might be some patients with a passed *H. pylori* infection, revealed by a positive serology, while there were no more bacteria present in the stomach. By comparison, it is worth underlining that 15.4% (n = 31) of patients in the control group were *H. pylori* positive. A relatively high *H. pylori* positivity in this group may be related to the fact that the control group included both the patients with normal mucosa and those with non-atrophic gastritis, susceptible of being *H. pylori* positive.

Our study has some limitations. First, the AIG group is relatively small. Even so, this condition is rare (~0.5–4.5% of the general population [5]). Second, we did not perform a complete blood count; as a result, we cannot assess the prevalence of anemia or its character (microcytic or macrocytic) related to micronutrient deficiencies. Third, the results of ferritin and vitamin B12 concentrations might have been skewed because some patients might have been under iron and vitamin B12 supplementation. However, the lower levels of these nutrients in AIG patients, despite potential supplementation, still reinforce the message of a common deficiency in these patients. Additionally, we did not collect data about patients' diet, other medical conditions, or medicine ingestion that may lead to micronutrient deficiencies (e.g., vegan diet, proton pump inhibitor treatment). Besides, additional iron indices were not assessed, like soluble transferrin receptor and transferrin saturation. Another limitation is the lack of assessment of other markers of vitamin B12 deficiency (methylmalonic acid and homocysteine) because serum concentrations of vitamin B12 do not always reflect its tissue concentration [30]. An important limitation is that *H. pylori* serology was assessed only once, despite the current guidelines indicating double testing. However, the serological test used from GastroPanel® is considered very accurate with the diagnostic accuracy of the *H. pylori* ELISA IgG with a sensitivity of 95.0% and specificity of 97.5% [31]. Our study has numerous strengths, including its multicentric and prospective design. Importantly, all patients underwent upper endoscopy; hence, all GPLs, including AIG, were confirmed histologically.

In conclusion, iron and vitamin B12 deficiencies are more commonly observed in patients with AIG compared to those with NAIG or control patients. Therefore, it is essential to screen for both iron and vitamin B12 deficiencies in AIG patients. These findings highlight the importance of addressing micronutrient deficiencies in the management of GPL, particularly in patients with autoimmune etiology.

Statement of Ethics

The study was conducted following the Declaration of Helsinki and approved by the Institutional Review Board (Comité de Protection des Personnes Ouest IV) on November 8, 2011, and registered on clinicaltrials.gov under the number NCT02624271. The bio-collection derived from the study was registered under the number DC-2011-1399. Written informed consent was obtained from all the patients.

Conflict of Interest Statement

M.O. received grants from Gilead, Angelini Pharma, and Takeda unrelated to the topic of this article.

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Author Contributions

Conceptualization and methodology, N.C., T.M.B., and M.O.; software and formal analysis, M.A.V.; validation, resources, and funding acquisition, N.C. and T.M.B.; investigation, E.B.C, D.M., J.B, D.Mo., D.Ma., J.F.M., D.T., D.L., J.M., C.H., R.J., and A.J.; data curation, M.A.V. and M.O.; writing – original draft preparation, M.O., T.M.B., N.C., and A.J.; writing – review and editing, M.O., T.M.B., and N.C.; supervision, T.M.B. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data are not publicly available due to the protection of patient's privacy. Further inquiries can be directed to the corresponding author.

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8. Summary

The presented doctoral dissertation consists of articles where different aspects of patients with GPL were tackled, including non-invasive markers for GPL diagnosis, autoantibodies, and micronutrient deficiencies.

Article 1 aimed to evaluate the diagnostic performance in detecting atrophic gastritis of serum pepsinogen (PGI and PGII) testing, using chemiluminescent enzyme immunoassay (CLEIA), and other potential biomarkers, including IL-6, HE-4, adiponectin, ferritin, and KL-6 also with CLEIA method. The accuracy of these biomarkers was compared to histology, which is considered the diagnostic gold standard. For the detection of moderate to severe corpus atrophic gastritis, the pepsinogen I/II ratio exhibited a sensitivity of 75.0% (95% CI 57.8–87.9) and a specificity of 92.6% (95% CI 88.2–95.8). Data from the literature show that pepsinogens alone do not perform well in detecting atrophic gastritis of the antrum. Therefore, the development of other makers is needed. IL-6, in the case of moderate to severe antrum atrophic gastritis, demonstrated a sensitivity of 72.2% (95% CI 46.5–90.3). Combining the pepsinogen I/II ratio with HE-4 yielded a sensitivity of 85.2% (95% CI 72.9–93.4) for detecting moderate to severe atrophic gastritis at any location. In conclusion, this study highlights the accuracy of PG testing through CLEIA for detecting corpus atrophic gastritis. Additionally, IL-6 and HE-4 may hold promise as valuable markers for detecting antrum AG. These findings offer potential insights into the early identification of individuals at risk for gastric cancer through serum biomarker assessments.

Article 2 aimed to compare the diagnostic performance of PGs with different methods, CLEIA and ELISA. The study showed that diagnostic performances of PG I for detecting corpus chronic atrophic gastritis were excellent, with sensitivity and specificity of 92.7% and 99.1% for ELISA and 90.5% and 98.2% for CLEIA, respectively. For AIG, corresponding values were 97.7% and 97.4% for ELISA and 95.6% and 97.1% for CLEIA. In multivariate analysis, PG levels were associated with the autoimmune origin (p<0.001) but not with the extent of the atrophic gastritis. In conclusion, pepsinogens are highly efficient for diagnosing corpus-limited CAG and discriminating AIG from *H. pylori*-induced gastritis. Additionally, both techniques, CLEIA and ELISA, are suitable for PG testing, regarding their excellent and comparable sensitivity and specificity.

Article 3 investigated the presence of autoantibodies in patients with gastric precancerous lesions (GPL) and control patients. 19 autoantibodies were tested (ANA, APCA, AIFA, and 16 myositis-associated antibodies). The results were compared among patients with GPL, including AIG, NAIG, and control patients. The study found that ANA positivity was significantly higher in patients with AIG (46.7%) compared to those with NAIG (29%) and control patients (27%), p=0.04. Female gender was associated with a higher likelihood of ANA positivity (OR 0.51 [0.31 - 0.81], p=0.005), while age and *H. pylori* infection did not significantly influence ANA positivity. Myositis-associated antibodies were found in 8.9% of AIG, 5.5% of NAIG, and 4.4% of control patients, with no significant differences among the groups (p=0.8). Higher APCA and AIFA positivity was confirmed in AIG, and these findings were not influenced by *H. pylori* infection, age, or gender in the multivariate analysis. In conclusion, this study reveals that ANA antibodies are more prevalent in AIG patients than in control patients, although the clinical significance of this observation is yet to be determined. Importantly, *H. pylori* infection did not appear to significantly impact the seropositivity of autoantibodies, including ANA, APCA, and AIFA. Furthermore, the positivity of myositis-associated antibodies was not increased in patients with GPL compared to control patients. In summary, the results of this study do not support the notion of an overrepresentation of common autoantibodies in patients with gastric precancerous lesions.

Article 4 examines the prevalence of micronutrient deficiencies, specifically vitamin B12 and iron, in patients with Atrophic Gastritis (AIG), Non-Atrophic Gastritis (NAIG), and control patients. The study found that the median vitamin B12 concentration was significantly lower in AIG (367.5 pg/mL, Q1, Q3: 235.5, 524.5) than in NAIG (445.0 pg/mL, Q1, Q3: 355.0, 565.0, p=0.001), and control patients (391.0 pg/mL, Q1, Q3: 323.5, 488.7, p=0.001). Vitamin B12 deficiency was most common in AIG (13.3%), followed by control (2.8%), and least common in NAIG patients (1.5%). Similarly, the median ferritin concentration was significantly lower in AIG (39.5 ng/mL, Q1, Q3: 15.4, 98.3 ng/mL) than in NAIG (80.5 ng/mL, Q1, Q3: 43.6, 133.9, p=0.04), and control patients (66.5 ng/mL, Q1, Q3: 33.4, 119.8, p = 0.007). Iron deficiency was observed twice as often in AIG (28.9%) than in NAIG and control patients (~12% in each group). After adjusting ferritin concentration for C-reactive protein (CRP) levels, iron deficiency remained more prevalent in AIG patients (33.3%), followed by control patients (18.4%), and NAIG patients (16.5%). Multivariate analysis indicated that AIG patients faced a higher risk of vitamin B12 deficiency (OR 11.52, [2.85-57.64] p=0.001) and iron

deficiency (OR 2.92 [1.32-6.30] p=0.007) compared to controls. In contrast, NAIG patients did not have an increased risk of developing those deficiencies compared to controls. Data from the literature show that *H. pylori* infection leads to vitamin B12 deficiency. In our study, *H. pylori* positivity did not affect the occurrence of either vitamin B12 or iron deficiency. Additionally, other factors like age and sex did not affect the occurrence of vitamin B12 or iron deficiency. These findings underscore the importance of screening for iron and vitamin B12 deficiencies, particularly in AIG patients, and emphasize the significance of managing micronutrient deficiencies in treating individuals with GPL.

9. Conclusions

Gastric cancer poses a significant threat when diagnosed in the advanced stage, emphasizing the critical role of early detection in reducing mortality. Given that GC typically follows GPL, there exists a valuable opportunity for proactive identification and appropriate monitoring of at-risk patients. The studies presented in this dissertation highlight the utility and effectiveness of serum markers, particularly pepsinogen, for specific categories of GPL patients, utilizing both ELISA and CLEIA diagnostic techniques (as demonstrated in articles 1 and 2). It also showed a promising diagnostic performance of different serum biomarkers, such as IL-6 and HE-4, in combination with pepsinogens, as suggested in Article 1. Despite the increased prevalence of anti-nuclear antibodies in GPL patients, the association of GPL with autoimmunity was inconclusive in article 3, warranting larger future studies for more robust conclusions. Article 4 reinforces the importance of micronutrient deficiencies, particularly iron deficiency, in patients with autoimmune gastritis, delivering a crucial message to the medical community. Moreover, the study did not confirm the anticipated association between vitamin B12 deficiency and H. pylori infection. Future research in the field of prevention of GC should focus on exploring innovative serum biomarkers, developing an algorithm to stratify patients in terms of their risk for developing GC, defining the modalities for screening patients with GC in different countries, and possibly including serum biomarkers in the GPL diagnosis to diminish the patient's burden.

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11. Statement of Ethics

The studies were conducted following the Declaration of Helsinki and approved by the Institutional Review Board (Comité de Protection des Personnes Ouest IV, France) on November 8, 2011, and registered on clinicaltrials.gov under the number NCT02624271. The bio-collection derived from the studies was registered under the number DC-2011-1399.

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13. Statement of authors

OŚWIADCZENIE

Tytuł: Atrophic Gastritis and Autoimmunity: Results from a Prospective, Multicenter Study

Autorzy: Osmola Małgorzata, Hémont Caroline, Chapelle Nicolas, Vibet Marie-Anne, Tougeron David, Moussata Driffa, Lamarque Dominique, Bigot-Corbel Edith, Masson Damien, Blin Justine, Leroy Maxime, Josien Regis, Mosnier Jean-François, Matysiak-Budnik Tamara

Wkład w powstanie publikacji: **Małgorzata Osmola**: tworzenie projektu badania, przegląd piśmiennictwa, praca laboratoryjna: przygotowaniu próbek do wykonania oznaczeń przeciwciał, wykonaniu oznaczeń przeciwciał, gromadzenie i zestawienie danych oraz przygotowania tekstu oryginalnego. Wkład procentowy (50%). **Caroline Hémont** tworzenie projektu badania, oznaczenia laboratoryjne, przygotowanie artykułu. Wkład procentowy (10%). **Nicolas Chapelle** tworzenie projektu i metodologii badania, pozyskanie funduszy, przygotowanie artykułu. Wkład procentowy (10%). **Marie-Anne Vibet** przygotowanie danych, wykonanie obliczeń i analizy statystycznej. Wkład procentowy (5 %). **Tamara Matysiak-Budnik** tworzenie projektu i metodologii badania, prowadzenie nadzoru nad projektem, przygotowanie artykułu. Wkład procentowy (10%). **David Tougeron** badanie pacjentów (1%), **Driffa Moussata** badanie pacjentów (1%), **Dominique Lamarque** badanie pacjentów (1%), **Edith Bigot-Corbel,** wykonanie oznaczeń laboratoryjnych (1%), **Damien Masson** wykonanie oznaczeń laboratoryjnych (1%), **Justine Blin** wykonanie oznaczeń laboratoryjnych (1%), **Maxime Leroy** przygotowanie analizy statystycznej (1%), **Regis Josien** opracowanie metodologii badania (1%)**, Jean-François Mosnier** wykonanie badań histopatologicznych (1%), **Jérôme Martin** tworzenie projektu i metodologii badania, przygotowanie artykułu (5%)

Tytuł: Iron and Vitamin B12 Deficiency in Patients with Autoimmune Gastritis and Helicobacter pylori Gastritis: Results from a Prospective Multicenter Study.

Autorzy: Osmola Małgorzata, Chapelle Nicolas, Vibet Marie-Anne, Bigot-Corbel Edith, Masson Damien, Hemont Caroline, Jirka Adam, Blin Justine; Tougeron David, Moussata Driffa, Lamarque Dominique, Josien Regis, Mosnier Jean-François, Martin Jérôme, Matysiak-Budnik Tamara.

Wkład w powstanie publikacji: **Małgorzata Osmola** współudział w tworzeniu projektu badania, przegląd piśmiennictwa, praca laboratoryjna: przygotowaniu próbek do wykonania oznaczeń, gromadzeniu i zestawieniu danych oraz przygotowania tekstu oryginalnego. Wkład procentowy (60%). **Nicolas Chapelle** tworzenie projektu i metodologii badania, pozyskanie funduszy, przygotowanie artykułu. Wkład procentowy (10%). **Marie-Anne Vibet**

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Tytuł: Serum Pepsinogens Combined with New Biomarkers Testing Using Chemiluminescent Enzyme Immunoassay for Non-Invasive Diagnosis of Atrophic Gastritis: A Prospective, Multicenter Study.

Autorzy: Chapelle Nicolas, Osmola Małgorzata, Martin Jérôme, Blin Justine, Leroy Maxime, Jirka Iva, Moussata Driffa, Lamarque Dominique, Olivier Raphael, Tougeron David, Hay-Lombardie Anne, Bigot-Corbel Edith, Masson Damien, Mosnier Jean-François, Matysiak-Budnik Tamara.

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Tytuł: Serum pepsinogens can help to discriminate between H. pylori-induced and auto-immune atrophic gastritis: Results from a prospective multicenter study.

Autorzy: Chapelle Nicolas, Martin Jérôme, Osmola Małgorzata, Hémont Caroline, Leroy Maxime, Vibet Marie-Anne, Tougeron David, Moussata Driffa, Lamarque Dominique, Bigot-Corbel Edith, Masson Damien, Blin Justine, Josien Regis, Mosnier Jean-François, Matysiak-Budnik Tamara.

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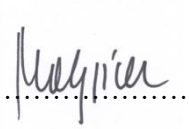
Jednocześnie wyrażam zgodę na wykorzystanie wyżej wymienionych prac jako część rozprawy doktorskiej lek. Małgorzaty Osmoli

Tamara Matysiak-Budnik



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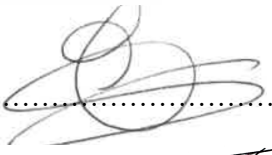

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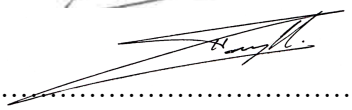
Nicolas Chapelle



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Marie-Anne Vibet

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Jérôme Martin

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STATEMENT

Title: Atrophic Gastritis and Autoimmunity: Results from a Prospective, Multicenter Study

Authors: Osmola Małgorzata, Hémont Caroline, Chapelle Nicolas, Vibet Marie-Anne, Tougeron David, Moussata Driffa, Lamarque Dominique, Bigot-Corbel Edith, Masson Damien, Blin Justine, Leroy Maxime, Josien Regis, Mosnier Jean-François, Matysiak-Budnik Tamara

Contribution to the publication: **Małgorzata Osmola** creating the study design, literature review, and laboratory work: preparing samples for antibody tests, performing antibody tests, collecting and compiling data, and preparing the original text. Percentage contribution (50%). **Caroline Hémont** creating the study design, laboratory tests, and preparing the article. Percentage contribution (10%). **Nicolas Chapelle** creating the research project and methodology, obtaining funds, preparing the article. Percentage contribution (10%). **Marie-Anne Vibet** preparing data, performing calculations and statistical analysis. Percentage contribution (5%). **Tamara Matysiak-Budnik** creating the research project and methodology, supervising the project, preparing the article. Percentage contribution (10%). **David Tougeron**, examination of patients (1%), **Driffa Moussata**, examination of patients (1%), **Dominique Lamarque**, examination of patients (1%), **Edith Bigot-Corbel**, performance of laboratory tests (1%), **Damien Masson**, performance of laboratory tests (1%), **Justine Blin** performance of the laboratory tests (1%), Maxime Leroy statistical analysis (1%), **Regis Josien** developing the study methodology (1%), **Jean-François Mosnier** performing histopathological tests (1%), **Jérôme Martin** creating the study design and methodology, article preparation (5%).

Title: Iron and Vitamin B12 Deficiency in Patients with Autoimmune Gastritis and Helicobacter pylori Gastritis: Results from a Prospective Multicenter Study.

Authors: Osmola Małgorzata, Chapelle Nicolas, Vibet Marie-Anne, Bigot-Corbel Edith, Masson Damien, Hemont Caroline, Jirka Adam, Blin Justine; Tougeron David, Moussata Driffa, Lamarque Dominique, Josien Regis, Mosnier Jean-François, Martin Jérôme, Matysiak-Budnik Tamara.

Contribution to the publication: **Małgorzata Osmola** participated in the creation of the research project, literature review, laboratory work: preparation of samples for the laboratory tests, collection, and compilation of data, and preparation of the original text. Percentage contribution (60%). **Nicolas Chapelle** creating the research project and methodology, obtaining funds, preparing the article. Percentage contribution (10%). **Marie-Anne Vibet** preparing data, performing statistical analysis. Percentage contribution (10%). **Tamara Matysiak-Budnik** creating the research project and methodology, supervising the project, preparing the article. Percentage contribution (10%). **Edith Bigot-Corbel**, performing laboratory determinations (1%), **Damien Masson** performing laboratory determinations (1%), **Caroline Hémont**

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Title: Serum Pepsinogens Combined with New Biomarkers Testing Using Chemiluminescent Enzyme Immunoassay for Non-Invasive Diagnosis of Atrophic Gastritis: A Prospective, Multicenter Study.

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Contribution to the publication: **Małgorzata Osmola**, review of the literature, collection and collation of data, and preparation of the original text. Percentage contribution (15%). **Nicolas Chapelle** creating the research project and methodology, obtaining funds, preparing the article. Percentage contribution (50%), **Jérôme Martin** preparation of the research methodology. percentage contribution (10%). **Tamara Matysiak-Budnik** creating the research project and methodology, supervising the project, preparing the article. Percentage contribution (10%). **Justine Blin** performing laboratory tests (1%), **Maxime Leroy** statistical analysis (1%), **Iva Jirka** collecting serum from patients (1%), **Driffa Moussata** examining patients (1%), **Dominique Lamarque** examining patients (1%), **Raphael Olivier** examining patients, **David Tougeron** examining patients (1%), **Anne Hay-Lombardie** performing laboratory tests (1%), **Edith Bigot-Corbel**, performing laboratory tests (1%), **Damien Masson** performing laboratory tests (1%), **Jean-François Mosnier** performing histopathological tests (1%)

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supervising the project, preparing the article. Percentage contribution (10%). **Marie-Anne Vibet,** statistical analysis (1%), **David Tougeron**, examination of patients (1%), **Driffa Moussata**, examination of patients (1%), **Dominique Lamarque**, examination of patients (1%), **Edith Bigot-Corbel**, laboratory tests (1%) , **Damien Masson** laboratory tests (1%), **Justine Blin** laboratory tests (1%), **Maxime Leroy** statistical analysis (1%), **Regis Josien** supervising laboratory work (1%), **Jean-François Mosnier** histopathological tests (1%)

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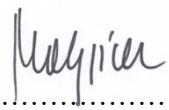
Tamara Matysiak-Budnik



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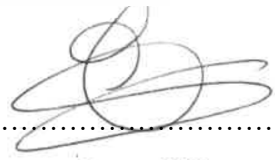
Caroline Hémont



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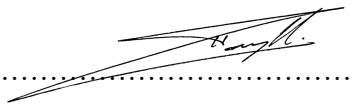
Nicolas Chapelle



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Marie-Anne Vibet

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Jérôme Martin

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OŚWIADCZENIE

Autorka: **Małgorzata Osmola**

Tytuł: Atrophic Gastritis and Autoimmunity: Results from a Prospective, Multicenter Study

Oświadczam, iż mój wkład w powstanie publikacji polegał na: współudziale w tworzeniu projektu badania, przeglądzie piśmiennictwa, pracy laboratoryjnej: przygotowaniu próbek do wykonania oznaczeń przeciwciał, wykonaniu oznaczeń przeciwciał, gromadzeniu i zestawieniu danych oraz przygotowania tekstu oryginalnego. Mój udział procentowy w przygotowaniu publikacji określam jako 50 %.

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