Sapienza Università di Roma

Facoltà di Medicina e Psicologia

Dottorato di Ricerca in Oncologia - XXXV Ciclo

Curriculum: Oncologia digestiva

Coordinatore: Prof.ssa Donatella Caserta

Tesi di Dottorato di Ricerca

Risk of gastric neoplasms in autoimmune atrophic gastritis and its relationship with gastric microbiota and immune pathways

Relatore:

Prof.ssa Edith Lahner

Dottorando:

Dr. Laura Conti

Anno Accademico 2021/2022

Index

Part-I (Introduction)

Epidemiology 1
Autoimmune atrophic gastritis 1
Gastric carcinogenesis 3
Gastric microbiota composition 4
Gastric mucosal immune pathways 5

Part-II

Aims of the project 7

Part-III (Results)

1 Studies on clinical-biochemical AAG features 8
1.1 Gastroesophageal reflux symptoms and microscopic esophagitis in a cohort of consecutive patients affected by atrophic body gastritis 11
1.2 Cell Blood Count Alterations and Patterns of Anaemia in Autoimmune Atrophic Gastritis at Diagnosis: A Multicentre Study 15
1.3 Seronegative autoimmune atrophic gastritis is more common in elderly patients 18
1.4 Time course of anti-parietal cell antibodies in autoimmune atrophic gastritis: a prospective study 18

2 Studies on endoscopic/histological AAG features and associated neoplastic risk 20
2.1 Narrow band imaging characteristics of gastric polypoid lesions: a single-centre prospective pilot study 23
2.2 Endoscopic surveillance at 3 years after diagnosis, according to European guidelines, seems safe in patients with atrophic gastritis in a low-risk region 26
2.3 Pseudopyloric Metaplasia Is Not Associated With the Development of Gastric Cancer .....

3 Studies on gastric microbiota composition and gastric mucosal immune pathways 29

3.1 Gastric microbiota composition in patients with corpus atrophic gastritis 29
3.2 Mucosal overexpression of thymic stromal lymphopoietin and proinflammatory cytokines in patients with autoimmune atrophic gastritis 32

Conclusions 34
References 35
Tables 39
Figures 45

PART-I

INTRODUCTION

Epidemiology of autoimmune atrophic gastritis

Autoimmune atrophic gastritis (AAG) is a slowly progressive organ-specific, immune-mediated condition that affects the corpus and fundus of the stomach (1). The prevalence of AAG in the general population is estimated to be as high as 2-5% (2). However, the true prevalence of AAG in the Italian general population is not known. AAG represents a pre-neoplastic condition. Patients affected by AAG are likely to develop several neoplasms, in particular type-1 neuroendocrine tumors in percentage variable from 0.4% to 7% and gastric cancer (GC) with an incidence ranging between 0% and 1.8% per year (3–5). The increased GC risk associated to AAG emphasizes the importance of diagnosing and monitoring these patients, as recommended by the last European guidelines on diagnosis and management of precancerous gastric conditions (MAPS II) (6). GC is the third leading cause of cancer mortality worldwide and the fifth for incidence. Incidence rates are the highest in Eastern Asia and Eastern Europe, while the incidence rate is low in Northern Europe and Northern America with rates similar to Africa's countries. The annual burden of GC is predicted to increase to»1.8 million new cases and »1.3 million deaths by 2040 (7).

Autoimmune atrophic gastritis

AAG is a chronic disease, characterized by atrophy of the oxyntic mucosa with subsequent hypochlorhydria, hypergastrinemia and deficiency of intrinsic factor

leading pernicious anemia at the late stage. Often, the positivity of autoantibodies against parietal cells (PCA) and/or intrinsic factor and the co-presence of autoimmune diseases as thyroid autoimmune disease or type 1 diabetes are associated (8,9).

PCA are IgG against the parietal cell H + /K + ATPase, a transmembrane proton pump, located in the intracellular secretory channels of parietal cells of the gastric oxyntic mucosa. Since their discovery, PCA have become the serological hallmark of AAG diagnosis (10–12), and they were also used to screen patients with other autoimmune disorders in which AAG is particularly prevalent (11,13).

Histologically AAG is characterized by gastric body atrophy, defined as replacement of oxyntic glands by metaplastic pyloric or intestinal glands according to the updated Sydney System (14). When this pathological process occurs, the subsequent reduced mass of specialized parietal cells can lead to impaired gastric acid and intrinsic factor secretion, eventually resulting in malabsorption of iron and vitamin B12 with consequent iron deficiency and/or pernicious anemia which may represent the sole clinical presentation of AAG (15,16).

From a clinical point of view, AAG has been traditionally considered a silent condition with an often-unspecific clinical presentation which may contribute together with other factors to the frequent and significant diagnostic delay (17,18). Other conditions, such as neuropathy, several autoimmune diseases or the presence of dyspepsia may be clinical clues raising the suspicion of AAG (19,20).

Gastric carcinogenesis

The incidence of gastric neoplasms is higher in patients with AAG compared to the general population. AAG has been associated with the development of two types of gastric neoplasms: intestinal type GC (incidence range between 0% and 1.8% per year) and type I gastric carcinoid (TIGC, percentage variable from 0.4% to 7%) (2,21). Considering GC, the intestinal subtype is due to a multistep process where the normal gastric mucosa is modified until it becomes carcinoma. This transformation is mostly due to the infection of *Helicobacter pylori* (Hp), a gram-negative bacterium that is considered as a class I carcinogen (22,23). The pathogenesis of the development of GC has been described by Correa and it is better known as "Correa cascade" (24).

In Hp-atrophic gastritis, the infection of Hp brings the normal mucosa to a chronic gastritis stage that represents the first stage of a transformation that could bring to develop gastric carcinoma. Colonization of Hp, normally starts in the antrum and then diffuse to corpus and fundus, where the chronic inflammation brings to the loss of gastric glands creating a stage of atrophy. The consequences of atrophic gastritis are hypochlorhydria and hypergastrinemia. Normal glandular tissue could be replaced by connective tissue or glandular structures inappropriate for location bringing to the stage of pseudo-pyloric (PPM) or intestinal metaplasia (IM). Atrophic gastritis and intestinal metaplasia are considered precancerous conditions on which dysplasia and GC could be developed.

Albeit AAG classical histopathological alterations consist of corporal-limited Hp-negative atrophic gastritis with a spared antrum, sometimes an active or previous

Hp infection and/or involved antral mucosa may be observed, showing overlapping features with multifocal atrophic gastritis mainly linked to Hp (2,10).

In AAG, the development towards GC depends on several factors such as conditions leading to an increased intragastric pH and oxidative stress, gastric microbiota composition, host factors such as the immune pathway, rather than environmental risk factors like smoke, alcohol, body mass index and diet.

An increased gastric pH and oxidative stress may be a consequence of Hp infection with the consequent persistent inflammatory infiltration of the corpus-fundus of gastric mucosa present in AAG. A recent study, which compared the human gastric microbiota in several hypochlorhydria states (Hp-induced atrophic gastritis, auto-immune atrophic gastritis and proton-pump inhibitor use), has observed a relatively higher microbial diversity in AAG patients, with samples dominated by Streptococcus, than in patients with Hp-related gastritis (25).

Indeed, gastric atrophy was associated with changes of the citric acid cycle (26), a biochemical pathway associated with gastric carcinogenesis, thus suggesting the possible crucial role of microbiota in gastric carcinogenesis (27).

Gastric microbiota composition

AAG is characterized by a progressive loss of acid-secreting parietal cells leading to hypo-achlorhydria. Due to this peculiar intra-gastric environment, gastric microbiota composition in individuals with AAG was first supposed and then recently reported to be different from subjects with a normal acidic healthy stomach. Recent data confirm the prominent role of Hp as the main bacterium

responsible for gastric disease and long-term complications (23). However, other bacteria than Hp, for example, Streptococci, were found in subjects who developed GC and in subjects at risk of this fearful complication, as well as those with autoimmune gastritis (28). The gastric microbiota analysis based on 16S rRNA gene-restricting high-throughput sequencing methods has permitted us to identify many unexpected or previously unknown bacteria in Hp-negative stomachs with 262 phylotypes representing 13 phyla (29–31). This technology allows us to analyze microbiota composition below the genus level, but only provides information on the bacterial presence, without any detail about bacterial functions or the vitality state of microorganisms (28). Based on this scenario, the future goal is to perform studies assessing the metabolically active bacteria of the stomach using many innovative and different methods such as reverse-transcribed 16S rRNA as an amplification template (32). However, data on gastric microbiota composition in AAG are still lacking and insights in this field may open new perspectives for potential prevention measures of malignant complication of AAG as modulation of the gastric microbiota (33).

Gastric mucosal immune pathways

Although improvements have been recently made in the clinical management of AAG, little is known regarding the immunological mechanisms underlying this condition. Most of the knowledge concerning the molecular pathways leading to mucosal damage in AAG is based on experimental models (34,35), while few data regarding humans are available. From mice AAG models, we learned that a

number of proinflammatory cytokines are overexpressed in the atrophic gastric corpus mucosa, including tumor necrosis factor (TNF)-a, interleukin (IL)-21, and IL-17A (36,37). Moreover, the thymic stromal lymphopoietin (TSLP) signaling seems to be altered in mice models of AAG, thus providing evidence for a regulatory role of this cytokine in this condition (38). A major turning point in the understanding of the pathogenesis of human AAG was the discovery of the major target autoantigen recognized by PCA, the serological marker of AAG (39). Of note, although previous studies showed a cytotoxic role of PCA against parietal cells in vitro, this is unlikely to happen in vivo because parietal cells are difficult to be accessed by these antibodies. Indeed, in a mice model, transferred human PCA were not able to induce an inflammatory response in the gastric corpus mucosa (40). Regarding data in humans, the study by D'Elios et al.(41) showed that H1/K1 ATPase induced in vitro proliferation of CD41T-cell clones derived from the gastric corpus mucosa of 5 patients with AAG. These cells were also found to produce TNF-a and interferon (IFN)-g, and they triggered immunoglobulin (Ig) production by activated B cells and plasma cells, causing cytotoxicity and apoptosis of gastric parietal cells. Nevertheless, a thorough characterization of the lamina propria mononuclear cells (LPMCs) infiltrating the gastric corpus mucosa of patients with AAG is still lacking, and a systematic dissection of the inflammatory pathways (i.e., cytokines) and tissue remodeling/damage pathways (i.e., matrix metalloproteinases [MMPs], transforming growth factor [TGF]-b1) implicated in the development of human AAG-related lesions is warranted.

PART-II

AIMS OF THE PROJECT

AAG is nowadays considered a precancerous condition with an increased neoplastic risk that may justify the need of endoscopic surveillance (6).

The chronic inflammatory state that persists through the course of AAG and the resulting impairment of gastric acid secretion create a background for the development of inflammatory and neoplastic proliferations in the gastric mucosa. Many questions regarding the natural history of AAG and the risk factors for the development of GC in patients affected by AAG are still opened.

The aims of this project are:

1. To investigate in depth the natural history of AAG focusing on the clinical and biochemical characteristics of patients with AAG at diagnosis and follow-up.
2. To assess the occurrence of gastric neoplasms in a cohort of AAG patients stratified according to risk groups when monitored by gastroscopy/histology at 3 year-intervals.
3. To investigate the gastric microbiota composition of AAG patients in relation to neoplastic complications.
4. To assess the possible role of immune-mediated pathways regarding gastric mucosa cytokine expression and lamina propria mononuclear cells (LPMCs) infiltrating the gastric corpus mucosa of patients affected by AAG.

PART-III

RESULTS

1 Studies on clinical-biochemical AAG features

1.1 Gastroesophageal reflux symptoms and microscopic esophagitis in a cohort of consecutive patients affected by atrophic body gastritis

During a 12 months period, we included 54 consecutive patients undergoing regular follow-up for AAG in our tertiary referral center. In each patient presence of upper gastrointestinal symptoms was assessed at the time of the outpatient visit through a standardized interview, evaluating type and frequency of GER typical (heartburn and regurgitation) and atypical symptoms (cough, no cardiac chest pain, pharyngeal globe, and dysphagia) (18). An EGD with multiple oesophageal and gastric biopsies was performed in each patient. Gastroesophageal reflux disease (GERD) was defined as the presence of typical and/or atypical reflux symptoms at least one-two times a week in the last 6 months (42). Presence of dyspepsia, defined by the presence of symptoms thought to originate in the gastro-duodenal region, with symptoms onset at least 6 months before, was also assessed. This was subdivided in subtype postprandial distress syndrome (PDS), in presence of bothersome postprandial fullness and/or early satiety at least several times per week, or epigastric pain syndrome (EPS), in presence of epigastric pain and/or burning at least once a week (43). EGD with multiple oesophageal (at least 4, two biopsies at 2 cm and two biopsies on the oesophageal side of the Z line) (44) and gastric biopsies according to update Sydney System mapping (14) was performed. Histologic evaluation and definition of microscopic

esophagitis regarding oesophageal samples, recognition and assessment of microscopic lesions related to GERD were performed assessing: (i) basal cell layer hyperplasia; (ii) papillary elongation; (iii) dilatation of intercellular spaces (DIS); (iv) intraepithelial eosinophils; (v) neutrophils and (vi) mononuclear cells [33,34]. Each microscopic lesion was scored (from 0 to 2) based on its severity. A combined severity score was obtained for each patient by summing up lesion scores and dividing by the number of lesion types assessed. As suggested in the Esohisto project, the calculation was restricted to four lesions (basal cell layer hyperplasia, papillary elongation, DIS and the presence of intraepithelial eosinophils) as these are the most informative elementary lesions. Scores 0–0.25 were considered as normal; 0.5–0.75 as 'mild esophagitis' and _1 as 'severe esophagitis' (45).

Of the 54 included patients, 20.4% (n=11) were male with a mean age of 57.6 ± 14 years. The mean BMI was 24.9 ± 4.1 kg/m2 with 37.5% of patients being overweight. 14.6% were active cigarette smokers. Patients received a diagnosis of ABG, at least one year before the inclusion in the current study, with a mean age from ABG diagnosis of 7.4 ± 6.6 years. At diagnosis, fasting gastrinaemia level was 651.2 ± 473.8 pg/ml and pepsinogen I was 13.9 ± 12.6 ng/l. Clinical features associated to ABG were: iron deficiency anaemia (56.6%), pernicious anaemia (30.2%), *H.p* infection (7.4%), the presence of gastric autoantibodies against parietal cells (81.5%), and autoimmune disorders (41.7%). First-degree family history for GC was reported in 11.1% of patients. The occurrence of GER symptoms and associated characteristics. One or more upper GI symptoms were reported in 70.4% of patients. At least one typical GER symptoms were reported in

24.1% (n=13) with 9.2% of patients complaining of heartburn and 18.5% regurgitation. One or more atypical GERD symptoms were reported in 44.4% (n=24) of patients, with 29.6% complaining of cough, 22.2% non-cardiac chest pain, 20.4% pharyngeal globe and 14.8% dysphagia. Only typical or atypical GER-related symptoms were reported in 24.1% (n=13) and 27.5% (n=15) of patients respectively; both typical and atypical GER-related symptoms were present in 16.7% (n=9) of patients. Concomitantly, PDS-like dyspepsia was reported in 40.7% (n=22) and EPS-like dyspepsia in 16.7% (n=9). Pernicious anaemia was less frequent in patients with typical GERD symptoms (7.7%) compared to patients without GERD (41.6%) (barely missed p=.05). Even if all patients showed low pepsinogen I levels, patients without GERD presented higher pepsinogen I levels compared to patients with typical GERD (8.9 ± 7.3 vs. 20.6 ± 14.8 mg/l, p=.04). No other differences regarding gender, age, BMI and other clinical features were found. Two (3.7%) out of 54 patients presented oesophageal lesions at endoscopy. One symptomatic male patient, aged 67 years, complaining of typical -heartburn and regurgitation- and atypical GERD symptoms – chest pain- presented at endoscopy an erosive esophagitis (LA-C according to Los Angeles classification) with a concomitant hiatal hernia. This patient presented concomitantly EPS-like dyspepsia. Another female patient, aged 80 years, complaining of heartburn and PDS-like dyspepsia, presented a short tongue of columnar lined mucosa at distal oesophagus with an area of intestinal metaplasia configuring a diagnosis of Barrett's oesophagus (C2M2, according to Prague classification). The first patient was overweight (BMI 27.7 Kg/m2) and the second one was obese (BMI 32.3

Kg/m2). The remaining patients showed no relevant oesophageal or gastric endoscopic findings. A mild microscopic esophagitis (ME) was reported in 49% and a severe ME in 24.5% of ABG patients. Comparing patients with or without ME, dyspeptic symptoms were less frequent in ABG patients with ME compared to those without (p=.05, barely missed). No significant differences regarding GERD prevalence were found among patients with or without ME. The prevalence of the single symptom in patients with or without ME is shown in **Table 1**. Cough was the only symptom significantly more frequent in patients with GER-related esophagitis. The occurrence of others GER-related symptoms was similar between the two groups.

Finally, these preliminary data showed that GERD is not infrequent in ABG being symptoms present in a quarter of patients, suggesting that ABG not exclude per se arising of oesophageal complaints. In ABG we found that microscopic esophagitis is a common finding, but further studies are needed to understand its clinical relevance.

1.2 Cell Blood Count Alterations and Patterns of Anaemia in Autoimmune Atrophic Gastritis at Diagnosis: A Multicentre Study

In this national multicentric study, four Italian tertiary referral centres for the diagnosis and management of AAG were included (Roma, Pavia, Milano, Aviano). AAG diagnosis was based on internationally agreed criteria in each centre (2). Relevant biochemical data at the time of AAG diagnosis (±1 month) were collected, including haemoglobin, MCV (normal range 80–98 femtoliter), RDW (normal range 11–15%), platelets (normal range 150,000–450,000/microliter),

serum vitamin B12 (deficient if < 200 ng/L), iron (deficient if < 55 ng/mL), ferritin (deficient if < 30 ng/mL), folate (deficient if < 4 ng/mL), homocysteine (increased if > 12 mol/L), and presence or absence of serum PCA. PCA were detected by either immunofluorescence or enzyme-linked immunosorbent assay (ELISA) techniques. Anaemia was classified according to the World Health Organisation (WHO), i.e., haemoglobin < 120 g/L in females and < 130 g/L in males living at sea level. Iron deficiency anaemia was defined as the presence of anaemia and low iron and ferritin levels, while pernicious anaemia was defined as the presence of macrocytic anaemia (or normocytic in case of dimorphic anaemia) and vitamin B12 and/or folate deficiency. Anaemia of chronic disease was inferred in case of ferritin> 100 ng/mL and iron < 55 ng/mL. A few patients with concomitant haematological disorders were excluded a priori, as it was not possible to ascertain the aetiology of red blood cell alterations.

Of the 654 included patients (mean age 59.2 ±13.8 years, F:M ratio = 2.3:1), 287 patients (43.9%) were from Pavia, 268 (41.0%) from Rome, 74 (11.3%) from Milan, and 25 (3.8%) from Aviano.

Female gender was predominant, 470 patients (71.8%) were aged > 50 years and 295 patients (46.8%) suffered from another concomitant autoimmune disease (251 patients; 39.9%). According to the Sydney–Houston criteria, the mean severity score of corpus atrophy (information available for 549/654 patients) was 2.6 ±0.6. Operative Link on Gastritis Assessment (OLGA) scores 1 and 2 were observed in 52 (9.4%) and 497 (90.5%) patients, respectively. Gastric corpus IM was present in 437 (79.6%) patients, with a mean severity score of 1.2 ±0.8, whereas Operative

Link on Gastric Intestinal Metaplasia Assessment (OLGIM) scores 1, 2, and 3 were present in 208 (37.8%), 225 (40.9%), and 4 (0.7%) patients, respectively. Pseudopyloric metaplasia was seen in 409 (62.5%) patients. Haematological alterations were the leading cause of AAG diagnosis (56.3%).

Anaemia, which was observed in 316 patients (48.3%; mean age 60.1 15.8 years, F:M ratio = 2.3:1), was mild (haemoglobin 95 g/L) in 194 patients (61.4%), moderate (haemoglobin 80–94 g/L) in 52 patients (16.4%), and severe (haemoglobin < 80 g/L) in 70 patients (22.2%). **Figure 1** reports the proportion of patients with and without anaemia (A), according to MCV (B), and subtypes of anaemia (C). **Table 2** reports all important demographic, clinical, haematological, and histological features of AAG patients according to the presence or absence of anaemia (any type). With regards to non-anaemic patients, MCV and RDW alterations were present in 76 (23.9%) and in 113 (39.1%) cases, respectively. A significantly higher prevalence of gastrointestinal symptoms, autoimmunity, and treated Hp infection was also seen in non-anaemic compared to anaemic patients (Table 2). Regardless of anaemia, vitamin B12 deficiency was observed in 291 (50.1%) patients and iron deficiency in 327 (57.8%) patients. Combined vitamin B12 and iron deficiency was present in 136 (25.1%) cases.

In all AAG patients, while normocytosis was similarly distributed between genders, microcytosis occurred more frequently in females (24.0% versus 15.5%; p = 0.0237) and macrocytosis more frequently in males (36.4% versus 20.3%; p < 0.0001). Pernicious anaemia (27.1% versus 12.4%; p = 0.0015) and vitamin B12 deficiency without anaemia (56.3% versus 46.8%; p = 0.0448) were more frequent

in males while iron deficiency anaemia (16.9% versus 10.0%; p = 0.0393) and iron deficiency without anaemia (64.4% versus 40.9%) were more frequent in females. Combined pernicious and iron deficiency anaemia was similarly distributed between genders (13.3% vs. 9.1%; p = 0.1970). Considering haematological alterations with regard to age (as a Continuous Variable), AAG patients with microcytosis were younger (54.7 ± 15.5 versus 61.1 ± 14.5 years; p < 0.0001), while those with macrocytosis were older (63.2 ±13.8 versus 58.5 ± 15.1; p = 0.0008). Patients with iron deficiency anaemia (56.8± 16.6 versus 60.2± 14.6 years; p = 0.0436) and combined pernicious and iron deficiency anaemia (55.9 ± 15.3 versus 60.2± 14.9 years; p = 0.0211) were younger, while patients with pernicious anaemia were older (63.0 ±14.6 versus 58.9± 14.9 years; p = 0.0144). At logistic regression analysis, anaemia (any type) was significantly associated with anisocytosis, thrombocytopenia, absence of gastrointestinal symptoms, and no PPI use. Moreover, iron deficiency anaemia was significantly associated with anisocytosis and absence of thrombocytopenia, while pernicious anaemia was significantly associated with anisocytosis, thrombocytopenia, and no history of Hp infection.

As result from our study, roughly half of AAG patients were anaemic, being pernicious anaemia and iron deficiency anaemia the most prevalent subtypes. The crude prevalence of pernicious anaemia was similar to that of iron deficiency anaemia but was different when adjusted for gender and age. Particularly, iron deficiency anaemia was more common in younger female patients, whereas pernicious anaemia was more common in older male patients. These findings may be explained by the slow evolution of AAG, that may take several years to

progress. It is reasonable to assume that iron deficiency characterises earlier disease stages, in which hypo-achlorhydria causes iron malabsorption, even if additional unknown immunological factors may have a role. Conversely, vitamin B12 is stored in large amounts, and it takes years for pernicious anaemia to develop. Surprisingly, there seems to be no correlation between anaemia and severity of histological damage, according to both OLGA and OLGIM classifications. Even patients showing severe gastric corpus atrophy might have been enrolled at any time after development of the histological changes.

In conclusion more attention should be paid to scant haematologic alterations. Prompt micronutrient supplementation is warranted in AAG patients, as could prevent (or revert) the development of anaemia.

1.3 Seronegative autoimmune atrophic gastritis is more common in elderly

This multicenter cross-sectional study aimed to characterize PCA-negative patients at AAG histological diagnosis comparing clinical, biochemical, and histological features between PCA positive and negative adult patients. Two Italian tertiary referral centers for the diagnosis and management of AAG (Roma, Pavia) were involved. In both centers, AAG diagnosis was made according to internationally agreed criteria. Gender, age at AAG diagnosis, family history of AAG or GC, smoking or alcohol habit, clinical presentation leading to AAG diagnosis such as autoimmune, cardiovascular, neurological and/or endocrinological comorbidities and serological features were evaluated. PCA was detected by IFI (INOVA Diagnostic, San Diego, CA) or ELISA (Quanta lite Gastric

Parietal cell Antibody ELISAs, INOVA Diagnostic). PCA negative or positive patients were defined as seronegatives/seropositives AAG patients respectively. Histological, biochemical, and clinical features collected at the time of AAG histological diagnosis were compared between the two groups.

Of the 516 included adult patients (mean age 59.6 ±12.8 years, F:M ratio = 2.2:1), 21.1% were seronegative. PCA were detected by IFI in 436 (84.4%) patients and by ELISA in 101 (21.5%) patients. **Table 3** shows the main clinical, biochemical and histological features stratified by PCA positivity and negativity. Seronegative patients were significant older than seropositive ones (65.9 ±14.1 versus 57.9 ±15.1 years, $p < 0.0\ 0\ 01$). As shown in **Figure 2**, significant differences between the two groups were present. The proportion of patients aged between 70- 79 years and greater than 80 years was higher in seronegative patients compared to positive ones (12.8% versus 5.1% and 38.5% versus 21.3%; $p < 0.005$), respectively. On the contrary, the proportion of patients aged between 40- 49 years was greater in seropositive patients compared to seronegatives (19.6% versus 3.7%; $p = 0.0\ 0\ 01$). Logistic regression analysis showed an increased prevalence of seronegativity with an odds ratio (OR) of 2.4 (95% CI 1.13–5.25) among AAG patients older than 50 years. The most frequent class of clinical presentation, i.e. hematological alterations, was similar in AAG patients with seropositivity and negativity. As far as regard hematological variables, the proportions of patients with iron deficiency and pernicious anemia at AAG diagnosis were not different between the two groups. Conversely, the endocrinological presentation was significantly more frequent in the seropositive group than in the seronegative one. Of the 516 AAG

patients, 208 patients presented at least one autoimmune disease. Autoimmune thyroid diseases were the most prevalent autoimmune disorders at AAG diagnosis with a higher proportion among seropositives compared to seronegatives (5.2% vs 0% p = 0.03 and 42% vs 27.1%; p = 0.0068). No significant differences were found between the two groups concerning gender, gastrointestinal or neuropsychiatric clinical presentation, positive family history of AAG or GC, co-presence and number of autoimmune associated diseases, co-presence of cardiovascular and neurological/psychiatric diseases. Regarding histological features, mild, moderate, and severe grade of corpus-fundus atrophy and presence/absence of intestinal or pseudopyloric metaplasia of corpus-fundus were similar in patients with seropositivity and negativity. Comparing mild with moderate-severe corpus-fundus atrophy, no significant differences were found between the two groups. At logistic regression, cofactors as gender, family history of AAG, presence of autoimmune, presence of pernicious anemia, iron deficiency anemia and severity of corpus-fundus atrophy at AAG diagnosis showed no significant association with the dependent variable.

In a sub-cohort of 101 AAG patients (mean age of 61.2 ±13.7 years, F:M ratio = 2:1), data of quantitative PCA levels tested by ELISA were available. Considering patients with seropositivity, a significant inverse correlation was observed between age at AAG diagnosis and PCA levels (rho = - 0.250; p = 0.0118), as shown in **Figure 3**. In our study, seronegative AAG seems to increase in older patients and this effect can be noticed in patients over 50 years and was even more evident in those over 70 years of age at AAG diagnosis. The role of age in

seronegative AAG was further supported by the significant inverse correlation between decreasing levels of PCA detected by ELISA and increasing age at diagnosis. The different time points of AAG natural history, in addition to the likely different duration of gastric disease of each included patient could explain the higher occurrence of seronegativity in patients older than 70 years of age. Seronegativity in older patients may be interpreted as a seroconversion occurring at a certain point of the natural history of AAG, likely because of the consumption and subsequent loss of the antigens (H + /K + ATPase) due to advanced atrophy of the oxyntic mucosa acting as stimulus for the antibody-producing plasma cells. In conclusion, seronegativity may occur in roughly 20% of AAG patients. This emphasizes the importance of histological evaluation to reach the diagnosis of AAG. AAG diagnosis should not rely only on serology, in particular in elderly individuals.

1.4 Time course of anti-parietal cell antibodies in autoimmune atrophic gastritis: a prospective study

A prospective study including 49 adult patients with AAG diagnosis was performed to assess the time course of PCA in the medium/long term among AAG patients and its possible relation with gastric histological atrophic damage progression at follow-up. Patients were included if they met the following inclusion criteria: i) histological diagnosis of AAG; ii) PCA tested by ELISA/IFI at both histological diagnosis and follow-up; iii) follow-up gastroscopy performed during the 2021st with concomitant serological PCA detection performed by

ELISA; iv) complete clinical data at AAG diagnosis and follow-up. For PCA ELISA manufacturer's cutoff was 20(RU)/mLIn. Patients with PCA tested by ELISA at both diagnosis and follow-up were divided on the basis of age at AAG diagnosis (≥50yrs=group1;<50 yrs=group2) and they were compared regarding clinical, biochemical, and histological features.

Amongst 49AAG patients (median age:59.5yrs, range22-84yrs), the median endoscopic/histological follow-up was 4.5years with a range of 3-16years. 65.3% of included patients were over 50 years old and 12.2% were seronegatives at AAG histological diagnosis. 5/6 PCA seronegative AAG patients were ≥ 50 years old at AAG diagnosis (F:M 3:3). Amongst 5/6 seronegative patients, the PCA seronegativity persisted and the last patient became seropositive at follow-up (median 3.5, range 3-7years). Of 43/49 PCA seropositives at diagnosis, 5 (F:M 4:1) became seronegative at follow-up and they were all over 50 years old. In 35/49 included AAG patients, PCA was tested by ELISA at both histological diagnosis and follow-up. Amongst 30/35AAG PCA seropositives, 4 became seronegative at follow-up, all of them aged over 50 years old and they did not present any significant worsening of corpus-fundus atrophic damage at follow-up endoscopy; in 6 patients the PCA value decreased of more than 20(RU)/mLIn; 14 patients had PCA seropositivity stably persistent (PCA changes:±20(RU)/mLIn) at follow-up and none had an increase of PCA greater than 20(RU)/mLIn at histological follow-up. In a sub-analysis a comparison between group 1 and group 2 showed a significant higher prevalence of autoimmune associated disorders in group1 than group 2 (p=0.04), while no other significant differences were found regarding

gender, PCA seronegativity at diagnosis, PCA seroconversion, PCA variations at follow-up and histological features at diagnosis and follow-up between the two groups.

In conclusion, in about one third of AAG included patients, the PCA value decreases or becomes negative at follow-up and all these patients are over 50 years old. Our study showed that PCA value may change over time suggesting that the natural history of AAG could be affected by the modification of this crucial marker.

2 Studies on endoscopic/histological AAG features and their associated neoplastic risk

2.1 Narrow band imaging characteristics of gastric polypoid lesions: a single-centre prospective pilot study

A prospective study including 40 consecutive adult patients (≥ 18 years) was performed to investigate the endoscopic NBI appearances of gastric polypoid lesion (GPL). Each included patients underwent a gastroscopy with high-resolution (HR)-NBI gastroscopes and presented at least one GPL. The identified GPL were endoscopically removed and sent for histological analysis. For the purpose of this study, we considered all GPL which at histology were identified as hyperplastic polyps (HP), adenomas, or type-1 gastric carcinoid (T1-GC); GPL which histologically were identified as normal mucosa, or atrophic gastritis with or without intestinal metaplasia, or fundic gland polyps (FGP), were not included. During gastroscopies if a GPL was found, images of NBI appearances were taken

and stored on a pen drive support in high-definition format. All GPL were identified, and number, location, size, and endoscopic characteristics were recorded. Two endoscopists blindly reviewed the digital images and registered the endoscopic NBI appearances on a specific check-list. Five variables were taken into consideration: the mucosal and vascular pattern, vascular thickness, and density as well as the presence of light-blue crest. GPL were then categorized in three different groups (HP, adenomas and T1-GC) using the histopathological evaluation as gold standard. Of included patients, 55% were females and the median age was 63 (range 36-85) years. Overall, 52 GPL were included: 29 (55.8%) HP; 18 (34.6%) T1-GC; 5 (9.6%) adenomas. The median size of GPL was 7 (range 2-35) mm. **Table 4** shows the NBI features of GPL. Forty-two (80.8%) presented regular circular mucosal pattern, 9 (17.3%) had tubule-villous and 1 (1.9%) irregular mucosal pattern. Only 2 GPL (3.8%) presented light blue crests. The vascular pattern was regular in 49 (94.2%) GPL, normal or thick in 50 (96.1%), with high vascular density in 49 (94.2%). In 15 (28.8%) GPL other features were recorded: nine (17.3%) presented a central erosion and 4 (7.7%) a demarcation line with a central erosion. Twenty-nine (55.8%) HP were found: 24 (82.8%) had regular circular mucosal pattern, while regular tubule-villous pattern was observed in 5 (17.2%). Only one (3.4%) presented light blue crest, while vascular pattern, thickness and density were normal for all HP but one (3.4%) which presented thin vessels. No other NBI features were observed. Eighteen (34.6%) T1-GC were found. Seventeen (94.4%) presented regular circular mucosal pattern and only one (5.6%) presented irregular mucosal pattern. Light blue crest was

observed only in one T1-GC (5.6%), while regular vascular pattern was observed in 15 (83.3%) and normal vascular thickness in 17 (94.4%). Vascular density was regular in 15 (83.3%), low in two (11.1%) and variable in one (5.6%). Furthermore, both endoscopists observed the presence of a central erosion in 14 (77.8%) and a clear demarcation line in the central part in 6 (33.3%) T1-GC. Five (9.6%) adenomas were found. Four (80.0%) had a tubule-villous mucosal pattern, while only one (20.0%) had regular circular mucosal pattern. Light blue- crests were absent in all of them. All the adenomas had regular vascular pattern, normal vascular thickness, and normal vascular density. No further NBI features were observed. Figure 3 shows examples of NBI appearance of a HP, a T1-GC, and an adenoma. As shown in **Table 4**, 80% of adenomas presented tubule-villous mucosal pattern (p=0.01 versus other lesions). This specific characteristic showed a sensitivity of 80.0% (95% CI 66.2 – 99.3), a specificity of 89.4% (95% CI 77.0 – 95.8), a PPV of 44.4% (95% CI 30.9 – 58.8) and a NPV of 97.7% (95% CI 87.8 – 99.8). The presence of a regular circular mucosal pattern was more frequently observed in HP and T1-GC compared to adenomas (p<0.001). The presence of a central erosion with or without demarcation line was more frequently observed in T1-GC (p<0.001 vs HP) and showed a sensitivity, specificity, PPV and NPV of 77.8% (95% CI 63.8 – 87.7), 97.1% (95% CI 86.9 – 99.6), 93.3% (95% CI 81.9 – 98.1) and 89.2% (95% CI 76.7 – 95.7), respectively. In conclusion, in the present pilot study the NBI analysis of the mucosal pattern seems to be effective to endoscopically discriminate between adenomas and HP, while the main characteristic of T1-GC seems to be the presence of a central erosion, sometimes with a clear demarcation

line. The endoscopic NBI characterization of GPL may contribute to optimize the management of these lesions.

2.2 Endoscopic surveillance at 3 years after diagnosis, according to European guidelines, seems safe in patients with atrophic gastritis in a low-risk region

A longitudinal cohort study was conducted between 2011 and 2019 as part of a surveillance program for early detection of gastric cancerous lesions in adult outpatients with corpus-involving AG in the referral center for AAG. The aim of the study was to prospectively investigate the occurrence of neoplastic lesions (i.e., gastric cancer, dysplasia, or type-1 gastric neuroendocrine tumours) at a 3-year follow-up in patients with autoimmune and multifocal AG. For this purpose, consecutive patients with histological diagnosis of autoimmune or multifocal AG underwent an endoscopic follow-up at 3 years interval (±6 months). Baseline and follow-up endoscopy were conducted with high-resolution (HR)- NBI endoscopes and target biopsies on suspicious endoscopic for IM or other lesions and standard biopsies according to updated Sydney System when the mucosa appeared endoscopically normal.

Overall, 160 patients were included (F 117(73.0%); median age 66 (35–87) years). Autoimmune and multifocal AG were present in 122(76.3%) and 38(23.7%) patients, respectively. At the baseline gastroscopy, 6 endoscopic lesions were detected and endoscopically removed which were characterized by type-1 gastric neuroendocrine tumour (n = 5) and low-grade dysplasia adenoma (n = 1). At the time of diagnosis, 14 patients (8.8%) were staged as OLGA I, 125 (78.1%) as OLGA

II, 17 (10.6%) as OLGA III, and 4 (2.5%) as OLGA IV. Considering the OLGIM classification, 23 (14.4%) patients did not have IM at the time of diagnosis and were considered OLGIM 0, while 49 (30.6%) patients were OLGIM I, 76 (47.5%) OLGIM II, 9 (5.6%) OLGIM III, and 3 (1.9%) OLGIM IV.

At the 3-year follow-up, 16(10.0%) patients presented 16 gastric neoplastic lesions: 3(18.7%) gastric cancers, 4(25.0%) low-grade dysplasia, 2(12.5%) low-grade dysplasia adenomas, 7 (43.7%) type-1 neuroendocrine tumours (**Figure 4).**

In these patients, OLGA and OLGIM III/IV stages were present in 4 (25%) and 1(6.3%), respectively; 11(69.0%) presented autoimmune AG, and all but one (93.7%) had parietal cells antibodies positivity (p = 0.026 vs patients without lesions). All lesions were endoscopically (87.5%) or surgically (12.5%) treated with favorable outcome. Age> 70 years was associated with a 9-fold higher probability of developing gastric epithelial neoplastic lesions (OR 9.6, 95CI% 1.2–79.4, p = 0.0359).

Characteristics of patients with gastric cancer

All three patients with GC were females aged be- tween 70 and 81 years. None of them had familiarity for GC or active or past infection of Hp. Two (66.7%) presented dyspepsia and pernicious anaemia was present in one (33.3%) patient at the time of diagnosis. None of them referred to new- onset upper gastrointestinal symptoms or other suspicious clinical signs of gastric neoplasia at weight loss, worsening of dyspeptic symptoms or new onset of iron deficiency anaemia. None of these three GC patients presented an advanced stage of OLGA/OLGIM (i.e. III/IV), nor at baseline, neither at the 3-year follow-up and all of them had an

autoimmune AG with positivity to parietal cells antibodies. One (33.3%) patient was treated with endoscopic submucosal dissection, with a final diagnosis of pT1a neoplasia, whilst the other two (66.7%) patients required surgery. One was a pT1b pN0 neoplasia and the other one was a pT2 pN0. All of them had a favorable outcome and all the patients are alive.

Characteristics of patients with type-1 gastric neuroendocrine tumours

Seven type-1 gastric neuroendocrine tumours were found at the 3-year follow-up. The females were four (57.1%) with a median age of 61 and a range between 39 and 81 years. One (4.3%) patient had familiarity for gastric cancer, none presented active Hp infection, whilst 1 (14.3%) had a previous infection already successfully treated. Three (43.0%) patients presented persisting dyspepsia and four (57.1%) had pernicious anaemia. Six (85.7%) patients had low OLGA stages at baseline and at follow-up and only one (14.3%) patient had OLGA III. All patients had low OLGIM stages at baseline and at follow-up. All the type-1 neuroendocrine tumours could be successfully removed by endoscopic mucosal resection or submucosal dissection.

Characteristics of patients with epithelial dysplasia

At the 3-year follow-up, six low-grade dysplasia were found: four on adenomatous polyps and two on flat mucosa. Females were five (83.3%) and the median age was 76 years with a range be- tween 68 and 87 years. None of them had familiarity for gas- tric cancer, two (33.3%) presented active Hp infection whilst the other two (33.3%) had a previous infection already successfully eradicated. Three (50.0%) patients presented persisting dyspepsia and four (66.7%)

had pernicious anaemia. Five (83.3%) patients had low OLGA/OLGIM stages, and only one (16.7%) patient had OLGA/OLGIM III.

This is the first prospective study investigating the appropriateness of the 3-year follow-up interval for the endoscopic surveillance in corpus-involving AG patients using electronic chromoendoscopy at baseline and follow-up, according to suggested guidelines. Our findings show that the 3-year interval endoscopic surveillance in AG as proposed by European guidelines seems to be safe to accurately detect treatable gastric neoplastic lesions. Furthermore, the current study confirms the higher risk of gastric neoplasms, GC and type 1 gastric neuroendocrine tumours in patients with corpus-involving AG, also in autoimmune AG, former considered at very low risk for GC and even being excluded from endoscopic surveillance in the first version of MAPS guidelines.

2.3 ***Pseudopyloric Metaplasia Is Not Associated with the Development of Gastric Cancer***

A longitudinal cohort study was conducted on consecutive patients affected by chronic atrophic gastritis (CAG) adhering to endoscopic-histological surveillance. Inclusion criteria were the histological diagnosis of CAG; complete personal, clinical, hematological-serological, endoscopic, and histopathological data of gastric biopsies and eventual epithelial gastric neoplastic lesions (GNL).

Of the baseline population of 510 patients (67.8% female, median age 61 years, and range 18–92 years), a follow-up population of 292 patients (57.3%) (74% female, median age 58.5 years, and range 23–88 years) with a median follow-up of 4.2

years (range 3–17 years) met the inclusion criteria. The follow-up period was equal to/longer than 6, 8, and 10 years in 92 patients (31.5%), 46 patients (15.7%), and 20 patients (6.8%), respectively. For this study, metaplastic atrophy either with pseudopyloric glands and/or intestinal glands in the corpus oxyntic mucosa biopsies was considered. Then, patients were stratified for the presence at baseline of pseudopyloric metaplasia (PPM) with or without concomitant Intestinal Metaplasia (IM) in corpus mucosa biopsies, and the occurrence of GNL at the longest available follow-up was assessed.

Of the 292 patients with CAG, corpus PPM without concomitant corpus IM (PPM group) was found in 62 patients (21.2%): female patients (85.5%) were preponderant, and the median age was 54.0 years (25–84); patients with corpus IM in the absence of PPM (IM group) were 66 (22.6%): female patients were 69.7% and the median age was 62.3 (32–88) years. Corpus PPM with the concomitant presence of corpus IM (PPM and IM group) was present in 164 patients (56.2%): female patients were 71.3% and the median age was 58.6 (23–84) years.

At the median follow-up of 4.2 (3–17) years, 9 patients (3.1%) with GNL were detected. Briefly, GC was diagnosed in 5 patients (1.7%) at 3, 3, 7, 7, and 8 years of follow-up and was histologically defined as intestinal-type adenocarcinoma according to the Lauren classification in all of them. Regarding the type of corpus metaplasia, in these 5 patients who developed GC, PPM without IM was not observed at both baseline and follow-up. All but one presented PPM with the concomitant presence of IM at both baseline and follow-up, whereas the remaining patients had IM without PPM at both baseline and follow-up.

In 4 patients, low-grade gastric dysplasia of intestinal type was detected at 3, 3, 3, and 8 years of follow-up. Concerning the type of corpus metaplasia, PPM without IM was not found in these patients: 2 patients had IM without PPM at both baseline and follow-up, 1 patient had IM without PPM at baseline and PPM with IM at the follow-up, and the remaining patient had PPM and IM at baseline and IM without PPM at the follow-up. The study population of 292 patients with CAG was divided into the following 3 groups: the evolution of the subtype of gastric corpus metaplasia from PPM to IM and PPM or IM alone (n 45, 15.4%), the evolution of the subtype of gastric corpus metaplasia from IM to IM and PPM or PPM alone (n 5 67, 22.9%), and the group without any variation of the subtype of gastric corpus metaplasia (n 5 180, 61.7%) not included in this analysis. As shown in **Table 5**, at univariate analysis, severe corpus atrophy at baseline was significantly more frequent in patients in the group evolving from PPM to IM when compared with those evolving from IM to PPM (57.8% vs 23.9%, P 5 0.003). At multivariate analysis, severe corpus atrophy was confirmed as an independent risk factor for gastric corpus metaplasia progression from PPM to IM (OR 4.4; 95% CI 1.9–9.9).

At the follow-up, GC was detected in 5 patients (1.7%) and gastric dysplasia (GD) in 4 patients (1.4%). In all these 9 patients with GC/GD at the follow-up, corpus IM was present at baseline and follow-up. Age <50 years (odds ratio [OR] 2.5), absence of pernicious anemia (OR 4.3), and absence of severe corpus atrophy (OR 2.3) were associated with corpus PPM without corpus IM.

In conclusion, in CAG patients characterized at baseline with corpus PPM in the absence of corpus IM at a median follow up of 4.2 years, GC or GD was not observed while the observed incident GC and GD were consistently associated with corpus IM. Corpus PPM without corpus IM was associated with younger age (<50 years), absence of pernicious anemia, and absence of severe corpus atrophy, suggesting a lower stage of progression of the disease. Corpus PPM alone may not be considered a precancerous lesion per se because the presence of corpus IM seems to be a necessary step for the occurrence of GC.

3 Studies on gastric microbiota composition and gastric mucosal immune pathways

3.1 Gastric microbiota composition in patients with corpus atrophic gastritis

This is a cross-sectional monocentric study conducted between December 2019 and February 2020 on consecutive patients with known histological diagnosis of CAG undergoing gastroscopy for gastric malignancy surveillance and patients undergoing gastroscopy for dyspepsia or anemia. Considering that the gastric acid is reduced in CAG patients, a potentially overgrowth of not-typical intra-gastric bacteria swallowed with food or saliva or deriving from the oral microbiota may occur. The aim of the study was to characterize the antrum/corpus composition of the gastric bacterial microbiota in CAG patients compared to controls without CAG. Included patients were classified into cases and controls according to the histological presence/absence of CAG diagnosis.

Patients matched the inclusion criteria for cases if they were: adults with histological diagnosis of corpus mucosa atrophy with or without involvement of the antral mucosa. Autoimmune-CAG diagnosis was based on the presence of corpus-fundus atrophy with antrum sparing with/without serum IgG PCA positivity (46); multifocal-CAG diagnosis was based on the presence of the antral and corpus/fundus mucosa atrophy associated with past or active Hp infection. Controls were composed of subjects without corpus mucosa atrophy. All patients with known diagnosis of CAG were off antisecretory drugs. Antibiotics were withdrawn at least three weeks before gastroscopy. Five cases with CAG and two controls were previously treated for Hp- infection, at least twelve months before enrolment in the study. All included patients were on their habitual Mediterranean diet with- out specific restrictions. In each patients, at least five gastric biopsies were obtained during gastroscopy, processed for histopathological evaluation according to the updated Sydney system (14). Genomic DNA from one antral and one corpus biopsy from each case (n = 23) and control (n = 32) was extracted. Gastric microbiota was assessed by sequencing hypervariable regions of the 16SrRNA gene. All the analyses on the gastric microbial diversity were performed by excluding the two samples of the Hp-positive case and the Hp-positive control to avoid confounding.

Of the 55 included subjects (median age 61.5 years, range 18–83), 23 (41.8%) were histologically diagnosed as having CAG (cases) and 32 (58.2%) as controls.

As shown in **Figure 5**, bacterial abundance and diversity were significantly lower in CAG cases than in controls ($p < 0.001$).

Overall, a total of 32 phyla were identified. At phyla level, Firmicutes were more frequent in cases and Bacteroidetes and Fusobacteria in controls ($p < 0.0\ 0\ 01$).

At the family level, a total of 261 gastric bacterial families could be identified, the most frequent were: Streptococcaceae, Prevotellaceae, Enterobacteriaceae, Neisseriaceae, Fusobacteriaceae. Streptococcaceae were more abundant in cases ($p < 0.0\ 0\ 01$), Prevotellaceae in controls ($p < 0.0\ 0\ 01$).

As shown in **Table 6** Streptococcus was significantly more frequent in CAG cases than in controls (16.02% vs 9.59%, $p < 0.0\ 0\ 01$), followed at lower percentages (< 2%) by Gemella, Klebsiella, Rothia, and Morganella.

Considering gastric bacterial microbiota profiles and CAG severity by OLGA/OLGIM stages, a positive correlation between the severity of both, gastric atrophy (OLGA score) and intestinal metaplasia (OLGIM score) and the abundance of the genera Streptococcus, Sphingosinicella, Idiomarina, Atlantibacter, Citrobacter, Enterobacter, Klebsiella, Kosakonia, and Morganella was found. The genus Streptococcus was positively correlated with severe OLGA/OLGIM stages linked to a higher risk of GC.

Furthermore, differences in the gastric microbiota profile at the phylum and at the genus level were observed between patients with autoimmune and those with multifocal CAG. In brief, at the phylum level, Firmicutes were found more frequently in patients with autoimmune-CAG than in those with multifocal-CAG (30.7% versus 20%, $p = 0.0103$), but Proteobacteria (28.4% versus 19.7%, $p = 0.0305$) and Cyanobacteria ($p = 0.0181$) were more frequent in patients with multifocal-CAG than in those with autoimmune-CAG.

In conclusion, the gastric bacterial microbiota in CAG cases prevalently Hp - negative at histology showed reduced abundance and complexity as typical for dysbiosis but was characterized by higher colonization of Firmicutes, in particular Streptococcus, which were increased in subjects with OLGA/OLGIM stages at higher risk of GC. Further studies are required to understand whether specific microbial profiling of patients with pre-neoplastic conditions as CAG might pave the way for the identification and the development of innovative prevention strategies of GC, as gastric microbiota modulation by probiotics treatment or functional food and oral hygiene programs.

3.2 Mucosal Overexpression of Thymic Stromal Lymphopoietin and Proinflammatory Cytokines in Patients With Autoimmune Atrophic Gastritis

This study, conducted in collaboration with San Matteo Hospital of Pavia, aims to assess immune mucosal alterations in patients with AAG. For this purpose, we investigated the lamina propria mononuclear cell (LPMC) populations and the mucosal expression of thymic stromal lymphopoietin (TSLP) and nicotinamide phosphoribosyl transferase (NAMPT) in AAG patients. Ex vivo cytokine production by organ culture biopsies, under different stimuli (short TSLP and zinc-l-carnosine), and the gastric vascular barrier through plasmalemma vesicle-associated protein-1 (PV1) were also assessed. Gastric corpus biopsies from 24 consecutive patients (median age 62 years, IQ range 56–67, 14 women) with an established diagnosis of AAG, undergoing EGD during the study period as part of their routinary surveillance, were enrolled. Gastric corpus biopsies of AAG

patients were collected with AAG age-matched and sex-matched healthy controls (HCs), and 14 patients with Hp infection (HP). In all cases, biopsy specimens were taken by the endoscopists in the upper third of the gastric corpus. Some of the biopsy samples were immediately fixed in a 3% buffered formalin, embedded in paraffin within 24 hours and processed according to the standard methods for histology, or embedded in OCT Tissue-Tek (Sakura Finetek, Torrance, CA), snap frozen, and processed for immunofluorescence. Finally, some biopsies were used for LPMC isolation and organ cultures or collected and stored in RNA later for RNA extraction. Peripheral blood was taken from the same patients at the time of endoscopy to isolate peripheral blood mononuclear cells (PBMCs).

Figure 6 shows that the percentage of CD451 LPMC was significantly higher in AAG in comparison with both HP and HC, with no significant difference between HC and HP (A). The percentage of B cells (CD191) was comparable among the 3 groups (B). In the subset of CD191 LPMC, the percentage of CD381 cells (plasma cells) was significantly higher in AAG compared with HC, whereas no significant difference was found between AAG and HP, and between HC and HP (C).

Within the subset of CD191 PBMC, the percentage of IgD1(D), IgM1(E), and IgG1(F) cells did not significantly differ among the 3 groups.

As presented in **Figure 7**, ex vivo production (organ culture supernatant concentration) of tumor necrosis factor (TNF)-a, interleukin (IL)-15, and transforming growth factor b1 was significantly higher in AAG compared with HC, whereas IL-33 was significantly lower in AAG in comparison with HC. No other significant differences were noticed regarding the remaining cytokines,

namely IFN-g, IL-6, IL-11, IL-17, IL-21, and IL-22. At immunofluorescence, both IL-7R and TSLP were more expressed in AAG compared with HC and HP, and short TSLP transcripts were significantly increased in AAG compared with HC. In the supernatants of AAG corpus mucosa, short TSLP significantly reduced TNF-a, while zinc-l-carnosine significantly reduced interferon-g, TNF-a, IL-21, IL-6, and IL-15. NAMPT transcripts were significantly increased in AAG compared with HC. A schematic representation of the possible contribution of the above-mentioned pathways to the pathogenesis of AAG is shown in **Figure 8**. In conclusion, plasma cells, proinflammatory cytokines, and altered gastric vascular barrier may play a major role in AAG. TSLP and NAMPT may represent potential therapeutic targets, while zinc-l-carnosine may dampen mucosal inflammation.

CONCLUSIONS

The studies conducted during this PhD project were meant to in-depth assess several clinical, biochemical, histological, and immunological aspects, not previously investigated, that can contribute to better understand the natural history of AAG as well as the neoplastic risk and the best management of this gastric precancerous condition. Considering the studies on gastric microbiota and immunological pathways in AAG, we can consider them as the first pieces of evidence, and they certainly cannot be interpreted as a point of arrival but should rather be viewed as a starting point for future research in this very complex and intriguing field in which much work is yet to be done. In the last few years, many pieces have been added to the knowledge puzzle, and future research is needed.

REFERENCES

1. Rugge M, Correa P, Dixon MF, Fiocca R, Hattori T, Lechago J, et al. Gastric mucosal atrophy: interobserver consistency using new criteria for classification and grading. Aliment Pharmacol Ther. 2002 Jul;16(7):1249–59.

2. Lahner E, Zagari RM, Zullo A, Di Sabatino A, Meggio A, Cesaro P, et al. Chronic atrophic gastritis: Natural history, diagnosis and therapeutic management. A position paper by the Italian Society of Hospital Gastroenterologists and Digestive Endoscopists [AIGO], the Italian Society of Digestive Endoscopy [SIED], the Italian Society of Gastroenterology [SIGE], and the Italian Society of Internal Medicine [SIMI]. Dig Liver Dis Off J Ital Soc Gastroenterol Ital Assoc Study Liver. 2019;51(12):1621–32.

3. Zhang H, Jin Z, Cui R, Ding S, Huang Y, Zhou L. Autoimmune metaplastic atrophic gastritis in chinese: a study of 320 patients at a large tertiary medical center. Scand J Gastroenterol. 2017 Feb;52(2):150–6.

4. Vannella L, Lahner E, Osborn J, Annibale B. Systematic review: gastric cancer incidence in pernicious anaemia. Aliment Pharmacol Ther. 2013 Feb;37(4):375–82.

5. Lahner E, Esposito G, Pilozzi E, Purchiaroni F, Corleto VD, Di Giulio E, et al. Occurrence of gastric cancer and carcinoids in atrophic gastritis during prospective long-term follow up. Scand J Gastroenterol. 2015 Jul;50(7):856–65.

6. Pimentel-Nunes P, Libânio D, Marcos-Pinto R, Areia M, Leja M, Esposito G, et al. Management of epithelial precancerous conditions and lesions in the stomach (MAPS II): European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter and Microbiota Study Group (EHMSG), European Society of Pathology (ESP), and Sociedade Portuguesa de Endoscopia Digestiva (SPED) guideline update 2019. Endoscopy. 2019 Apr;51(4):365–88.

7. Morgan E, Arnold M, Camargo MC, Gini A, Kunzmann AT, Matsuda T, et al. The current and future incidence and mortality of gastric cancer in 185 countries, 2020–40: A population-based modelling study. eClinicalMedicine [Internet]. 2022 May 1 [cited 2022 Oct 17];47. Available from: https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(22)00134-1/fulltext

8. De Block CEM, De Leeuw IH, Van Gaal LF. Autoimmune gastritis in type 1 diabetes: a clinically oriented review. J Clin Endocrinol Metab. 2008 Feb;93(2):363–71.

9. Lahner E, Centanni M, Agnello G, Gargano L, Vannella L, Iannoni C, et al. Occurrence and risk factors for autoimmune thyroid disease in patients with atrophic body gastritis. Am J Med. 2008 Feb;121(2):136–41.

10. Neumann WL, Coss E, Rugge M, Genta RM. Autoimmune atrophic gastritis--pathogenesis, pathology and management. Nat Rev Gastroenterol Hepatol. 2013 Sep;10(9):529–41.

11. Bizzaro N, Antico A. Diagnosis and classification of pernicious anemia. Autoimmun Rev. 2014 May;13(4–5):565–8.

12. Rusak E, Chobot A, Krzywicka A, Wenzlau J. Anti-parietal cell antibodies - diagnostic significance. Adv Med Sci. 2016 Sep;61(2):175–9.

13. Lahner E, Norman GL, Severi C, Encabo S, Shums Z, Vannella L, et al. Reassessment of intrinsic factor and parietal cell autoantibodies in atrophic gastritis with respect to cobalamin deficiency. Am J Gastroenterol. 2009 Aug;104(8):2071–9.

14. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol. 1996 Oct;20(10):1161–81.

15. Massironi S, Zilli A, Elvevi A, Invernizzi P. The changing face of chronic autoimmune atrophic gastritis: an updated comprehensive perspective. Autoimmun Rev. 2019 Mar;18(3):215–22.

16. Lenti MV, Lahner E, Bergamaschi G, Miceli E, Conti L, Massironi S, et al. Cell Blood Count Alterations and Patterns of Anaemia in Autoimmune Atrophic Gastritis at Diagnosis: A Multicentre Study. J Clin Med. 2019 Nov 15;8(11).

17. Lenti MV, Miceli E, Cococcia S, Klersy C, Staiani M, Guglielmi F, et al. Determinants of diagnostic delay in autoimmune atrophic gastritis. Aliment Pharmacol Ther. 2019;50(2):167–75.

18. Carabotti M, Lahner E, Esposito G, Sacchi MC, Severi C, Annibale B. Upper gastrointestinal symptoms in autoimmune gastritis: A cross-sectional study. Medicine (Baltimore). 2017 Jan;96(1):e5784.

19. Miceli E, Lenti MV, Padula D, Luinetti O, Vattiato C, Monti CM, et al. Common features of patients with autoimmune atrophic gastritis. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2012 Jul;10(7):812–4.

20. Lahner E, Carabotti M, Esposito G, Hassan C, Zullo A, Annibale B. Occurrence and predictors of metaplastic atrophic gastritis in a nation-wide consecutive endoscopic population presenting with upper gastrointestinal symptoms. Eur J Gastroenterol Hepatol. 2018;30(11):1291–6.

21. Lahner E, Esposito G, Pilozzi E, Purchiaroni F, Corleto VD, Di Giulio E, et al. Occurrence of gastric cancer and carcinoids in atrophic gastritis during prospective long-term follow up. Scand J Gastroenterol. 2015 Jul;50(7):856–65.

22. Bravo D, Hoare A, Soto C, Valenzuela MA, Quest AF. Helicobacter pylori in human health and disease: Mechanisms for local gastric and systemic effects. World J Gastroenterol. 2018 Jul 28;24(28):3071–89.

23. Correa P. Helicobacter pylori and gastric carcinogenesis. Am J Surg Pathol. 1995;19 Suppl 1:S37-43.

24. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res. 1992 Dec 15;52(24):6735–40.

25. Butcher LD, den Hartog G, Ernst PB, Crowe SE. Oxidative Stress Resulting From Helicobacter pylori Infection Contributes to Gastric Carcinogenesis. Cell Mol Gastroenterol Hepatol. 2017 May;3(3):316–22.

26. Annibale B, Capurso G, Lahner E, Passi S, Ricci R, Maggio F, et al. Concomitant alterations in intragastric pH and ascorbic acid concentration in patients with Helicobacter pylori gastritis and associated iron deficiency anaemia. Gut. 2003 Apr;52(4):496–501.

27. Parsons BN, Ijaz UZ, D'Amore R, Burkitt MD, Eccles R, Lenzi L, et al. Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of Helicobacter pylori-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use. PLoS Pathog. 2017 Nov;13(11):e1006653.

28. Engstrand L, Graham DY. Microbiome and Gastric Cancer. Dig Dis Sci. 2020 Mar;65(3):865–73.

29. Rajilic-Stojanovic M, Figueiredo C, Smet A, Hansen R, Kupcinskas J, Rokkas T, et al. Systematic review: gastric microbiota in health and disease. Aliment Pharmacol Ther. 2020 Mar;51(6):582–602.

30. Conti L, Borro M, Milani C, Simmaco M, Esposito G, Canali G, et al. Gastric microbiota composition in patients with corpus atrophic gastritis. Dig Liver Dis Off J Ital Soc Gastroenterol Ital Assoc Study Liver. 2021 Jun 8;S1590-8658(21)00248-6.

31. Lahner E, Conti L, Annibale B, Corleto VD. Current Perspectives in Atrophic Gastritis. Curr Gastroenterol Rep. 2020 Jun 15;22(8):38.

32. Wu WM, Yang YS, Peng LH. Microbiota in the stomach: new insights. J Dig Dis. 2014 Feb;15(2):54–61.

33. Capurso G, Lahner E. The interaction between smoking, alcohol and the gut microbiome. Best Pract Res Clin Gastroenterol. 2017 Oct;31(5):579–88.

34. Alderuccio F, Sentry JW, Marshall ACJ, Biondo M, Toh BH. Animal models of human disease: experimental autoimmune gastritis--a model for autoimmune gastritis and pernicious anemia. Clin Immunol Orlando Fla. 2002 Jan;102(1):48–58.

35. Field J, Biondo MA, Murphy K, Alderuccio F, Toh BH. Experimental autoimmune gastritis: mouse models of human organ-specific autoimmune disease. Int Rev Immunol. 2005 Apr;24(1–2):93–110.

36. Martinelli TM, van Driel IR, Alderuccio F, Gleeson PA, Toh BH. Analysis of mononuclear cell infiltrate and cytokine production in murine autoimmune gastritis. Gastroenterology. 1996 Jun;110(6):1791–802.

37. Tu E, Ang DKY, Bellingham SA, Hogan TV, Teng MWL, Smyth MJ, et al. Both IFN-γ and IL-17 are required for the development of severe autoimmune gastritis. Eur J Immunol. 2012 Oct;42(10):2574–83.

38. Nishiura H, Kido M, Aoki N, Iwamoto S, Maruoka R, Ikeda A, et al. Increased susceptibility to autoimmune gastritis in thymic stromal lymphopoietin receptor-deficient mice. J Immunol Baltim Md 1950. 2012 Jan 1;188(1):190–7.

39. De Aizpurua HJ, Cosgrove LJ, Ungar B, Toh BH. Autoantibodies cytotoxic to gastric parietal cells in serum of patients with pernicious anemia. N Engl J Med. 1983 Sep 15;309(11):625–9.

40. Inada M, Glass GB. Effect of prolonged administration of homologous and heterologous intrinsic factor antibodies on the parietal and peptic cell masses and the secretory function of the rat gastric mucosa. Gastroenterology. 1975 Aug;69(2):396–408.

41. D'Elios MM, Bergman MP, Azzurri A, Amedei A, Benagiano M, De Pont JJ, et al. H(+),K(+)-atpase (proton pump) is the target autoantigen of Th1-type cytotoxic T cells in autoimmune gastritis. Gastroenterology. 2001 Feb;120(2):377–86.

42. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R, Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. Am J Gastroenterol. 2006 Aug;101(8):1900–20; quiz 1943.

43. Tack J, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR, et al. Functional gastroduodenal disorders. Gastroenterology. 2006 Apr;130(5):1466–79.

44. Fiocca R, Mastracci L, Milione M, Parente P, Savarino V, Gruppo Italiano Patologi Apparato Digerente (GIPAD), et al. Microscopic esophagitis and Barrett's esophagus: the histology report. Dig Liver Dis Off J Ital Soc Gastroenterol Ital Assoc Study Liver. 2011 Mar;43 Suppl 4:S319-330.

45. Schneider NI, Plieschnegger W, Geppert M, Wigginghaus B, Hoess GM, Eherer A, et al. Validation study of the Esohisto consensus guidelines for the recognition of microscopic esophagitis (histoGERD Trial). Hum Pathol. 2014 May;45(5):994–1002.

46. Conti L, Lenti MV, Di Sabatino A, Miceli E, Galli G, Cazzato M, et al. Seronegative autoimmune atrophic gastritis is more common in elderly patients. Dig Liver Dis Off J Ital Soc Gastroenterol Ital Assoc Study Liver. 2020 May 30;

TABLES

Table 1 GERD-related symptoms in ABG patients with or without microscopic esophagitis.

	No microscopic esophagitis	Mild-severe microscopic esophagitis	*p* value
Typical GERD	15.4	22.2	.709
Heartburn	0	8.3	.555
Regurgitation	15.4	16.7	1
Atypical GERD (alone)	15.4	30.6	.466
Typical and atypical GERD symptoms	7.7	19.4	.663
Cough	7.7	38.9	**.042**
Globus	7.7	22.2	.411
Chest pain	15.4	22.2	.719
Dysphagia	15.4	16.7	1

When not otherwise indicated, data are expressed as the total number (percentage).
Statistically significant values are highlighted in bold.

Table 2 Clinical, haematological, and histological variables of patients with autoimmune atrophic gastritis (AAG) according to the presence or absence of anaemia (any type).

	Presence of Anaemia	Absence of Anaemia	*p*-Value
Female gender	220/459 (47.9)	239/459 (52.1)	0.8916
Male gender	96/195 (49.2)	99/195 (50.8)	0.9563
Age (years) mean ± SD	60.1 ± 15.8	59.3 ± 13.9	0.5061
Age groups (years)			
≥80	31 (10.0)	10 (3.0)	Trend 0.5463
70–79	74 (23.9)	82 (24.8)	
60–69	64 (20.6)	89 (26.8)	
50–59	47 (15.2)	73 (22.0)	
40–49	61 (19.7)	41 (12.3)	
30–39	24 (7.7)	32 (9.6)	
≤29	9 (2.9)	5 (1.5)	
Current or past smoking	103/300 (34.3)	114/324 (35.2)	0.8894
Gastrointestinal symptoms	32/302 (10.6)	75/310 (24.2)	<0.0001
Endocrinological disorders	7/302 (2.3)	16/312 (5.1)	0.1050
Cardiovascular disease	178/301 (59.1)	188/317 (59.3)	0.9674
Neuropsychiatric disorders	80/297 (26.9)	90/313 (28.7)	0.6816
Autoimmune comorbidities	130/305 (42.6)	165/325 (50.8)	0.0491
Autoimmune thyroid disease	108/303 (35.6)	143/325 (44.0)	0.0399
Family history of AAG	19/296 (6.4)	33/314 (10.5)	0.1136
Family history of gastric cancer	6/296 (2.0)	9/314 (2.9)	0.6838
PPI use prior to diagnosis	63/216 (29.2)	97/223 (43.5)	0.0025
Previous *H. pylori* infection	26/276 (9.4)	61/311 (19.6)	0.0008
Macrocytosis	108/303 (35.6)	48/317 (15.1)	<0.0001
Microcytosis	105/303 (34.6)	28/317 (8.8)	<0.0001
Normocytosis	90/303 (29.7)	241/317 (76.0)	<0.0001
Vitamin B_{12} deficiency	167/278 (60.1)	124/303 (40.9)	<0.0001
Iron deficiency	163/280 (58.2)	164/286 (57.3)	0.9007
Folate deficiency	24/165 (14.5)	10/201 (4.9)	0.0031
Vitamin B_{12} and iron deficiency	72/263 (27.4)	64/279 (22.9)	0.2749
Increased RDW	227/277 (81.9)	113/289 (39.1)	<0.0001
Thrombocytopenia	69/294 (23.5)	23/310 (7.4)	<0.0001
Thrombocytosis	6/294 (2.0)	7/310 (2.2)	0.9231
Intestinal metaplasia	246/310 (79.3)	258/332 (77.7)	0.6814
Pseudopyloric metaplasia	196/301 (65.1)	194/317 (61.2)	0.3547
OLGA 1 OLGA 2	22/269 (8.2) 247/269 (91.8)	30/280 (10.7) 250/280 (89.3)	Trend 0.3851
OLGIM 0 OLGIM 1 OLGIM 2 OLGIM 3	56/269 (20.8) 102/269 (37.9) 111/269 (41.3) 0 (0)	56/280 (20.0) 106/280 (37.9) 114/280 (40.7) 4/280 (1.4)	Trend 0.6349

Abbreviations: OLGA, Operative Link for Gastritis Assessment; OLGIM, Operative Link on Gastric Intestinal Metaplasia Assessment; PPI, proton pump inhibitor; RDW, red blood cell distribution width; SD, standard deviation. Percentages were calculated after exclusion of patients with missing data. $p < 0.0001$ were more frequent in females. Combined pernicious and iron deficiency anaemia was similarly distributed between genders (13.3% versus 9.1%; $p = 0.1970$). Thrombocytopenia was nearly two-fold more common in males compared to females (21.2% versus 12.7%; $p = 0.0108$). Finally, folate deficiency (16% versus 6.5%; $p = 0.007$) and hyperhomocysteinaemia (63.2% versus 45.9%; $p = 0.0011$) were both more frequent in males.

Table 3 Clinical, serological, and histological features with respect to positivity or negativity of parietal cell antibodies (PCA).

Presence of:	Positivity to PCA *n* =407	Negativity to PCA *n* =109 (21.1)	*p-value*
Female gender	(78.9)70.3	63.3	0.2
Age, years, mean ± SD	57.9 ± 15.1	65.9 ± 14.1	<0.0001
Clinical features leading to AAG diagnosis:			
- gastrointestinal	5.3	14.7	0.99
- hematological	61.48	62.38	0.95
- neurological / psychiatric	18.42	20.2	0.78
- endocrinological	5.2	0	0.03
Co-presence of autoimmune diseases	47.8	36.1	0.04
First-degree family history of AAG	9.9	6.4	0.08
Patterns of anemia:			
- pernicious anemia	27.4	31.4	0.48
- iron deficiency anemia	26.3	32	0.29
Severity of corpus atrophy			
- mild	9.3	7.3	0.53
- moderate	18.9	26.6	0.08
- severe	71.7	66.1	0.29

Table 4 Narrow band imaging (NBI) features of gastric polypoid lesions; *p<0.012 versus adenomas; °p=0.003 versus adenomas; # versus hyperplastic polyps and type-1 gastric carcinoids p=0.01; ^ versus hyperplastic polyps p<0.001.

NBI features	**Hyperplastic polyps** **n= 29 (55.8%)**	**Adenomas** **n= 5 (9.6%)**	**Type-1 gastric carcinoids** **n= 18 (34.6%)**
Mucosal pattern			
Regular circular	**24 (82.8%)***	**1 (20%)**	**17 (94.4%)°**
Regular tubulo-villous	**5 (17.2%)**	**4 (80%)#**	**0**
Irregular	**0**	**0**	**1 (5.6%)**
Light blue crest			
Present	**1 (3.4%)**	**0**	**1 (5.6%)**
Absent	**28 (96.6%)**	**5 (100%)**	**17 (94.4%)**
Vascular pattern			
Regular	**29 (100%)**	**5 (100%)**	**15 (83.3%)**
Irregular	**0**	**0**	**3 (16.7%)**
Vascular thickness			
Normal or thick	**28 (96.6%)**	**5 (100%)**	**17 (94.4%)**
Thin or ultrathin	**1 (3.4%)**	**0**	**1 (5.6%)**
Vascular density			
High	**29 (100%)**	**5 (100%)**	**15 (83.3%)**
Low	**0**	**0**	**2 (11.1%)**
Variable	**0**	**0**	**1 (5.6%)**
Other features			
Central erosion	**1 (3.4%)**	**0**	**8 (44.4%)^**
Central erosion + demarcation line	**0**	**0**	**6 (33.3%)^**

Table 5 Comparison of clinical, serological, and histological features in patients for the evolution of subtypes of gastric metaplasia at the follow-up compared with baseline.

	Evolution of PPM to PPM and IM or IM	Evolution of IM to PPM and IM or PPM	*P*
Total	67 (22.9)	45 (15.4)	
Female	55 (82.1)	34 (75.6)	0.4035
Age, yr, median (range)	57.7 (27–88)	59.3 (27–81)	
Smoking	25 (37.3)	16 (35.6)	0.8505
Familiarity for gastric cancer	4 (6.0)	2 (4.4)	0.7264
Dyspepsia	28 (41.8)	21 (46.7)	0.6117
Autoimmune gastritis	59 (88.1)	39 (86.7)	0.8278
Autoimmune thyroid disease	27 (40.3)	22 (48.9)	0.3711
Parietal cell antibodies	41 (61.2)	33 (73.3)	0.2207
Hp infection[a]	31 (46.3)	18 (40.0)	0.4299
Iron-deficiency anemia	19 (28.4)	15 (33.3)	0.3446
Pernicious anemia	22 (32.8)	21 (46.7)	0.1419
Corpus atrophy severity score[b]			
Mild atrophy	14 (20.9)	5 (11.1)	0.1004
Moderate atrophy	37 (55.2)	14 (31.1)	0.0124
Severe atrophy	16 (23.9)	26 (57.8)	0.0003

It has been supposed that gastric corpus metaplasia may evolve (33,34) through a stepwise passage from PPM to both PPM and IM or from both to IM alone; analogously, it may evolve in the opposite direction from IM to both subtypes or to PPM alone.
Data were expressed as n (%) of the total.
Hp, *Helicobacter pylori*; IM, intestinal metaplasia; PPM, pseudopyloric metaplasia.
[a]Positivity at histology and/or serology and/or previous eradication treatment.
[b]According to the updated Sydney system.

Table 6 Taxonomy at the genus level in corpus atrophic gastritis cases and controls. The corresponding phyla are also indicated. Only those taxa showing a statistically significant difference between cases and controls and a mean percentage of >0.01 were reported.

		Mean%		Std. Deviation				
Taxonomy (genus)	Taxonomy phylum	Controls	Cases	Controls	Cases	% absolute	% relative	p-value
Streptococcus	Firmicutes	9.59	16.02	5.56	8.21	−6.43	−40.15	0.000
Gemella	Firmicutes	0.95	1.45	0.57	1.33	−0.50	−34.51	0.017
Klebsiella	Proteobacteria	0.00	0.39	0.00	0.89	−0.39	−99.70	0.004
Rothia	Actinobacteria	0.76	1.04	0.68	0.84	−0.28	−26.56	0.058
Morganella	Proteobacteria	0.00	0.11	0.01	0.28	−0.10	−97.35	0.013
Idiomarina	Proteobacteria	0.00	0.04	0.00	0.13	−0.04	Abs in ctr	0.043
Enterobacter	Proteobacteria	0.00	0.04	0.01	0.12	−0.03	−95.50%	0.047
Eggerthia	Firmicutes	0.01	0.00	0.02	0.00	0.01	1359.61%	0.034
Mycoplasma	Tenericutes	0.01	0.00	0.02	0.01	0.01	166.45%	0.026
Fretibacterium	Synergistetes	0.01	0.00	0.01	0.00	0.01	674.93%	0.001
U. m. of Clostridiales vadinBB60 group family	Firmicutes	0.01	0.00	0.03	0.01	0.01	527.32%	0.054
Rhodopseudomonas	Proteobacteria	0.01	0.00	0.02	0.01	0.01	308.61%	0.035
Atopostipes	Firmicutes	0.01	0.00	0.03	0.00	0.01	5470.25%	0.041
Centipeda	Firmicutes	0.01	0.00	0.03	0.01	0.01	482.98%	0.042
Kingella	Proteobacteria	0.01	0.00	0.03	0.01	0.01	295.59%	0.008
Rikenellaceae RC9 gut group	Bacteroidetes	0.01	0.00	0.03	0.01	0.01	332.39%	0.004
Eikenella	Proteobacteria	0.02	0.01	0.03	0.02	0.01	180.88%	0.029
Peptococcus	Firmicutes	0.01	0.00	0.03	0.01	0.01	607.77%	0.002
Olsenella	Actinobacteria	0.02	0.00	0.04	0.01	0.01	274.65	0.014
Butyrivibrio 2	Firmicutes	0.02	0.00	0.04	0.01	0.01	381.31	0.011
U. m. of Lachnospiraceae family	Firmicutes	0.02	0.00	0.04	0.01	0.01	498.66	0.019
U. m. of Veillonellaceae family	Firmicutes	0.02	0.00	0.03	0.01	0.01	537.73	0.000
Dialister	Firmicutes	0.03	0.02	0.04	0.03	0.01	78.98	0.015
U. m. of Leptotrichiaceae family	Fusobacteria	0.02	0.00	0.06	0.00	0.01	1257.77	0.045
Lachnospiraceae NK4A136 group	Firmicutes	0.02	0.01	0.05	0.02	0.02	222.35	0.047
Delftia	Proteobacteria	0.02	0.00	0.05	0.01	0.02	455.46	0.015
Lautropia	Proteobacteria	0.03	0.02	0.05	0.03	0.02	96.13	0.033
Eubacterium xylanophilum group (Lachnospiraceae family)	Firmicutes	0.03	0.02	0.06	0.03	0.02	115.72	0.035
Selenomonas	Firmicutes	0.02	0.01	0.03	0.01	0.02	366.73	0.000
Filifactor	Firmicutes	0.05	0.02	0.08	0.05	0.02	115.00	0.039
Johnsonella	Firmicutes	0.03	0.00	0.06	0.01	0.03	531.70	0.003
Tannerella	Bacteroidetes	0.03	0.01	0.06	0.01	0.03	413.64	0.001
Oribacterium	Firmicutes	0.06	0.03	0.09	0.03	0.03	105.45	0.014
Catonella	Firmicutes	0.05	0.02	0.08	0.03	0.03	147.69	0.007
Lachnoanaerobaculum	Firmicutes	0.07	0.04	0.08	0.05	0.03	85.38	0.008
Treponema 2	Spirochaetes	0.05	0.01	0.06	0.02	0.03	323.71	0.000
Solobacterium	Firmicutes	0.08	0.04	0.13	0.05	0.04	84.13	0.050
Lawsonella	Actinobacteria	0.04	0.00	0.14	0.00	0.04	8406.00	0.029
Mogibacterium	Firmicutes	0.10	0.06	0.10	0.06	0.04	64.36	0.008
Selenomonas 3	Firmicutes	0.07	0.03	0.10	0.03	0.04	158.73	0.004
Pseudomonas	Proteobacteria	0.10	0.05	0.17	0.05	0.05	98.01	0.041

Abs = absent; ctr =controls.
Data are expressed in percentages.

FIGURES

Figure 1 The study population includes 654 adult patients suffering from AAG. The figure shows the proportion of patients with and without anaemia (A), the distribution of mean corpuscular volume (MCV) in anaemic patients (B), and subtypes of anaemia (C). The category "Others" includes chronic kidney failure and neoplastic diseases. Abbreviations: ACD, anaemia of chronic disease; IDA, iron deficiency anaemia; PA, pernicious anaemia.

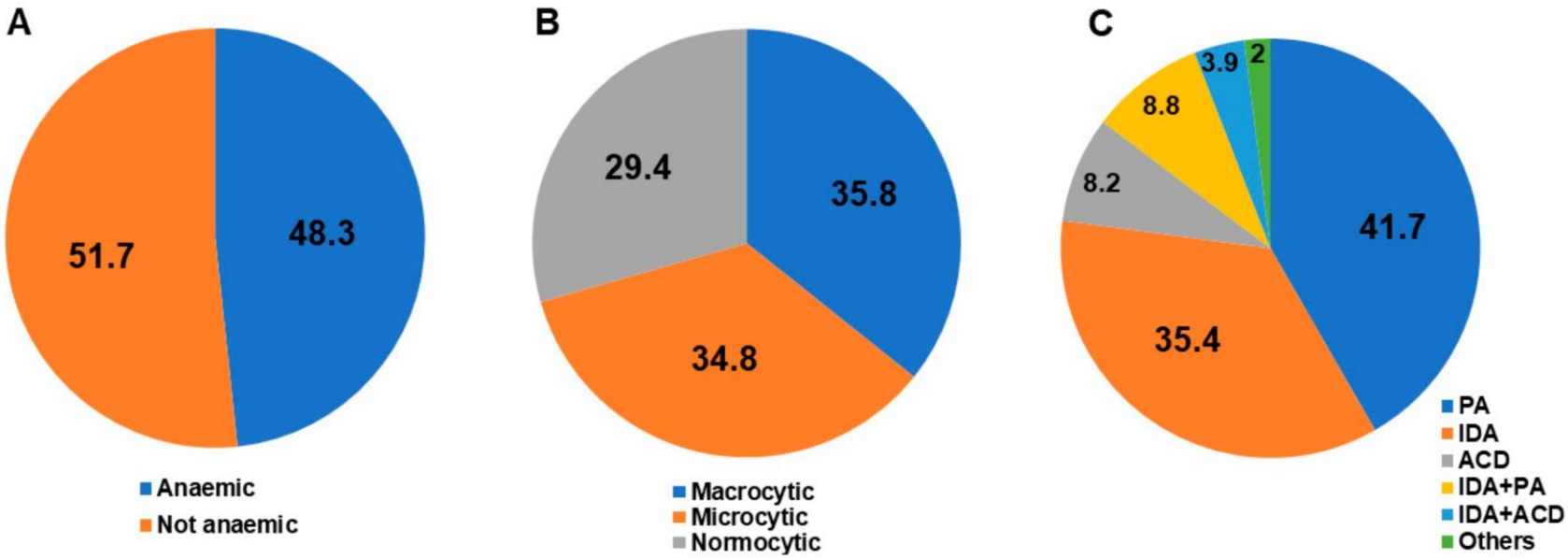


Figure 2 Age groups of patients are plotted with regard to anti-parietal cell antibodies positivity (n = 407, 78.9%) or negativity (n = 109, 21.1%). Legend: 1 ≤ 29 yrs; 2 ≥ 30≤39 yrs; 3 ≥ 40 ≤ 49 yrs; 4 ≥ 50 ≤ 59 yrs; 5 ≥ 60 ≤ 69 yrs; 6 ≥ 70 ≤ 79 yrs; 7 ≥ 80 yrs
3∗ p< 0,0001; 6∗ p<0,0004; 7∗ p<0.0088.

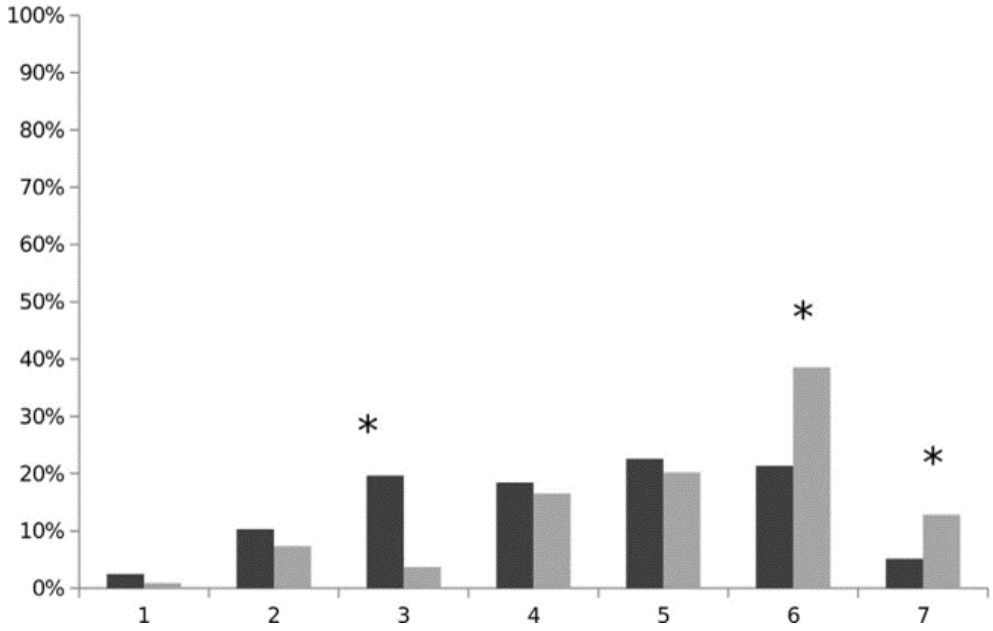


Figure 3 Inverse correlation between age at AAG diagnosis (n = 101 patients) and PCA positivity Spearman's coefficient of rank correlation (rho) = −0.250, p = 0.0118; ∗∗∗n = 3 outliers.

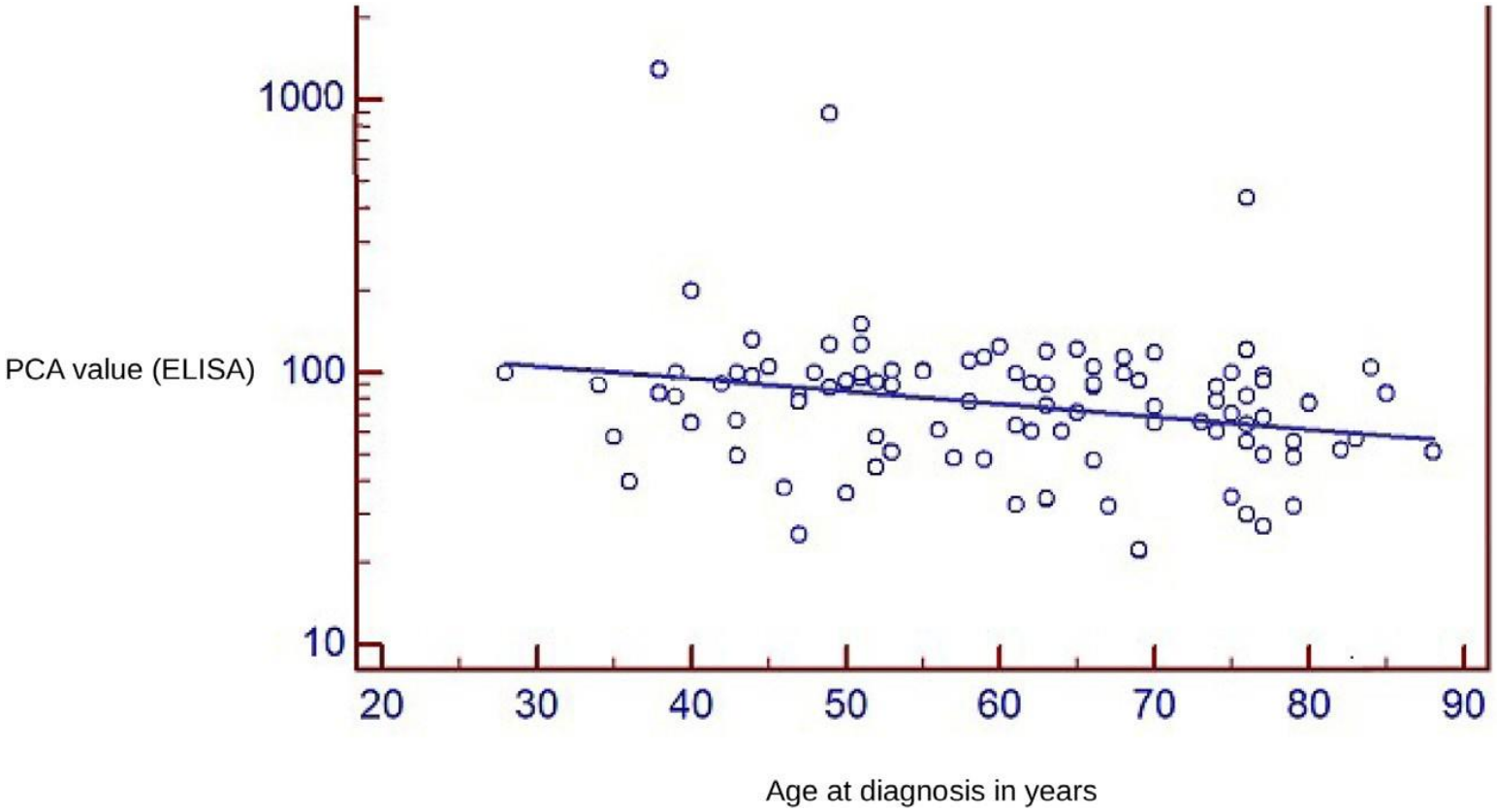


Figure 4 Examples of lesions at 3-year follow-up in white light and Narrow Band Imaging.

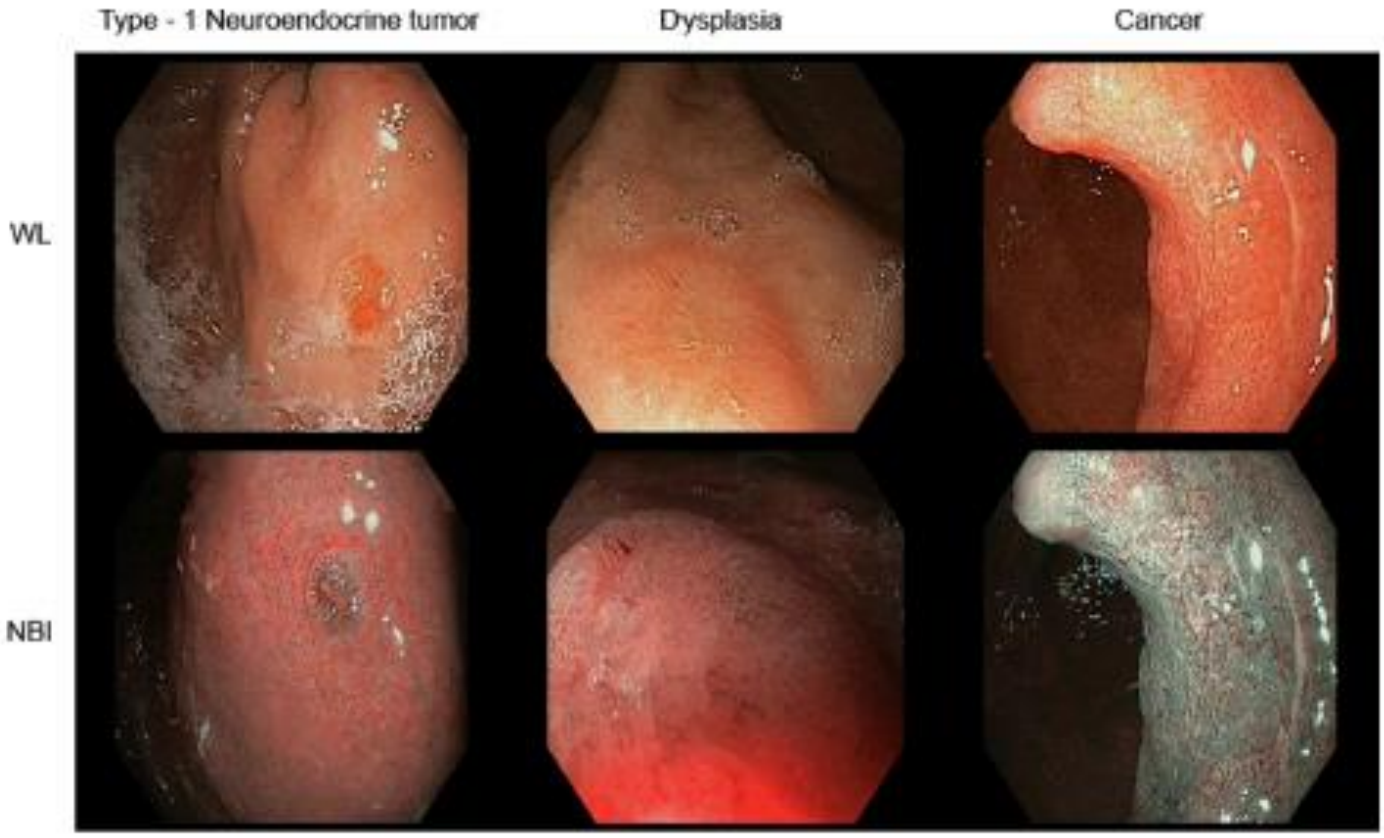
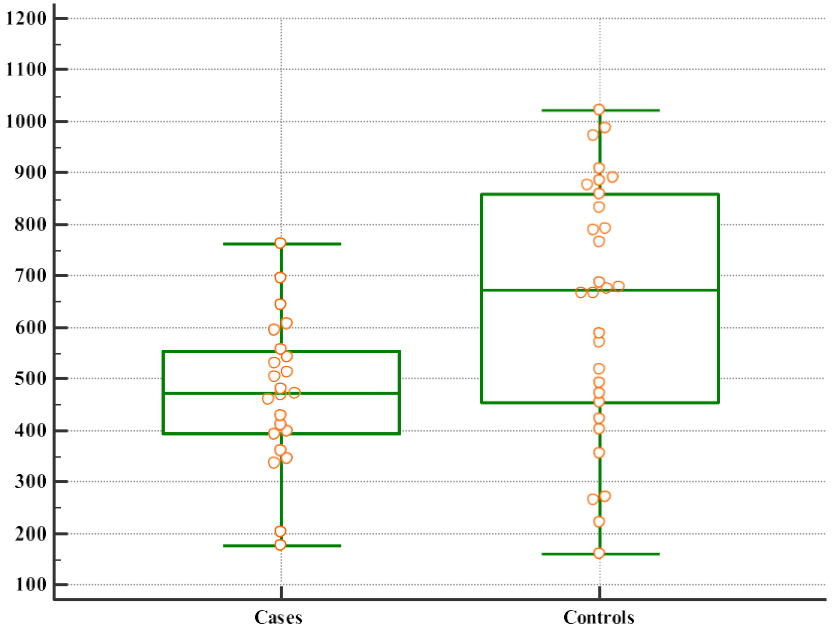


Figure 5 Alpha-diversity of gastric microbiota in the corpus mucosa: comparison between operational taxonomic units (OTUs) (13.333 reads) in cases with corpus atrophic gastritis and controls with a histologically normal corpus mucosa (on the left) and antral mucosa (on the right). The alpha-diversity was represented by a box-and-whisker plot. The bottom and top of the box were the first and third quartiles and the band inside the box was the median. The ends of the whiskers represented the minimum and maximum of all the data of the sample. A statistically significant difference was shown ($p < 0.0093$). Shannon index H was 3.0 in cases with corpus atrophic gastritis and in controls ($p < 0.05$).



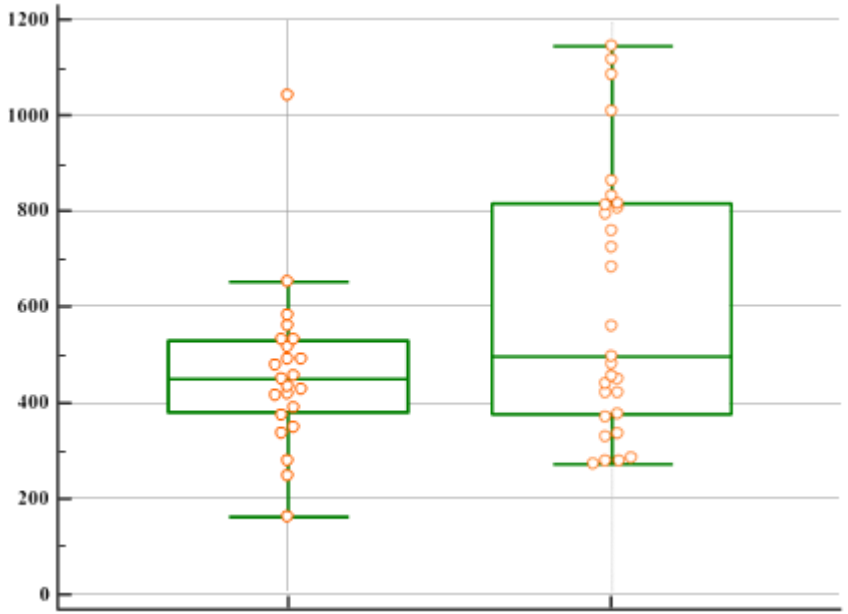


Figure 6 Whisker plot showing characterization by flow cytometry of CD451 (a), CD191 (b), CD381 (c), immunoglobulin (Ig) D1 (d), IgM1 (e), and IgG1 (f) lamina propria mononuclear cells (LPMCs) isolated from the gastric corpus of 26 healthy controls (HCs), 24 patients with autoimmune atrophic gastritis (AAG), and 14 patients with Helicobacter pylori gastritis (HP). Box and whisker plot represents the median, upper and lower quartiles, and minimum/ maximum value, while the symbol "1" is the mean. The dots, when present, represent the outliers. *P , 0.01; **P , 0.001; ****P , 0.00001.

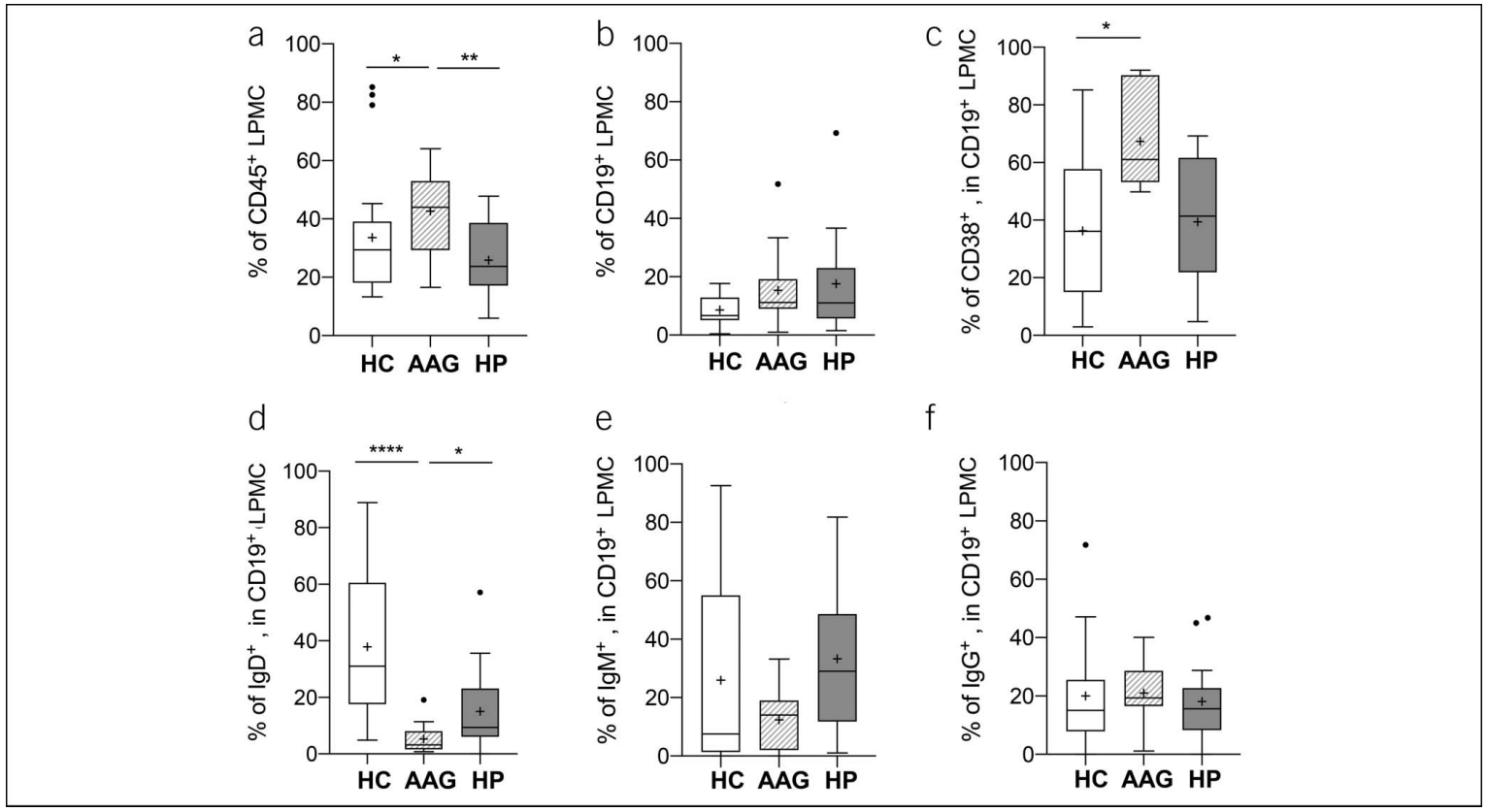


Figure 7 *Ex vivo* levels of cytokines, expressed in pg/mL, in the supernatants of gastric corpus mucosa biopsies collected from 24 patients with autoimmune atrophic gastritis (AAG) compared with 26 healthy controls (HCs), cultured for 24 hours in the absence of stimuli. Box and whisker plot represents themedian, upper and lower quartiles, and minimum/maximum value

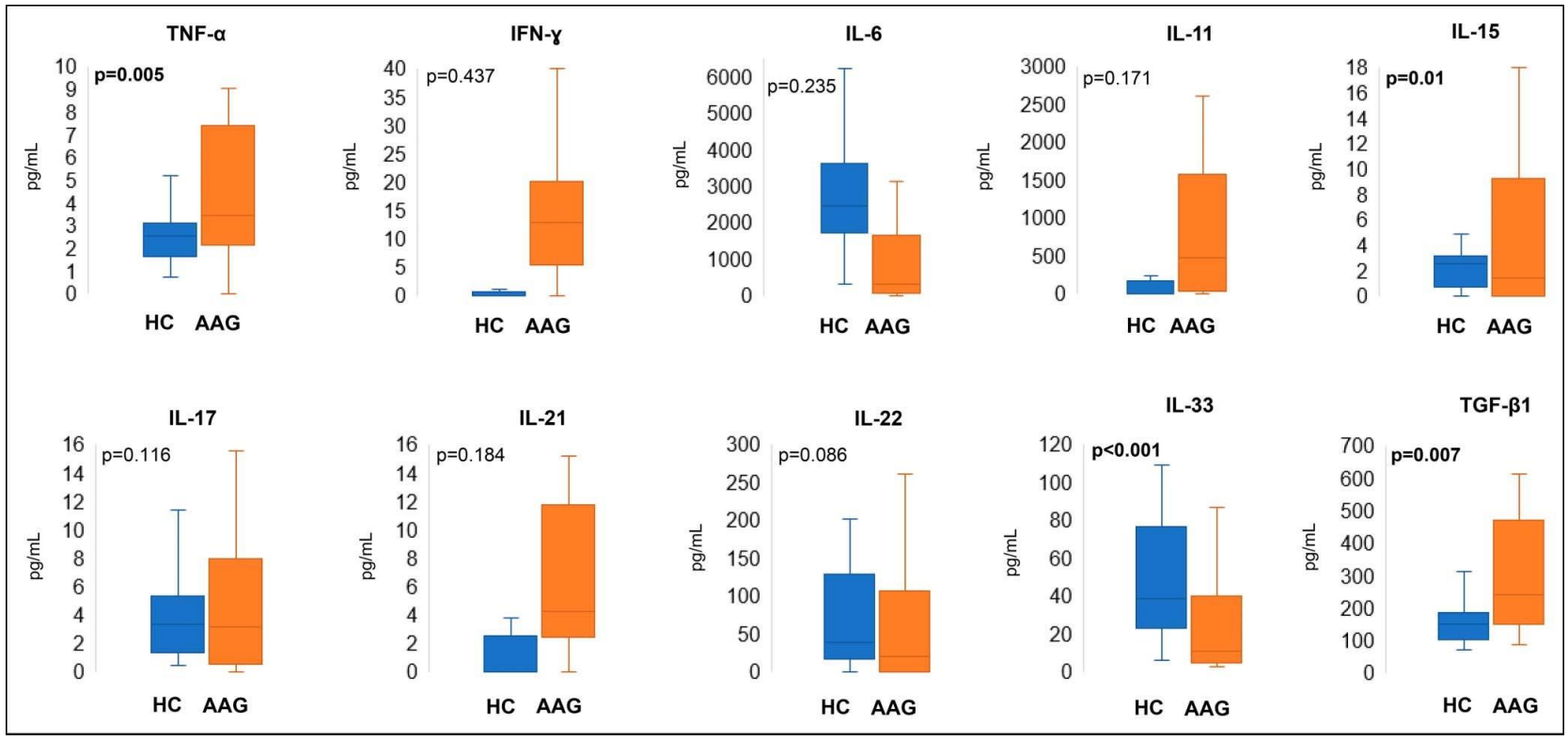


Figure 8 Summary of the potential pathogenetic mechanisms in autoimmune atrophic gastritis (AAG), according to the results found in the study. The oxyntic glands are made of different cell types including epithelial cells (e.g., mucous neck cells and chief cells) and parietal cells. The H1/K1 ATPase can be found on parietal cells, and its beta subunit constitutes the major autoantigen recognized by the T-cell receptor (TCR) located on T cells, through the major histocompatibility complex (MHC) II. Subsequently, autoreactive CD41 T cells produce tumor necrosis factor (TNF)-a, interleukin (IL)-33, and IL-15, all promoting a cascade which determines epithelial cell apoptosis and atrophy. Autoreactive Tcells also stimulate B cells that differentiate into plasma cells producing PCA and IFA. TGF-b1 is also produced by Tcells; it stimulates different lamina propria mononuclear cells (LPMCs) that in turn release matrix metalloproteinase 3 (MMP3), favoring fibrosis and tissue remodeling. The whole inflammatory process may potentially be counteracted by thymic stromal lymphopoietin (TSLP), which is produced by epithelial cells, acting on the TSLP receptor (TSLPR) located on T cells. Finally, the reduced expression of plasmalemma vesicle 1 (PV1) may lead to a lower gastric vascular permeability, maybe as a consequence of hypo/achlorhydria that favors bacterial overgrowth.