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# Objective Evidence of Gastro-Esophageal Reflux Disease is Rare in Patients with Autoimmune Gastritis

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## ABSTRACT

**Background & Aims**: Patients with autoimmune atrophic gastritis (AAG) often complain of acid reflux symptoms, despite the evidence of hypo-achlorhydria. Rome IV criteria are used to define functional esophageal disorders. Our aim was to characterize gastroesophageal reflux disease (GERD) phenotypes in patients with AAG.

**Methods**: Between 2017-2018, 172 AAG patients were evaluated at Gastro-Oncology outpatient clinic of University of Padua. Of them, 38 patients with reflux symptoms underwent high-resolution manometry (HRM) and multichannel intraluminal impedance-pH monitoring (MII-pH). Seventy-six AAG consecutive patients asymptomatic for gastroesophageal reflux were selected as age and gender matched controls. Serum biomarkers (pepsinogens, gastrin-17 and *Helicobacter pylori* antibodies), upper endoscopy, histology and clinical data were compared.

**Results**: Out of 38/172 (22%) AAG patients with reflux symptoms, 2/38 had a GERD diagnosis based on abnormal esophageal acid exposure and 6/38 had a major motility disorder (i.e. outflow obstruction). Among the 30/38 patients with normal endoscopic findings, 9/30 had reflux hypersensitivity, 19 functional heartburn, 1 functional globus, 1 functional chest pain according to the Rome IV criteria. Antral atrophy, advanced corpus atrophy and OLGA stage were more frequent in controls than in reflux patients (p=0.01, p=0.031, p=0.01, respectively). No differences were found for serum biomarkers and symptom presentation. Most of the patients received proton pump inhibitors (PPIs) treatment (87%), with a minority (34%) reporting clinical benefit.

**Conclusions**: Reflux symptoms are relatively common in AAG patients, but a firm diagnosis of GERD is rare (5%), whereas most of the patients have a functional disorder. PPI treatment is mostly clinical ineffective and should not be largely indicated.

**Key words:** gastroesophageal reflux disease – autoimmune gastritis – Rome IV – proton pump inhibitors – functional gastrointestinal disorders.

**Abbreviations**: AAG: autoimmune atrophic gastritis; AET: acid exposure time; APCA: anti-parietal cell antibodies; ECL: enterochromaffin-like; EGDS: esophagogastroduodenoscopy; GERD: gastro-esophageal reflux disease; *Hp: Helicobacter pylori*; HRM: high-resolution manometry; $H_2RA$: $H_2$ receptor antagonist; IFA: anti-intrinsic factor antibodies; IRP: integrated relaxation pressure; LES: lower esophageal sphincter; MII-pH: multichannel intraluminal impedance-pH monitoring; Pg: pepsinogen; PPI: proton pump inhibitor; RH: reflux hypersensitivity; SI: symptom index; SAP: symptom association probability.

## INTRODUCTION

Autoimmune gastritis (AAG) is a chronic, immune-mediated inflammatory disease, involving the body and fundus of the stomach [1]. The epidemiology of AAG is largely unknown due its almost asymptomatic course, with an estimated prevalence ranging from 2 to 5% [2] and with an even higher prevalence of anti-parietal cell antibodies (APCA) [3]. Autoimmune gastritis is mediated by a $CD4^+$ Th1 cells immune response with APCA targeting the $H^+/K^+$ ATPase, leading to destruction of parietal cells [4], and anti-intrinsic factor antibodies (IFA), impairing cobalamin absorption [1]. The destruction of gastric body glands leads to reduced acid secretion with consequent hypergastrinemia and low levels of pepsinogen (Pg) I [5, 6]. These changes elicit two important clinical manifestations of AAG: dyspeptic symptoms, due to the reduction/absence

of acid output, and anemia, caused by the reduced iron bioavailability [7] and the impaired cobalamin absorption [8].

The diagnosis of AAG is made by esophago-gastroduodenoscopy (EGDS) with biopsy sampling and histological evidence of body/fundus atrophy. Histology is characterised by a) the presence in gastric body mucosa of lympho-epithelial infiltrate into the *lamina propria*; b) the loss of proper gastric glandular cells and replacement by intestinal-type or by pyloric-type glands; c) progressive enterochromaffin-like (ECL) cells hyperplasia, potentially up to carcinoid type I development [9-11]. The use of the OLGA staging system to score the presence of atrophy is recommended [12, 13].

Gastroesophageal reflux disease (GERD) is one of the most frequent benign disorders of the upper gastrointestinal tract [14]. Its worldwide prevalence ranges between 2.5-25.9% [15]. Gastroesophageal reflux disease is due to the incompetence of the lower esophageal sphincter (LES), anatomic alterations or motility disorders [15, 16], and is characterized by acid reflux, essential for the onset of GERD, but with weakly acidic or alkaline reflux also potentially associated with esophageal symptoms [17, 18].

An indirect diagnosis of GERD can be obtained by endoscopic findings, but most of the patients do not present any mucosal injury at upper endoscopy [19]. Thus, to date, the gold standard technique for gastroesophageal reflux burden assessment is represented by the multichannel intraluminal impedance-pH (MII-pH) monitoring, allowing the characterization of reflux episodes, and the identifications of patients affected by functional versus organic disorder [20-22]. Indeed, the recently revised Rome IV criteria define "functional esophageal conditions" the presence of esophageal symptoms in the absence of endoscopic lesions, negative MII-pH and normal high-resolution manometry (HRM). Moreover, Rome IV criteria defined reflux hypersensitivity (RH) (normal acid exposure and positive symptoms association) being an independent disorder in which visceral hypersensitivity and peripheral or central sensitization play a major important role [23].

Despite the low acid secretion, AAG patients present gastroesophageal reflux symptoms and the mechanisms underlying them are poorly known. We hypothesized that most of them were of functional basis and therefore we aimed to objectively assess the prevalence of GERD in AAG patients and to characterize their reflux symptoms, also correlating them with specific differences in the serological markers routinely used to evaluate gastric function.

## METHODS

In this observational prospective study, we included patients with previous or recent diagnosis of AAG seen in our Gastro-Oncology outpatient clinic for their regular follow-up between 2017 and 2018. The study protocol was approved by the Ethics Committee of our hospital (n 46093) and it was conducted according to the declaration of Helsinki. All patients enrolled signed a written informed consent.

During the outpatient visits, an accurate history regarding their symptoms (dyspeptic, atypical and typical reflux symptoms) was collected, and all patients presenting typical and atypical reflux symptoms, as assessed by using validated questionnaires [24-26], were asked to undergo MII-pH monitoring and HRM. All patients had performed within one year from the recruitment an EGDS for the gastric cancer surveillance in atrophic gastritis as suggested by published guidelines [12], or for confirming the diagnosis of AAG. Biopsy sampling was performed according to the Update Sydney System, with OLGA staging defined by expert pathologists confirming body and fundic area-limited atrophic gastritis. Positive APCA, IFA, gastrin above normal levels (ELISA Test), in the absence of PPI administration, and Pg I serum levels below normal also contributed to the diagnosis. Presence of *Helicobacter pylori* (*Hp*) infection was assessed in all patients by appropriate staining in gastric biopsies (Hematoxylin and Eosin, Alcian-Blue and Periodic Acid Schiff stain and Giemsa). Previous infection was looked for collecting an accurate history with regard to previous gastric findings, serological data, fecal *Hp* antigen (enzyme immunoassay), and previous *Hp* eradication.

All data regarding concurrent pathologies, lifestyle, laboratory tests (Pg I, Pg II, gastrin, chromogranin, antibodies anti-*Hp* or fecal *Hp* antigen), diagnostic tests, histology and therapy were collected. In particular, symptomatic patients were interviewed for: type of symptoms: reflux related (i.e. heartburn, acid regurgitation, chest pain, hoarseness, cough, asthma, globus, dysphagia, dental erosions) and dyspepsia (epigastric pain or burning, belching, post prandial fullness, early satiety, nausea, vomiting, bloating), for symptoms intensity (severe, moderate, mild, no symptom) and frequency (daily, more than 2 times/week, sometimes, never) according to a 4-points Lickert scale and for medication use, in particular proton pump inhibitor (PPIs), $H_2$ receptor antagonist ($H_2$RAs), anti-acids, prokinetics and alginate-based compounds.

For each GERD symptomatic AAG patient (cases), we selected two consecutive AAG patients asymptomatic for reflux and without endoscopic signs of GERD, paired for age and gender, to compare endoscopic and clinical parameters (controls).

Esophageal pressures were measured in supine position after 6 hours of fasting. The 4.2 mm diameter catheter used had been assembled with 36 circumferential sensors spaced at 1 cm (Medtronic, Los Angeles, CA, USA) and the instrument had been calibrated to 0 and 300 mmHg applying an external pressure before the recording. The recording protocol included at least 5 minutes of basal registration to evaluate the gastro-esophageal junction (GEJ) and at least 10 water swallows (5 ml), every 30 seconds, to evaluate the esophageal peristalsis [27, 28]. Data acquisition, visualization and analysis were performed by Manoview analysis software (Medtronic, Duluth, GA, USA). In summary, after the LES localization (superior and inferior margins), mean basal pressure and integrated relaxation pressure (IRP) were evaluated [28, 29]. Esophago-gastric junction morphology was also defined according to Chicago v. 3 classification [30]. The mean pressure range of upper esophageal sphincter (UES) was 34-104 mmHg and the residual mean pressure <12 mmHg. Other measured parameters for esophageal body motility and peristalsis propagation accuracy were [30]: the frontal contraction speed, the distal latency (calculated as the time between the release of the UES and the

contractile deceleration point), the contractile distal integral and the percentage of normal, premature, and failed peristaltic waves, during the 10 swallows performed by the patient.

Multi-channel intraluminal impedance and pH monitoring were performed by using a 2.1 mm catheter (Sandhill Scientific Inc, Colorado, USA). The impedance record was obtained at 3, 6, 7, 9, 15 and 17 cm over the LES proximal border, while the pH electrode was located at 5 cm above the LES. During the 24h-study patients were asked to eat 3 times/day, to remain in upright position during the day and to indicate the recumbent period at night-time (max 8 h). Patients were also asked to take a symptoms diary and to push the "event" bottom in case of reflux symptoms. Medications (PPI or $H_2$RA) were interrupted at least 20 days prior to testing. During the wash-out period only alginates on an as-needed basis were allowed [31].

Data recorded were analyzed by the BioView Analysis software (Sandhill Scientific Inc) and reflux episodes were studied with an IT algorithm Autoscan (Sandhill Scientific Inc). All tracings were analyzed manually by an expert observer. Reflux episodes were characterized on the basis of their impedance changes (i.e. acid vs. non-acid) and physical (i.e., liquid, gaseous and mixed-gaseous) composition, according to the revised Porto classification [32, 33]. For symptoms analysis both weakly acidic acid and alkaline refluxes were considered as "non-acid reflux" episodes. Acid exposure time (AET) was defined as the total time with pH < 4, divided by the total time of monitoring and a percentage time of esophageal acid exposure <4.2% was considered as normal. A correlation between symptoms and reflux episodes was calculated [18, 33] by using Symptom Index (SI, positive if ≥ 50%) and Symptom Association Probability (SAP, positive >95%). The examination was considered positive for GERD in case of abnormal AET and/or SI and/or SAP. With this in mind, 3 conditions were highlighted: a) GERD in case of AET greater than 4.2%; b) RH: normal acid exposure but positive correlation between acid/non-acid reflux episodes and symptoms; c) functional disorder: normal acid exposure and no correlation between acid/non-acid episodes and symptoms (i.e. heartburn, regurgitation, chest pain, dysphagia, and globus).

Thus, on the basis of MII-pH, HRM and endoscopy results, AAG patients with reflux symptoms were classified according to the Rome IV criteria regarding the presence of functional esophageal disorders (no endoscopic signs of disease, no mechanical obstructions and negative MII-pH and HRM).

Data distribution was non-parametric (Kolmogorov-Smirnov 1 sample test). Quantitative variables were analyzed using the Mann-Whitney U test (MW) and the Kruskall-Wallis (KW) tests. Categorical variables were analyzed using the $\chi^2$ or Fisher exact test depending on the sample size. Results were considered significant with p values <0.05. Statistical analyses were performed using Stat Direct Statistical Software.

## RESULTS

### Demographic and clinical features

One hundred and seventy-two patients with AAG diagnosis were considered. Among them, 38 patients (22%) presented reflux symptoms (cases) and underwent additional functional investigations, whereas 76 consecutive asymptomatic AAG patients were selected as controls. The demographics and clinical features of enrolled patients are described in Table I.

**Table I.** Demographic and clinical features of AAG patients with and without reflux symptoms

| | AAG patients with reflux symptoms (n=38) | AAG patients without reflux symptoms (n=76) | p value |
|---|---|---|---|
| Male, n (%) | 9 (24) | 18 (24) | 1 |
| Fermale, n (%) | 29 (76) | 58 (76) | 1 |
| Mean age ± SD | 59 ± 13 | 57 ± 11 | 0.266 |
| Mean BMI ± SD | 25.4 ± 6.4 | 24.2 ± 3.9 | 0.076 |
| Anti parietal cells ab, n (%) | 36 (96) | 74 (97) | 0.471 |
| Anti intrinsic factor ab, n (%) | 22 (58) | 46 (61) | 0.787 |
| *Hp* infection, n (%) | 1 (3) | 0 (0) | 0.333 |
| Presence of autoimmune comorbidity, n (%) | 23 (61) | 47 (62) | 0.318 |

AAG: autoimmune atrophic gastritis; ab: antibodies; BMI: body mass index; *Hp: Helicobacter pylor*i; SD: standard deviation.

### HRM and MII-pH data

According to the Chicago classification v. 3.0, 24/38 (63%) had normal peristalsis, whereas 37% had various motility disorders, including 8 minor disorders (n=7 weak peristalsis, and n=1 hypertensive peristalsis), and 6 major disorders (n=6 EGJ outflow obstruction).

The main MII-pH monitoring parameters are reported in Table II. Two out of 38 patients (5.3%) had GERD, whereas 9 (24.7%) presented features compatible with RH, with an overall AET lower than 4.0%, but a positive symptoms-reflux association. Among these 9 patients, 6 had a RH to non-acid reflux, 2 to mixed reflux and 1 to acid reflux. The remaining 27 patients (71%) had negative findings at pH monitoring. In terms of reflux characteristics, as shown in Table III, non-acid refluxes were significantly more frequent of acid refluxes in all AAG patient and in functional patients.

Overall, 2/38 patients (5.3%) had GERD, 9/38 (24.7%) had RH, and 6/38 (13%) had a major motility disorder. The remaining 21 had normal manometry and normal MII-pH.

### Endoscopy and histologic data

At endoscopic examination, none of the control subjects showed esophageal mucosal injuries. Among cases, only 1/38 patients (3%) presented endoscopic and histological evidence of Barrett's esophagus (p=0.333). None had evidence of esophagitis.

Hiatal hernia was present in 17/38 (45%) cases, and in 13/76 (17%) controls (OR=3.9; 95%CI: 1.49-10.3, p=0.003). Eight out of 38 patients (21%) had evidence of duodenal-gastric biliary reflux, with no difference between cases and controls.

More advanced corpus atrophy (grade 2-3 vs 0-1) and higher OLGA stage (stage 2-3 vs 0-1), were more frequent in controls and in Rome IV patients than in GERD patients (p=0.015 and p=0.042, respectively). Antrum atrophy resulted more frequent in controls than in AAG patients with reflux symptoms (p=0.031).

**Table II**. Main impedance-pH monitoring parameters recorded in AAG patients reporting reflux symptoms with and without GERD diagnosis

| | AAG patients with GER symptoms (n=38) | AAG patients with GERD (n=2) | AAG patients with outflow obstruction (n=6) | AAG patients with functional esophageal disorder (n=30) |
|---|---|---|---|---|
| Acid exposure time pH (%) | 0.4 (0-1.1) | 5.3 (4.7-5.9) | 0.5 (0-0.8) | 0.35 (0-1.1) |
| Gastric time pH < 4 (%) | 0 (0.75-13) | 50.3 (1.3-99.3) | 12.9 (3,5-86) | 0 (0-0.02) |
| Mean acid clearing time (s) | 62 (0-119) | 449 (136-771) | 21 (0-103) | 54.5 (0-110.7) |
| Acid reflux, n | 4 (0-12) | 20.5 (12-29) | 7 (0,5-15) | 4 (0-10.5) |
| Non-acid reflux, n | 28 (11.5-51) | 30 (7-53) | 11 (9-20) | 37.5 (16,7-57.5) |
| Total reflux, n | 37 (18.5-63.5) | 52 (36-68) | 22 (13.5-29) | 43.5 (17.5-65.5) |
| Proximal extended reflux, n | 12 (6-31.2) | 22 (18-26) | 8 (5.5-9.5) | 20 (6-33.5) |

Data are expressed as median values (25th-75th percentiles or min-max value). AAG: autoimmune atrophic gastritis; GER: gastro-esophageal reflux; GERD: gastro-esophageal reflux disease

**Table III**. Features of refluxes in patients with GERD diagnosis, Rome IV, and all AAG patients presenting reflux symptoms

| | N of acid refluxes | N of non-acid refluxes | p |
|---|---|---|---|
| GERD patients (n=2) | 20.5 | 30 | 0,666 |
| Rome IV patients (n=30) | 4 | 37.5 | < 0.0001 |
| AAG with GER symptoms (n=38) | 4 | 28 | < 0.0001 |

For abbreviations see Table II.

No significant differences among analyzed groups for enterochromaffin-like cells hyperplasia, intestinal metaplasia and pseudo-pyloric metaplasia were found. Only one patient had on-going *Hp* infection and a concomitant diagnosis of functional heartburn. No difference was found in terms of history of *Hp* infection.

**Stratification of cases according to Rome IV criteria**

Thirty eight patients (79%) fulfilled the Rome IV criteria and were eligible for a functional esophageal disorder diagnosis. Fig. 1 summarizes the features of all AAG patients with reflux symptoms with respect all investigations carried out (MII-pH, EGDS, HR-M).

No differences were found with respect to reflux symptoms type and frequency among GERD and patients with functional esophageal disorders (Table IV). Examining dyspeptic symptoms there was no difference between GERD, functional patients and the control group, not even for symptom intensity.

With respect to the PPI use, 84% of cases (n=32) already were on PPI treatment (p<0.0001), but only 34% of cases (n=11) reported a benefit with >50% of symptom relief on reflux symptoms. Patients belonging to the GERD group had significant benefits on dyspeptic or reflux symptoms from treatment when compared with patients with functional esophageal disorders (with p=0.059, at the limit of statistical significance).

No significant differences were found comparing the serum levels of PgI, PgII, PgI/PgII, gastrin, and chromogranin A for each considered sub-group of patients.

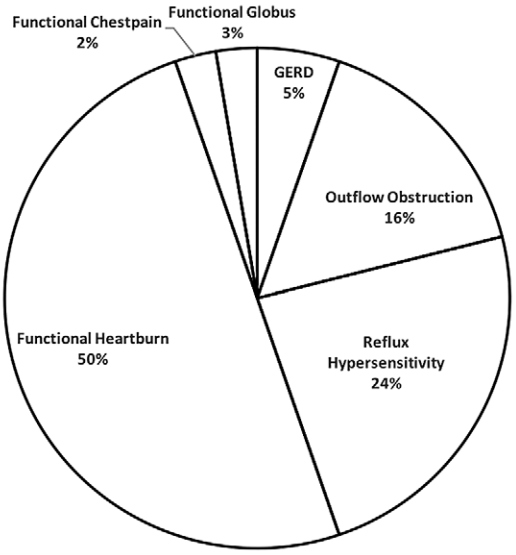


**Fig. 1.** Features of AAG patients with reflux symptoms with respect all investigations (MII-pH, EGDS, HRM) and Rome IV criteria

## DISCUSSION

In AAG, the antibodies targeted to parietal cells induce a progressive body and fundus atrophy, with consequently hypo-achlorhydria and reduced pepsin activation. These alterations, in turn, may cause the development of dyspeptic symptoms and anemia (microcytic sideropenic anemia, or macrocytic anemia related to cobalamin deficiency). However, GERD symptoms are common in AAG patients and various studies reported on their prevalence. According to these studies, patients with AAG complain of GERD symptoms in about 17-24% of the cases [34-36], an unexpectedly high number of patients when considering the low acid output determined by this disease. Moreover, several patients consume PPIs due to the presence of symptoms suggestive of GERD. With this premise, we aimed to evaluate the prevalence of GERD and functional esophageal disorders in AAG by using objective testing and adopting the last version of the Rome criteria (Rome IV) to define the presence of functional conditions. Our findings demonstrate that 22% of AAG patients have reflux symptoms but only few

**Table IV**. Frequency distribution of reflux and dyspeptic symptoms in AAG patients

| | AAG patients with GER symptoms (n=38) | AAG patients with GERD (n=2) | AAG patients with functional esophageal disorders (n=30) | AAG patients with outflow obstruction (n=6) | AAG patients without reflux symptoms (n 76) |
|---|---|---|---|---|---|
| Reflux symptoms | | | | | |
| Heartburn | 21 (55%) | 2 (100%) | 17 (57%) | 2 (33%) | - |
| Acid regurgitation | 24 (63%) | 2 (100%) | 18 (60%) | 4 (67%) | - |
| Chest pain | 16 (42%) | 0 (0%) | 14 (47%) | 2 (33%) | - |
| Belching | 23 (61%) | 1 (50%) | 18 (60%) | 4 (67%) | - |
| Cough | 16 (42%) | 1 (50%) | 13 (43%) | 2 (33%) | - |
| Hoarseness | 16 (42%) | 1 (50%) | 14 (47%) | 1 (17%) | - |
| Asthma | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | - |
| Dental Erosion | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | |
| Dyspeptic symptoms | | | | | |
| Epigastric pain | 15 (39%) | 0 (0%) | 13 (43%) | 2 (33%) | 40 (53%) |
| Bloating | 22 (58%) | 1 (50%) | 17 (57%) | 5 (83%) | 37 (49%) |
| Postprandial fulness | 21 (55%) | 0 (0%) | 17 (57%) | 4 (67%) | 41 (54%) |
| Early satiety | 7 (18%) | 0 (0%) | 7 (23%) | 0 (0%) | 26 (34%) |
| Nausea | 12 (32%) | 1 (50%) | 9 (30%) | 2 (33%) | 28 (37%) |
| Vomiting | 3 (1%) | 0 (0%) | 3 (10%) | 0 (0%) | 6 (8%) |

For abbreviations see Table II.

(5%) had a real GERD diagnosis, whereas the majority (79%) of them has a functional esophageal disorder.

Gastroesophageal reflux disease is a multifactorial entity, with prevalence ranging around 20% in the general population. Patients present either typical (i.e. heartburn, acid regurgitation) or atypical (cough, chest pain, hoarseness, belching, asthma) symptoms [37]. A non-invasive GERD diagnosis can be made on the basis of the symptoms presented by patients and/or prescribing an empirical anti-secretive therapy. Endoscopy can be diagnostic as well, by the detection of GERD-related lesions, whose prevalence however is rare. Thus, MII-pH is considered the gold standard for an objective diagnosis of GERD, through the demonstration of pathologic esophageal acid exposure and/or positive correlation between reflux episodes and symptoms [38]. Moreover, in clinical practice, HRM is commonly performed before MII-pH to exclude major esophageal motility disorders and to correctly localize lower esophageal sphincter. For this reason, to define the prevalence of GERD and functional esophageal disorders in an AAG population reporting reflux symptoms, we investigated a large group of AAG patients with endoscopy, HRM, and MII-pH, observing that only 5% of our cases were affected by objectified GERD. Endoscopic signs of GERD, with a histological diagnosis of Barrett esophagus, were detected in only one patient, who had also a pathological acid reflux at pH-MII. No patient had esophagitis. This result is in line with data reported by Carabotti et al. [36] who found a prevalence of erosive esophagitis in 3.7% of patients with AAG who underwent upper endoscopy. Tenca et al. [34], on the contrary, reported a diagnosis of GERD in 24% of AAG patients with reflux symptoms who underwent MII-pH. However, these authors included in the GERD definition also patients with RH (i.e. the majority of their GERD subjects), who were re-classified by the Rome IV criteria as affected by a functional esophageal condition. In general, it is estimated that about 60% of the patients reporting typical GERD-like symptoms are affected by true GERD (i.e. erosive esophagitis at upper endoscopy and/or abnormal acid exposure at MII-pH), whereas 40% of them may present with a functional condition (i.e. reflux hypersensitivity and/or functional heartburn). Therefore, the prevalence of functional esophageal disorders among our AAG patients appear higher than in the general population [19]. We hypothesized that this could be related to the chronic activation of some immune pathways responsible for esophageal symptom elicitation and perception, but further studies are required to confirm this.

As recently confirmed by Tenca et al. [34] non-acid reflux seems to be relevant in AAG patients, being more frequent than acid reflux ($p<0.0001$). This is related to the pathophysiology of AAG, that present a reduced acid gastric output. As a matter of fact, 36/38 patients (94.7%) had a normal AET. In confirmation, the analysis of histopathological features showed a more advanced body atrophy and a higher OLGA stage in controls and in Rome IV patients vs GERD ($p=0.015$ and $p=0.0423$, respectively) and a more frequent antrum atrophy in controls patients than in AAG patients with reflux symptoms. These results suggests that atrophy could be a protective factor from GERD [39, 40] and the presence of a conserved antral-body feedback, with an acid secretion at least in part preserved is connected with reflux even in AAG patients.

However, some patients (79%) present GERD symptoms even in the absence of any pathologic feature (physio-pathologic or endoscopic). This category has been recently classified according to Rome IV criteria with a labelling of "functional esophageal disorders". Indeed, patients with negative endoscopy, absence of major peristalsis disorders and a non-pathologic MII-pH can be classified according to Rome IV criteria as affected by functional heartburn, functional chest-pain, and functional globus, with most of the patient in

our cohort being in the functional heartburn category (64% of functional patients).

Type of symptoms and levels of serum biomarkers such as gastrin or PgI are apparently not of any help in discriminating the type of reflux in AAG, leading to the conclusion that these patients, when symptomatic, need a complete instrumental work-up for a correct diagnosis and a proper treatment to be settled. Indeed, an interesting finding of this study was that, despite a low intrinsic acid secretion, 84% of patient with reflux symptoms we recruited were already treated with PPIs, but a significant benefit of that treatment was obtained only in a minority of patients (34%). Symptoms relief was significantly more frequent in GERD patients when compared with patients with functional esophageal disorders (p=0.059). Response to PPIs in AAG patients with functional esophageal disorders can be due to an additional reduction of gastric secretion.

At esophageal HRM, 37% of patients presented some kind of peristalsis disorders mostly represented by minor changes, but with major peristalsis disorders, such as outflow obstruction, in 16% of the cases. EGJ outflow obstruction is defined by Chicago Criteria v. 3.0 as "an elevated median IRP with evidence of intact or weak peristalsis, such that the criteria of achalasia are not met". This disorder could have several etiologies as incompletely expressed or early achalasia, esophageal wall stiffness (infiltrative disease or cancer), ischemic conditions of the distal esophagus, or hiatal hernia. However, the clinical meaning of this finding is unclear, given various studies reporting the normalization of this finding over time or lack of symptoms correlation. Hence this finding needs further investigation to clarify its etiology [30].

Strengths of our study were the prospective design of the study, with a relative large number of patients included, considered the prevalence of AAG in the general population. Moreover, our patients underwent a complete diagnostic work-up (EGDS, pH-MII, HRM, serum biomarkers, clinical evaluations). The possible limitation was the lack of a control group of asymptomatic AAG patients who performed pH-MII and HRM.

## CONCLUSIONS

This study indicates that although AAG patients may complain of reflux symptoms despite the peculiar no-acidic context, a very low prevalence of objective GERD can be demonstrated. In most of the cases, reflux symptoms have a functional origin, and the majority of these patients can be classified with respect to Rome IV criteria as functional esophageal disorders. In this context, our data indicate that the use of anti-secretive drugs should always been considered with caution, not being by any mean the first line treatment in AAG patients. These patients should indeed be managed in a multidisciplinary setting, and adequately investigated to identify those harboring acid reflux who could benefit from PPI, in order to reducing the PPI inappropriate use.

**Conflicts of interest:** None to declare.

**Authors' contributions:** V.P., G.M., E.S., F.F. conceived the study. V.P., C.O. collected data. E.S. interpreted pH-MII and HRM studies. F.F. performed the endoscopies. M.F. and M.R. performed the histopathological analysis. V.P., G.M. analyzed the data and drafted the manuscript. V.P. corrected the manuscript. E.S., F.F critically revised the paper.

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