Clinical Implications of Low-grade Duodenal Eosinophilia in Functional Dyspepsia

A Prospective Real-life Study

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Background: Functional dyspepsia (FD) is a multifactorial disorder with no targeted therapy. Duodenal eosinophilia and low-grade inflammation are potential pathogenic mechanisms. However, the impact of duodenal eosinophils (D-EO) histologic evaluation in real-life clinical practice was not explored.

Aim: To evaluate the clinical utility of D-EO and low-grade inflammation in FD in real-life practice.

Materials and Methods: A multicenter prospective study was conducted. A total of 636 patients who meet Rome-III criteria were selected before upper endoscopy and 516 patients were included after normal endoscopy were assessed. Clinical parameters, *Helicobacter pylori* (*H. pylori*), and duodenal histology were evaluated.

Results: FD subtypes were 231 (45%) patients who had epigastric pain syndrome (EPS), 168 (33%) postprandial distress syndrome (PDS), and 117 (22%) EPS/PDS overlap. Two hundred fifty-nine (50.3%) patients were *H. pylori*$^+$. Histologic duodenal grading of chronic inflammation and intraepithelial lymphocytes showed no difference between FD subtypes. Increased in D-EO densities (>10 per high power field) was significant in PDS compared with EPS and EPS/PDS overlap subtypes. The odds ratio of PDS in subjects with duodenal eosinophilia densities was 2.28 (95% CI, 1.66-3.14; $P<0.0001$), adjusting for age, gender, *H. pylori* and nonsteroidal anti-inflammatory drug the odds ratio was 3.6 (95% CI, 2.45-5.28; $P<0.0001$). receiver operating characteristic curve analysis further demonstrated that low-grade duodenal eosinophilia, in particular *H. pylori*$^-$, was highly accurate for PDS with the area under the curve 0.731 compared with *H. pylori*$^+$ area under the curve 0.598. Furthermore, low-grade duodenal eosinophilia was significantly correlated with treatment response under 4 to 6 weeks of proton pump inhibitor therapy.

Conclusion: Our findings suggest that low-grade duodenal eosinophilia is associated with PDS subtype non-*H. pylori* FD patients and could be a useful marker of treatment response.

Key Words: functional dyspepsia, duodenal inflammation, duodenal eosinophilia, Helicobacter pylori

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Functional dyspepsia (FD) is a highly prevalent disease affecting ~10% of the general adult population,[1] but might vary according to country and criteria used to define its presence.[2] Characteristic symptoms of FD include epigastric pain, epigastric burning, postprandial fullness, or early satiety, present for at least 6 months with no structural disease on a routine investigation.[3] FD is classified according to the Rome criteria,[3,4] which includes: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS). Indeed, FD is a chronic functional gastrointestinal disorder with no cure; therefore, it affects the quality of life,[5] increase health care cost, and impair work productivity.[6]

Several neurogastroenterological mechanisms have been proposed to explain FD symptom subtypes, however, the pathophysiology is not fully clarified.[2] In recent studies, no correlation was found between gastric motor disorders with either of the 2 subtypes PDS and EPS.[7] These findings raise the possibility that primary gastric dysfunction is not the main trigger of FD symptom subtypes.[8] In the last decade, the theoretical basis of the concept of FD has begun to change, in light of new data focus on the duodenum.[9] It was observed that in FD patients the duodenal mucosa was characterized by an increase in eosinophils count [10] and degranulation,[11] mast cells degranulation,[12] and impaired permeability[12] with submucosal neural dysfunction.[13] Recently, in a small open-label study using proton pump inhibitor (PPI) therapy, changes in duodenal eosinophils (D-EO) were associated with clinical efficacy in FD.[14] These findings suggest that duodenal inflammation is an important pathogenic feature in FD. The aim of this study was to evaluate the diagnostic and clinical utility of D-EO and low-grade inflammation in FD subtypes in real-life clinical practice.

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The authors declare that they have nothing to disclose.

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MATERIALS AND METHODS

Patients with FD symptoms based on Rome-III criteria who underwent upper endoscopy at IOT Medical Center, Simes Medical Institute (Posadas City, Province of Misiones) and University Hospital San Juan Bautista (Santo Tomé City, Province of Corrientes), Argentina were evaluated in this prospective observational study. We consecutively recruited patients with newly diagnosed FD who were scheduled to undergo upper gastrointestinal endoscopy from January 2014 to November 2020. The criteria for inclusion were: (1) age between 18 and 70 years, (2) symptoms meeting Rome-III criteria, and (3) unremarkable endoscopic findings. The criteria for exclusion before upper endoscopy were: (1) progressive, severe diseases requiring active medical management (eg, advanced congestive heart failure, uncontrolled diabetes, decompensated cirrhosis, end-stage renal failure, neurological disease, advanced cancer, or psychiatric disorder), (2) those with a bleeding tendency or taking warfarin, aspirin, or antiplatelet drugs, (3) medical conditions known to increase peripheral and tissue eosinophilia (inflammatory bowel disease, celiac disease, vasculitis, connective tissue disease, hypereosinophilia syndrome, active infection and transplantation), (4) atopic disease such as food allergies (milk, eggs, peanuts, tree nuts, fish, shellfish, fruit, and vegetables), asthma, allergic rhinitis or drug reaction, and eczema, (5) patients with prior history of *H. pylori* infection, (6) patients who had taken an antibiotic and corticosteroids drugs within the past 3 weeks, and (7) history of previous significant gastrointestinal pathology (gastro-esophageal reflux disease and peptic ulcer disease), and history of gastrointestinal surgery (except appendectomy, cholecystectomy, and hernia repair). Patients receiving PPI or nonsteroidal anti-inflammatory drugs (NSAID) were advised to suspend 14 days before endoscopy. The criteria for exclusion after upper endoscopy were: (1) evidence of active peptic ulcer disease or gastro-esophageal erosive esophagitis, (2) evidence of malignant gastric disease, (3) signs of celiac disease, and (4) not available duodenal biopsies. The study was approved by the local ethical committee. Written informed consent was obtained from all participants

Abdominal Symptom Evaluation

All participants completed the simplified abdominal symptom questionnaire, which contains the severity of abdominal symptoms (encompassing how the symptoms affect dairy activities by a numeric analog scale from 0 to 5).[10] FD patients satisfied the Rome-III criteria for the past 3 months with symptom onset at least 6 months before diagnosis.[3] The diagnostic criteria for FD included 1 or more of the following: early satiety, postprandial fullness, epigastric pain, or epigastric soreness. In this case, early satiety and postprandial fullness were defined as experiencing symptoms at least 3 times/week, and epigastric pain or epigastric soreness were defined as experiencing symptoms at least 1 time/week.[3] FD was divided into 2 subtypes depending on the symptoms: EPS is associated with epigastric pain or epigastric soreness and PDS is associated with early satiety or postprandial fullness. Those who meet both criteria were classified as EPS/PDS overlap syndrome

Endoscopy

All recruited participants underwent upper gastrointestinal endoscopy performed by experienced endoscopists (F.J.B. and S.A.). Biopsy specimens were collected from the lesser curvature of the gastric body (2 biopsies), lesser curvature of gastric antrum (2 biopsies), and the second portion of the duodenum (D2) (3 biopsies) using a Radial Jaw 3 forceps (Boston Scientific). Only patients with normal endoscopy findings were included.

Histopathologic Analysis

Biopsies were fixed in 10% formalin and processed to paraffin embedding for hematoxylin and eosin (HE) staining by routine methods. Gastric pathology was recorded as per the Sydney system.[15] Duodenal biopsies were reviewed by 2 pathologists (C.V. and E.K.) under coded identification without knowledge of the clinical data. The presence of *H. pylori* was assessed on gastric biopsies using Giemsa staining in all patients. Duodenal inflammation was assessed semiquantitatively on HE slides for the presence and severity of microscopic duodenitis according to the Updated Sidney Criteria[15,16] (Fig. 1). For the purpose of this study, to determine D-EO at D2 lamina propria, 5 nonoverlapping fields on the slides (40× field, high power field (HPF)) were selected with the highest eosinophils density and quantified, data is expressed as mean × HPF, grading as follows: mild: <10, moderate: ≥10 to <20, and marked: ≥20. Although there are no universally accepted criteria for defining abnormal eosinophil densities counts in lamina propria, we graded semiquantitatively each by 10 × HPF based on previous data of Whitington and Whitington,[17] Kokkonen et al,[18] Lowichik and Weinberg,[19] Kalach et al,[20] Lee et al,[21] Leite et al[22] and Genta et al[23] where a threshold of >10 or >20 D-EO per HPF was used to discriminate higher duodenal eosinophil densities. Duodenal architecture and villous/crypt ratio were assessed to exclude celiac disease[24] and intraepithelial lymphocytes were quantified per 100 enterocytes in 3 to 5 villi on HPF and grading: very mild: 0 to 9, mild: ≥10 to <20, moderate: ≥20 to <40, and marked: ≥40.

Statistical Analysis and Sample Size

Sample size calculation was performed under the assumption that the prevalence of duodenal eosinophilia in FD subtypes PDS of 47% and EPS of 32%.[25] We calculated with 80% of power and an alpha level of 0.05, that at least 166 patients for each FD subtype arm would be needed for the study.

Data were presented as mean ± SD or median interquartile range or number of subjects (% of total) as appropriate. Differences between groups were analyzed by the students' *t* test or analysis of variance for normal distribution and the Wilcoxon rank sum test or Kruskal Wallis test for non-normal distribution. Categorical values were compared using χ^2 tests. The relationship between duodenal eosinophilia and clinical variables or subtypes of FD was examined by the logistic regression model. Youden index was assessed by receiver operating characteristic curve analysis to evaluate the optimal cutoff point for diagnostic accuracy of duodenal eosinophil densities in lamina propria. A comparison of 2 areas under the curves (AUCs) was done using the DeLong test. Data were analyzed using SPSS 22.0 and Med-Calc, and a 2-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Study Population

Six hundred thirty-six patients who meet Rome-III criteria were evaluated before upper endoscopy and 516

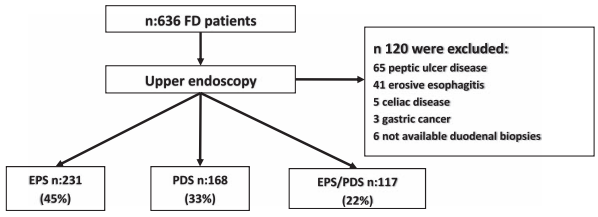

FIGURE 1. Study flowchart. FD, EPS, and PDS. EPS indicates epigastric pain syndrome; FD, functional dyspepsia; PDS, postprandial distress syndrome.

were included after upper endoscopy for analysis. A flowchart of the study and baseline characteristics of patients are seen in Figure 1 and Table 1. The cohort of patients was comprised of 309 (60%) women and 207 (40%) men with a median age of 51 (interquartile range, 20.5) years. The FD subtypes of patients were as follows: 231 (45%) patients had EPS, 168 (33%) PDS, and 117 (22%) were EPS/PDS overlap. Two hundred fifty-nine patients (50.3%) were *H. pylori*$^+$. In relation to clinical comorbidities: 117 (22.8%) patients had type-II diabetes mellitus/insulin resistance, 152 (29.6%) patients had hypertension, mean body mass index (BMI) was 26.83 ± 4.23 and 102 (19.7%) patients had obesity (BMI ≥ 30 kg/m^2), regular smoking was present in 132 (25%) patients, alcohol consumption daily or occasional was referred by 279 (54%) subjects. NSAID and PPI consumption were detected and discontinued before upper endoscopy in 220 (42%) and 147 (28%) patients respectively (Table 1).

Functional Dyspepsia Subtypes and Clinical Variables

There were no differences in FD subtypes at BMI, obesity, diabetes/insulin resistance, hypertension, PPI, smoking, alcohol use, and prior cholecystectomy (Table 1). EPS subjects were significantly younger (median 49 y), had increased *H. pylori* prevalence (69%), NSAID consumption (52%), and underwent appendectomy than PDS and EPS/PDS overlap subjects (Table 1). There was a significantly more proportion of female patients in PDS (68%) than in EPS (56%) and EPS/PDS overlap (55%) subtypes.

Functional Dyspepsia Subtypes and Histologic Grading Duodenal Inflammation

Histologic duodenal grading of chronic inflammation showed no difference between FD subtypes (Fig. 2). Also, no difference in FD subtypes was observed at D2 intraepithelial lymphocytes. However, D-EO densities were significantly increased in PDS subjects compared with EPS and EPS/PDS overlap subtypes (Fig. 2). To determine the best cutoff of D-EO densities in PDS patients, a Youden index analysis was performed, and D-EO of >10 per HPF had a sensitivity of 56% and specificity of 73% with a positive predictive value of 50% and negative predictive value of 78% (Fig. 3A). Indeed, 57% of PDS subjects had D-EO of >10 per HPF compared 33% in EPS and 16% in EPS/PDS overlap subtypes ($P<0.05$) (Fig. 2). The odds ratio (OR) for PDS versus non-PDS FD subtypes with D-EO was 2.28 (95% CI, 1.66-3.14; $P<0.0001$). After adjusting for age, gender, *H. pylori,* and NSAID the OR was 3.6 (95% CI, 2.45-5.28; $P<0.0001$). These results showed that D-EO densities, D-EO of >10 per HPF, are associated with the PDS subtype.

Duodenal Eosinophilia and Clinical Variables

Using the cutoff D-EO of >10 per HPF (Fig. 4), increase in D-EO densities was observed in 189 subjects with FD (36.6%). No difference was observed in age, gender, BMI, obesity, diabetes, hypertension, smoking, alcohol, prior cholecystectomy, and appendectomy between FD patients with or without low-grade duodenal eosinophilia (Table 2). D-EO was not increased in those who used NSAID (Table 2). More proportion of low-grade duodenal eosinophilia patients was positive for *H. pylori* (64%, $P<0.05$) (Table 2). To evaluate the accuracy of the D-EO cutoff for a diagnosis of PDS based on *H. pylori* infection status a comparison of Youden index analysis was done (Fig. 3A). The receiver operating characteristic curves for D-EO cutoff point of >10 per HPF for *H. pylori*$^+$ had a sensitivity of 63% and specificity of 58% with a positive predictive value of 28% and negative predictive value of 85%, for *H. pylori*$^-$ had a sensitivity of 53% and specificity of 96% with a positive predictive value of 90% and negative predictive value of 71%. An AUC comparison showed that

TABLE 1. FD Subtypes and Clinical Variables

Variable	Total (n 516); n (%)	EPS (n: 231); n (%)	PDS (n: 168); n (%)	EPS/PDS (n: 117); n (%)	*P**
Age (IQR)	51 (20.5)	49 (18)*	54 (19)	51 (19.3)	0.001
Gender (F)	309 (60)	131 (56)	114 (68)*	64 (55)	<0.05
BMI (SD)	26.83 (4.23)	26.9 (4.4)	26,5 (4.2)	27.2 (4)	ns
Obesity	102 (19.7)	45 (19)	30 (18)	27 (23)	ns
Helicobacter pylori$^+$	259 (50.3)	159 (69)*	53 (31)	47 (40)	<0.05
NSAID	220 (43)	120 (52)*	47 (28)	53 (45)	<0.05
Diabetes	117 (22.8)	50 (22)	41 (24)	26 (22)	ns
Hypertension	152 (29.6)	63 (27)	53 (31)	36 (31)	ns
Smoking	132 (25)	63 (27)	42 (25)	27 (23)	ns
Alcohol	279 (54)	137 (59)	83 (49)	60 (51)	ns
Appendectomy	146 (28)	82 (35)*	41 (24)	23 (20)	<0.05
Cholecystectomy	85 (16)	35 (15)	32 (19)	19 (16)	ns

*Indicates $P < 0.05$.

Data are expressed as mean (+/− SD), median (IQR) or percentage (%) of total. For continuous variables, analysis of variance or Kruskal Wallis test was used for normal or non-normal distributed data. χ^2 test for categorical variables.

BMI indicates body mass index; EPS, epigastric pain syndrome; FD, functional dyspepsia; IQR, interquartile range; NS, non-significant difference; NSAID, nonsteroidal anti-inflammatory drug, PSD; postprandial distress syndrome.

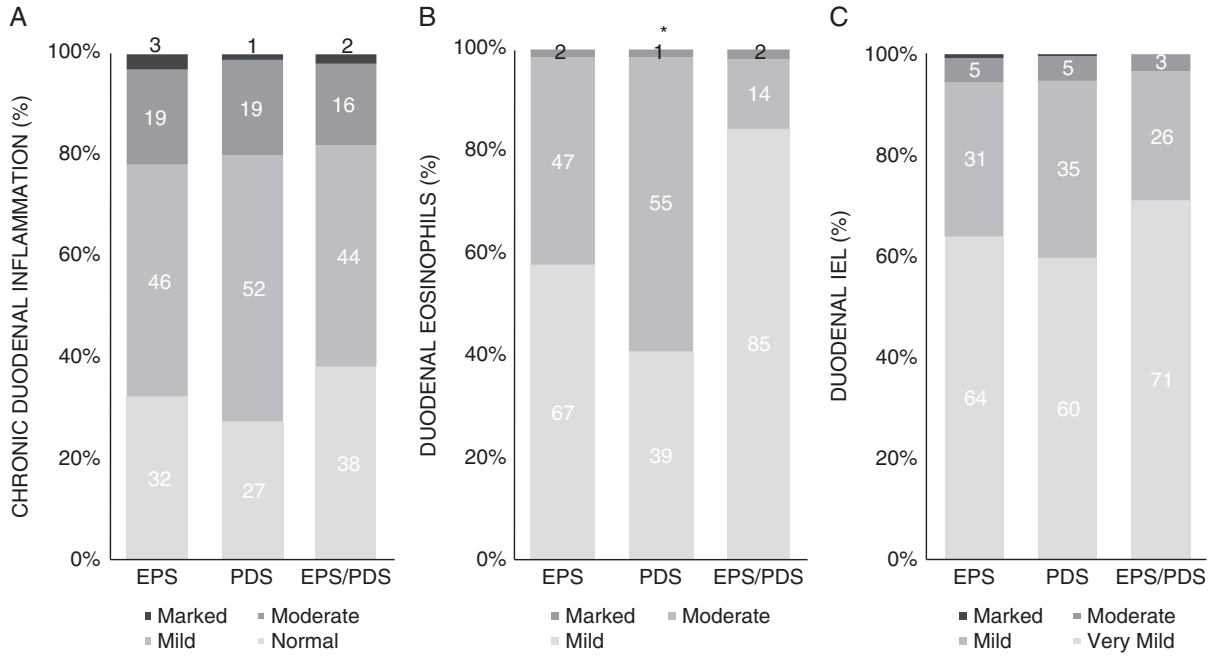

FIGURE 2. FD subtypes and histologic grading of duodenal inflammation at second portion of duodenum. A, Chronic duodenal inflammation. B, Duodenal eosinophils (D-EO) count in lamina propria. C, Duodenal IEL. Data are expressed as percentage of total. * indicates $P < 0.05$; EPS, epigastric pain syndrome; IEL, intraepithelial lymphocyte; PDS, postprandial distress syndrome.

duodenal eosinophilia in *H. pylori*$^-$ was significantly best accurate for PDS with AUC 0.731 (± 0.032, SE) than *H pylori*$^+$ PDS patients AUC 0,598 (± 0.045, SE) ($P = 0.016$) (Figs. 3B and C). These findings suggest that low-grade duodenal eosinophilia is associated with the PDS subtype predominantly in *H. pylori*$^-$ FD patients.

Next, we evaluated the clinical impact of increased D-EO densities in PDS *H. pylori*$^-$ FD management. Indeed, using the simplified abdominal symptom questionnaire (0 to 5), a significantly higher score in early satiety ($P = 0.0045$) was associated with increased D-EO densities but not with postprandial fullness (Fig. 5A). Moreover, we evaluated whether an increase duodenal eosinophil densities affect the efficacy of the standard of care of FD[26] (Pantoprazole 40 mg or Esomeprazole 40 mg once daily 30 minutes before the first daily meal and nutritional counseling for 4 to 6 wk). Of 116 PDS *H. pylori*$^-$ subjects, 96 completed the questionnaire before upper endoscopy and after 4 to 6 weeks of the standard of care therapy. Significantly more proportion of patients resolved PDS symptoms early satiety and postprandial fullness in the group of high D-EO densities (early satiety score, 0 to 1; 69.8%; $P = 0.0028$; postprandial fullness score, 0 to 1;73%; $P = 0.0003$) compared with low D-EO (early satiety score, 0 to 1; 37.2 %; postprandial fullness score, 0 to 1; 34%) (Fig. 5B). Overall, our observations indicates that duodenal eosinophil evaluation could be a useful predictor of PDS symptoms alleviation under PPI therapy.

DISCUSSION

In this study, we investigated the potential diagnostic and clinical impact of D-EO and low-grade inflammation in FD subtypes in real-life clinical practice. The results of this

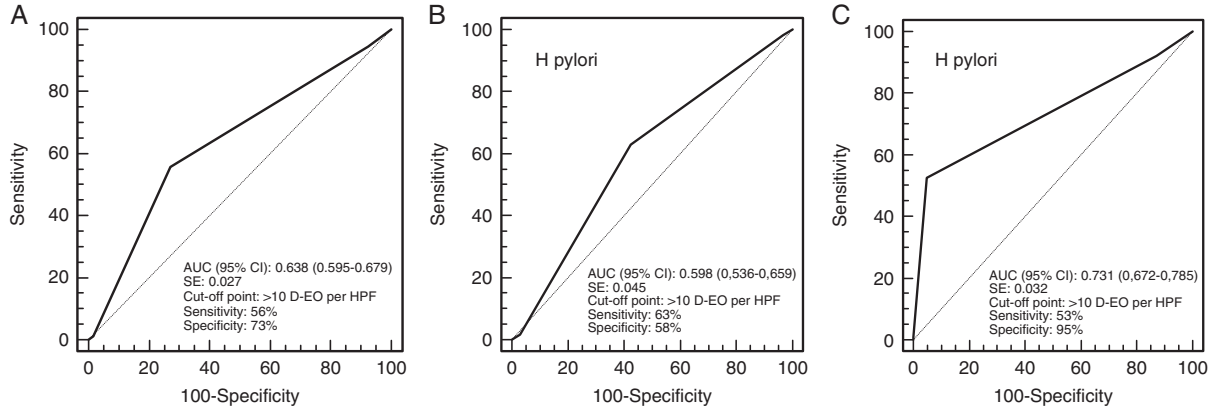

FIGURE 3. Youden index analysis by receiver operating characteristic curves of D-EO in PDS diagnosis. A, PDS cohort. B, PDS *H. pylori*$^+$. C, PDS *H. pylori*$^-$. AUC indicates area under the curve; D-EO, duodenal eosinophils.

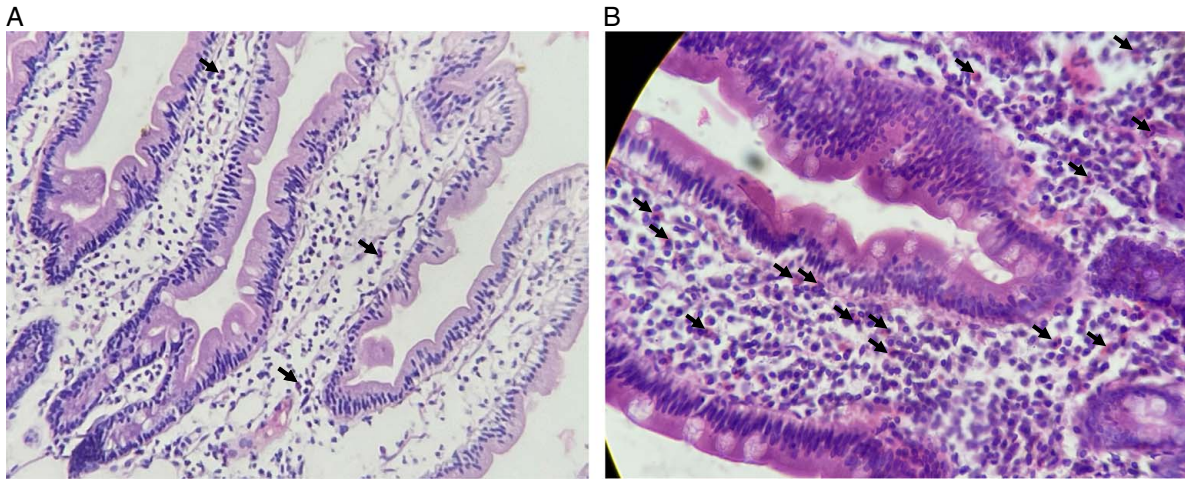

FIGURE 4. D-EO density in lamina propria adjacent to glands. A, Low D-EO density, <10 D-EO per HPF. B, High D-EO density, >10 D-EO per HPF. Arrows indicates eosinophil cell.

prospective observational study suggest that low-grade duodenal eosinophilia is associated with FD in the *H. pylori*$^{-}$ PDS subtype. Moreover, we observed that increased D-EO densities may be a marker of PPI therapy-mediated alleviation of dyspeptic symptoms in PDS such as early satiety and postprandial fullness. Thus, we provide real-life evidence for the clinical application of duodenal eosinophil in FD patients.

The etiopathogenesis of FD is considered multifactorial including associations with gut-brain axis such as psychological distress, altered microbiota, postinfectious gastroenteritis, and *H. pylori*, although dyspeptic symptoms associated with *H. pylori* by Kyoto consensus may also be considered as a separate entity.[27] Indeed, abnormalities of gastric function including impaired accommodation or hypersensitivity to distention and delayed emptying have been reported in FD, but these changes correlate poorly with symptoms.[7] Emerging data increasingly point towards the duodenum as the key integrator in dyspepsia symptom generation and it has been proposed that gastric motor dysfunction may be attributed to disordered duodeno-gastric feedback and low-grade inflammation.[28] Talley et al proposed a new model of disease that is initiated by internal and external triggers that induce gastrointestinal barrier dysfunction leading to a low-grade duodenal inflammation characterized by an innate immune response with increased activation of eosinophils and mast cells.[9,10,29,30] Emergent studies have reported that duodenal low-grade inflammation and increased small intestinal homing T cells correlate with delayed gastric emptying and dyspeptic symptom severity.[12,31] Moreover, recent observation correlates D-EO or mast cells activation and calcium transient amplitudes to high K+ or electrical stimulation in FD patients.[13] Duodenal eosinophilia and mast cell degranulation seems to be related to an early step in triggering and sustained visceral hypersensitivity and altered motor control, but is not clear what is causing this phenomenon in a group of FD patients but not in others. Emerging data suggest that childhood environmental factors (herbivore pet),[32] luminal triggers (eg, food, microbiota, and bile acids),[25,33] increased duodenal acid exposure[34] and systemic factors including stress (cortisol)[35] may explain duodenal mucosal hyperpermeability and low-grade inflammation mediated an inhibitory duodeno-gastric motor reflex. It was hypothesized that eosinophils secondary to duodenal acid or food allergy accumulate in some patients with FD, and degranulate releasing a variety of cytokines, neuro-mediators, and lipid mediators, which in turn induces vagal M2 receptor dysfunction and stimulates the smooth muscle.[36] Indeed, eosinophil degranulation but no mean eosinophil count was independently associated with FD (OR: 1.74; 95% CI: 1.08, 2.78; $P = 0.02$) and early satiety (OR: 2.04; 95% CI: 1.26, 3.30; $P = 0.004$).[11] Also, in a cohort of FD patients followed for 10 years a significant association of duodenal eosinophilia in the second part of duodenum with new onset symptomatic gastroesophageal reflux disease was observed especially in the PDS subtype at baseline.[37] Whether mean eosinophil count or degranulation may be the surrogate marker of this low-grade cytokine-mediated pathogenesis of FD symptoms is a matter of debate that need multi-center and multi-ethnic studies.

In our study, we observed a correlation of increased duodenal eosinophilia densities (low-grade duodenal

TABLE 2. Duodenal Eosinophilia and Clinical Variables

Variable	High D-EO (189); n (%)	Low D-EO (327); n (%)	*P*
Age (IQR)	52 (19)	51 (21)	ns
Gender (F)	105 (55)	204 (62)	ns
BMI (SD)	26.6 (4.03)	26.9 (4.35)	ns
Obesity	32 (17)	70 (21)	ns
Helicobacter pylori^{+}	121 (64)*	138 (42)	<0.05
PPI	56 (30)	91 (28)	ns
NSAID	69 (36)	151 (46)*	<0.05
Diabetes	37 (19)	80 (24)	ns
Hypertension	52 (27)	100 (30)	ns
Smoking	50 (26)	82 (25)	ns
Alcohol	112 (59)	168 (51)	ns
Appendectomy	61 (32)	85 (26)	ns
Cholecystectomy	27 (14)	58 (18)	ns

*Indicates $P < 0.05$.

Data are expressed as mean (+/− SD), median (IQR) or percentage (%) of total. For continuous variables, *t* test or Wilcoxon rank sum test was used for normal or non-normal distributed data. χ^2 test for categorical variables.

BMI indicates body mass index; D-EO, duodenal eosinophils; IQR, interquartile range; NS, non-significant difference; NSAID, nonsteroidal anti-inflammatory drug; PPI, proton pump inhibitor.

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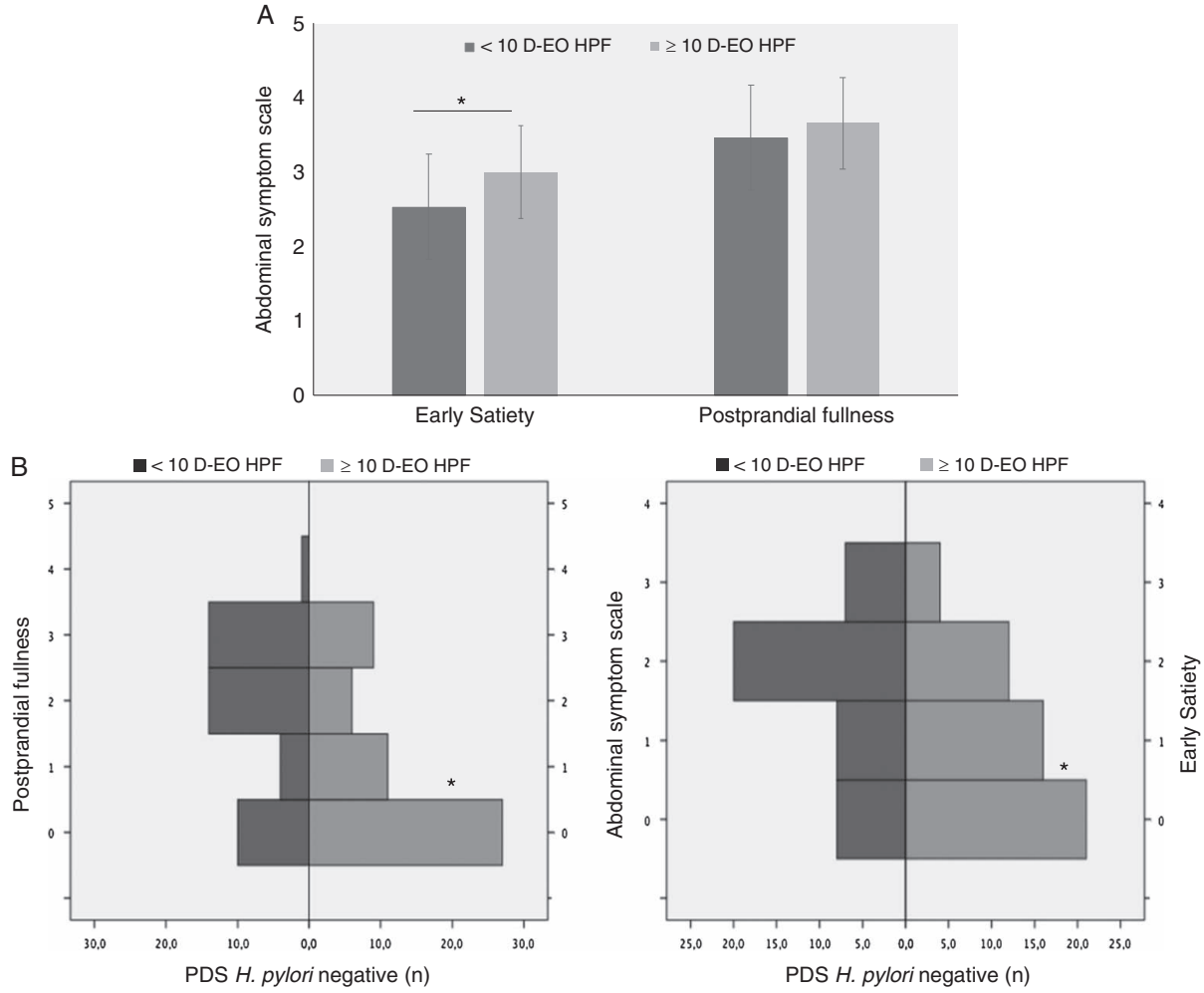

FIGURE 5. Abdominal symptom questionnaire (analog scale from 0 to 5) and D-EO in PDS *H. pylori*$^-$. A, Early satiety and postprandial fullness symptom scale before upper endoscopy, bars expressed as mean (error bars +/−, SD), *t* test or Wilcoxon rank sum test was used for normal or non-normal distributed data, * indicates $P < 0.05$. B, Early satiety and postprandial fullness symptom scale after 4 to 6 weeks of standard of care, bars expressed n of subjects in each symptom scale. Responders were categorized as 0 to 1, nonresponders as > 1. χ^2 test was used, * indicates $P < 0.05$. D-EO indicates duodenal eosinophils; PDS, postprandial distress syndrome.

eosinophilia) at D2 in the PDS subtype but not in EPS or EPS/PDS overlap. This association remains significant after adjusting to age, gender, *H. pylori,* and NSAID consumption. Our observations are in accord with previous observations, where duodenal eosinophilia was associated with PDS,[37] early satiety, and postprandial fullness.[25,38] Indeed, in a recent large cross-sectional study, no significant association between duodenal eosinophilia and FD was observed, but the presence of duodenal eosinophilic degranulation was significantly associated with early satiety.[11] Noteworthy, as a proof of concept in a budesonide placebo-controlled trial, by decreasing duodenal eosinophilia was significantly associated with a reduction in postprandial fullness and early satiety symptoms but not with the full spectrum of FD.[39] Another superb trial from the Leuven group showed that by reducing duodenal eosinophilia under PPI therapy a significant amelioration of full-spectrum FD symptoms was addressed.[14] Moreover, a recent Cochrane meta-analysis showed a trend for higher efficacy of PPI in the PDS versus EPS FD subtype.[40] Perhaps low-grade duodenal inflammation could be relevant to stratify FD for clinical management in high or low duodenal eosinophil densities. Indeed, in our cohort, we observed that high duodenal eosinophil densities in FD *H. pylori*$^-$ showed an AUC more accurately for PDS and was associated with marked symptom improvement under PPI therapy. Although the correlation between increased D-EO densities and symptoms subtypes in FD does not prove causality, confirmation from mechanistic and international prospective studies is needed to clarify the clinical role of low-grade duodenal eosinophilia in FD subtypes management to apply in clinical practice.

The strength of this study is this a prospective evaluation from a well-sample-sized defined FD cohort based on Rome-III criteria with normal upper endoscopy in a real-life clinical setting. More than 98% of the duodenal biopsies were evaluated. Pathologists were blinded to medical history before evaluation. We performed a clinical follow-up of symptom evaluation, at baseline and after 4 to 6 weeks of PPI therapy.

A limitation of the study pertains to the population studied, no healthy volunteer was included, histologic

evaluation, duodenal samples from D2 only, psychological factors were not evaluated and validated questionnaires were not used. Our cohort comprised the north-eastern Argentinean population where a high prevalence of *H. pylori* is expected. Indeed, a prevalence of 50% of *H. pylori*$^{+}$ was observed and could explain the high proportion of EPS patients rather than PDS in this cohort. Because this is an observational study, the mechanism of increased D-EO densities in *H. pylori*$^{-}$ PDS but not in EPS patients was not explored, this observation merits further investigation. In our histologic evaluation, using a semiquantitative grading scale of D-EO densities, a cutoff of D-EO >10 /HPF was ascertained by Youden index analysis and is in accord with Talley et al[10] where clusters of >10 D-EO were significantly associated with nonulcer dyspepsia. In this study, we choose to evaluate D2 based on previous data where duodenal eosinophilia and degranulation were observed in FD subjects predominantly at D2 when compared with healthy controls.[38,41] No specific eosinophil immunostaining was performed in our study, however high agreement of eosinophil counts by HE versus major basic protein immunostaining was observed by Talley et al,[10] making the HE approach an acceptable tool for eosinophil evaluation in real-life clinical practice.

In conclusion, the results of this prospective observational real-life setting study suggest that duodenal eosinophilia is associated with FD in the PDS subtype and revealed that duodenal eosinophilia is a useful histologic marker of PPI-therapy response in PDS non-*H. pylori* FD patients.

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