Helicobacter pylori infection does not protect against eosinophilic esophagitis: results from a large multicenter case-control study

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OBJECTIVES: **Rising trends in eosinophilic esophagitis (EoE) have been repeatedly linked to declining *Helicobacter pylori (H. pylori)* infection, mostly in retrospective studies. We aimed to prospectively evaluate this inverse association.**

METHODS: **Prospective case-control study conducted in 23 centers. Children and adults naïve to eradication therapy for *H. pylori* were included. Cases were EoE patients, whereas controls were defined by esophageal symptoms and <5 eos/HPF on esophageal biopsies. *H. pylori* status was diagnosed by non-invasive (excluding serology) or invasive testing off proton pump inhibitor (PPI) therapy for 2 weeks. Atopy was defined by the presence of IgE-mediated conditions diagnosed by an allergist.**

RESULTS: **808 individuals, including 404 cases and 404 controls (170 children) were enrolled. Overall *H. pylori* prevalence was 38% (45% children vs. 37% adults, *p* 0.009) and was not different between cases and controls (37% vs. 40%, *p* 0.3; odds ratio (OR) 0.97; 95% confidence interval (CI) 0.73–1.30), neither in children (42% vs. 46%, *p* 0.1) nor in adults (36% vs. 38%, *p* 0.4). Atopy (OR 0.85; 95%CI 0.75–0.98) and allergic rhinitis (OR 0.81; 95%CI 0.68–0.98) showed a borderline inverse association with *H. pylori* infection in EoE patients. This trend was not confirmed for asthma or food allergy.**

CONCLUSIONS: ***H. pylori* infection was not inversely associated with EoE, neither in children nor in adults. A borderline inverse association was confirmed for atopy and allergic rhinitis, but not asthma of food allergy. Our findings question a true protective role of *H. pylori* infection against allergic disorders, including EoE.**

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INTRODUCTION

Eosinophilic esophagitis (EoE), first described in the early 90s, represents a rising chronic, immune/antigen-mediated disease, characterized clinically by symptoms related to esophageal dysfunction and histologically by T-helper (Th)2-mediated eosinophil-predominant inflammation [1, 2]. EoE affects typically

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children and young adults in westernized countries, with a strong male predominance, increased risk of familial heritability and higher rate of concomitant atopic disorders [2–4]. Currently, EoE is the most common cause of dysphagia and food impaction in children and adults under 50 years-old [2, 3]. The reasons for the rapid increase in incidence and prevalence of EoE, especially over the past decade, remain unclear.

Helicobacter pylori (H. pylori) infects approximately 50% of the global population, with a wide variation between regions and countries [5]. Prevalence is highest in Africa, Latin America, and Asia (54–80%), but lowest in Northern Europe, Northern America and Oceania (16–37%) [5]. Primary infection mostly occurs during childhood and inadequate sanitation practices, low social class, and crowded or high-density living conditions seem to be related to a higher prevalence of *H. pylori* infection [5]. *H. pylori* colonizes gastric mucosa and usually elicits a gastric Th1/Th17 type of immune response [6]. Since its formal characterization in the early 1980s and subsequent association with several conditions (peptic ulcer disease, chronic gastritis, gastric cancer, and MALT lymphoma), *H. pylori* prevalence has markedly decreased in westernized countries [e.g., United States (28%) [7], Japan (18%) [8], Sweden (15%) [9]].

Therefore, an inverse association between rising EoE and declining *H. pylori* patterns has been repeatedly reported in literature over the past five years [10–14]. The rationale behind this association, proven in a murine model [15], is that the immunomodulatory properties of *H. pylori*, polarizing the immune system towards a Th1 response, may confer protection against Th2-mediated allergic disorders. As such, a protective role of *H. pylori* infection against the development of EoE has been suggested. Because of the limited number of studies and potential methodological issues (retrospective design, small sample size, inadequate *H. pylori* diagnostic methods), prospective large-scale multicenter studies are still required to clarify the proposed association between *H. pylori* and EoE. We aimed to further investigate this association through a large multicenter prospective and controlled evaluation of *H. pylori* status in EoE patients.

METHODS

This study was a prospective multicenter case-control study conducted in Spain (16 centers), Italy (5 centers), France (1 center), and Colombia (1 center) from December 2014 to January 2017. All patients gave their written informed consent for endoscopic procedures and study enrolment. The investigations were conducted according to the principles expressed in the Declaration of Helsinki. Approval from the Institutional Review Board was obtained from each participating institution.

Patients

Consecutive patients older than 2 yrs-old with a diagnosis of EoE, defined by updated evidence-based guidelines [2] (symptoms of esophageal dysfunction plus $\geq$15 eosinophilis per high power field (eos/HPF) in esophageal biopsies) and naïve to EoE therapy were eligible for enrollment. Patients were recruited from outpatient gastroenterology clinics or endoscopy suites. Symptoms of esophageal dysfunction were food refusal, reflux-like symptoms, vomiting, abdominal pain for toddlers and young children and food impaction, dysphagia, heartburn and chest pain for older children, adolescent and adults [2].

Controls

Controls were required to be individuals who also were undergoing upper endoscopy, mostly due to suspicion of EoE, in whom esophageal biopsies were obtained. Accordingly, the control group was made up of consecutive patients older than 2 yrs-old with similar symptoms of esophageal dysfunction but eosinophilic inflammation $<$5 eos/HPF in esophageal biopsies taken during endoscopic procedures.

Assessment of clinic, endoscopic, and histologic data

For all included cases and controls, data on demographics (age, gender, smoking habit), atopic background, symptoms of esophageal dysfunction, endoscopic features of EoE (rings, furrows, exudates, edema, strictures, narrow caliber esophagus, crepe paper esophagus) [16], the presence of distal erosive esophagitis, graded according to Los Angeles classification, and histological features (peak proximal and peak distal eosinophil counts) at the index endoscopy were obtained. Atopy in both groups was defined by the presence of any of the following disorders: asthma, rhinoconjunctivitis, food allergy, oral allergy syndrome, atopic dermatitis, anaphylaxis, urticaria or angioedema, registered in clinical records and diagnosed by an allergy specialist.

All endoscopic procedures were performed with either topic pharyngeal anesthesia or propofol-based sedation, according to patient preference, by board-certified gastroenterologists and pediatricians. Using conventional grasping forceps, at least six biopsy specimens were taken separately from the distal and proximal esophagus. Mucosal biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin for histologic examination, performed by senior gastrointestinal pathologists with expertise in EoE at each center. Peak eosinophil count was determined in the area of highest density of eosinophils and esophageal eosinophilia was defined upon the presence of 15 or more eos/HPF in at least one field [1, 2].

Data collection

Study data were collected and managed using REDCap electronic data capture tools hosted at the Spanish Gastroenterological Association (AEG): AEG-REDCap (https://redcap.aegastro.es/) [17]. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: (1) an intuitive interface for validated data entry; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for importing data from external sources.

H. pylori status

All included individuals, both cases and controls, were required to be naïve for *H. pylori* eradication therapy. EoE patients and controls with previous eradication regimens for *H. pylori* were

excluded. The diagnosis of *H. pylori* infection was based on positivity to non-invasive tests [^{13}C-urea breath test (UBT), monoclonal stool antigen test] or invasive tests (rapid urease test or histology) during endoscopic procedures. Serology was not accepted as a diagnostic test. All tests were performed with patients off antibiotics or bismuth for 4 weeks and off proton pump inhibitor (PPI) therapy for 2 weeks [18]. When rapid urease test was selected as diagnostic test, one biopsy was taken from the corpus and one from the antrum. As for histology, two biopsies from each location were taken. In patients with negative invasive tests, a ^{13}C-UBT or monoclonal stool antigen test was recommended to further exclude *H. pylori* infection. If patients were on active PPI therapy during the endoscopic procedure, a further non-invasive test off PPI therapy was performed.

Eradication of *H. pylori* infection was defined as a negative ^{13}C-UBT with citric acid at least 4 weeks after completion of treatment. ^{13}C-UBT was performed after an overnight fast and PPI discontinuation at least 2 weeks before [18].

Management of EoE

According to the current guidelines [2], patients were classified as responders to PPI therapy when they achieved symptom improvement and histologic remission, defined by <15 eos/HPF, after an 8-week course of double-dose PPI therapy. In patients unresponsive to PPI therapy, dietary or topic swallowed steroids were offered.

Endpoints

The primary endpoint of the study was to assess the prevalence of *H. pylori* infection in a large cohort of pediatric and adult EoE patients, compared to a group of symptomatic controls. Secondary aims were to evaluate differences in terms of *H. pylori* prevalence between children and adults, atopic and non-atopic and responders and non-responders to PPI therapy.

Statistical analysis

The SPSS 21.0 (IBM, Armonk, NY) statistical analysis package was used. Categorical variables were described with percentages and continuous variables were described with mean (standard deviation) or median (range), as appropriate. Associations between categories were tested with χ^2 (with Fisher's correction when necessary) and continuous data were assessed using the two-sample *t*-test or the Mann–Whitney *U*, for parametric and non-parametric data, respectively. For multiple comparisons, analysis of variance and Student–Newman–Keuls multiple comparisons were performed. A multiple logistic regression analysis was performed using variables with $P < 0.1$ in the univariate analysis. We used a backward modeling strategy, and the log likelihood ratio was used for model comparison. The magnitude of the effect is described with an odds ratio (OR) and 95% confidence interval (CI). *P* values < 0.05 were considered statistically significant. With previous studies reporting a 3.2% maximum difference in *H. pylori* prevalence between cases and controls, a sample size of 830 patients was required to be 90% sure than the limits of a two-sided 90% CI will exclude a difference between groups of more than 3.2.

RESULTS

Baseline characteristics

From an initial set of 1243 eligible individuals, a total of 808 (404 cases, 404 controls) were included in the study, of whom 170

Table 1 Baseline characteristic of the study population, comparing cases to controls

	EoE *n*=404	Controls *n*=404	*p*
Age (years, mean and range)	35 (2–77)	37 (2–83)	*0.2*
Male gender	299 (74%)	287 (71%)	*0.1*
Caucasian	397 (98%)	389 (96%)	*0.5*
Smoking habit	37 (9%)	65 (16%)	*0.02*
Atopic disorders	283 (70%)	127 (31%)	*<0.001*
Rhinoconjunctivitis	211 (52%)	106 (26%)	*<0.001*
Asthma	163 (40%)	73 (18%)	*<0.001*
Food allergy	120 (29%)	26 (6%)	*<0.001*
Atopic dermatitis	62 (15%)	18 (4%)	*<0.001*
Symptoms (children)			
Epigastric/abdominal pain	167 (41%)	311 (77%)	*<0.001*
Heartburn/regurgitation	175 (43%)	103 (25%)	*<0.001*
Dysphagia	190 (47%)	33 (8%)	*<0.001*
Nausea/vomiting	99 (24%)	77 (19%)	*0.3*
Food bolus impaction	125 (31%)	9 (2%)	*<0.001*
Symptoms (adults)			
Dysphagia	283 (70%)	235 (58%)	*0.008*
Food bolus impaction	240 (59%)	59 (14%)	*<0.001*
Heartburn/regurgitation	175 (43%)	148 (36%)	*0.01*
Epigastralgia	59 (14%)	73 (18%)	*0.04*
Regurgitation	41 (10%)	46 (11%)	*0.7*
Chest pain	45 (11%)	29 (7%)	*0.05*
Nausea/vomiting	24 (6%)	33 (8%)	*0.3*
Endoscopic findings			
Normal endoscopic appearance	65 (16%)	271 (67%)	*<0.001*
Rings	202 (50%)	33 (8%)	*<0.001*
Longitudinal furrows	223 (55%)	11 (2%)	*<0.001*
Edema	304 (75%)	9 (2%)	*<0.001*
Whitish exudates	138 (34%)	9 (2%)	*<0.001*
Reflux esophagitis	29 (7%)	65 (16%)	*0.002*
Stricture	61 (15%)	13 (3%)	*0.001*
Crepe paper esophagus	25 (6%)	0 (0%)	*0.04*
Peptic ulcer disese	5 (1%)	14 (3%)	*0.3*
Esophageal eosinophilia			
Proximal esophagus	43 (0–190)	0.7 (0–2)	*<0.001*
Distal esophagus	46 (0–192)	1.2 (0–4)	*<0.001*

Statistically significant *P* values appear in italics

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individuals (21%) were younger than 14 years-old. The number of patients included from the main recruiting centers is shown as a Supplementary file **1**. Reasons for exclusion were refusal to participate ($n = 241$) and previous eradication regimens for *H. pylori* infection ($n = 194$). Cases and controls were comparable in terms of age and gender. Atopic disorders, dysphagia/food impaction, typical EoE endoscopic findings and esophageal eosinophilia were all significantly more frequently found in EoE patients. On the contrary, epigastric and abdominal pain, normal endoscopic appearance or reflux esophagitis were more common in the control group. Predominant symptoms in pediatric EoE patients were mixed (dysphagia, heartburn, epigastric/abdominal pain, and regurgitation), being dysphagia and food impaction the leading symptoms in adult EoE patients. As for controls, epigastric/abdominal pain was the most common symptom leading to endoscopy referral in children, whereas dysphagia and heartburn were the most frequent complaints in adults. Compared baseline characteristics are shown in Table **1**.

Prevalence of *H. pylori*

Overall analysis. Overall, 312/808 individuals (38%) were diagnosed with *H. pylori* infection at baseline. *H. pylori* prevalence was significantly higher in children than in adults [77/170 (45%) vs. 235/638 (37%), p 0.009]. The flow chart of patients during the *H. pylori* diagnosis process is displayed in Fig. **1**. As for *H. pylori* prevalence, there were no differences between Spanish (43%) and Italian (39%) included cases and controls.

Cases and controls. No significant differences in *H. pylori* infection prevalence were identified between cases and controls in an overall analysis [cases 151/404 (37%) vs. controls 161/404 (40%), p 0.3], in children [cases 36/85 (42%) vs. controls 39/85 (46%), p 0.1], and adult population [cases (115/319 (36%) vs. controls 122/319 (38%), p 0.3]. These data are summarized in Fig. **2**.

Atopic conditions. Atopic individuals overall had a lower *H. pylori* infection prevalence when compared to non-atopic individuals (non-atopic 42% vs. atopic 34%, p 0.01; OR 0.85; 95%CI 0.75–0.98). Further sub-analyses confirmed this trend in adult patients, but not in children. As for allergic rhinitis, an inverse association with *H pylori* infection was confirmed in an overall analysis (non-rhinitis 41% vs. rhinitis 34%, p 0.01; OR 0.81; 95%CI 0.67–0.98), but could not be replicated in further sub-analyses. No inverse associations were observed neither for asthma nor for IgE-mediated food allergy. These data are shown in Table **2**.

Response to PPI therapy. Response to PPI therapy was documented in 191 (47%) EoE patients. Prevalence of *H. pylori* infection was similar regardless of response to PPI therapy (39% responders vs. 35% non-responders, p 0.2), or the presence of reflux esophagitis (37% yes vs. 42% no, p 0.2).

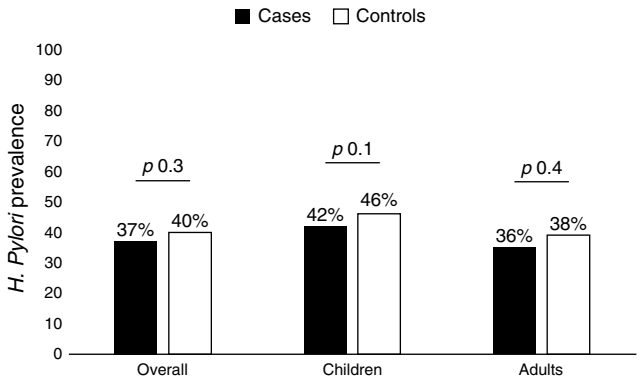

Fig. 2 Prevalence of *H. pylori* infection in the study population broken down by type of patient and age range

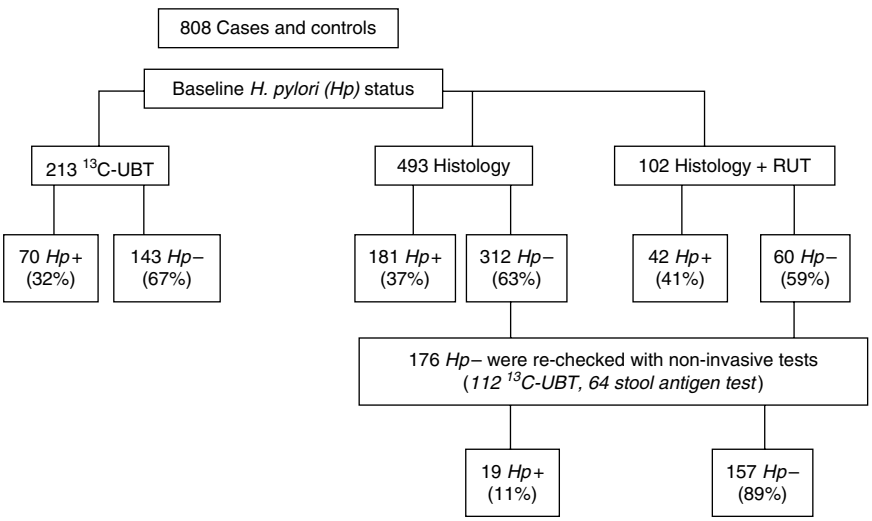

Fig. 1 Flow chart of patients during the study. RUT rapid urease test, UBT urea breath test

Table 2 Subanalyses evaluating the prevalence of *H. pylori* infection depending on the atopic status and the presence of different allergic conditions

	H. pylori prevalence		Odds ratio (95% confidence interval)	*p* value
	Atopic	Non-atopic		
Overall	34%	42%	***OR 0.85 (95% CI 0.75–0.98)***	***0.01***
Controls	36%	43%	OR 0.72 (95% CI 0.51–1.03)	0.04
Pediatric controls	44%	46%	OR 1.08 (95% CI 0.42–2.7)	0.4
Adult controls	34%	41%	***OR 0.86 (95% CI 0.76–0.99)***	***0.02***
Cases	36%	45%	OR 0.89 (95% CI 0.78–1.01)	0.04
Pediatric cases	48%	43%	OR 0.97 (95% CI 0.58–1.23)	0.07
Adult cases	32%	44%	***OR 0.84 (95% CI 0.73–0.98)***	***0.01***
	Rhinitis	Non-rhinitis		
Overall	33%	41%	***OR 0.81 (95% CI 0.67–0.98)***	***0.01***
Controls	28%	43%	***OR 0.63 (95% CI 0.40–0.97)***	***0.02***
Pediatric controls	35%	41%	OR 0.92 (95% CI 0.73–1.06)	0.1
Adult controls	33%	39%	OR 0.97 (95% CI 0.65–1.44)	0.2
Cases	34%	41%	OR 0.91 (95% CI 0.71–1.23)	0.1
Pediatric cases	41%	43%	OR 1.2 (95% CI 0.58–1.8)	0.5
Adult cases	35%	40%	OR 0.95 (95% CI 0.73–1.18)	0.2
	Asthma	Non-asthma		
Overall	37%	38%	OR 1.04 (95% CI 0.76–1.43)	0.4
Controls	37%	39%	OR 0.99 (95% CI 0.62–1.24)	0.2
Pediatric controls	42%	44%	OR 1.1 (95% CI 0.47–1.41)	0.3
Adult controls	36%	40%	OR 0.92 (95% CI 0.69–1.15)	0.1
Cases	36%	39%	OR 0.99 (95% CI 0.61–1.19)	0.2
Pediatric cases	46%	43%	OR 0.96 (95% CI 0.58–1.23)	0.2
Adult cases	36%	38%	OR 1.2 (95% CI 0.49–1.38)	0.3
	Food allergy	Non food allergy		
Overall	36%	39%	OR 1.12 (95% CI 0.79–1.60)	0.3
Controls	37%	42%	OR 0.91 (95% CI 0.68–1.09)	0.1
Pediatric controls	44%	46%	OR 1.23 (95% CI 0.43–1.74)	0.4
Adult controls	36%	40%	OR 0.98 (95% CI 0.73–1.26)	0.2
Cases	37%	41%	OR 0.94 (95% CI 0.7–1.16)	0.2
Pediatric cases	48%	43%	OR 0.89 (95% CI 0.68–1.18)	0.1
Adult cases	37%	39%	OR 1.3 (95% CI 0.57–1.86)	0.4

Statistically significant *P* values appear in bold italics

DISCUSSION

The present study is the first large multicenter prospective study evaluating the relationship between EoE and *H. pylori* infection. Contrary to all previous reports, *H. pylori* infection was present in a similar proportion of cases and controls, questioning an inverse protective association of *H. pylori* against EoE. We do humbly believe that data showing parallel declining *H. pylori* infection and rising incidence of EoE might merely reflect a coincidental divergent trend between diseases, rather than a causal relationship. EoE was first described in the early 90s [19] and since, incidence and prevalence have steadily grown [2, 3]. Conversely, *H. pylori* eradication regimens were also developed during the early 90s [20, 21] and their latter widespread use has been shown enormously effective to reduce peptic ulcer disease [22] and gastric cancer incidence in high-risk populations [23].

A weak inverse association between *H. pylori* and atopy and rhinitis was observed in the present study, but this could not be demonstrated for asthma or food allergy. In line with our findings for

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EoE, meta-analyses and studies questioning the inverse association between *H. pylori* infection and atopic disorders have lately been reported [24–27]. More specifically, discrepant results have been lately reported in studies and meta-analyses regarding the proposed inverse association between asthma and *H. pylori* infection, supporting [28–33] or discarding [34–36] this hypothesis. A more recent review on this topic has suggested that *H. pylori*, rather than providing protection against atopic conditions, may merely be a marker of socio-economic status and hygiene, well known to be also directly associated with atopic conditions [37]. Therefore, rising allergic disorders may be influenced by a more general change in human microecology, like gut microbiota alterations related to better hygiene, antibiotic use, and changes in dietary habits. In this complex scenario, *H. pylori* declining is likely to represent a mere epiphenomenon [38]. Two recent studies conducted in Finland and The Netherlands have shown conflicting inverse or positive associations depending on either ethnic background or socioeconomic environment, hinting at the existence of potentially missed confounders driving this association [35, 39].

Likewise, numerous methodological deficiencies were identified in previous case-control studies suggesting an inverse association between *H. pylori* infection and EoE [11–14] (Table 3). The two biggest studies, from the same group of researchers [11, 14], performed a retrospective analysis of pathological databases. However, the protocol for taking gastric biopsies was not provided [11] (or stated that only 3.9% of biopsy sets were Sydney System compliant [14]), and no information on previous PPI discontinuation before biopsies was available. These data about PPI withdrawal were not provided in the remaining study, which also used histology as a diagnostic test [12].Therefore, there might be concerns about *H. pylori* false negatives in these studies. In the first big studies [10, 11], cases were defined by any esophageal eosinophilia, without using the specific histological threshold of 15 eos/HPF. Additionally, data on previous eradication regimens [10–14] or esophageal symptoms [10–14] were missing in some patients. As for the latest prospective study, a small sample size, the use of serology as a diagnostic test for *H. pylori* infection and the lack of esophageal biopsies in the control group question the reproducibility of these results. Validated IgG antibodies cannot discriminate whether *H. pylori* infection is present or past, and this may result in a significant proportion of false positives. In this regard, a protective role for *H. pylori* can only be possible via early colonization, that would eventually protect against further development of allergic disorders during the adulthood. However, by using serology and limiting included patients to adults, one cannot truly ascertain this temporal association.

More importantly, the majority of these studies found an extremely low prevalence of *H. pylori* infection, ranging from 3 to 8.6% in EoE cases [11–14], excepting the Kalixanda study (16%) [10]. In a recent comprehensive meta-analysis assessing the global prevalence of *H. pylori* infection, the lowest *H. pylori* prevalence rates were reported in Switzerland (18.9%), Denmark (22.1%), New Zealand (24.0%), Australia (24.6%), and Sweden (26.2%) [23]. Even these lowest figures are five to ten times higher than the prevalence estimated for *H. pylori* in the four previous case-control studies [11–14]. In the aforementioned meta-analysis, estimates for prevalence of *H. pylori* infection in Western Europe and North America were 34.3% and 37.1%, respectively [23]. As such, our overlapping prevalence rates in the present study (37% cases, 40% controls) match much more with a realistic scenario for *H. pylori* infection, compared to previous case-control studies [10–13]. Prevalence data in Spain and Italy were estimated to be

Table 3 Comparative analysis of all case-control studies evaluating the relationship between *H. pylori* infection and either esophageal eosinophilia or EoE

First author, country, year of publication	Study design, *H. pylori* diagnosis, population, sample size (cases/controls)	Data on previous eradication regimens for *H. pylori*	Definition of cases	Esophageal biopsies controls	*H. pylori* prevalence cases	*H. pylori* prevalence controls	*p* value or odds ratio (95% CI)
Ronkainen J, Finland, 2007 [10]	Prospective, histology, Children/adults, 48/952	Not provided	Any esophageal eosinophilia	Yes	16.7%	33%	p 0.04
Dellon ES, US, 2011 [11]	Retrospective, histology, children/adults, 5767/56,301	Not provided	Any esophageal eosinophilia	Yes	4.8%	6.8%	0.69 (0.77–0.87)
Elitsur Y, US, 2014 [12]	Retrospective, histology/RUT, children, 62/904	Not provided	>15 eos/HPF + Lack of response to PPI therapy	Yes	3%	6.5%	0.096 (0.013–0.72)
Von Arnim U, Germany, 2016 [13]	Prospective, serology, adults, 58/116	Not provided	Esophageal symptoms + >15 eos/HPF	No	5.2%	37.9%	0.24 (0.11–0.50)
Sonnenberg A, US, 2016 [14]	Retrospective, histology, Children/adults, 25,969/284,552	Not provided	Dysphagia + >15 eos/HPF	Yes	4.5%	7.3%	0.77 (0.73–0.8)
Present study	Prospective, UBT/RUT/ StoolAg/ histology, children/ adults, 404/404	All cases and controls were naïve for eradication regimens	Esophageal symptoms + >15 eos/HPF	Yes	37%	40%	0.97 (0.73–1.30)

CI confidence interval, *eos/HPF* eosinophils per high power field, *RUT* rapid urease test, *StoolAg* stool antigen, *UBT* urea breath test

56 and 55% in the aforementioned meta-analysis [23], but were around 40% in the present study, in line with recent studies suggesting a progressive declining in *H. pylori* prevalence in developed countries [7–9].

Our study also first assessed a similar lack of association between *H. pylori* and EoE in both responders and non-responders to PPI therapy. This finding is an additional argument supporting the lack of relevant differences between EoE patients responders and non-responders to PPI therapy, whom should be all included within the EoE spectrum [2]. Strengths to the present study include a large number of cases and control prospectively recruited at multiple centers, including both children and adults, exclusion of patients with previous *H. pylori* eradication treatments, protocolized esophageal and gastric biopsies in both cases and controls, detailed clinical information and adequate previous PPI discontinuation before *H. pylori* testing, widening the diagnostic approach with non-invasive testing. As for potential limitations, concerns with the control group should be acknowledged. Atopy (31%), rhinitis (26%), and asthma (18%) in controls were much more common than that expected for a standard healthy population. Control group was made up of symptomatic patients with minimal to no esophageal eosinophilia on esophageal biopsies, so a potential explanation is that increasing index of suspicion for EoE has led to more endoscopy referral in atopic patients with any kind of upper gastrointestinal symptom, especially epigastric/abdominal pain. No specific questionnaires were used to evaluate EoE symptoms and different diagnosed means were utilized to test *H. pylori* infection, which might have biased the comparative analysis.

In conclusion, no inverse relationship was observed between *H. pylori* infection and EoE in a large prospective multicenter case-control study. Similarly, a borderline association was observed for atopy and allergic rhinitis, but not for asthma or food allergy. In line with recent evidence, our findings question a true protective role of *H. pylori* infection against allergic disorders. Therefore, *H. pylori* infection may merely act as a weak surrogate for socio-economic status and hygiene, rather than modulate the immune host response. Opposite to the *H. pylori* infection hypothesis, we speculate that evolving environmental and microbial exposures, microbiota changes related to better hygiene, antibiotic use or dietary habits, as well as differences in ethnicity and socioeconomic conditions, are likely more plausible reasons to understand the rising trends of atopic conditions over the past two decades.

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CONFLICT OF INTEREST

Guarantor of the article: Javier Molina-Infante.

Specific author contributions: JM-I: study concept and design, patient recruitment, acquisition of data, statistical analysis, manuscript draft and revision. ES, CG-J, RP, IM, OB, AP-G, AM, JA, AP, CG-A, NA, AMV, PB-G, MM-P, MP, NDB, VAC, JLD-J, RG-R, CJ, EM, JAP-D, AP-A, ST, VO, FZ, AP-G: patient recruitment, acquisition of data and critical revision of the manuscript. JPG and AJL: patient recruitment, statistical analysis, manuscript draft and revision.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ An inverse association between rising eosinophilic esophagitis (EoE) and declining *H. pylori* infection has been repeatedly reported.
- ✓ Most studies are retrospective and/or hampered by methodological issues.

WHAT IS NEW IN HERE

- ✓ In the first large prospective case-control study, no inverse association was found between *H. pylori* and EoE.
- ✓ Minimal or no inverse association was observed between *H. pylori* and atopic conditions.
- ✓ Our findings question a true protective role of *H. pylori* infection against allergic disorders, including EoE.

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Supplementary Table 1. Number of cases and controls included from the main recruiting centers in the study

	N
Caceres	138
Tomelloso	114
Madrid-Puerta de Hierro	82
Padua	69
Milan	65
Madrid-Gregorio Marañon	43
Barcelona-Terrassa	54
Zaragoza	37
Barcelona-Viladecans	34
Valladolid	33
Barcelona-Sant Pau	31
Marbella	29