Duodenal Eosinophils and Mast Cells in Functional Dyspepsia: A Systematic Review and Meta-Analysis of Case-Control Studies

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BACKGROUND & AIMS: **This study explored the link between duodenal eosinophils and mast cells in patients with functional dyspepsia (FD).**

METHODS: **MEDLINE (PubMed) and Embase electronic databases were searched until June 2021 for case-control studies reporting duodenal eosinophils and mast cells in FD. Pooled standardized mean difference (SMD), odds ratio, and 95% CIs of duodenal eosinophils and mast cells in FD patients and controls were calculated, using a random-effects model.**

RESULTS: **Twenty-two case-control studies with 1108 FD patients and 893 controls were identified. Duodenal eosinophils (SMD, 1.29; 95% CI, 0.85–1.73; $P = .0001$) and mast cells (SMD, 2.11; 95% CI, 1.14–3.07; $P = .0001$) were increased in FD patients compared with controls. Substantial heterogeneity was found ($I^2 = 93.61$, $P = .0001$; and $I^2 = 96.69$, $P = .0001$, respectively) and visual inspection of funnel plots confirmed publication bias. Degranulation of duodenal eosinophils was significantly higher in FD patients compared with controls (odds ratio, 3.78; 95% CI, 6.76–4.48; $P = .0001$), without statistically significant heterogeneity. We conducted a sensitivity analysis for duodenal eosinophils, by including only high-quality studies, and the results remained unchanged (SMD, 1.73; 95% CI, 1.06–2.40; $P = .0001$), with substantial heterogeneity. Postinfectious FD patients had increased duodenal eosinophils compared with controls (SMD, 3.91; 95% CI, 1.32–6.51; $P = .001$) and FD patients without any history of infection (SMD, 1.42; 95% CI, 0.88–1.96; $P = .001$). *Helicobacter pylori*–negative FD patients had significantly higher duodenal eosinophils compared with controls (SMD, 3.98; 95% CI, 2.13–5.84; $P = .0001$), with substantial heterogeneity. No significant difference in duodenal eosinophils was seen according to FD subtypes.**

CONCLUSIONS: **This meta-analysis suggests a link between duodenal microinflammation and FD. However, the quality of evidence is very low, largely owing to the unexplained heterogeneity and serious risk of publication bias in all comparative analyses. Thus, causality remains uncertain and further studies are required.**

Keywords: Functional Dyspepsia; Functional Gastrointestinal Disorders; Irritable Bowel Syndrome; Eosinophils; Mast Cells; Systematic Review; Meta-Analysis.

Abbreviations used in this paper: **EPS, epigastric pain syndrome; FD, functional dyspepsia; FGID, functional gastrointestinal disorder; GDP, gross domestic product; HPF, high-power field; IBS, irritable bowel syndrome; NCOS, Newcastle–Ottawa scale; OR, odds ratio; PDS, postprandial distress syndrome; PI-FD, postinfectious functional dyspepsia; PPI, proton pump inhibitor; SMD, standardized mean difference.**

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Functional dyspepsia (FD), now conceptualized as a disorder of gut–brain interaction,[1] is a symptom-based diagnosis in the absence of organic gastrointestinal lesions explaining the gastrointestinal presentation. FD is one of the most common functional gastrointestinal disorders (FGIDs), with a pooled prevalence rate of 7.2%.[2] Two major subgroups of FD are recognized: postprandial distress syndrome (PDS), with postprandial fullness or early satiation, and epigastric pain syndrome (EPS), with epigastric pain and/or burning. In clinical practice, FD often overlaps with another common FGID, irritable bowel syndrome (IBS),[3] characterized predominantly by pain and related bowel symptoms. The pathophysiology of both FD and IBS is multifactorial and gastroduodenal motor and sensory dysfunction, impaired mucosal integrity, low-grade mucosal immune activation, gut microbial dysbiosis, and dysregulation of the gut–brain axis all have been implicated.[4]

In recent years, there has been a focus on the proximal duodenum as a site of microinflammation in FD.[5] Talley et al[6] coined the term "duodenal eosinophilia" when they found that duodenal eosinophil counts were increased significantly in patients with nonulcer dyspepsia, especially those with early satiety, compared with controls. Since then, several studies have reported that both duodenal eosinophil and mast cell counts[7,8] and their degranulation[7] are increased in FD. However, other studies have failed to find a similar link between symptoms and duodenal eosinophils and/or mast cells in FD patients with or without IBS.[9–11] Moreover, in a recent study including 136 Japanese patients with FD, duodenal eosinophilia was limited to patients with postinfectious FD (PI-FD).[12]

Thus far, there is no universally accepted normal range of eosinophil counts in the second part of the duodenum that could serve as a threshold for diagnosing duodenal eosinophilia across populations. Confounders such as techniques for counting eosinophils, time after a meal, site of duodenal biopsy, genetic, seasonal, geographic, and environmental including diet variation, and influence of concomitant *Helicobacter pylori* infection, acid suppression with proton pump inhibitors (PPIs), and pathologic expertise and interest may affect the densities of duodenal eosinophilic infiltration reported.

We thus conducted a systematic review and meta-analysis to (1) determine and compare the prevalence of duodenal eosinophils and mast cells in FD patients, including those with PI-FD and FD subtypes and controls; (2) explore the link between cell counting techniques, criteria for FD diagnosis, and variations in prevalence of duodenal eosinophils; (3) assess the effect of *H pylori* infection, PPI use, and seasonal variation on the prevalence of duodenal eosinophils; and (4) assess the effect of geographic and related socioeconomic factors (countries stratified according to their gross domestic product [GDP]) on the prevalence of duodenal eosinophils in FD patients and controls.

What You Need to Know

Background

Duodenal eosinophils and mast cells have been implicated in the pathophysiology of functional dyspepsia (FD).

Findings

Based on our systematic review and meta-analysis, involving 22 case-control studies, duodenal eosinophils and mast cells are increased significantly in FD and the proportion of degranulated eosinophils and mast cells is higher in FD compared with controls. However, we noted substantial clinical heterogeneity, potentially owing to the lack of standardized histologic assessments or inappropriate controls.

Implications for patient care

Increased (degranulated) duodenal eosinophils and mast cells are found in patients with FD. These findings may identify a subgroup of FD patients with a distinct pathophysiology. However, the quality of evidence is low, and more data are required.

Materials and Methods

Protocol and Registration

This systematic review and meta-analysis meets the preferred reporting items for systematic reviews and meta-analysis statement requirements.[13,14] The protocol for this Systematic Review was registered prospectively with International Prospective Register of Systematic Reviews (PROSPERO) (CRD42021255431).

Search Strategy

Electronic databases, including PUBMED, MEDLINE (OvidSP), and EMBASE, were searched from initiation (1966) up to June 2021 for all studies assessing duodenal eosinophils and mast cells in FD patients with or without IBS. The literature search strategy is outlined in Figure 1, and was conducted with the assistance of our librarian. The search strategy for MEDLINE is outlined in Supplementary Figure 1. For further details see the Supplementary Materials and Methods.

Selection of Studies

Two authors (T.F. and A.S.) independently screened abstracts and titles. Abstracts were eliminated if the study did not investigate the association between duodenal eosinophils and mast cells and FD or FGIDs. Full texts of the remaining articles were retrieved and reviewed. Eligibility criteria for study inclusion are provided in Table 1 and detailed in the Supplementary

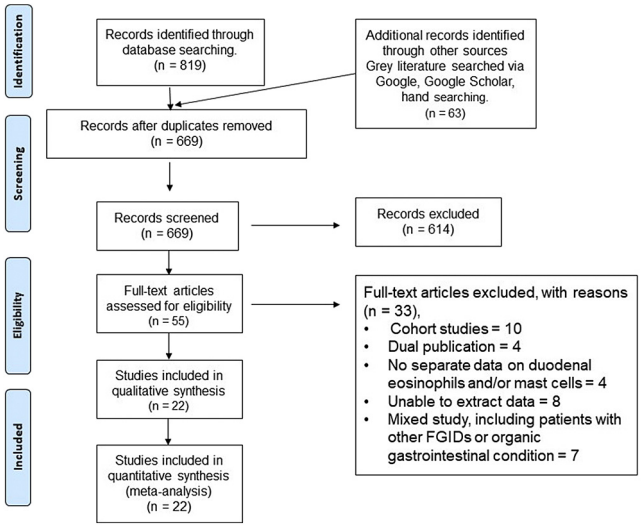

Figure 1. Preferred reporting items for systematic reviews and meta-analysis statement requirements (PRISMA) flow diagram. FGID, functional gastrointestinal disorder.

materials. The studies that were excluded are detailed in, Supplementary Table 1. Disagreements between reviewers were resolved by mutual consensus after reference to the original published report.

Data Extraction and Quality Assessment

Data were extracted independently by 2 authors (T.F. and A.S.) with discrepancies resolved by reference to the source publication. Data were entered into a Microsoft Excel spreadsheet (2010 Professional edition; Microsoft Corp, Redmond, WA). The variables extracted are detailed in the Supplementary materials. The quality of the case-control included studies that were assessed using the Newcastle–Ottawa scale (NCOS), judging the selection of the study groups, the comparability of groups, and ascertainment of exposure of interest, to assign a maximum score of 9 stars.[15]

Table 1. Eligibility Criteria for the Studies Included in the Systematic Review and Meta-Analysis

Eligibility criteria
Case-control studies published as full articles in peer-reviewed journals or conference abstracts
Adults or children with a presumed diagnosis of functional dyspepsia (FD) with or without irritable bowel syndrome based on a questionnaire, or meeting specific diagnostic criteria[a]
Control group, referred to as controls, included healthy asymptomatic controls as well as patient controls, including patients undergoing evaluation for unexplained gastrointestinal syndromes (eg, anemia, dysphagia, Barrett's esophagus, diarrhea, and so forth)
Clinically validated methods to diagnose duodenal eosinophils and mast cells
Participants not specially selected

[a]Rome criteria.[1,51–53]

Data Analysis

Standardized mean difference (SMD) and 95% CIs[16] were calculated to estimate the difference between eosinophils and mast cells in FD patients compared with controls. This was followed by calculating the pooled estimates of prevalence and odds ratios (ORs) and 95% CIs of duodenal eosinophils and mast cell degranulation in FD patients and their respective controls. Details on subgroup and sensitivity analysis are detailed in the Supplementary materials.

Analyses for the association between duodenal eosinophils and mast cells in FD patients and descriptive analyses were performed using the Comprehensive Meta-Analysis Software (Biostat, Inc, Englewood, NJ) (version 3.3.070; NJ), outlined in the Supplementary file.

Results

Selection Outcome

The initial literature search showed 882 publications. Of these, 55 published articles appeared to be relevant for the study question and were retrieved for further evaluation. Of these, 33 were excluded for various reasons, leaving 22 eligible case-control studies[6–12,17–31] (Figure 1 Supplementary Table 1). The characteristics of all the studies in the current meta-analysis including the methodology pertaining to diagnosis of duodenal eosinophils and mast cells, patient characteristics, and geographic region are outlined in Tables 2 and 3 and Supplementary Tables 2 and 3. The summary of findings of the systematic review and meta-analysis has been outlined in Table 4.

Duodenal Eosinophils in Functional Dyspepsia Patients

Overall, the 22 suitable case-control studies (19 in adults and 3 in pediatric populations) included 915 adult and 193 pediatric FD patients and 828 adult and 65 pediatric controls. When all adult and pediatric studies were combined, FD patients had increased eosinophils in the second part of the duodenum as compared with controls (SMD, 1.29; 95% CI, 0.85–1.73; $P = .0001$) (Figure 2). Although there was considerable heterogeneity between the studies ($I^2 = 93.61$; $P = .0001$), and the effect for pediatric studies was not significant, the 95% CIs of the SMD for adult and pediatric studies overlapped. Visual inspection of the funnel plot (Supplementary Figure 2) showed asymmetry, suggesting the possibility of publication bias consistent with results of the Egger test ($P = .0004$).

Duodenal Mast Cells in Functional Dyspepsia Patients

Eight[7–9,11,20,23,24,30] studies included in this systematic review and meta-analysis and 2 additional

Table 2. Characteristics of Studies, Including Diagnostic Criteria and Assessment of Duodenal Eosinophils in FD and Controls

Study no	Study	Country	Study population	Patients with FD, n	Criteria for FD diagnosis	EPS/PDS				Types of controls	Method of eos counting (cells/mm^2 or HPF)	Duodenal (D2) eos count in FD, means (±SD)	Duodenal (D2) eos count in controls, means (±SD)
						EPS, n	PDS, n	overlap, n	Controls, n				
1	Chaudhari et al,[17] 2017	India[a]	Adult	50	Rome III	NA	NA	NA	30	Non-FD disease controls	5 HPF	8.1 (±5.4)	1.9 (±0.4)
2	Du et al,[11] 2016	China[a]	Adult	96	Rome III	NA	NA	NA	24	Healthy controls	5 HPF	11.6 (±5.6)	11.0 (±6.2)
3	Futagami et al,[12] 2010	Japan[b]	Adult	27[c]	Rome III	12[c]	15[c]	NA	20	Healthy controls	mm^2	4.0 (±1.2)	1.3 (±0.7)
4	Genta et al,[10] 2018	United States[b]	Adult	44	Physician based diagnosis	NA	NA	NA	214	Non-FD disease controls	5 HPF	8.5 (±7.2)	8.2 (±6.3)
5	Halland et al,[18] 2019	United States[b]	Adult	17	Rome III and Rome IV	NA	NA	NA	10	Healthy controls	mm^2	6.2 (±1.5)	4.3 (±1.1)
6	Lee et al,[19] 2016	South Korea[b]	Pediatric	43	Rome III	NA	NA	NA	19	Non-FD disease controls	5 HPF	13.4 (±5.3)	9.6 (±5.9)
7	Lee et al,[20] 2019	South Korea[b]	Adult	51	Rome III	15	11	NA	35	Healthy controls	5 HPF	42.1 (±27.7)	26.4 (±23.0)
8	Leite et al,[21] 2020	Brazil[a]	Adult	42	Rome III	26	16	NA	21	Healthy controls	5 HPF	11.1 (±6.1)	14.7 (±11.0)
9	Sakar et al,[22] 2020	Bangladesh[a]	Adult	42	Rome III	NA	NA	NA	42	Non-FD disease controls	5 HPF	5.4 (±2.1)	3.9 (±2.2)
10	Taki et al,[23] 2019	Japan[b]	Adult	35	Rome III	NA	25	NA	31	Healthy controls	3 HPF	21.3 (±18.5)	17.0 (±5.1)
11	Talley et al,[6] 2007	Sweden[b]	Adult	51	Rome II	NA	NA	NA	48	Healthy controls	5 HPF	34.6 (±16.9)	18.6 (±10.5)
12	Vanheel et al,[8] 2014	Belgium[b]	Adult	15[d]	Rome III	NA	NA	NA	15	Healthy controls	7 HPF	28.3 (±3.0)	17.8 (±1.8)
13	Vanheel et al,[24] 2018	Belgium[b]	Adult	24	Rome III	NA	NA	NA	37	Healthy controls	mm^2	57.2 (±3.3)	42.6 (±3.6)
14	Walker et al,[25] 2014	Australia[b]	Adult	33	Rome II	NA	NA	NA	22	Non-FD disease controls	mm^2	12.1 (±2.6)	10.2 (±2.8)
15	Wang et al,[7] 2015	China[a]	Adult	141	Rome III	NA	NA	NA	39	Healthy controls	5 HPF	5.0 (±1.3)	4.2 (±1.3)
16	Wauters et al,[30] 2021	Belgium[b]	Adult	47[e]	Rome IV	3	15	10	30	Healthy controls	mm^2	78.5 (±4.0)	27.2 (±2.1)
17	Bafutto et al,[26] 2012 (A)	Brazil[a]	Adult	36	Rome III	NA	NA	NA	9	Healthy controls	5 HPF	14.2 (±7.4)	8.4 (±3.4)
18	Binesh et al,[9] 2012	Iran[a]	Adult	25	Physician-based diagnosis	NA	NA	NA	27	Non-FD disease controls	5 HPF	14.5 (±5.6)	18.3 (±10.8)
19	Pignataro et al,[27] 2011 (A)	South America[a]	Adult	50	Rome III	NA	NA	NA	50	NA	NA	43.8 (±5.8)	38.5 (±4.9)

Table 2. Continued

Study no	Study	Country	Study population	Patients with FD, n	Criteria for FD diagnosis	EPS, n	PDS, n	EPS/PDS overlap, n	Controls, n	Types of controls	Method of eos counting (cells/mm^2 or HPF)	Duodenal (D2) eos count in FD, means (±SD)	Duodenal (D2) eos count in controls, means (±SD)
20	Ronkainen et al,[28] 2019[f]	Sweden[b]	Adult	89	Rome III	27	71	9	124	Healthy controls	5 HPF	32.0 (±17.0)	22.4 (±13.6)
21	Waulters et al,[29] 2017	Australia[b]	Pediatric	36	Rome III	NA	NA	NA	36	Non-FD disease controls	mm^2	37.6 (±16.3)	19.1 (±8.4)
22	Singh et al,[31] 2018	United States[b]	Pediatric	114	Rome IV	NA	NA	NA	10	Non-FD disease controls	5 HPF	27.0 (±12.8)	17.7 (±6.1)

(A), abstract; D2, second part of the duodenum; eos, eosinophils; EPS, epigastric pain syndrome; FD, functional dyspepsia; HPF, high-power field; NA, not available; PDS, postprandial distress syndrome.
[a]Countries with a gross domestic product (GDP) ≤$30,000 US.
[b]Countries with a GDP >$30,000 US.
[c]Patients with postinfectious FD were excluded.
[d]Vanheel et al[8] included 3 patients with postinfectious FD and no separate data were provided for the duodenal eosinophils in FD patients and in those with postinfectious FD.
[e]Twenty-five of 47 FD patients who were PPI naïve were included in the analysis.
[f]Includes the cohort reported in 2007 (Talley et al).[6] For the analyses, all the eosinophil counts are expressed as eos/HPF. To convert the counts from eos/HPF to eos/mm^2, we used a coefficient of 4.22.

studies[32,33] reporting duodenal mast cells in FD patients and controls were included in this subgroup analysis (Table 3). Overall, in FD patients, duodenal mast cells were increased significantly compared with controls (SMD, 2.11; 95% CI, 1.14–3.07; $P = .0001$) (Supplementary Figure 3), however, considerable heterogeneity was noted between the studies included in the analysis ($I^2 = 96.69$; $P = .0001$). Visual inspection of the forest plot showed asymmetry (Supplementary Figure 4), suggesting the possibility of publication bias consistent with results of the Egger test ($P = .003$).

Influence of Selection Criteria for Controls, and Risk of Bias on the Duodenal Eosinophils and Mast Cells in Functional Dyspepsia Patients and Controls

High-quality studies with low risk of bias. The majority (13 of 22; 59%) of the case-control studies were of high quality, defined as a score of 6 or higher using the NCOS (Supplementary Table 4). Including only high-quality studies, 13 studies yielded an increased duodenal eosinophil count in FD patients compared with controls (SMD, 1.73; 95% CI, 1.06–2.40; $P = .0001$) (Supplementary Figure 5). However, there was still considerable heterogeneity between the studies included in this analysis ($I^2 = 95.12$; $P = .0001$). Moreover, visual inspection of the funnel plot (Supplementary Figure 6) indicated the possibility of publication bias, confirming the results of the Egger test ($P = .002$). Thus, conducting a sensitivity analysis according to the quality of the studies did not reduce the heterogeneity or the risk of publication bias.

Healthy controls. Although many case-control studies used healthy controls, 8 studies included other patient groups (eg, patients referred for investigation of unexplained gastrointestinal symptoms, iron-deficiency anemia, and so forth), referred to as non-FD disease controls (Table 2). Including case-control studies with only healthy asymptomatic controls[6–8,11,12,18,20,21,23,24,26–28,30] (Table 2), the SMD for increased eosinophils in FD patients compared with controls was even higher at 1.88 (95% CI, 1.16–2.60; $P = .0001$) (Supplementary Figure 7) compared with that seen in the primary analysis. Once again, considerable heterogeneity ($I^2 = 95.68$; $P = .0001$) was found among the studies and visual inspection of the funnel plot (Supplementary Figure 8) showed asymmetry, in keeping with the results of the Egger test ($P = .0032$).

Diagnostic Criteria for Functional Dyspepsia Diagnosis and Duodenal Eosinophils and Mast Cells

For the diagnosis of FD, 15, 3, and 2 studies used Rome III, Rome IV, and Rome II criteria, respectively.

Table 3. Characteristics of Studies, Including Diagnostic Criteria and Assessment of Duodenal Mast Cells in FD and Controls

No	Study	Country	Patients with FD, n	Controls, n	Method of mast cell staining	Method of mast cell counting	Duodenal (D2) mast cell count in FD patients, means (±SD)	Duodenal (D2) mast cell count in controls, means (±SD)
1	Du et al,[11] 2016	China	96	24	Tryptase	/5 HPF	13.6 (±2.9)	14.8 (±2.4)
2	Lee et al,[20] 2019	South Korea	51	35	c-Kit (CD117)	/5 HPF	57.7 (±24.5)	48.1 (±22.1)
3	Taki et al,[23] 2019	Japan	35	31	c-Kit (CD117)	/3 HPF	20.0 (±4.0)	17.7 (±4.1)
4	Vanheel et al,[8] 2014	Belgium	15	15	Tryptase	/7 HPF	48.5 (±3.1)	30.5 (±2.3)
5	Vanheel et al,[24] 2018	Belgium	24	37	Tryptase	/mm^2	92.6 (±4.9)	63.7 (±4.9)
6	Walker et al,[32] 2009	Sweden	51	48	c-Kit (CD117)	/5 HPF	160.0 (±78.0)	143.0 (±37.0)
7	Wang et al,[7] 2015	China	141	39	Toluidine blue	/5 HPF	23.6 (±2.6)	20.3 (±3.5)
8	Wauters et al,[30] 2021	Belgium	47	30	c-Kit (CD117)	/mm^2	111.6 (±5.5)	68.3 (±4.5)
9	Binesh et al,[9] 2012	Iran	25	27	Giemsa staining	/5 HPF	9.8 (±6.9)	7.5 (±5.5)
10	Dizdar at al,[33] 2010	Norway	28	19	Naphthol AS-D chloroacetate/ diazonium salt pararosaniline/ Mayer's hematoxylin	/mm^2	3.1 (±0.2)	3.0 (±0.3)

NOTE. For the analyses, all the mast cell counts are expressed as mast cells/HPF; 25 of 47 FD patients, who were PPI naïve, were included in the analysis. To convert the counts from mast cells/HPF to mast cells/mm^2, we used a coefficient of 4.22.
D2, second part of the duodenum; FD, functional dyspepsia; HPF, high-power field.

Two studies used physician-based diagnostic criteria (Table 2). The largest (and statistically significant) difference in duodenal eosinophils between FD patients and controls was observed for studies using Rome IV criteria (SMD, 3.63; 95% CI, 2.30–4.97; P = .0001) (Supplementary Figure 9), followed by those using Rome III criteria (SMD, 1.18; 95% CI, 0.65–1.70; P = .0001) and Rome II criteria (SMD, 0.92; 95% CI, -0.49 to 2.34; P = .201). There was substantial heterogeneity (I^2 = 98.41; P = .0001; and I^2 = 90.86; P = .0001) in the subanalyses including studies using Rome IV and Rome III criteria, respectively, and no heterogeneity (I^2 = 28.19; P = .238) in analysis using Rome II criteria for diagnosing FD. No significant difference was seen in the eosinophils in FD patients and controls using the physician-based diagnosis (SMD, -0.19; 95% CI, -1.60 to 1.22; P = .792), with moderate heterogeneity in the studies included in this analysis (I^2 = 54.48; P = .138).

Degranulation of Duodenal Eosinophils and Mast Cells in Functional Dyspepsia Patients

Five studies[6,7,11,20,24] reported on degranulation of duodenal eosinophils and mast cells in FD patients (Supplementary Table 5), however, data from 1 study[20] could not be extracted. The OR for degranulation of duodenal eosinophils was significantly higher in FD patients compared with controls (OR, 3.78; 95% CI, 6.76–4.48; P = .0001) (Supplementary Figure 10), with no statistically significant heterogeneity between the studies (I^2 = 0; P = .895). Although not statistically significant, degranulation of duodenal mast cells was higher in FD patients compared with controls (OR, 2.09; 95% CI, 0.35–12.69; P = .422) (Supplementary Figure 11), with substantial heterogeneity between the studies (I^2 = 64.59; P = .06).

Functional Dyspepsia Subtypes and Duodenal Eosinophils and Mast Cells

Four studies[12,21,28,30] reported on the duodenal eosinophils in different FD subtypes (Supplementary Table 6), while data from 1 study[28] could not be extracted. There was no significant difference in the number of duodenal eosinophils (SMD, 0.10; 95% CI, -0.56 to 0.76; P = .761) in FD patients with PDS as compared with those with EPS (Supplementary Figure 12). There was moderate heterogeneity in the studies included in the analysis (I^2 = 45.92; P = .157). Similarly, no significant difference was seen in only 1 study[30] that reported on duodenal mast cells in FD subtypes (SMD, 0.116; 95% CI, -1.13 to 1.36; P = .855).

Table 4. Summary of Findings of the Outcomes Reported in This Systematic Review and Meta-Analysis

	Studies, n	FD, n	Controls, n	Prevalence rates of duodenal eosinophils in FD patients compared with controls, SMD (95% CI)	Assessment of heterogeneity between studies
All case-control studies	22	1108	893	1.29 (0.85–1.73), $P = .0001$, **$P = .0004$**	$I^2 = 93.61$; $P = .0001$
Only high-quality, case-control studies	13	440	437	1.73 (1.06–2.40), $P = .0001$ **$P = .002$**	$I^2 = 95.12$; $P = .0001$
Case-control studies including only healthy controls	13	671	433	1.88 (1.16–2.60), $P = .0001$, **$P = .0032$**	$I^2 = 95.68$; $P = .0001$
Case-control studies including only adult FD patients	19	915	828	1.36 (0.87–1.85), $P = .0001$, **$P = .0009$**	$I^2 = 94.40$; $P = .0001$
Case-control studies including only pediatric FD patients	3	193	65	0.98 (0.49–1.46), $P = .001$	$I^2 = 54.23$; $P = .113$
Case-control studies including only studies counting eos/HPF	15	874	688	0.62 (0.31–0.93), $P = .0001$, **$P = .212$**	$I^2 = 84.87$; $P = .0001$
Case control studies including only studies counting eos/mm^2	7	220	191	3.82 (2.01–5.63), $P = .001$	$I^2 = 96.95$; $P = .0001$
Case-control studies including only *H pylori*–negative FD patients and controls	6	278	170	3.98 (2.13–5.84), $P = .0001$	$I^2 = 97.37$; $P = .0001$
Case-control studies including only PI-FD patients	2	PI-FD (n = 45	29	3.91 (1.32–6.51), $P = .001$	$I^2 = 89.84$; $P = .002$
Case-control studies including only IBS patients	2	IBS (n = 81	67	0.024 (-0.75 to 0.79), $P = .951$	$I^2 = 79.71$; $P = .026$
Case-control studies including countries with low GDP	8	482	242	0.48 (-0.23 to 1.19), $P = .188$	$I^2 = 85.15$; $P = .0001$
Case-control studies including countries with high GDP	14	626	651	1.80 (1.23–2.35), $P = .0001$, **$P = .0002$**	$I^2 = 95.22$; $P = .0001$
Case-control studies assessing duodenal mast cells in FD patients	10	513	305	2.11 (1.14–3.07), $P = .0001$, **$P = .0030$**	$I^2 = 96.69$; $P = .0001$
Case-control studies assessing duodenal eosinophils in FD subtypes (PDS compared with EPS)	3	FD (n = 205 EPS (n = 68 PDS (n = 117	195	0.10 (-0.56 to 0.76), $P = .761$	$I^2 = 45.92$; $P = .157$
Case-control studies including only studies using Rome II for FD diagnosis	2	84	70	0.92 (-0.49 to 2.34), $P = .201$	$I^2 = 28.19$; $P = .238$
Case-control studies including only studies using Rome III for FD diagnosis	15	777	532	1.18 (0.65–1.70), $P = .0001$, **$P = .011$**	$I^2 = 90.86$; $P = .0001$
Case-control studies including only studies using Rome IV for FD diagnosis	3	178	50	3.63 (2.30–4.97), $P = .0001$	$I^2 = 98.41$; $P = .0001$
Case-control studies including only studies using physician-based criteria for FD diagnosis	2	69	241	-0.19 (-1.60 to 1.22), $P = .792$	$I^2 = 54.48$; $P = .138$

NOTE. Bolded *P* values indicate results from the Egger test.

CI, confidence interval; eos, eosinophil; EPS, epigastric pain syndrome; FD, functional dyspepsia; GDP, gross domestic product; HPF, high-power field; IBS, irritable bowel syndrome; PDS, postprandial distress syndrome; PI-FD, postinfectious functional dyspepsia; SMD, standardized mean difference.

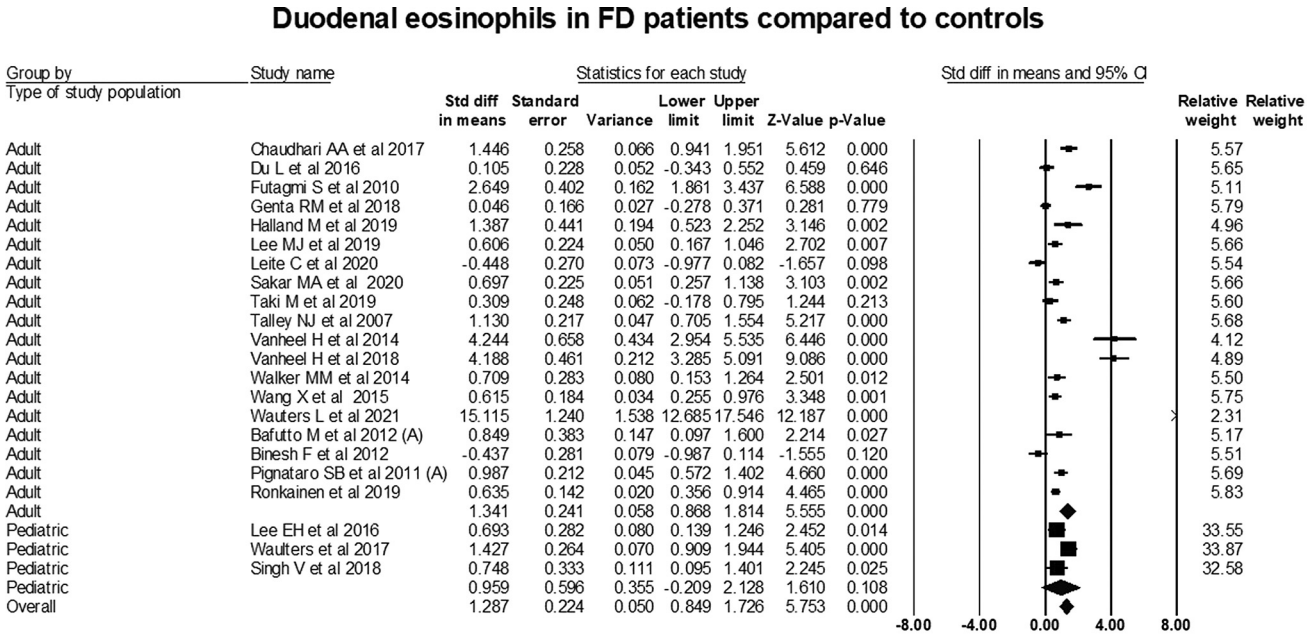

Figure 2. Forest plot of case-control studies showing duodenal eosinophils in functional dyspepsia (FD) patients and controls. FD patients showed increased numbers of duodenal eosinophils (standardized mean difference [SMD], 1.29; 95% CI, 0.85–1.73; $P = .0001$; $I^2 = 93.61$; $P = .0001$). Std diff, standardized difference.

Postinfectious Functional Dyspepsia and Duodenal Eosinophils

Four studies[12,20,26,33] reported on duodenal eosinophils in PI-FD (Supplementary Table 7), however, data from 2 studies[20,33] could not be extracted. Duodenal eosinophils were increased significantly in patients with PI-FD compared with controls (SMD, 3.91; 95% CI, 1.32–6.51; $P = .001$) (Supplementary Figure 13), with considerable heterogeneity in the analysis ($I^2 = 89.84$; $P = .002$). Moreover, patients with PI-FD had increased duodenal eosinophils as compared with those without PI-FD (SMD, 1.42; 95% CI, 0.88–1.96; $P = .001$) (Supplementary Figure 14), with no significant heterogeneity among the studies ($I^2 = 34.95$; $P = .215$).

Comparison of Duodenal Eosinophils and Mast Cells in Irritable Bowel Syndrome, Functional Dyspepsia Patients, and Controls

Four studies[19,20,28,32] reported duodenal eosinophils and mast cells in FD and IBS patients separately (Supplementary Table 8), however, data from 2 studies[20,28] could not be extracted. No difference in duodenal eosinophils in IBS patients as compared with controls (SMD, 0.024; 95% CI, -0.75 to 0.79; $P = .951$) (Supplementary Figure 15), with substantial heterogeneity among the studies ($I^2 = 79.71$; $P = .026$), was found. Duodenal eosinophils were not significantly higher in FD patients compared with those with IBS (SMD, 0.87; 95% CI, -0.34 to 2.07; $P = .159$) (Supplementary Figure 16), with substantial heterogeneity in the analysis ($I^2 = 91.58$; $P = .001$). Only 1 study[32] that reported on duodenal mast cells in IBS patients found duodenal mast cells were increased significantly in IBS patients compared with FD patients and controls (data not shown).

Comparison of Duodenal Eosinophils in Functional Dyspepsia Patients and Controls, Stratified According to Technique of Counting Eosinophils

Fifteen studies[6–12,17–23,26,28] counted eosinophils per high-power field (HPF) and 6 studies[12,18,24,25,29,30] counted eosinophils per square millimeter. Using both counting techniques, duodenal eosinophils were increased in FD patients compared with controls. The duodenal eosinophils in FD patients were higher when counted per mm^2 (SMD, 3.82; 95% CI, 2.01–5.63; $P = .001$) (Supplementary Figure 17*A*) compared with when counted per HPF (SMD, 0.62; 95% CI, 0.31–0.93; $P = .0001$) (Supplementary Figure 17*B*). Moreover, there was substantial heterogeneity between the studies included in both the analyses ($I^2 = 96.95$; $P = .0001$ for counts/mm^2 and $I^2 = 84.87$; $P = .0001$ for counts/HPF).

Association Between H pylori *Status in Functional Dyspepsia Patients and Duodenal Eosinophils*

Overall, 6 studies[8,22,24,29–31] excluding *H pylori*–positive FD patients and controls found significantly

increased duodenal eosinophils in FD patients compared with controls (SMD, 3.98; 95% CI, 2.13–5.84; $P = .0001$) (Supplementary Figure 18), however, there was considerable heterogeneity between the studies ($I^2 = 97.37$; $P = .0001$). Information about the *H pylori* status of FD patients and controls is outlined in Supplementary Tables 2 and 9. In a subgroup analysis including 3 studies,[11,23,27] no significant difference was found in the duodenal eosinophils in *H pylori*–positive FD patients and *H pylori*–positive controls (SMD, 0.44; 95% CI, -0.51 to 1.39; $P = .364$) (Supplementary Figure 19), with considerable heterogeneity between the studies ($I^2 = 75.30$; $P = .017$). Similarly, no significant difference was found in the duodenal eosinophils in *H pylori*–positive FD patients and *H pylori*–negative FD patients (SMD, 0.44; 95% CI, -0.510 to 1.390; $P = .364$), with moderate heterogeneity between the studies ($I^2 = 56.90$; $P = .098$).

Effect of Proton Pump Inhibitors on Duodenal Eosinophils and Mast Cells in Functional Dyspepsia Patients

Overall, 5 studies[8,18,28–30] looked at the association between PPI use in FD patients and duodenal eosinophils, however, 4 studies were excluded from the analysis because complete data could not be extracted (Supplementary Table 2). The study by Wauters et al[30] found that PPI therapy was associated with a significant reduction in duodenal eosinophils in FD patients (eosinophil pretreatment, 331.07 ± 16.93 vs post-treatment, 182.63 ± 22.62; $P < .001$), while an inverse association was seen in the control eosinophils (pre-treatment, 114.6 ± 8.83 vs post-treatment, 229.22 ± 21.0; $P < .001$).

Prevalence of Duodenal Eosinophils in Functional Dyspepsia Patients and Controls in Different Geographic Regions, Stratified According to Gross Domestic Product

To assess the variation in duodenal eosinophilia in FD in different geographic regions, we conducted a subgroup analysis of countries stratified according to GDP per capita (high [>30,000 USD per capita] and low [≤30,000 USD per capita]) (Table 2). In case-control studies, the difference in duodenal eosinophils in FD patients compared with controls was significantly higher in countries with a high GDP (SMD, 1.80; 95% CI, 1.23–2.35; $P = .0001$) (Supplementary Figure 20), whereas in countries with low GDP the difference between FD patients and controls failed statistical significance (SMD, 0.48; 95% CI, -0.23 to 1.19; $P = .188$) (Supplementary Figure 20) and the 95% CIs of the SMD did not overlap, suggesting a statistically significant difference between studies from high and low GDP countries. Overall substantial heterogeneity was noted for the analyses for countries with high and low GDP ($I^2 = 95.22$; $P = .0001$ and $I^2 = 85.15$; $P = .0001$, respectively). Visual inspection of the funnel plot showed asymmetry, in keeping with the results of the Egger test ($P = .0002$).

Discussion

Duodenal microinflammation is a potential mechanism in the pathophysiology of FD.[34] This systematic review and meta-analysis identified 22 published peer-reviewed, case-control studies from 12 different countries with 1108 FD patients and 893 controls. This was a large pooled analysis of case-control studies exploring the link between duodenal eosinophils and mast cells in FD patients, FD subtypes, PI-FD, and controls. Overall, the data suggest a significant increase in the duodenal eosinophils (SMD, 1.29; 95% CI, 0.85–1.73) and mast cells (SMD, 2.11; 95% CI, 1.14–3.07) in FD patients compared with controls. Furthermore, FD patients had significantly higher degranulation of duodenal eosinophils, but not of mast cells, when compared with controls. The largest (significant) differences in duodenal eosinophils were found in *H pylori*–negative FD patients and controls (SMD, 3.98; 95% CI, 2.13–5.84) and for the comparison of PI-FD with controls (SMD, 3.91; 95% CI, 1.32–6.51). In contrast, the data show no significant difference in duodenal eosinophil counts between FD subtypes. There were considerable variations in duodenal eosinophil counts in FD patients and controls across different geographic regions, and differences between FD patients and controls were larger in countries with a high GDP per capita.

Our analysis also suggests that the diagnostic criteria for FD may influence the magnitude of the difference of duodenal eosinophils between FD patients and controls. The largest (and statically significant) effects are seen for Rome IV followed by Rome III and Rome II, while no significant effect was seen for physician-based diagnosis. Subgroup analysis for various diagnostic criteria (physician-based vs various versions of the Rome criteria) showed that the SMD increases with the more recent versions of the Rome criteria with CIs not overlapping when the SMD for Rome IV is compared with Rome II or Rome III. This effect is consistent across all studies (Supplementary Figure 9). Symptoms are required to be more frequent or severe to meet Rome IV criteria. Thus, the data suggest that increased eosinophils might be associated with more severe disease manifestations. This highlights the need for strict criteria for FD diagnosis and suggests that Rome IV included patients with more frequent (and potentially more severe) symptoms compared with Rome II and Rome III.

There is substantial heterogeneity and a high risk of publication bias across studies included in the primary analysis and a majority of the subgroup analyses in this systematic review and meta-analysis. Although well-defined and universally accepted thresholds for

diagnosing duodenal eosinophilia and/or mast cells are lacking, we compared mean eosinophil numbers per HPF of FD patients and controls. Thus, the heterogeneity might be explained by lack of uniform selection criteria for cases and controls, the use of non-FD dyspepsia patients as controls in 9 of 22 studies, and a lack of uniform counting techniques to measure eosinophils and mast cells. It also needs to be noted that a small number of studies have shown strong effects in relation to the difference between patients and controls, thus substantially contributing to the overall differences in the mean eosinophil counts between FD patients and controls. We believe the wide range in duodenal eosinophil prevalence in FD patients and controls is at least partly explained by these factors. To address this, we conducted a sensitivity analysis: by separately examining case-control studies with healthy controls, as well as restricting the analysis only to those studies with high-quality NCOS assessment scores (ie, with a relatively low risk of bias). However, conducting a sensitivity analysis did not reduce the heterogeneity or risk of bias. Thus, the high heterogeneity scores and high risk of bias could be explained at least partially by the inherent limitations of the studies included in this systematic review and meta-analysis.

One of the key findings of this systematic review and meta-analysis was the association of degranulation of eosinophils or mast cells and FD. We found a significant 3-fold higher degranulation of eosinophils and a nonsignificant but still 2-fold higher degranulation of mast cells in FD patients compared with controls. Although there was no heterogeneity regarding eosinophil degranulation, there was substantial heterogeneity for mast cell degranulation, suggesting that there are a multitude of thus far unaccounted factors (including diet, infections, allergens) that may influence the degranulation of these inflammatory cells.[35] This is an important finding because the physiologic effects of eosinophils and mast cells are dependent not only on the cell density, but also modified by the extent of cell degranulation reflecting release of cytokines or other substances stored in granules.[36] Similarly increased numbers and proportions of activated mast cells have been observed in the small intestine of patients with diarrhea-dominant IBS. Eosinophils interact directly and indirectly with the enteric nervous system through crosstalk with mast cells, which is called the *eosinophil–mast cell axis*. Initially, mast cells induce eosinophils to migrate into the mucosa, and these in turn may activate mast cells via specific mediators and growth factors, causing proliferation, maturation, and degranulation.[37] Mediators released from activated mast cells and eosinophils lead to neural stimulation and smooth muscle contraction, which ultimately may lead to the generation of abdominal pain and bloating. Indeed, duodenal neuronal structural and functional abnormalities that correlate with the inflammatory infiltrate have been reported in FD.[38] Collectively, eosinophils and mast cells and activation or degranulation of these cells resulting in local release of cytokines and neurotransmitters are potentially, if not likely, involved in the development of visceral hypersensitivity, and their interaction may be a leading cause of symptoms of functional gastrointestinal diseases.[39] This could have therapeutic implications.

As part of the data analysis, patients with different FD types (EPS vs PDS), were compared because a link was reported previously between duodenal eosinophils and cardinal PDS symptoms, early satiety and postprandial fullness,[6,28] but not with EPS. However, the subgroup analysis did not find any significant link between duodenal eosinophils or mast cells and FD subtypes. This may question the current pathophysiologic concept of PDS.[6,28] A subset of FD patients develop dyspepsia symptoms after an acute gastroenteritis.[40] There has been an increasing focus to characterize the small intestinal immune cells in patients with PI-FD. PI-FD has been associated with increased numbers of eosinophil and macrophages in the duodenal mucosa compared with non PI-FD.[12] The subgroup analysis comparing PI-FD vs non–PI-FD showed (across the different studies) very consistent and statistically significantly higher SMD in duodenal eosinophils in PI-FD, pointing toward a role of gastrointestinal infections for the increase of duodenal eosinophils in FD patients. Our meta-analysis also found that patients with PI-FD had significantly increased duodenal eosinophils compared with healthy controls and non–PI-FD patients. This is an important finding and points toward the possibility that duodenal low-grade inflammation is due to PI-FD. However, unlike the well-established association between gastrointestinal infections and IBS,[41] these findings must be interpreted with caution because only limited studies with very small sample sizes (including 45 patients with PI-FD and 29 controls) were available for this subgroup analysis.

Only 2 studies included in this systematic review and meta-analysis looked at duodenal eosinophils and mast cells in both FD and IBS patients separately. Our results show duodenal mast cells, but not eosinophil counts, were statistically higher in FD patients compared with IBS patients. Interestingly, Cremon et al[42] showed colonic mucosal mast cell infiltration in IBS patients was associated with abdominal bloating and dysmotility-like dyspepsia.

Comparing *H pylori*–positive and *H pylori*–negative subjects, significantly higher duodenal eosinophil counts were observed in *H pylori*–negative FD patients compared with controls, while no significant difference was observed in *H pylori*–positive FD patients compared with *H pylori*–positive controls, and the CIs of the SMD for both analyses did not overlap. Other studies already have shown that a *H pylori* infection is associated with increased eosinophils in the gastric mucosa in FD patients and controls.[25,43] Taken together, this suggests that *H pylori* infection may up-regulate gastric and duodenal eosinophil counts in FD patients and controls and therefore no significant difference in duodenal

eosinophil counts was found in *H pylori*–positive FD patients when compared with *H pylori*–positive controls.

Eosinophils are reacting to the status quo: food/plant antigens/microbiome, which is a dynamic process. Therefore, there are multiple variables accounting for clinical heterogenicity as well as differing clinical criteria for clinical diagnosis. We also observed that duodenal eosinophils in FD patients (compared with controls) were higher when eosinophils were counted per square millimeter compared with when counted per HPF. This might be caused by variability in the size of the microscopic field, selection of fields (eosinophils may be numerous near lymphoid follicles), and criteria for including cells in the count. Indeed, some studies included all eosinophils seen while others only included those with a fully visible nucleus.[44]

Seasonal variations and their effect on duodenal eosinophils have been explored, but the results have been inconsistent. Järbrink-Sehgal et al[45] observed that samples collected in the fall had significantly lower mean duodenal eosinophils than those collected in the spring, summer, and winter. However, Walker et al[46] found no link between duodenal eosinophilia and biopsy specimens taken in the autumn/winter seasons and the spring/summer seasons. Genetic susceptibility to atopy also has a bearing, FD and atopy are linked. None of the studies included in this systematic review and meta-analysis looked at the link between seasonal variation and duodenal eosinophilia.[47]

PPI often is considered a first-line treatment before diet and topical steroids in the treatment of eosinophilic esophagitis, previously (and erroneously) referred to as PPI-responsive esophageal eosinophilia. The benefit of PPI therapy in PPI-responsive esophageal eosinophilia may be secondary to anti-inflammatory effects rather than antisecretory properties.[48] Recently, Wauters et al[30] showed that treatment with PPIs reduced not only symptoms and duodenal eosinophilia, but also the higher mast cell infiltration and mucosal permeability in FD patients, although in healthy volunteers PPI use was associated with increased duodenal eosinophils.

Lower GDP was associated with a smaller difference of duodenal eosinophils between FD and controls, while the reverse was true for countries with a high GDP. It can be speculated that this is related to seasonal or environmental factors including the influence of diet and the background prevalence of allergic and atopic conditions or parasitic infestations. Against this background there are suggestions[10] that eosinophilic duodenitis should be reported as a histopathologic finding rather than a self-standing disease. The priority remains to determine the range of normal eosinophils in the United States and other populations while accounting for a variety of confounders. A recent US study reported normal duodenal eosinophil and mast cell values and a high discovery rate of eosinophilic duodenitis in patients with functional gastrointestinal symptoms.[49]

A previous meta-analysis included case-control as well as prevalence studies[50] and reported higher gastroduodenal eosinophil and mast cells in FD patients compared with controls. Significant heterogeneity was found among the studies included in the primary and subgroup analyses. In the current meta-analysis, we excluded prevalence studies because they can have limited internal validity resulting from both bias and confounding. Moreover, the available literature points toward significant variations in the prevalence of duodenal eosinophils in FD patients linked to geographic, environmental, and methodologic factors. We only included case-control studies to minimize the effect of these confounders and critically compare the eosinophil and mast cell counts in FD patients with local controls. While updating the previous findings,[50] we systematically explored the reasons for the high heterogeneity and high risk of bias also seen previously. We analyzed other important factors such as the links between eosinophils and mast cells in PI-FD, and the degranulation of eosinophils and mast cells in FD patients compared with controls. We also assessed the effects of cell counting techniques, and the influence of medications such as PPIs and environmental factors including *H pylori* status, seasonal variation, or GDP per capita. Nevertheless, we need to acknowledge some limitations. There is a lack of valid and universally accepted thresholds for diagnosing duodenal eosinophilia, and a lack of standardized methods of counting and reporting mast cells and eosinophils. In addition, the various studies included healthy asymptomatic subjects as well as patients with a variety of diseases as controls. It also is worth noting the small sample sizes of some case-control studies, with fewer than 50 participants per arm, and some subgroup analyses, which are based on a small number of studies.

In conclusion, this systematic review and meta-analysis does suggest that duodenal eosinophil and mast cell counts are increased in FD, and the proportion of degranulation of eosinophils also is higher in FD compared with controls. In particular, an increase of duodenal eosinophils is found in patients with PI-FD compared with other FD patients, emphasizing the role of low-grade duodenal inflammation in PI-FD. Our meta-analyses found no association between duodenal immune cells and specific FD subtypes. Duodenal mast cells were increased in IBS patients compared with those with FD and controls. Seasonal and geographic variations and environmental factors are likely to influence the intramucosal immune cells in the gastrointestinal tract. However, most of the comparative analysis showed substantial heterogeneity and risk of bias and there was substantial clinical heterogeneity, most likely owing to the lack of uniform selection criteria for cases and controls, lack of an established threshold above which duodenal eosinophilia can be diagnosed, lack of standardization of cell counting methods, and considerable variation of duodenal eosinophil and mast cell counts in FD patients and controls. Thus, the overall quality of

evidence was low, and the results need to be interpreted with caution.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2022.01.014

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Nicholas J Talley, MD, PhD, FRACP, FAHMS (Conceptualization: Supporting; Writing – original draft: Supporting; Writing – review & editing: Supporting)

Gerald Johannes Holtmann, MD, PhD, MBA (Conceptualization: Equal; Data curation: Supporting; Formal analysis: Supporting; Methodology: Supporting; Software: Supporting; Supervision: Supporting; Validation: Equal; Writing – original draft: Supporting; Writing – review & editing: Supporting)

Conflicts of interest
These authors disclose the following: Gerald Holtmann has served on the advisory boards of Australian Biotherapeutics, Glutagen, and Bayer; received research support from Bayer, Abbott, Pfizer, Janssen, Takeda, and Allergan; serves on the Boards of the West Moreton Hospital and Health Service, Queensland, UQ Healthcare, Brisbane, and the Gastro-Liga, Germany, and is Chair of the West Moreton Hospital and Health Service Board Quality and Safety Committee; holds a patent for the Brisbane aseptic biopsy device; serves as Editor of the Gastro-Liga Newsletter; and serves on the Research Committee of the Royal Australasian College of Physicians; Nicholas J. Talley has received personal fees from Allakos, Aviro Health, Antara Life Sciences, Arlyx from Bayer, Danone, Planet Innovation, Takeda, Viscera Labs, twoXAR, Viscera Labs, Dr Falk Pharma, Censa, Cadila Pharmaceuticals, Progenity, Inc,

Sanofi-aventis, Glutagen, ARENA Pharmaceuticals, IsoThrive, BluMaiden, HVN National Science Challenge; has received nonfinancial support from HVN National Science Challenge NZ outside the submitted work; holds the following patents: Biomarkers of IBS licensed (#12735358.9-1405/2710383 and #12735358.9-1405/2710384), Licensing Questionnaires Talley Bowel Disease Questionnaire licensed to Mayo/Talley, Nestec European Patent licensed, and Singapore Provisional Patent Nanyang Technological University Ref: TD/129/17 "Microbiota Modulation Of BDNF Tissue Repair Pathway" issued and copyright Nepean Dyspepsia Index 1998; is the Editor-in-Chief of *Medical Journal of Australia*, a Section Editor for *Up to Date*, *Precision and Future Medicine*, is a member of Australian Medical Council (2016-2019), MBS Review Taskforce (2016-2020), National Health and Medical Research Council Principal Committee, Research Committee (2016-2021), Asia Pacific Association of Medical Journal Editors (current), and GESA Board Member (2017-2019); judged research grants for Avant Foundation; and is a member of the advisory board of the International Foundation for Functional GI Disorders. Nikhil Thapar has received consultancy and speaker fees from Takeda and Danone/Nutricia; is a council member of the European Society of Paediatric Gastroenterology, is a committee member of Hepatology and Nutrition (2015-2019), Asia Pan-Pacific Society of Paediatric Gastroenterology, and Hepatology and Nutrition Gastroenterology Committee; and is the associate editor for the *Journal of Paediatric Gastroenterology* and *Nutrition*. The remaining authors disclose no conflicts.

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Supplementary Materials and Methods

Search Strategy

The initial search was not limited to specific languages so that we could capture all appropriate studies. A further advanced search was conducted. Grey literature was searched with Google and Google Scholar, and the Snowball method also was used to identify all relevant articles. The literature search was conducted with the help of our librarian.

Selection of Studies

Eligibility criteria for included studies were as follows: case-control studies, recruiting unselected subjects meeting diagnostic criteria for FD, reported the prevalence of eosinophils and mast cells in the second part of the duodenum using clinically validated methods in FD patients with or without concomitant IBS, and compared the prevalence of duodenal eosinophils and mast cells in FD vs controls. The diagnosis of FD was based on the clinical assessment, questionnaire data, or specific symptom-based criteria, including the Rome criteria. Studies not reporting original data, prevalence studies, those reporting on mixed populations of FGIDs with no separate data on FD, or those that did not use clinically validated methods to measure duodenal eosinophils and mast cells in FD were excluded. Conference abstracts that provided available data also were included in the study. Individuals in the control group included healthy asymptomatic controls as well as patient controls, including patients undergoing evaluation for unexplained gastrointestinal syndromes (eg, iron-deficiency anemia, dysphagia, Barrett's esophagus, and so forth).

Data Extraction and Quality Assessment

During the data collection process, the following data were extracted from the studies: the author, the year of the study, study design, country, source of controls, mean age, sex, technique for staining and counting duodenal eosinophils and mast cells, frequency of degranulation of duodenal eosinophils and mast cells, site of biopsies, predefined cut-off criteria for diagnosing duodenal eosinophilia, prevalence and mode of diagnosis of *H pylori* gastritis, concurrent use of PPI, and exclusion criteria for the patient and the control groups. In addition, for all FD patients, data regarding the mode of diagnosis of FD and the presence of concomitant IBS, FD subtypes, and postinfectious FD overlap with the other FGIDs was recorded. If cell counts were reported using multiple ways such as per square millimeter and per HPF, the count per HPF was recorded. If cell counts were reported as the mean cell count and peak cell count, only the mean cell count was recorded.

Data Analysis

Data were recorded as means and SD. The median value and range were transformed to means and SD.[1] The interquartile range or the 5th and 95th percentile ranges were converted to SD through the following formula: SD $= 0.7413 \times$ (values at 75th percentile $-$ values at 25th percentile) or SD $=$ (values at 95th percentile $-$ values at 5th percentile)/(2×1.645).[2] In an initial step, case numbers of FD patients and controls (using various methods of counting eosinophils) in the respective cohorts were determined. All cell counts were converted to counts per 1 HPF to enable direct comparison. To convert the counts from eos/mm^2 to eos/HPF we used a coefficient of 4.22.[3,4] A threshold of $30,000 purchase power adjusted GDP per capita was used to categorize studies that were conducted in high- or low-GDP countries.[5] Subgroup analysis stratified by method of counting eosinophils and mast cells, adult or pediatric studies, criteria for FD diagnosis, GDP, FD subtypes, postinfectious FD, presence of IBS, effect of PPI, and effect of *H pylori* were conducted. Finally, we performed a sensitivity analysis including only high-quality studies, reporting the prevalence of duodenal eosinophils in FD patients with their respective controls.

Analyses for the association between duodenal eosinophils and mast cells in FD patients and descriptive analyses were performed using the Comprehensive Meta-Analysis Software (version 3.3.070; NJ). A random-effects model[6] was chosen to appropriately account for variability in the summary estimate. The statistical package Comprehensive Meta-Analysis Software used logit transformation of proportions and the variance of the logit to estimate pooled event rates within groups and to compare event rates between groups. Between-study variation was evaluated using the Cochrane test[7] and was quantified through the I^2 index, in which values close to 100 indicate substantial variation between studies while values close to zero indicate minimal between-study variation. Standard approaches (Egger test and inspection of funnel plots) were applied to identify potential publication biases. Furthermore, either the chi-squared test $P < .10$ or $I^2 > 50\%$ indicated substantial heterogeneity.

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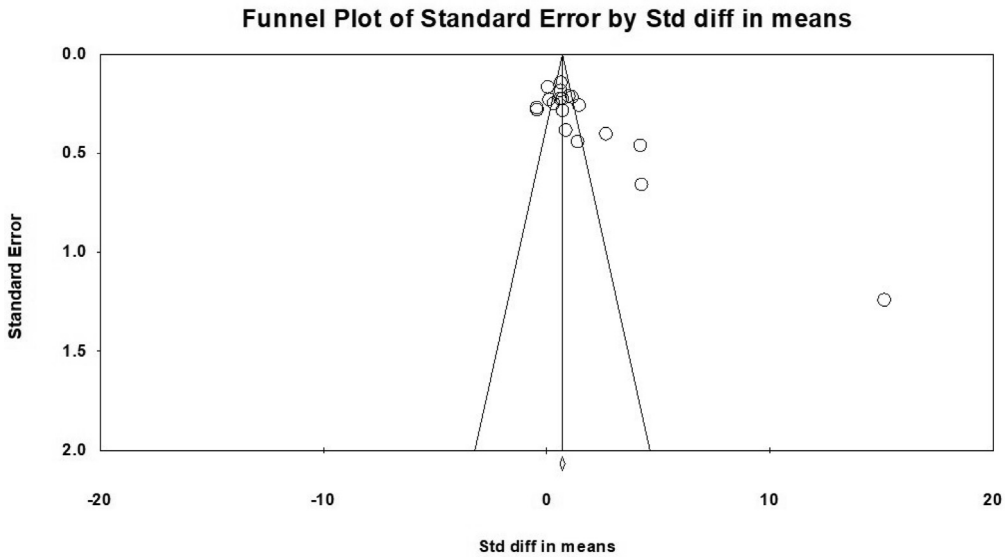
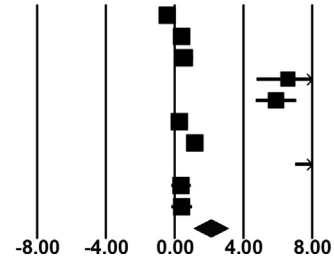
Database search strategy MEDLINE(PubMed)
1. dyspepsia* [ti] OR "FD" [ti] OR functional gastrointestinal disorder*[ti] OR "FGID" [ti] OR "pseudo-ulcer syndrome"[ti] OR duodenal disorder* [ti] OR intestinal functional disease* [ti]
2. functional dyspepsia* [tiab] OR "FD" [tiab] OR functional gastrointestinal disorder* [tiab] OR "FGID" [tiab] OR "pseudo-ulcer syndrome" [tiab] OR duodenal disorder* [tiab] OR intestinal functional disease* [tiab] OR Dyspepsia [MeSH Terms]
3. dyspepsia [MeSH Major Topic]
4. eosinophil* [ti] OR "EGIDs" [ti] OR mast cells* [ti] OR mastocytosis* [ti]
5. eosinophil* [tiab] OR "EGIDs" [tiab] OR mast cells* [tiab] OR mastocytosis [tiab] OR eosinophils [MeSH Terms] OR mast cells [MeSH Terms]
6. eosinophils [MeSH Major Topic] OR mast cells [MeSH Major Topic]
7. gastroesophageal reflux* [ti] OR esophagitis* [ti] OR oesophagus* [ti] OR irritable bowel syndrome* [ti] OR "GORD" [ti] OR “IBS” [ti] OR “fabry disease” [ti]
8. #1 AND #4
9. #2 AND #5
10. #9 NOT #7
11. #3 AND #6
12. #8 OR #10 OR #11
Limit: 01/2001-02/2021
Filters applied: MEDLINE
PubMed: 141
Article title ti, abstract ab, topic MeSH Terms, main topic MeSH Major Topic

Supplementary Figure 1. Search strategy for MEDLINE. MeSH, Medical Subject Headings.

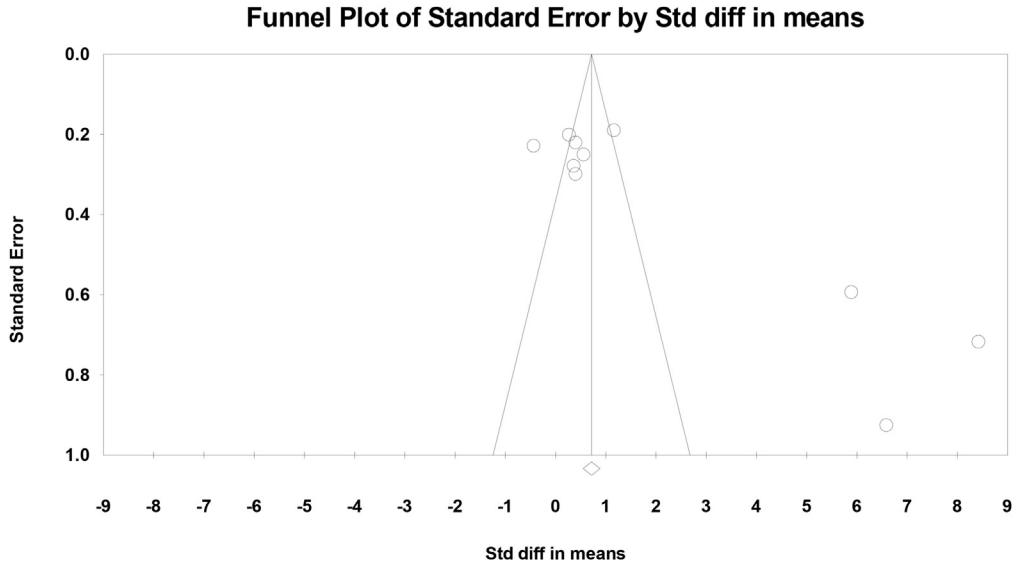


Supplementary Figure 2. Funnel plot of duodenal eosinophils in functional dyspepsia patients and controls. Std diff, standard difference.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Du L et al 2016	-0.427	0.230	0.053	-0.878	0.023	-1.858	0.063
Lee MJ et al 2019	0.408	0.222	0.049	-0.027	0.842	1.838	0.066
Taki M et al 2019	0.568	0.252	0.063	0.075	1.061	2.259	0.024
Vanheel H et al 2014	6.595	0.926	0.858	4.779	8.410	7.119	0.000
Vanheel H et al 2018	5.898	0.595	0.354	4.732	7.064	9.915	0.000
Walker MM et al 2009	0.276	0.202	0.041	-0.120	0.672	1.365	0.172
Wang X et al 2015	1.172	0.191	0.037	0.797	1.546	6.129	0.000
Wauters L et al 2021	8.430	0.718	0.516	7.022	9.838	11.735	0.000
Binesh F et al 2012	0.370	0.280	0.078	-0.178	0.919	1.323	0.186
Dizdar et al 2010	0.408	0.300	0.090	-0.180	0.997	1.360	0.174
	2.106	0.493	0.243	1.140	3.072	4.273	0.000

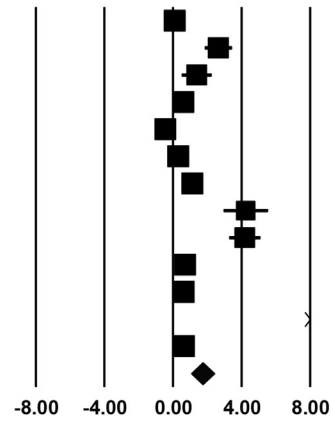


Supplementary Figure 3. Forest plot of studies showing duodenal mast cells in functional dyspepsia (FD) patients and controls (standardized mean difference [SMD], 2.11; 95% CI, 1.14–3.07; $P = .0001$) ($I^2 = 96.69$; $P = .0001$). Std diff, standard difference.

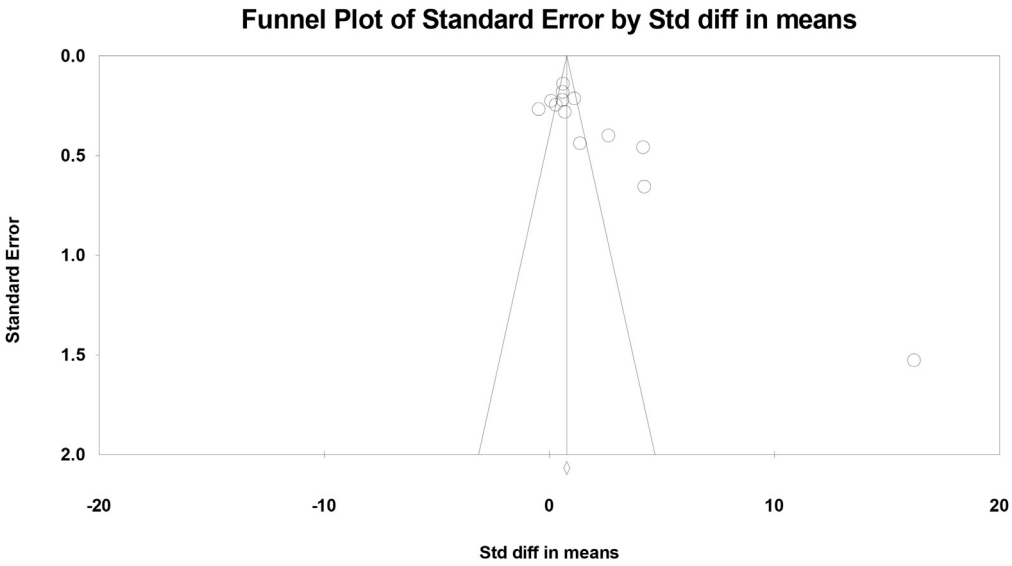


Supplementary Figure 4. Funnel plot of studies showing duodenal mast cells in functional dyspepsia (FD) patients and controls. Std diff, standard difference.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Du L et al 2016	0.105	0.228	0.052	-0.343	0.552	0.459	0.646
Futagmi S et al 2010	2.649	0.402	0.162	1.861	3.437	6.588	0.000
Halland M et al 2019	1.387	0.441	0.194	0.523	2.252	3.146	0.002
Lee MJ et al 2019	0.606	0.224	0.050	0.167	1.046	2.702	0.007
Leite C et al 2020	-0.448	0.270	0.073	-0.977	0.082	-1.657	0.098
Taki M et al 2019	0.309	0.248	0.062	-0.178	0.795	1.244	0.213
Talley NJ et al 2007	1.130	0.217	0.047	0.705	1.554	5.217	0.000
Vanheel H et al 2014	4.244	0.658	0.434	2.954	5.535	6.446	0.000
Vanheel H et al 2018	4.188	0.461	0.212	3.285	5.091	9.086	0.000
Walker MM et al 2014	0.709	0.283	0.080	0.153	1.264	2.501	0.012
Wang X et al 2015	0.615	0.184	0.034	0.255	0.976	3.348	0.001
Wauters L et al 2021	16.224	1.529	2.338	13.227	19.221	10.610	0.000
Ronkainen et al 2019	0.635	0.142	0.020	0.356	0.914	4.465	0.000
	1.734	0.342	0.117	1.063	2.405	5.066	0.000

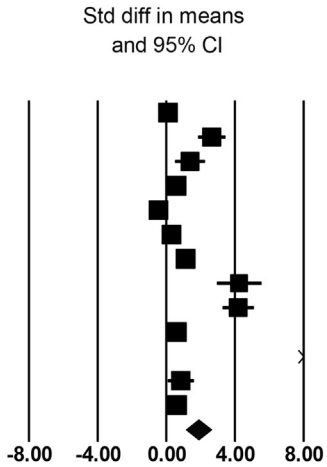


Supplementary Figure 5. Forest plot of studies showing duodenal eosinophils in functional dyspepsia (FD) patients and controls, including only high-quality studies (standardized mean difference [SMD], 1.73; 95% CI, 1.06–2.40; $P = .0001$) ($I^2 = 95.12$; $P = .0001$). Std diff, standard difference.

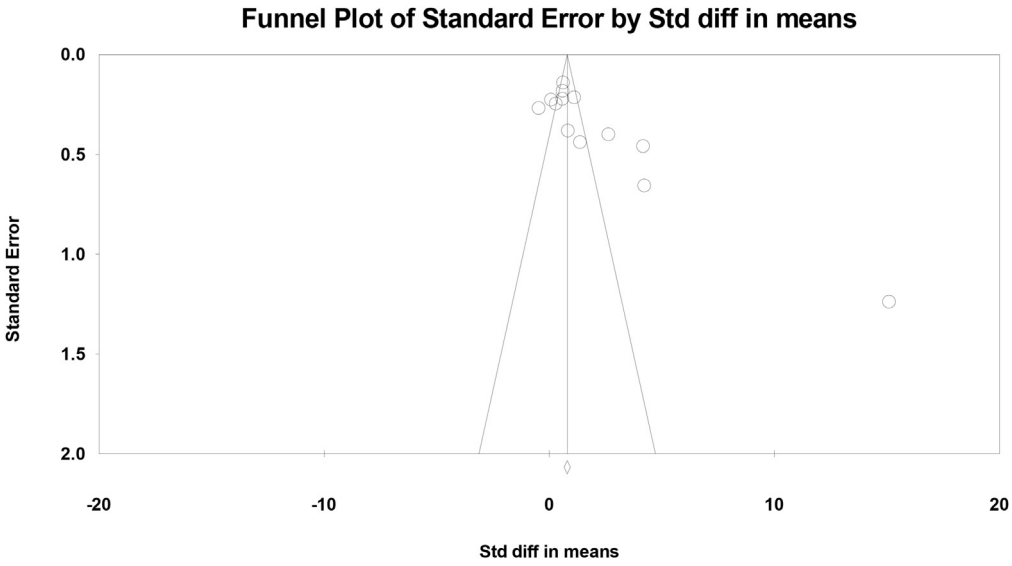


Supplementary Figure 6. Funnel plot of studies showing duodenal eosinophils in functional dyspepsia patients and controls, including only high-quality studies. Std diff, standard difference.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
			Statistics for each study				
Du L et al 2016	0.105	0.228	0.052	-0.343	0.552	0.459	0.646
Futagmi S et al 2010	2.649	0.402	0.162	1.861	3.437	6.588	0.000
Halland M et al 2019	1.387	0.441	0.194	0.523	2.252	3.146	0.002
Lee MJ et al 2019	0.606	0.224	0.050	0.167	1.046	2.702	0.007
Leite C et al 2020	-0.448	0.270	0.073	-0.977	0.082	-1.657	0.098
Taki M et al 2019	0.309	0.248	0.062	-0.178	0.795	1.244	0.213
Talley NJ et al 2007	1.130	0.217	0.047	0.705	1.554	5.217	0.000
Vanheel H et al 2014	4.244	0.658	0.434	2.954	5.535	6.446	0.000
Vanheel H et al 2018	4.188	0.461	0.212	3.285	5.091	9.086	0.000
Wang X et al 2015	0.615	0.184	0.034	0.255	0.976	3.348	0.001
Wauters L et al 2021	15.115	1.240	1.538	12.685	17.546	12.187	0.000
Bafutto M et al 2012 (A)	0.849	0.383	0.147	0.097	1.600	2.214	0.027
Ronkainen et al 2019	0.635	0.142	0.020	0.356	0.914	4.465	0.000
	1.881	0.367	0.135	1.161	2.600	5.125	0.000

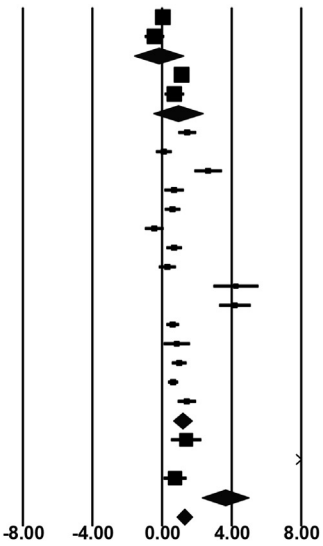


Supplementary Figure 7. Forest plot of studies showing duodenal eosinophils in functional dyspepsia (FD) patients and healthy controls (standardized mean difference [SMD], 1.88; 95% CI, 1.16–2.60; $P = .0001$) ($I^2 = 95.68$; $P = .0001$). Std diff, standard difference.



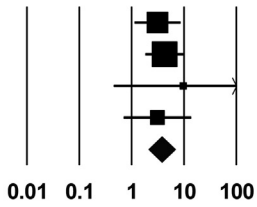
Supplementary Figure 8. Funnel plot of studies showing duodenal eosinophils in functional dyspepsia patients and healthy controls. Std diff, standard difference.

Group by FD diagnosis	Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
PBD	Genta RM et al 2018	0.046	0.166	0.027	-0.278	0.371	0.281	0.779
PBD	Binesh F et al 2012	-0.437	0.281	0.079	-0.987	0.114	-1.555	0.120
PBD		-0.189	0.718	0.516	-1.597	1.219	-0.263	0.792
Rome II	Talley NJ et al 2007	1.130	0.217	0.047	0.705	1.554	5.217	0.000
Rome II	Walker MM et al 2014	0.709	0.283	0.080	0.153	1.264	2.501	0.012
Rome II		0.922	0.722	0.522	-0.493	2.338	1.277	0.201
Rome III	Chaudhari AA et al 2017	1.446	0.258	0.066	0.941	1.951	5.612	0.000
Rome III	Du L et al 2016	0.105	0.228	0.052	-0.343	0.552	0.459	0.646
Rome III	Futagmi S et al 2010	2.649	0.402	0.162	1.861	3.437	6.588	0.000
Rome III	Lee EH et al 2016	0.693	0.282	0.080	0.139	1.246	2.452	0.014
Rome III	Lee MJ et al 2019	0.606	0.224	0.050	0.167	1.046	2.702	0.007
Rome III	Leite C et al 2020	-0.448	0.270	0.073	-0.977	0.082	-1.657	0.098
Rome III	Sakar MA et al 2020	0.697	0.225	0.051	0.257	1.138	3.103	0.002
Rome III	Taki M et al 2019	0.309	0.248	0.062	-0.178	0.795	1.244	0.213
Rome III	Vanheel H et al 2014	4.244	0.658	0.434	2.954	5.535	6.446	0.000
Rome III	Vanheel H et al 2018	4.188	0.461	0.212	3.285	5.091	9.086	0.000
Rome III	Wang X et al 2015	0.615	0.184	0.034	0.255	0.976	3.348	0.001
Rome III	Bafutto M et al 2012 (A)	0.849	0.383	0.147	0.097	1.600	2.214	0.027
Rome III	Pignataro SB et al 2011 (A)	0.987	0.212	0.045	0.572	1.402	4.660	0.000
Rome III	Ronkainen et al 2019	0.635	0.142	0.020	0.356	0.914	4.465	0.000
Rome III	Waulters et al 2017	1.427	0.264	0.070	0.909	1.944	5.405	0.000
Rome III		1.178	0.268	0.072	0.653	1.703	4.400	0.000
Rome IV	Halland M et al 2019	1.387	0.441	0.194	0.523	2.252	3.146	0.002
Rome IV	Wauters L et al 2021	15.115	1.240	1.538	12.685	17.546	12.187	0.000
Rome IV	Singh V et al 2018	0.748	0.333	0.111	0.095	1.401	2.245	0.025
Rome IV		3.634	0.680	0.462	2.303	4.966	5.348	0.000
Overall		1.287	0.224	0.050	0.849	1.726	5.752	0.000



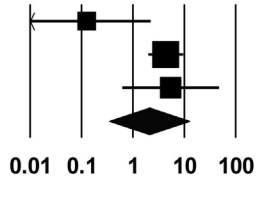
Supplementary Figure 9. Forest plot of studies showing duodenal eosinophils in functional dyspepsia (FD) patients and controls, according to criteria for diagnosing FD. Std diff, standard difference.

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Du L et al 2016	3.128	1.143	8.560	2.220	0.026
Wang X et al 2015	4.280	1.839	9.959	3.374	0.001
Talley NJ et al 2007	9.706	0.456	206.391	1.457	0.145
Vanheel H et al 2018	3.111	0.707	13.689	1.501	0.133
	3.779	2.111	6.764	4.476	0.000



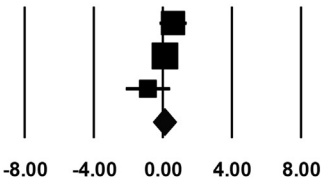
Supplementary Figure 10. Forest plot of studies showing degranulation of duodenal eosinophils in functional dyspepsia (FD) patients compared with controls (odds ratio [OR], 3.78; 95% CI, 6.76–4.48; $P = .0001$) ($I^2 = 0$; $P = .895$).

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Du L et al 2016	0.126	0.007	2.201	-1.419	0.156
Wang X et al 2015	4.357	2.003	9.474	3.713	0.000
Vanheel H et al 2018	5.429	0.627	47.022	1.536	0.125
	2.092	0.345	12.686	0.803	0.422



Supplementary Figure 11. Forest plot of studies showing degranulation of duodenal mast cells in functional dyspepsia (FD) patients compared with controls (odds ratio [OR], 2.09; 95% CI, 0.35–12.69; $P = .422$) ($I^2 = 64.59$; $P = .06$).

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Futagmi S et al 2010	0.595	0.396	0.157	-0.181	1.370	1.503	0.133
Leite C et al 2020	0.127	0.318	0.101	-0.497	0.750	0.398	0.690
Wauters L et al 2021	-0.862	0.649	0.421	-2.133	0.410	-1.328	0.184
	0.102	0.336	0.113	-0.557	0.762	0.304	0.761

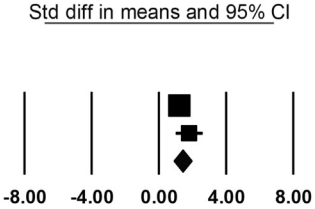


Supplementary Figure 12. Forest plot of studies showing duodenal eosinophils in functional dyspepsia (FD) patients with postprandial distress syndrome (PDS) compared with those with epigastric pain syndrome (EPS) (standardized mean difference [SMD], 0.10; 95% CI, -0.56 to 0.76; $P = .761$) ($I^2 = 45.92$; $P = .157$). Std diff, standard difference.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Futagmi S et al 2010	5.22	0.57	0.33	4.10	6.34	9.14	0.00
Bafutto M et al 2012 (A)	2.58	0.62	0.39	1.36	3.79	4.15	0.00
	3.91	1.32	1.75	1.32	6.51	2.95	0.00

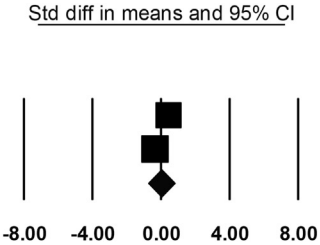
Supplementary Figure 13. Forest plot of studies showing duodenal eosinophils in patients with postinfectious functional dyspepsia (FD) and controls (standardized mean difference [SMD], 3.91; 95% CI, 1.32–6.51; $P = .001$) ($I^2 = 89.84$; $P = .002$). Std diff, standard difference.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Futagmi S et al 2010	1.22	0.23	0.06	0.76	1.68	5.21	0.00
Bafutto M et al 2012 (A)	1.80	0.40	0.16	1.01	2.59	4.46	0.00
	1.42	0.27	0.08	0.88	1.96	5.18	0.00

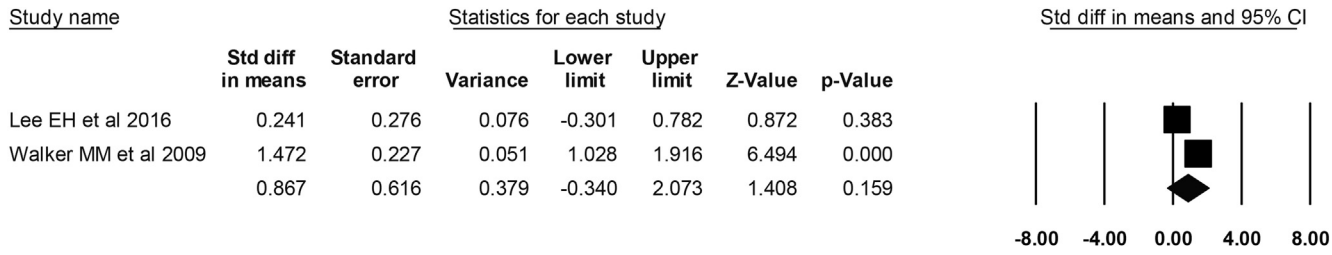


Supplementary Figure 14. Forest plot of studies showing duodenal eosinophils in postinfectious functional dyspepsia (FD) patients compared with those with non-postinfectious FD (standardized mean difference [SMD], 1.42; 95% CI, 0.88–1.96; $P = .001$), ($I^2 = 34.95$; $P = .215$). Std diff, standard difference.

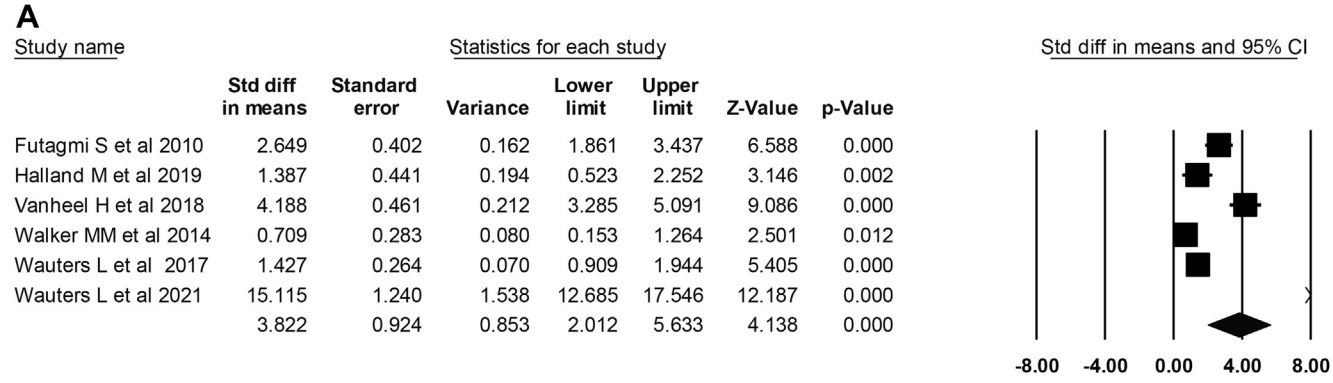
Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Lee EH et al 2016	0.438	0.282	0.079	-0.114	0.990	1.555	0.120
Walker MM et al 2009	-0.347	0.214	0.046	-0.767	0.072	-1.622	0.105
	0.024	0.392	0.154	-0.745	0.792	0.061	0.951

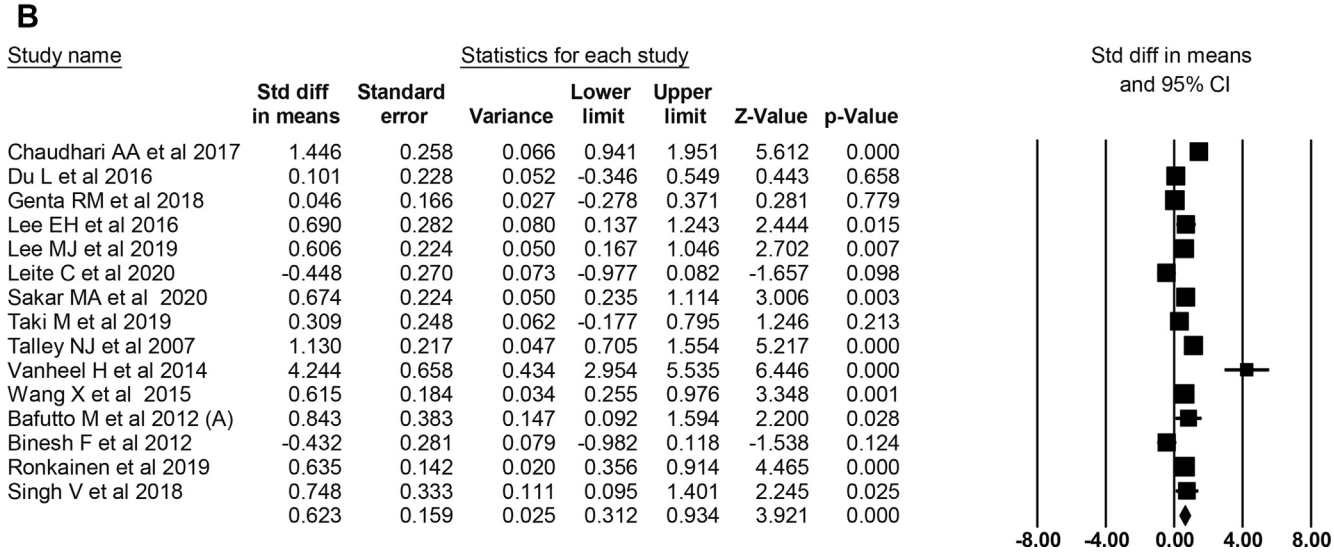


Supplementary Figure 15. Forest plot of studies showing duodenal eosinophils in patients with irritable bowel syndrome (IBS) and controls (standardized mean difference [SMD], 0.024; 95% CI, -0.75 to 0.79; $P = .951$) ($I^2 = 79.71$; $P = .026$). Std diff, standard difference.



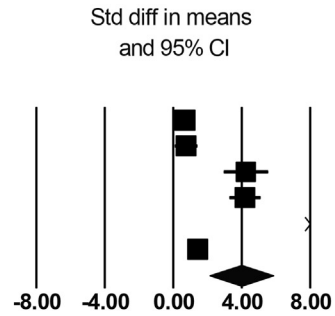
Supplementary Figure 16. Forest plot of studies showing duodenal eosinophils in patients with functional dyspepsia (FD) compared with those with irritable bowel syndrome (IBS) (standardized mean difference [SMD], 0.87; 95% CI, -0.34 to 2.07; $P = .159$) ($I^2 = 91.58$; $P = .001$). Std diff, standard difference.





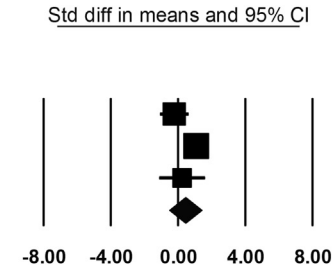
Supplementary Figure 17. (*A*) Forest plot of studies showing duodenal eosinophils in functional dyspepsia (FD) patients and controls, stratified according to counting technique, measuring eosinophils/mm^2 (standardized mean difference [SMD], 3.82; 95% CI, 2.01–5.63; $P = .001$) ($I^2 = 96.95$; $P = .0001$). (*B*) Forest plot of studies showing duodenal eosinophils in FD patients and controls, stratified according to counting technique, measuring eosinophils/HPF (SMD, 0.62; 95% CI, 0.31–0.93; $P = .0001$) ($I^2 = 84.87$; $P = .0001$). Std diff, standard difference.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Sakar MA et al 2020	0.685	0.225	0.050	0.245	1.125	3.051	0.002
Singh V et al 2018	0.748	0.333	0.111	0.095	1.401	2.245	0.025
Vanheel H et al 2014	4.244	0.658	0.434	2.954	5.535	6.446	0.000
Vanheel H et al 2018	4.188	0.461	0.212	3.285	5.091	9.086	0.000
Wauters L et al 2021	15.115	1.240	1.538	12.685	17.546	12.187	0.000
Waulters et al 2017	1.427	0.264	0.070	0.909	1.944	5.405	0.000
	3.984	0.946	0.895	2.130	5.837	4.212	0.000

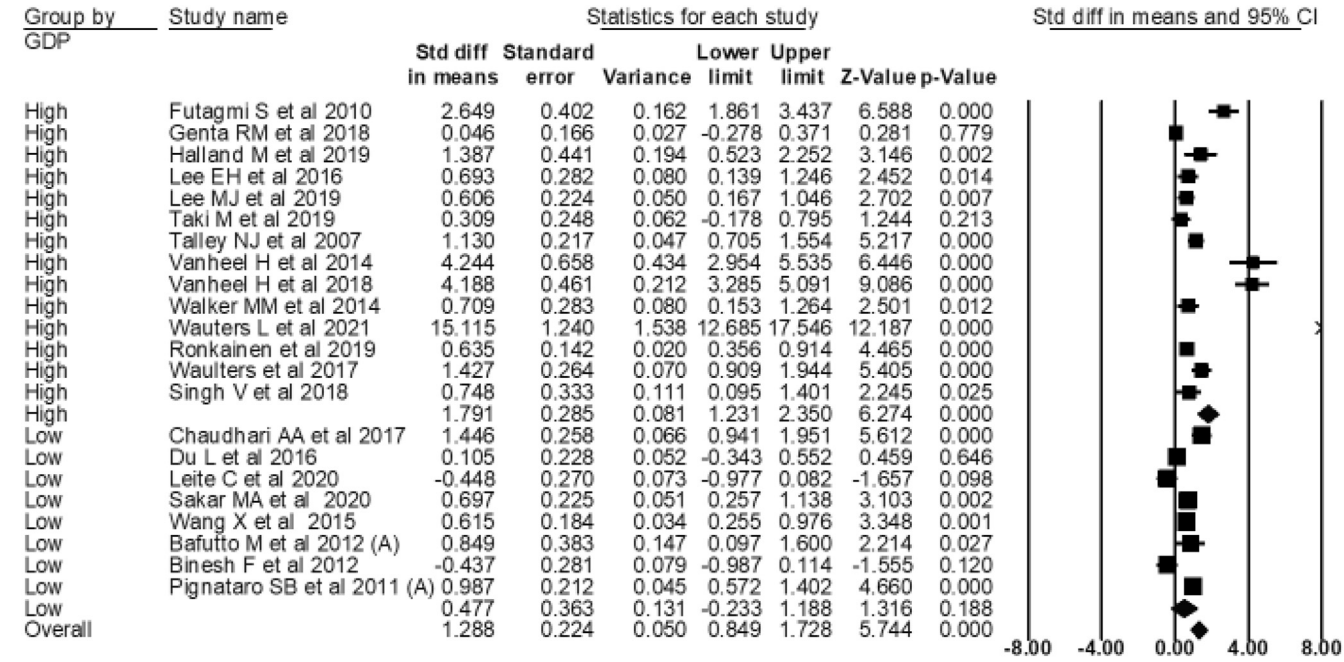


Supplementary Figure 18. Forest plot of studies showing duodenal eosinophils in *H pylori*–negative functional dyspepsia (FD) patients and controls (standardized mean difference [SMD], 3.98; 95% CI, 2.13–5.84; $P = .0001$) ($I^2 = 97.37$; $P = .0001$). Std diff, standard difference.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Du L et al 2016	-0.224	0.429	0.184	-1.064	0.616	-0.522	0.602
Pignataro S et al 2011 (A)	1.084	0.214	0.046	0.664	1.503	5.059	0.000
Taki M et al 2019	0.242	0.692	0.479	-1.115	1.599	0.350	0.727
	0.440	0.485	0.235	-0.510	1.390	0.908	0.364



Supplementary Figure 19. Forest plot of studies showing duodenal eosinophils in *H pylori*–positive functional dyspepsia (FD) patients compared with *H pylori*–positive controls (standardized mean difference [SMD], 0.44; 95% CI, -0.51 to 1.39; $P = .364$) ($I^2 = 75.30$; $P = .001$). Std diff, standard difference.



Supplementary Figure 20. Forest plot of studies showing duodenal eosinophils in functional dyspepsia (FD) patients and controls, stratified according to countries with high (>$30,000 US) and low (≤$30,000 US) GDP. Studies conducted in countries with a higher GDP had an increased prevalence of duodenal eosinophilia in FD patients compared with controls (standardized mean difference [SMD], 1.80; 95% CI, 1.23–2.35; $P = .0001$) ($I^2 = 95.22$; $P = .0001$) compared with those in countries with a lower GDP (SMD, 0.48; 95% CI, -0.23 to 1.19; $P = .188$) ($I^2 = 85.15$; $P = .0001$). Std diff, standard difference.

Supplementary Table 1. Exclusion Criteria for Studies Excluded From the Systematic Review and Meta-Analysis

Full-text articles excluded, with reasons (n = 33)
Cohort studies = 10[31,33–40,42]
Dual publication = 4[17,18–20]
No separate data on duodenal eosinophils and/or mast cells = 4[21–24]
Unable to extract data = 8[20,25–31]
Mixed study, including patients with other FGIDs or organic gastrointestinal condition = 7[32,33–38]

FGID, functional gastrointestinal disorder.

Supplementary Table 2. Assessment of Risk Factors and Methodology Pertaining to Diagnosis of Duodenal Eosinophils in FD, in the Studies Included in the Systematic Review and Meta-Analysis

No	Study	PPI use	Method for *H pylori* staining	Mode of *H pylori* diagnosis	*H pylori* status	Information regarding effect of seasonal variation on eosinophil count	Information regarding blinding of pathologist	Site of duodenal biopsy	Method for eosinophil staining	Cut-off criteria for duodenal eosinophilia	Method for counting eosinophils, eos counts/HPF or mm^2 in FD patients
1	Chaudhari et al,[39] 2017	NA	Giemsa, methylene blue	Rapid urease test using CLO kit test on gastric biopsy specimens during endoscopy	24/50 FD patients were positive	NA	NA	Duodenal	H&E	Up to 15/5 HPF	Eosinophils were counted in 5 consecutive nonoverlapping HPF in lamina propria,** expressed as 40.7/5 HPF
2	Du et al,[40] 2016	NA	NA	C13 urea breath test and gastric histology	25/96 FD patients, 7/24 controls were positive	NA	2 independent pathologists, no information regarding blinding	Duodenal bulb and D2	H&E, MBP	NA	Eosinophils were counted in 5 consecutive nonoverlapping HPF and expressed per 5 HPFs,** expressed as 58/5 HPF
3	Futagami et al,[41] 2010	NA	NA	Both urea breath test and gastric histology	NA	NA	2 independent blinded pathologists	Duodenal bulb and D2	H&E	NA	Eosinophils were evaluated per mm^2 in duodenal specimens, 16.9/mm^2
4	Genta et al,[3] 2018	NA	NA	Gastric histology	25/370 total study patients were positive	NA	NA	Duodenum	NA	NA	Eosinophils were counted in 3 HPF with the highest density of eosinophils, and mean expressed per HPF, 8.5/HPF

Supplementary Table 2. Continued

No	Study	PPI use	Method for *H pylori* staining	Mode of *H pylori* diagnosis	*H pylori* status	Information regarding effect of seasonal variation on eosinophil count	Information regarding blinding of pathologist	Site of duodenal biopsy	Method for eosinophil staining	Cut-off criteria for duodenal eosinophilia	Method for counting eosinophils, eos counts/HPF or mm^2 in FD patients
5	Halland et al,[42] 2019	All patients were on a PPI	NA	NA	2/22 FD patients were positive	NA	Blinded pathologist	D2	H&E	NA	Eosinophils were counted in 5 nonoverlapping HPFs, expressed counts/mm^2, 26/mm^2
6	Lee et al,[43] 2016	NA	Giemsa	Histology and rapid urease testing on gastric biopsy	NA	NA	2 independent blinded pathologists	Descending duodenum	H&E	>10/5 HPF for children	Average of 5 HPF, 13.4/5 HPF
7	Lee et al,[44] 2019	Acid-suppressing medication ceased 1 month prior	Giemsa	Rapid urease test or histology	20/51 FD patients, 13/35 controls were positive	NA	Blinded pathologist	Duodenum	H&E, MBP	NA	Mean number of eosinophils/5 HPF, 42.1/5 HPF
8	Leite et al,[45] 2020	NA	Giemsa	Rapid urease test during endoscopy	26/42 FD patients, 8/21 controls were positive	NA	2 independent blinded pathologists	D2	H&E	NA	Mean number of eosinophils/5 HPF, 11.9/5 HPF
9	Sakar et al,[46] 2020	PPI ceased 2 weeks prior	NA	C13 urea breath test	Excluded	NA	1 blinded pathologist	D2	H&E	Up to 22/5 HPF	Eosinophil count was performed in 5 randomly selected nonoverlapping HPF,** 22/5 HPF
10	Taki et al,[47] 2019	NA	NA	Serum antibody, rapid urease tests or urea breath tests, and endoscopic findings	25/28 FD patients, 28/31 controls were positive	NA	Blinded pathologist	D2	MBP	NA	Eosinophil count was performed in 3 randomly selected nonoverlapping HPF, expressed as cells/HPF, 21.3/HPF

Supplementary Table 2. Continued

No	Study	PPI use	Method for *H pylori* staining	Mode of *H pylori* diagnosis	*H pylori* status	Information regarding effect of seasonal variation on eosinophil count	Information regarding blinding of pathologist	Site of duodenal biopsy	Method for eosinophil staining	Cut-off criteria for duodenal eosinophilia	Method for counting eosinophils, eos counts/HPF or mm^2 in FD patients
11	Talley et al,[48] 2007	NA	Warthin Starry	Positive culture or on histology	13/51 FD patients, 14/48 controls were positive	NA	2 independent blinded pathologists	D1, D2	H&E, MBP	Up to 22/5 HPF	Eosinophil count was performed in 5 randomly selected HPF, expressed as mean, 35.6/5 HPF
12	Vanheel et al,[49] 2014	6 FD patients were on acid-suppressive therapy	NA	NA	Excluded	NA	Blinded pathologist	D2	MBP	NA	Eosinophil count was performed in 7 representative nonoverlapping HPF, expressed as mean, 28.3/7 HPF
13	Vanheel et al,[50] 2018	NA	NA	NA	Excluded	NA	Blinded pathologist	D2	MBP	NA	Eosinophil count was performed in 7 representative nonoverlapping HPF, expressed as cells/mm^2, 241.5/mm^2.
14	Walker et al,[51] 2014	NA	Giemsa	Gastric histology	5/33 FD patients, 4/22 controls were positive	NA	2 independent blinded pathologists	D1, D2	H&E	NA	Eosinophils were quantified by counting 5 nonoverlapping HPF, expressed cells/mm^2, 51/mm^2

Supplementary Table 2. Continued

No	Study	PPI use	Method for *H pylori* staining	Mode of *H pylori* diagnosis	*H pylori* status	Information regarding effect of seasonal variation on eosinophil count	Information regarding blinding of pathologist	Site of duodenal biopsy	Method for eosinophil staining	Cut-off criteria for duodenal eosinophilia	Method for counting eosinophils, eos counts/HPF or mm^2 in FD patients
15	Wang et al,[52] 2015	NA	NA	NA	42/141 FD patients, 10/39 controls were positive	NA	2 independent blinded pathologists	D1, D2	H&E, MBP	NA	Eosinophils were counted in 5 randomly nonselected HPF, expressed as number per 5 HPF,[a] 24.8/5 HPF
16	Wauters et al,[53] 2021	PPI as an intervention on symptoms and mucosal inflammation in FD	Giemsa	NA	Excluded	NA	Blinded pathologist	D2	H&E	NA	Eosinophils were counted per mm^2 by dividing the number of eosinophils into 3 separate regions, of which the mean was calculated, 331.07/mm^2
17	Bafutto et al,[54] 2012 (A)	None of the study subjects were on a PPI	Giemsa	NA	NA	NA	2 independent blinded pathologists	Proximal duodenum	H&E	NA	Eosinophils were counted in 5 randomly nonselected HPF, expressed as mean, 14.2/5 HPF
18	Binesh et al,[55] 2012	NA	Giemsa	Gastric histology	NA	NA	Blinded pathologist	D1	H&E	NA	Eosinophils were counted in 5 randomly nonselected HPF expressed as median, 16/5 HPF

Supplementary Table 2. Continued

No	Study	PPI use	Method for *H pylori* staining	Mode of *H pylori* diagnosis	*H pylori* status	Information regarding effect of seasonal variation on eosinophil count	Information regarding blinding of pathologist	Site of duodenal biopsy	Method for eosinophil staining	Cut-off criteria for duodenal eosinophilia	Method for counting eosinophils, eos counts/HPF or mm^2 in FD patients
19	Pignataro et al,[56] 2011 (A)	NA	NA	NA	All subjects were *H pylori* positive	NA	NA	Duodenal bulb and D2	NA	NA	NA
20	Ronkainen et al,[57] 2019	PPI use was not different between cases and controls	Warthin Starry	Histology and/or culture detected *H pylori*	71/213 FD patients and controls were positive	NA	Blinded pathologist	Duodenal biopsy	H&E	24/5 HPF in D2	Eosinophils were counted in 5 randomly selected HPF expressed as mean, 32/5 HPF
21	Waulters et al,[58] 2017	PPI use was not different between cases and controls	Giemsa	Gastric histology	Excluded	NA	Blinded pathologist	Distal duodenum	H&E	NA	Eosinophil counts were expressed per mm^2, 151/mm^2
22	Singh et al,[59] 2018	NA	NA	Gastric histology	Excluded	NA	2 independent pathologists, no information regarding blinding	Duodenum	H&E	Up to 20/5 HPF	Eosinophils counted in 5 consecutive HPF and expressed as mean, 27.0/5 HPF

(A), abstract; CLO test, *Campylobacter*-like organism test; D1, first part of the duodenum; D2, second part of the duodenum; FD, functional dyspepsia; HPF, high-power field; MBP, maltose-binding protein; NA, not available; PPI, proton pump inhibitor.

[a]Studies that were converted to counts per 1 HPF during analysis to enable direct comparison.

Supplementary Table 3. Demographic Characteristics and Exclusion Criteria for the Studies Included in This Systematic Review and Meta-Analysis

No	Study	Mean age of FD patients, *y*	Mean age of controls, *y*	Proportion of females in FD patients, n (%)	Proportion of females in controls, n (%)	Exclusion criteria
1	Chaudhari et al,[39] 2017	34.2	36.3	18 (36)	NA	History of PUD, gastrointestinal malignancy, previous gastric surgery, drug intake, and upper gastrointestinal bleeding
2	Du et al,[40] 2016	47.2	45.2	NA	NA	Progressive severe diseases requiring medical management, medical conditions known to be associated with tissue and peripheral eosinophilia, atopic disease, history of gastrointestinal pathology (GERD and PUD), and GI surgery except hernia repair, appendisectomy, and cholecystectomy
3	Futagami et al,[41] 2010	48.8	46.2	55 (54.4)	6 (30)	Severe heart, renal, pulmonary, liver cirrhosis, systemic illness, malignant disease, those with a history of gastroduodenal surgery, NSAID use, or anticoagulation use
4	Genta et al,[3] 2018	NA	53[a]	NA	NA	Applied the following criteria for normal: (1) no clinical history of any condition known to affect the small intestine including peptic ulcer, celiac disease, and autoimmune enteritis; (2) no reported history of a previous duodenal biopsy for whatever reason; (3) endoscopic description of a normal duodenum; specifically, cases with any mention of a definite or suspected duodenal abnormality, including polyps or nodules, erythema, duodenitis, villous atrophy or flattening, scalloping, erosions or ulcers, or strictures, were excluded; (4) no eosinophilic conditions of other portions of the gastrointestinal tract, and no reported history of parasitic infections All patients who had a history of upper gastrointestinal surgery for whatever reason were excluded The 5 most common indications for gastroscopy included GERD, dysphagia, dyspepsia, diarrhea, and anemia
5	Halland et al,[42] 2019	39	34	17 (77)	8 (80)	A personal history of a systemic or gastrointestinal eosinophilic disorder including eosinophilic esophagitis or gastroenteritis, celiac disease, history of prior gastric or esophageal surgery, and pregnant and/or lactating females, patients with current or recent (within 30 days) use of NSAIDs, or olmesartan
6	Lee et al,[43] 2016	12.2	10.6	51 (48.6)	11 (57.9)	Significant allergy, including food allergy, asthma, atopic dermatitis, and allergic rhinitis, or if they had underlying organic diseases that can cause gastrointestinal symptoms

Supplementary Table 3. Continued

No	Study	Mean age of FD patients, *y*	Mean age of controls, *y*	Proportion of females in FD patients, n (%)	Proportion of females in controls, n (%)	Exclusion criteria
7	Lee et al,[44] 2019	35.8	44.8	42 (82.4)	29 (82.9)	(1) Patients with organic diseases such as reflux esophagitis, peptic ulcer, erosive gastroduodenitis, and malignancies that could cause upper gastrointestinal symptoms similar to FD; (2) patients with dementia, apoplexy, or mental disease who could not complete the Korean version of the bowel disease questionnaire; (3) patients with uncontrolled diabetes, end-stage renal failure, decompensated liver cirrhosis, or terminal cancer that might induce upper gastrointestinal symptoms; (4) patients who had undergone major abdominal surgery except appendectomy; (5) patients with a bleeding tendency or taking warfarin, aspirin, or antiplatelet drugs; (6) patients with a parasitic infection, allergy, urticaria, atopic disease, or asthma that may induce eosinophilia or taking medications for such diseases; (7) patients with a history of idiopathic eosinophilic gastrointestinal disease or overt hypereosinophilic syndrome; (8) patients who had taken, within the past month, medications such as iodine, sulfonamides, nitrofurantoin, angiotensin-converting enzyme inhibitors, or cephalosporin that may induce eosinophilia; and (9) patients who had taken an antibiotic and NSAIDs or been administered an acid-secretion inhibitor within the past month
8	Leite et al,[45] 2020	NA	NA	NA	NA	Excluded patients with predominant symptoms of heartburn or irritable bowel syndrome; alarm symptoms; history of peptic ulcer, upper gastrointestinal tract surgery, or biliary colic; previous treatment for eradication of *H pylori*; known allergies to study medication; serious comorbidities; or alcohol or drug abuse Use of antibiotics or bismuth during the 4 weeks before enrollment, proton pump inhibitors during the 2 weeks before enrollment, or treatment with histamine-2–receptor blockers in the week before enrollment were not permitted We also excluded women of childbearing potential; patients unable to answer the study questionnaires; patients with endoscopic findings other than gastritis, duodenitis, or hiatal hernia; and patients unwilling or unable to provide consent

Supplementary Table 3. Continued

No	Study	Mean age of FD patients, *y*	Mean age of controls, *y*	Proportion of females in FD patients, n (%)	Proportion of females in controls, n (%)	Exclusion criteria
9	Sakar et al,[46] 2020	32.8	29.9	24 (57.1)	23 (54.8)	(1) Blood eosinophilia; (2) medical conditions known to increase peripheral and tissue eosinophil count (inflammatory bowel disease, celiac disease, vasculitis, connective tissue disease, hypereosinophilic syndrome, active infection, drugs, and transplantation); (3) progressive, severe diseases requiring active medical management (eg, advanced cardiac, liver, renal, or neurologic disease, advanced cancer). (4) history of significant gastrointestinal pathology (GERD and PUD), and history of gastrointestinal surgery (except appendicectomy, cholecystectomy, hernia repair); (5) positive for *H pylori*; and (6) patients with FD associated with overlapping gastroesophageal reflux symptoms or symptoms of IBS
10	Taki et al,[47] 2019	52.5	58.3	23 (92)	15 (48.4)	Use of NSAIDs, corticosteroid, antiallergy, or other immunosuppressive drugs
11	Talley et al,[48] 2007	53.4	53.4	24 (47.1)	27 (56.2)	Nonulcer dyspepsia, gastroesophageal reflux symptoms, or IBS
12	Vanheel et al,[49] 2014	28.9	28.4	10 (66.7)	10 (66.7)	Intake of NSAIDs, corticosteroids, or other immunosuppressive drugs in the preceding 6 months; diabetes or celiac disease; first-degree family members with type 1 diabetes, celiac disease, or IBD Specific IgE antibodies for cereal mix, nut mix, seafood mix, and food mix were measured in peripheral blood to assess food allergies, and participants were considered potentially positive when at least 1 of the IgE antibodies exceeded 1 U/mL
13	Vanheel et al,[50] 2018	30.5	58	16 (66.7)	18 (48.6)	As described for the study listed in the previous row
14	Walker et al,[51] 2014	46.3	58	21 (63.6)	13 (59.1)	Overt or medical conditions known to increase peripheral and tissue eosinophilia (celiac disease, IBD, vasculitis, connective tissue disorder, hypereosinophilic syndrome, active infection, and transplantation) Patients with GERD or PUD, known to increase peripheral and tissue eosinophilia, were excluded (IBD, celiac disease, vasculitis, connective tissue disease, hypereosinophilia syndrome, active infection, and transplantation) Patients with no known organic disease or IBS, reflux esophagitis, or PUD
15	Wang et al,[52] 2015	46.2	47.4	105 (74.5)	26 (66.7)	Outside age 18–65 years, history of gastrointestinal surgery, history of PUD, GERD, malignancy, pancreatic biliary disease, anaphylaxis
16	Wauters et al,[53] 2021	31.7	31.3	24 (86)	21 (70)	No active psychiatric, atopic, inflammatory, or metabolic conditions

Supplementary Table 3. Continued

No	Study	Mean age of FD patients, *y*	Mean age of controls, *y*	Proportion of females in FD patients, n (%)	Proportion of females in controls, n (%)	Exclusion criteria
17	Bafutto et al,[54] 2012 (A)	NA	NA	NA	NA	Atopic or allergic diseases; use of any medication, drugs, tobacco, or alcohol; GERD; IBS; and organic diseases All patients underwent blood and urine tests, ova, and parasite testing
18	Binesh et al,[55] 2012	31.72[a]	31.72[a]	17[b] (32.7[+])	17[b] (32.7[+])	No organic disease found during gastroscopy
19	Pignataro et al,[56] 2011 (A)	52.58	45.38	36 (72)	30 (60)	Patients with a history of allergy, asthma, parasitic diseases, diabetes, and intake of NSAIDs
20	Ronkainen et al,[57] 2019	62.1[a]	NA	141[b] (66.2	141[b] (66.2	NA
21	Waulters et al,[58] 2017	13.6	10.5	20 (56)	19 (53)	Exclusion criteria included the use of leukotriene receptor antagonists or corticosteroids in the past month and/or histamine H2-receptor antagonists in the past 2 weeks before the endoscopic procedure Additional exclusion criteria were conditions associated with eosinophilia, including IBD, celiac or connective tissue disease, vasculitis, hypereosinophilia, active infection (parasites and *Helicobacter*-like organisms on biopsy specimens or positive stool cultures), and transplantation
22	Singh et al,[59] 2018	NA	NA	NA	NA	NA

(A), abstract; FD, functional dyspepsia; GERD, gastroesophageal reflux disease; GI, gastrointestinal; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; NA, not available; NSAID, nonsteroidal anti-inflammatory drug; PUD, peptic ulcer disease.
[a]Overall mean age for FD patients and controls.
[b]Overall females for FD patients and controls.

Supplementary Table 4. Newcastle–Ottawa Scale for Assessment of Quality of Case-Control Studies Assessing the Prevalence of Duodenal Eosinophils in Patients With FD With or Without Concomitant IBS Included in the Systematic Review and Meta-Analysis

	1[39]	2[40]	3[41]	4[3]	5[42]	6[43]	7[44]	8[45]	9[46]	10[59]	11[47]	12[48]	13[49]	14[50]	15[51]	16[52]	17[53]	18[54]	19[55]	20[56]	21[57]	22[58]
Selection																						
Is the case definition adequate?	*	*	*	-	-	*	*	*	*	*	*	*	*	*	*	*	*	*	-	*	*	*
Representativeness of the cases	-	*	*	*	-	-	-	*	-	-	-	*	*	*	*	*	-	-	-	-	*	-
Selection of controls	-	*	*	-	*	-	*	*	-	-	*	*	*	*	-	*	*	-	-	-	*	-
Definition of controls	-	*	-	-	*	-	*	*	-	-	*	*	*	*	-	-	*	*	-	-	*	-
Comparibility																						
Study controls for single factor	*	*	*	-	*	*	*	-	*	-	*	*	*	*	*	*	*	-	*	*	*	*
Study controls for additional factors	*	*	*	-	*	*	*	-	*	-	*	*	*	*	*	*	*	-	-	-	*	*
Exposure																						
Ascertainment of exposure	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Same method of ascertainment for cases and controls	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Nonresponse rate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Overall quality score (maximum = 9)	5	8	7	3	6	5	7	6	5	3	7	8	8	8	6	7	7	4	3	4	8	5

NOTE. Each asterisk represents whether individual criterion within the subsection was fulfilled. Detailed assessment of quality of case–control studies included in the systematic review and meta-analysis:

1. Chaudhari et al[39]: This case-control study comprised 50 patients with FD defined by the Rome III criteria and 30 age- and sex-matched controls. The controls comprised patients in whom esophagogastroduodenoscopy was performed for indications other than FD (non-FD disease controls). Eosinophils were counted in 5 consecutive nonoverlapping HPF in the lamina propria.
2. Du et al[40]: Consecutive patients newly diagnosed with FD defined by the Rome III criteria, and asymptomatic controls who were scheduled to undergo upper gastrointestinal endoscopy as part of an annual health examination or surveillance of gastrointestinal metaplasia. There were no significant differences in age, frequency of smoking, alcohol drinking, and education between the 2 groups, as well as *H pylori*–positivity rate. All participants completed a group of questionnaires containing basic characteristics, Hospital Anxiety and Depression Scale, and a simplified abdominal symptom questionnaire, which contains the frequency and severity of abdominal symptoms.
3. Futagami et al[41]: FD patients, postinfectious FD patients, and 20 healthy volunteers were consecutively enrolled according to the Rome III criteria. The prevalence of symptoms in controls was not reported. Patients all had undergone a diagnostic upper gastrointestinal endoscopy and abdominal ultrasonography for dyspeptic symptoms. No information was provided about the demographic differences between cases and controls.
4. Genta et al[3]: Retrospective study in which the diagnosis of FD patients was determined by the treating physician and controls included non-FD disease controls, referred for the investigation of anemia, diarrhea, dyspepsia, and the "incidental biopsy." These are biopsy specimens taken during esophagogastroduodenoscopy in patients who may have had other indications (eg, gastroesophageal reflux disease, dysphagia, dyspepsia, or the exclusion of *H pylori*).
5. Halland et al[42]: All 22 patients had rumination syndrome, according to the Rome III and IV criteria, 17 of 22 patients with rumination syndrome had dyspeptic symptoms. All patients were on a proton pump inhibitor. Controls (n = 10) were asymptomatic, age- and sex-matched, who were either healthy volunteers or underwent investigation for iron-deficiency anemia. None of the controls reported any gastrointestinal symptoms.
6. Lee et al[43]: Pediatric patients with abdominal pain–functional gastrointestinal disorders who visited the pediatric gastroenterology outpatient clinics were recruited retrospectively and classified based on the Rome III criteria. Controls were "historic controls" from another study.[61] This study examined pediatric patient slides retrospectively and included them if there was no diagnostic abnormality observed during endoscopy and if the final clinical and pathologic diagnosis did not involve gastrointestinal disease at the time of the study. The majority of the patients had a final diagnosis of a functional gastrointestinal disorder. Cases and controls were age- and sex-matched.
7. Lee et al[44]: Study subjects were patients with FD who satisfied the Rome III criteria, sampled from the outpatient clinic of the referral center. The control group was sampled among those who did not have any gastrointestinal symptoms, had received endoscopy for anemia, or a screening test. The mean age of the FD group was significantly younger than that of the controls, but no differences were detected in sex, body mass index, smoking habits, alcohol use, prevalence of diabetes mellitus, hypertension, chronic viral hepatitis, or medication history between the 2 groups.
8. Leite et al[45]: The functional dyspeptic patients (cases) in this investigation participated in the randomized double-blind study Heroes Trial (Helicobacter Eradication Relief of Dyspeptic Symptoms; ClinicalTrials.gov number NCT00404534). Individuals aged 18 or older who fulfilled the Rome III criteria for FD were included. The control group consisted of individuals with no symptoms in the gastrointestinal tract selected among donors for the blood bank at the same hospital.
9. Sakar et al[46]: Patients with FD who satisfied the Rome III criteria sampled from the outpatient clinic of the referral center. The control group included subjects without complaints of dyspepsia and referred for upper gastrointestinal endoscopy for reasons other than dyspepsia (unexplained anemia, suspected celiac disease, and surveillance endoscopy). There was no significant difference in age, sex, body mass index, or frequency of smoking between the 2 groups.

10. Singh et al[59]: Cases were selected from a database of FD patients (diagnosed according to Rome III criteria), meeting Rome IV criteria. To be placed in the "normal" control group, subjects were identified by searching pathology records for the keywords "no diagnostic abnormality" in stomach and duodenum samples and "constipation." No information was provided about the differences regarding demographic characteristics for cases and controls.
11. Taki et al[47]: FD patients were diagnosed according to the Japanese version of the Rome III diagnostic questionnaire for IBS and FD. Controls included healthy individuals, and there was no information regarding symptom assessment in controls. Sex, age, and rates of *H pylori* infection did not differ between the patients with FD and controls.
12. Talley et al[48]: Cases included patients with nonulcer dyspepsia, diagnosed according to the Rome II criteria, who did not have gastroesophageal reflux symptoms or IBS. Controls were randomly selected subjects who were symptom-free in the endoscoped population; they did not have nonulcer dyspepsia, gastroesophageal reflux symptoms, or IBS. There were no differences between cases and controls with regard to *H pylori* status and demographic characteristics.
13. Vanheel et al[49]: Patients meeting Rome III criteria for FD were recruited prospectively at the outpatient clinic of the Department of Gastroenterology at University Hospitals Leuven, a tertiary care referral center. Age- and sex-matched healthy volunteers were recruited by a mailing list after exclusion of gastrointestinal symptoms or a history of gastrointestinal disease. The study had a very small sample size, with 15 patients in each group.
14. Vanheel et al[50]: Patients meeting Rome III criteria for FD were recruited prospectively from the outpatient clinic of the Department of Gastroenterology at University Hospitals Leuven, a tertiary care referral center. Healthy volunteers, as the control group, were recruited by a mailing list after exclusion of gastrointestinal symptoms or a history of gastrointestinal disease. There was no significant difference in sex or age between both groups, but body mass index was lower in the FD patient group ($P = .01$).
15. Walker et al[51]: This study included consecutive patients referred for gastroscopy, including cases as subjects with symptoms of FD according to the Rome II criteria and controls as those with no symptoms of FD (endoscoped for iron deficiency and suspected celiac disease). There was no significant difference between cases and controls with regard to demographic characteristics and *H pylori* status.
16. Wang et al[52]: FD patients meeting Rome III criteria were recruited consecutively from the Department of Gastroenterology. Consecutive age-matched healthy volunteers were recruited as controls during the study period. No information about symptoms in the control group was provided.
17. Wauters et al[53]: This was a prospective intervention case-control study in which cases included patients with predominant FD symptoms, diagnosed according to the Rome IV criteria. Age- and sex-matched healthy volunteers without gastrointestinal symptoms were recruited as controls by advertisement.
18. Bafutto et al[54]: Published as an abstract with the following limitations. A previous clinical evaluation was performed in adult patients (age, $\geq$18 y) with FD, postinfectious FD, and controls. The FD diagnosis was made based on the Rome III criteria. Controls included asymptomatic patients. The study had a very small sample size for the postinfectious FD (10 patients) and control group (9 subjects). No information about the demographic differences between cases and controls was provided.
19. Binesh et al[55]: Cases included adult patients with FD, and normal endoscopy and controls included patients who had no dyspepsia and no abnormality on their endoscopy. Regarding *H pylori* infection, the prevalence of *H pylori* infection in the case and control groups was similar. No information regarding other demographic characteristics for controls was provided.
20. Pignataro et al[56]: Published as an abstract with the following limitations. FD patients were diagnosed according to the Rome III criteria and no information was provided about the control group. The cases and controls were matched for sex.
21. Ronkainen et al[57]: Participants were selected randomly from the national Swedish population register and surveyed in 1998 to 2001 by a validated abdominal symptom questionnaire. A case-control study on all available FD cases (diagnosed according to Rome III criteria) with histologic evaluation of the duodenum at baseline (n = 89) vs healthy controls (n = 124) was performed. Cases and controls were matched for multiple demographic characters.
22. Waulters et al[58]: Retrospective study that included FD patients, defined according to the Rome III criteria. Controls included subjects with nonerosive reflux disease, nonorganic dysphagia, and rumination syndrome. The mean age of the FD patients was higher compared with controls, but there were no difference in other demographic characteristics.
FD, functional dyspepsia; HPF, high-power field; IBS, irritable bowel syndrome.

Supplementary Table 5. Characteristics of Studies Assessing Degranulation of Duodenal Eosinophils and Mast Cells in FD and Controls

No	Study	FD, n	Controls, n	Duodenal eos degranulation in FD patients, n (%)	Duodenal eos degranulation in controls, n (%)	Method for eos staining	Duodenal eos degranulation in FD patients, mean (±SD)	Duodenal eos degranulation in controls, mean (±SD)	Duodenal mast cell degranulation in FD patients, n (%)	Duodenal mast cell degranulation in controls, n (%)	Method for mast cell staining	Duodenal mast cell degranulation in FD patients, mean (±SD)	Duodenal mast cell degranulation in FD patients, mean (±SD)
1	Du et al,[40] 2016	96	24	49 (51)	6 (25)	H&E, MBP	10.7 (7.7)	5.3 (0.9)	83 (86.5)	24 (100)	Tryptase	13.6 (±2.9)	14.8 (±2.4)
2	Wang et al,[52] 2015	141	39	74 (52.5)	8 (20.5)	H&E, MBP	NA	NA	89 (63.1)	11 (28.2)	Toluidine blue	NA	NA
3	Talley et al,[48] 2007	15	5	7 (46.7)	0 (0)	H&E, MBP	NA	NA	NA	NA	NA	NA	NA
4	Vanheel et al,[50] 2018	17 (eos)[a] 20 (MC)	25 (eos)[a] 36 (MC)	14 (82.4)	15 (60)	MBP	NA	NA	19 (95)	28 (77.8)	Tryptase	NA	NA

eos, eosinophil; FD, functional dyspepsia; MBP, major basic protein; MC, mast cell; NA, not available.
[a]In the Vanheel et al[50] study, analysis for duodenal eosinophil degranulation was performed on 17 FD patients and 25 controls, and for duodenal mast cell degranulation analysis was performed on 20 FD patients and 36 controls.

Supplementary Table 6. Characteristics of Studies Assessing Duodenal Eosinophils in FD Subtypes and Controls

No	Study	Country	Patients with FD, n	EPS, n	PDS, n	EPS/PDS, n	Controls, n	Method of eos counting	Duodenal (D2) eos count in patients with FD, mean (±SD)	Duodenal (D2) eos count in EPS, mean (±SD)	Duodenal (D2) eos count in PDS, mean (±SD)	Duodenal (D2) eos count in EPS/PDS, mean (±SD)	Duodenal (D2) eos count in controls, mean (±SD)
1	Futagami et al,[41] 2010[a]	Japan	27	12[b]	15[b]	NA	20	mm^2	4.0 (±1.2)	3.6 (±1.0)	4.3 (±1.3)	NA	1.3 (±0.7)
2	Leite et al,[45] 2020[a]	Brazil	42	26	16	NA	21	5 HPF	11.1 (±6.1)	10.9 (±6.2)	11.8 (±8.4)	NA	14.7 (±11.0)
3	Wauters et al,[53] 2021[a]	Belgium	28	3	15	10	30	mm^2	78.5 (±4.0)	89.7 (±26.2)	69.0 (±23.7)	92.9 (±23.9)	27.2 (±2.1)
4	Ronkainen et al,[57] 2019	Sweden	89	27	71	9	124	5 HPF	32.0 (±17.0)	NA	NA	NA	22.4 (±13.6)

FD: Functional dyspepsia; EPS: epigastric pain syndrome; PDS: post prandial distress syndrome; HPF: high power field; eos: eosinophils; NA: not available; D2: second part of the duodenum.

[a]Studies that could be included in the subgroup analysis.

[b]Patients with postinfectious FD were excluded. For the analyses, all the eosinophil counts are expressed as eos/HPF. To convert the counts from eos/HPF to eos/mm^2, we used a coefficient of 4.22.

Supplementary Table 7. Characteristics of Studies Assessing Duodenal Eosinophils in Patients After Infectious FD and Controls

No	Study	Country	Patients with PI-FD, n	Patients with FD, n	Controls, n	Method of eos counting	Duodenal (D2) eos count in patients with PI-FD, mean (±SD)	Duodenal (D2) eos count in patients with FD, mean (±SD)	Duodenal (D2) eos count in controls, mean (±SD)
1	Futagami et al,[41] 2010[a]	Japan	35	55	20	mm^2	5.3 (±0.8)	4.0 (±1.2)	1.3 (±0.7)
2	Lee et al,[44] 2019	South Korea	5	51	35	5 HPF	NA	42.1 (±27.7)	26.4 (±23.0)
3	Bafutto et al,[54] 2012 (A)[a]	Brazil	10	36	9	5 HPF	28.8 (±10.4)	14.2 (±7.4)	8.4 (±3.4)
4	Dizdar et al,[32] 2010	Norway	28	28	19	mm^2	NA	NA	NA

(A), abstract; D2, second part of the duodenum; eos, eosinophils; FD, functional dyspepsia; HPF, high-power field; NA, not available; PI-FD, postinfectious functional dyspepsia.

[a]Studies that could be included in the subgroup analysis. For the analyses, all the eosinophil counts are expressed as eos/HPF. To convert the counts from eos/HPF to eos/mm^2, we used a coefficient of 4.22.

Supplementary Table 8. Characteristics of Studies Assessing Duodenal Eosinophils and Mast Cells in Patients With IBS, FD, and Controls

No	Study	Country	Patients with IBS, n	Patients with FD, n	Patients with IBS/FD overlap, n	Controls, n	Method of eos and mast cell counting	Method for eos staining	Method for mast cell staining	Duodenal (D2) eos count in IBS patients, mean (±SD)	Duodenal (D2) mast cell count in IBS patients, mean (±SD)	Duodenal (D2) eos count in FD patients, mean (±SD)	Duodenal (D2) mast cell count in FD patients, mean (±SD)
1	Lee et al,[43] 2016[a]	South Korea	40	43	2	19	5 HPF	NA	NA	12.1 (±5.6)	NA	13.4 (±5.3)	NA
2	Lee et al,[44] 2019	South Korea	0	51	11	35	5 HPF	H&E, MBP	c-Kit (CD117)	NA	NA	42.1 (±27.7)	57.7 (±24.5)
3	Walker et al,[17] 2009[a]	Sweden	41	51	NA	48	5 HPF	H&E	c-Kit (CD117)	15.5 (±6.6)	243 (±93)	33.5 (±17.2)	160.0 (±78.0)
4	Ronkainen et al,[57] 2019	Sweden	0	89	13	124	5 HPF	H&E	NA	NA	NA	32.0 (±17.0)	NA

NOTE. For the analyses, all the mast cell counts are expressed as eos/HPF. To convert the counts from eos/HPF to eos/mm^2, we used a coefficient of 4.22.
D2, second part of the duodenum; eos, eosinophil; FD, functional dyspepsia; HPF, high-power field; IBS, irritable bowel syndrome; MBP, major basic protein; NA, not available.
[a]Studies that could be included in the subgroup analysis.

Supplementary Table 9. Characteristics of Studies Assessing Duodenal Eosinophils in FD and Controls, According to the Prevalence of *H pylori*

No	Study	Country	FD, n	Controls, n	Method of *H pylori* staining	Mode of *H pylori* diagnosis	Method of eos counting	*H pylori*–positive FD patients, n	*H pylori*–positive controls, n	D2 eos count in *H pylori*–positive FD patients, mean (±SD)	D2 eos count in *H pylori*–negative FD patients, mean (±SD)	D2 eos count in *H pylori*–positive controls, mean (±SD)	D2 eos count in *H pylori*–negative controls, mean (±SD)
1	Chaudhari et al,[39] 2017	India	50	30	Giemsa, methylene blue	Rapid urease test using CLO kit, gastric histology	5 HPF	24	NA	NA	NA	NA	NA
2	Du et al,[40] 2016[a]	China	96	24	NA	C13 urea breath test and gastric histology	5 HPF	25	7	49 (±28)	61.2 (±27.7)	55.9 (±39.5)	54.8 (±28.3)
3	Genta et al,[3] 2018	United States	44	214	NA	Gastric histology	5 HPF	NA	NA	NA	NA	NA	NA
4	Halland et al,[42] 2019	United States	17	10	NA	NA	mm^2	2	NA	NA	NA	NA	NA
5	Lee et al,[44] 2019	South Korea	51	35	Giemsa	Rapid urease test or histology	5 HPF	20	13	45.6	39.6	28.9	25
6	Leite et al,[45] 2020	Brazil	42	21	Giemsa	Rapid urease test during endoscopy	5 HPF	26	8	13.2[b]	8.1[c]	NA	NA
7	Taki et al,[47] 2019	Japan	35	31	NA	Serum antibody, rapid urease tests or urea breath tests, and histology	3 HPF	7	3	19 (±13.2)	21.6 (±8.5)	16.1 (±7.2)	17.3 (±7.0)
8	Talley et al,[48] 2007	Sweden	51	48	Warthin Starry	Positive culture or histology	5 HPF	13	14	NA	NA	NA	NA
9	Walker et al,[51] 2014	Australia	33	22	Giemsa	Gastric histology	mm^2	5	4	NA	NA	NA	NA
10	Wang et al,[52] 2015	China	141	39	NA	NA	5 HPF	42	10	NA	NA	NA	NA

Supplementary Table 9. Continued

No	Study	Country	FD, n	Controls, n	Method of *H pylori* staining	Mode of *H pylori* diagnosis	Method of eos counting	*H pylori*–positive FD patients, n	*H pylori*–positive controls, n	D2 eos count in *H pylori*–positive FD patients, mean (±SD)	D2 eos count in *H pylori*–negative FD patients, mean (±SD)	D2 eos count in *H pylori*–positive controls, mean (±SD)	D2 eos count in *H pylori*–negative controls, mean (±SD)
11	Zhao et al,[60] 2013	China	215	0	Warthin Starry	C13 urea breath test and gastric histology	5 HPF	84	NA	28.8 (±15.6)	31.3 (±15.9)	NA	NA
12	Binesh et al,[55] 2012	Iran	25	27	Giemsa	Gastric histology	5 HPF	NA	NA	15[d]	16[d]	NA	NA
13	Pignataro et al,[56] 2011 (A)[a]	South America	50	50	NA	NA	NA	50	50	43.8 (±4.8)	NA	38.5 (±4.9)	NA
14	Ronkainen et al,[57] 2019	Sweden	89	124	Warthin Starry	Histology and/or culture detected *H pylori*	5 HPF	71[e]	NA	NA	NA	NA	NA

(A), abstract; CLO, *Campylobacter*-like organism; D2, second part of the duodenum; eos, eosinophil; FD, functional dyspepsia; HPF, high-power field; NA, not available.
[a]Studies that were included in the analysis.
[b]Combined median of D2 eosinophil count for all *H pylori*–positive FD patients and controls.
[c]Combined median of D2 eosinophil count for all *H pylori*–negative FD patients and controls.
[d]Eosinophils expressed as median with no range provided.
[e]Seventy-one of 213 patients with FD and controls were positive for *H pylori*.