Supplementary Appendix

Dellon ES, et. al., Determination of Biopsy Yield That Optimally Detects Eosinophilic Gastritis and/or Duodenitis in a Randomized Trial of Lirentelimab

Table of Contents

Supplemental Methods

Study oversight

The ENIGMA study was performed in accordance with the Declaration of Helsinki and in compliance with Good Clinical Practice Guidelines, institutional review boards and applicable laws. All patients gave written informed consent before entry. An independent data monitoring committee provided additional oversight.

Symptoms assessment and endoscopy and biopsy protocols

Patient symptoms were assessed daily using an electronic patient-reported outcome (PRO) questionnaire, developed in accordance with the Food and Drug Administration Guidance on PRO Measures.(1) Endoscopic features in the stomach were scored by individual investigators according to the Eosinophilic Gastritis Endoscopic Reference System (EG-REFS), developed by the Consortium of Eosinophilic Gastrointestinal Disease Researchers to assess endoscopic findings in patients with EG; it has not been validated in a large study or evaluated for its ability to identify patients with EG.(2, 3)

Additional biopsy and histopathologic evaluation details

Biopsy specimens were processed by a central laboratory, where they were embedded in paraffin, and 5 μm-thick sections were prepared for staining. Eosinophils and mast cells were identified by hematoxylin and eosin and anti-tryptase immunohistochemistry,(4) respectively. Immunohistochemistry was performed with a mouse anti-human mast cell tryptase primary antibody (Clone AA1, Dako M7052, Cambridge, UK) followed by a peroxidase-labeled anti-mouse polyclonal secondary antibody, and counterstained with hematoxylin. Each biopsy specimen was first examined at low power magnification (40X and 100X) to evaluate for proper orientation and for the presence of lesions (such as *H pylori* infection, celiac disease, neoplasia) that would make the subject ineligible for the study. Under this magnification, the morphologic features of gastric and duodenal biopsy specimens other than eosinophilic inflammation were graded according to the Updated Sydney System and The Marsh Scale Classification, respectively.(5, 6)

Acceptable specimens were then examined at medium power magnification (200X) to detect areas with the highest eosinophil density, before switching to high power magnification (400X) to count eosinophils. The first high-power field (hpf; area of 0.237 mm^2) (400X) in which eosinophils were counted was selected from the area with the highest density; the remaining hpfs were selected from non-

overlapping areas, which could be, but were not required to be, adjacent to the first hpf, depending on the distribution of eosinophils in the specimen (**Figure S1**). A minimum of 5 non-overlapping hpfs were evaluated per biopsy specimen, except in the event that the specimen was insufficient in size to allow for the evaluation of 5 independent fields. In that event, eosinophils were counted in as many non-overlapping fields as were available; in no case were overlapping fields counted.

To define the number of biopsies that would be required to optimize diagnostic yield in clinical practice, we assessed the number of biopsies, of the 8 gastric and 4 duodenal biopsies collected per subject, that had ≥30 eosinophils (eos)/hpf. An EG subject with 8 gastric biopsies meeting this criterion was classified as requiring ≥1 biopsy to detect EG, as any 1 of the 8 gastric biopsies would result in a positive diagnosis. Conversely, an EG subject with just 1 gastric biopsy meeting the criterion was classified as requiring 8 gastric biopsies to detect EG, because it was not possible to predict which of the 8 gastric biopsies would meet the threshold number of tissue eosinophils. Similar analyses were performed for subjects with eosinophilic duodenitis (EoD) and cumulative percentages of subjects with various minimum numbers of positive biopsies were calculated.

A countable eosinophil (one that was included in the eos/hpf counts) was defined as a cell filled with eosinophilic granules and at least 1 identifiable portion of the nucleus. We previously determined that the methods advocated by some to limit the counts to cells in which both lobes of the nucleus are visible results in a gross underestimation of tissue eosinophilia.(7) Histologic sections 5 μm thick often contain eosinophils that have been cut perpendicular to their bilobate nucleus, preventing the visualization of both lobes. Details on our approach have been presented in detail.(8)

Mast cells are more evenly distributed than the eosinophils, which are patch in gastric and duodenal mucosae, so a slightly different approach was used to count mast cells. The 5 non-overlapping hpfs were selected by starting with a full field at the left end of each biopsy fragment and then proceeding towards its right end. Countable mast cells that were stained by tryptase, with either an intact membrane or with extruding granules and a clearly recognizable cell structure. Isolated or aggregated tryptase-stained granules were not counted.

Statistical analyses

Histologic findings, endoscopic appearance, and symptoms were summarized using descriptive statistics. Pearson's correlation and the 2-sample *t* test were used to evaluate correlations and continuous variables, respectively. For all analyses, statistical significance was set at $P \leq .05$.

Supplemental References

1. FDA. Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims: Health and Human Services; 2009 [Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/patient-reported-outcome-measures-use-medical-product-development-support-labeling-claims.

2. Hirano I, Collins MH, King E, Sun Q, Abonia JP, Bonis PA, et al. Prospective evaluation of a novel endoscopic activity assessment system for eosinophilic gastritis. Gastroenterology. 2019;156:S71-2.

3. Shoda T, Wen T, Caldwell JM, Collins MH, Besse JA, Osswald GA, et al. Molecular, endoscopic, histologic, and circulating biomarker-based diagnosis of eosinophilic gastritis: Multi-site study. J Allergy Clin Immunol. 2020;145(1):255-69.

4. Reed CC, Genta RM, Youngblood BA, Wechsler JB, Dellon ES. Mast Cell and Eosinophil Counts in Gastric and Duodenal Biopsies From Patients With and Without Eosinophilic Gastroenteritis. Clin Gastroenterol Hepatol. 2020.

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

6. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol. 1999;11(10):1185-94.

7. Koutri E, Patereli A, Noni M, Gutierrez-Junquera C, Gonzalez-Lois C, Oliva S, et al. Distribution of eosinophils in the gastrointestinal tract of children with no organic disease. Ann Gastroenterol. 2020;33(5):508-15.

8. Genta RM, Sonnenberg A, Turner K. Quantification of the duodenal eosinophil content in adults: a necessary step for an evidence-based diagnosis of duodenal eosinophilia. Aliment Pharmacol Ther. 2018;47(8):1143-50.

Table S1. EG-REFS Scoring Criteria

	Fundus	Body	Antrum
Granularity	0 none 1 fine 2 course	0 none 1 fine 2 course	0 none 1 fine 2 course
Erosion/ ulceration	0 none 1 less than 5 erosions 2 5 or more erosions 3 shallow/superficial ulceration(s) 4 deep/excavated ulceration (ulceration <25% surface area of specified location) 5 deep/excavated ulceration (ulceration 25%–50% surface area of specified location) 6 deep/excavated ulceration (ulceration >50% surface area of specified location)	0 none 1 less than 5 erosions 2 5 or more erosions 3 shallow/superficial ulceration(s) 4 deep/excavated ulceration (ulceration <25% surface area of specified location) 5 deep/excavated ulceration (ulceration 25%–50% surface area of specified location) 6 deep/excavated ulceration (ulceration >50% surface area of specified location)	0 none 1 less than 5 erosions 2 5 or more erosions 3 shallow/superficial ulceration(s) 4 deep/excavated ulceration (ulceration <25% surface area of specified location) 5 deep/excavated ulceration (ulceration 25%–50% surface area of specified location) 6 deep/excavated ulceration (ulceration >50% surface area of specified location)
Raised lesion (nodularity)	0 none 1 mild (raised focal nodules) 2 severe (raised nodules with greater height from width)	0 none 1 mild (raised focal nodules) 2 severe (raised nodules with greater height from width)	0 none 1 mild (raised focal nodules) 2 severe (raised nodules with greater height from width)
Erythema	0 none 1 mild (pink) 2 severe (red/hemorrhagic)	0 none 1 mild (pink) 2 severe (red/hemorrhagic)	0 none 1 mild (pink) 2 severe (red/hemorrhagic)
Friability/ bleeding	0 none 1 mild (contact bleeding) 2 severe (spontaneous bleeding)	0 none 1 mild (contact bleeding) 2 severe (spontaneous bleeding)	0 none 1 mild (contact bleeding) 2 severe (spontaneous bleeding)
Folds	0 none 1 thickened folds	0 none 1 thickened folds	0 none 1 thickened folds
Pyloric stenosis	N/A	N/A	0 none 1 present (inability to pass diagnostic 8-10 mm endoscope)

Table S2. Characteristics of Subjects With and Without Prior History of EG/EoD

	Met EG/EoD Criteria n=72	Prior History of EG/EoD n=57	No Prior History n=15	*P* value[a]
Mean age, years (range)	42 (1874)	40 (18–68)	48 (20–74)	NS
Female sex, n (%)	43 (60%)	33 (58%)	10 (67%)	NS
White, n (%)	66 (92%)	52 (91%)	14 (93%)	NS
Weight, mean (range), kg	82 (47–171)	81 (47–171)	88 (59–136)	NS
Total symptom score at baseline, mean±SD	31±14	31±14	32±13	NS
History of asthma, allergic rhinitis, atopic dermatitis, and/or food allergy	48 (67%)	37 (65%)	11 (73%)	NS
Absolute eosinophil count				
Mean ±SD	654±951	791±1026	133±84	.016
Subjects with ≥250/μl, n (%)	45 (63%)	43 (75%)	2 (13%)	<.001
Subjects with ≥500/μl, n (%)	26 (36%)	26 (46%)	0	<.001
Prior history, n (%)				
Eosinophilic gastritis and/or duodenitis (EG/EoD)	57 (79%)	57 (100%)	0	<.001
Functional gastrointestinal disorder (irritable bowel syndrome, functional abdominal pain, functional diarrhea, or functional constipation)	24 (33%)	17 (30%)	7 (47%)	NS
Gastroesophageal reflux (GER), acid reflux, or heartburn	24 (33%)	16 (28%)	8 (53%)	NS
Peptic ulcer	9 (13%)	8 (14%)	1 (7%)	NS
Chronic gastritis/duodenitis	4 (6%)	0 (0%)	4 (27%)	<.001
Physician-guided treatment, n (%)				
Proton pump inhibitor	35 (49%)	26 (46%)	9 (60%)	NS
Diet modification	11 (15%)	9 (16%)	2 (13%)	NS
Low-dose systemic corticosteroid[b]	7 (10%)	7 (12%)	0	NS
Swallowed topical corticosteroid	7 (10%)	7 (12%)	0	NS

[a]Comparison of subjects with and without prior history of EG and/or EoD (EG/EoD). *P* values were calculated for continuous variables and for categorical variables using the 2-sample *t* test and χ^2 test, respectively.

[b]Prednisone ≤10 mg daily or equivalent as a pre-existing regimen and taken throughout the study.

NS, not significant

Table S3. Number of Positive Biopsies in Subjects With and Without History of EG/EoD

	All Subjects	History of EG/EoD	No History	*P* value[b]
Subjects with EG, n	45	38	7	
Biopsies with ≥5 positive hpfs[a], mean±SD	2.7±2.3	2.9±2.4	1.0±0.6	<.001
Biopsies with ≥1 positive hpf[a], mean±SD	4.5±2.2	4.9±2.1	2.0±1.2	<.001
Total positive hpfs[a] across biopsies, mean±SD	18.3±10.7	19.8±10.7	7.7±4.1	<.001
Subjects with EoD, n	62	50	12	
Biopsies with ≥3 positive hpfs[a], mean±SD	2.2±1.2	2.2±1.2	1.9±1.3	.449
Biopsies with ≥1 positive hpf[a], mean±SD	3.1±1.0	3.2±0.9	2.9±1.2	.477
Total positive hpfs[a] across biopsies, mean±SD	11.1±5.2	11.5±5.0	9.4±5.6	.254

[a]Positive hpf defined as ≥30 eos/hpf

[b]Comparison of subjects with and without prior history of EG/EoD. *P* values were calculated from 2-sample *t* test.

Table S4. Number of Positive Biopsies in Affected Regions

	All Subjects	Only 1 Region Affected (Stomach or Duodenum)	Both Regions Affected (Stomach + Duodenum)	*P* value[b]
Subjects with EG, n	45	10	35	
Biopsies with ≥5 positive hpfs[a], mean ±SD	2.6±2.3	2.3±1.6	2.7±2.5	.501
Biopsies with ≥1 positive hpf[a], mean ±SD	4.4±2.3	3.7±2.4	4.7±2.2	.271
Total positive hpfs[a] across biopsies, mean ±SD	17.9±10.9	15.6±9.3	18.5±11.4	.414
Subjects with EoD, n	62	27	35	
Biopsies with ≥3 positive hpfs[a], mean ±SD	2.2±1.2	1.9±1.1	2.4±1.3	.065
Biopsies with ≥1 positive hpf[a], mean ±SD	3.1±1.0	3.2±0.9	3.1±1.0	.685
Total positive hpfs[a] across biopsies, mean ±SD	11.1±5.2	7.6±2.5	8.5±3.2	.202

[a]Positive hpf defined as ≥30 eos/hpf

[b]Comparison of subjects with 1 vs both regions affected. *P* values were calculated using 2-sample *t* test

Table S5. Number of Positive Biopsies in Subjects With or Without Steroid Use

	All Subjects	Subjects Receiving Steroids[b]	Subjects Not Receiving Steroids	*P* value[c]
Subjects with EG, n	45	10	35	
Biopsies with ≥5 positive hpfs[a], mean ±SD	2.6±2.3	2.2±2.4	2.8±2.3	.475
Biopsies with ≥1 positive hpf[a], mean ±SD	4.4±2.3	4.6±2.3	4.4±2.3	.810
Total positive hpfs[a] across biopsies, mean ±SD	17.9±10.9	16.6±11.5	18.3±10.9	.669
Subjects with EoD, n	62	12	50	
Biopsies with ≥3 positive hpfs[a], mean ±SD	2.2±1.2	2.2±1.6	2.2±1.2	1.00
Biopsies with ≥1 positive hpf[a], mean ±SD	3.1±1.0	3.1±1.1	3.1±0.9	1.00
Total positive hpfs[a] across biopsies, mean ±SD	11.1±5.2	10.9±6.2	11.1±5.0	.906

[a]Positive hpf defined as ≥30 eos/hpf

[b]Systemic corticosteroids or swallowed topical steroid capsules

[c]Comparison of subjects receiving vs not receiving steroids. *P* values were calculated using the 2-sample *t* test.

Table S6. High-Powered Fields Required to Detect EG/EoD

Biopsy Type	Number of hpfs Required	Subjects with Positive Result[1]	Subjects with Negative Result[2]	True Positives[3]	False Positives	True Negatives	False Negatives	Sensitivity	Specificity	PPV	NPV
Gastric (n=88)	5	45	43	45	0	43	0	100%	100%	100%	100%
	4	45	43	45	0	43	0	100%	100%	100%	100%
	3	46	42	45	1	42	0	100%	98%	98%	100%
	2	48	40	45	3	40	0	100%	93%	94%	100%
	1	52	36	45	7	36	0	100%	84%	87%	100%
Duodenal (n=87)	3	62	62	0	0	25	0	100%	100%	100%	100%
	2	64	62	2	2	23	0	100%	92%	97%	100%
	1	64	62	2	2	23	0	100%	92%	97%	100%

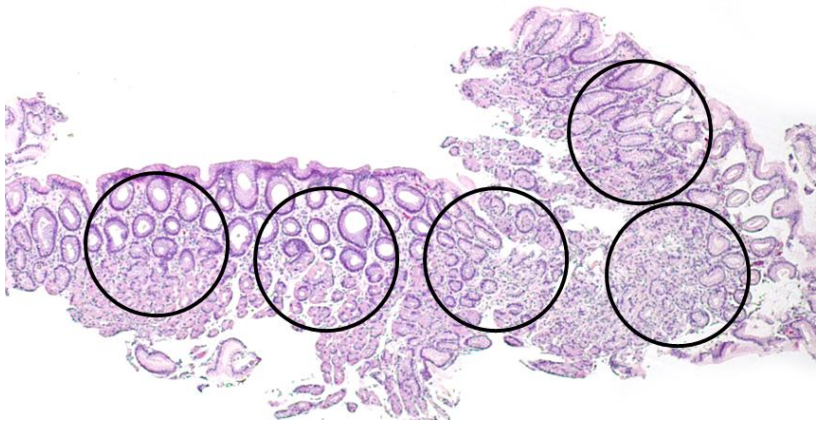
Notes: A positive result was defined as ≥30 eosinophils/high-powered field (hpf) in all hpfs specified; a negative result was defined as <30 eos/hpf in at least 1 of the specified hpfs; a gastric true positive was defined as a patient with ≥30 eos/hpf in 5 hpfs in the stomach; a duodenal true positive was defined as a patient with ≥30 eos/hpf in 3 hpfs in the duodenum.

Of the 88 symptomatic subjects biopsied, 1 did not have adequate duodenal biopsy specimens and thus could not be assessed for duodenal involvement.

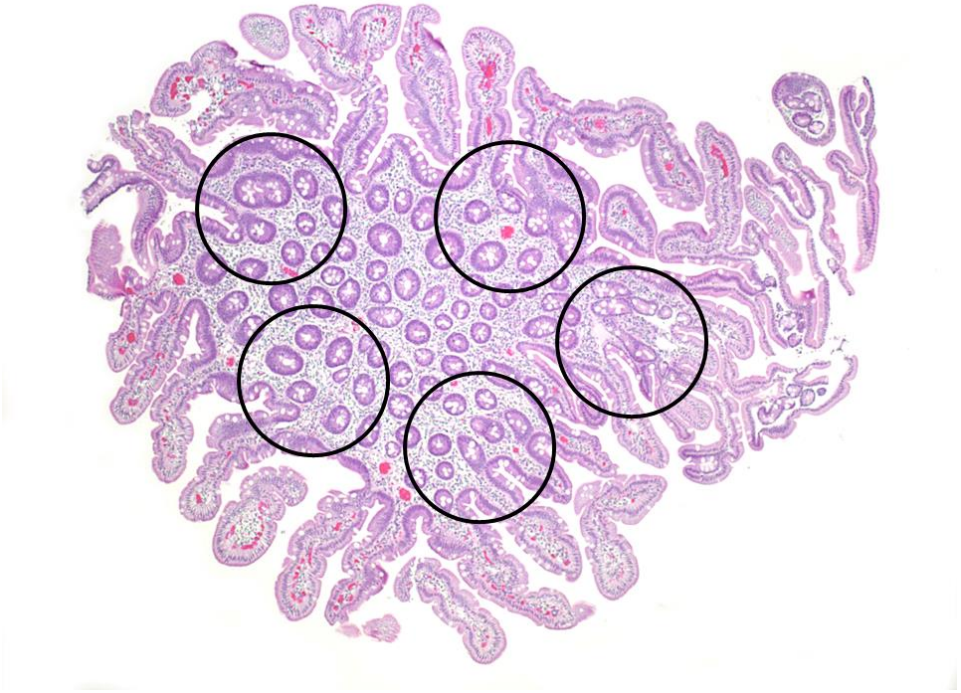
PPV, positive-predictive value; NPV, negative-predictive value

Figure S1. Representative Images of 5 Non-Overlapping Fields per Specimen

A.

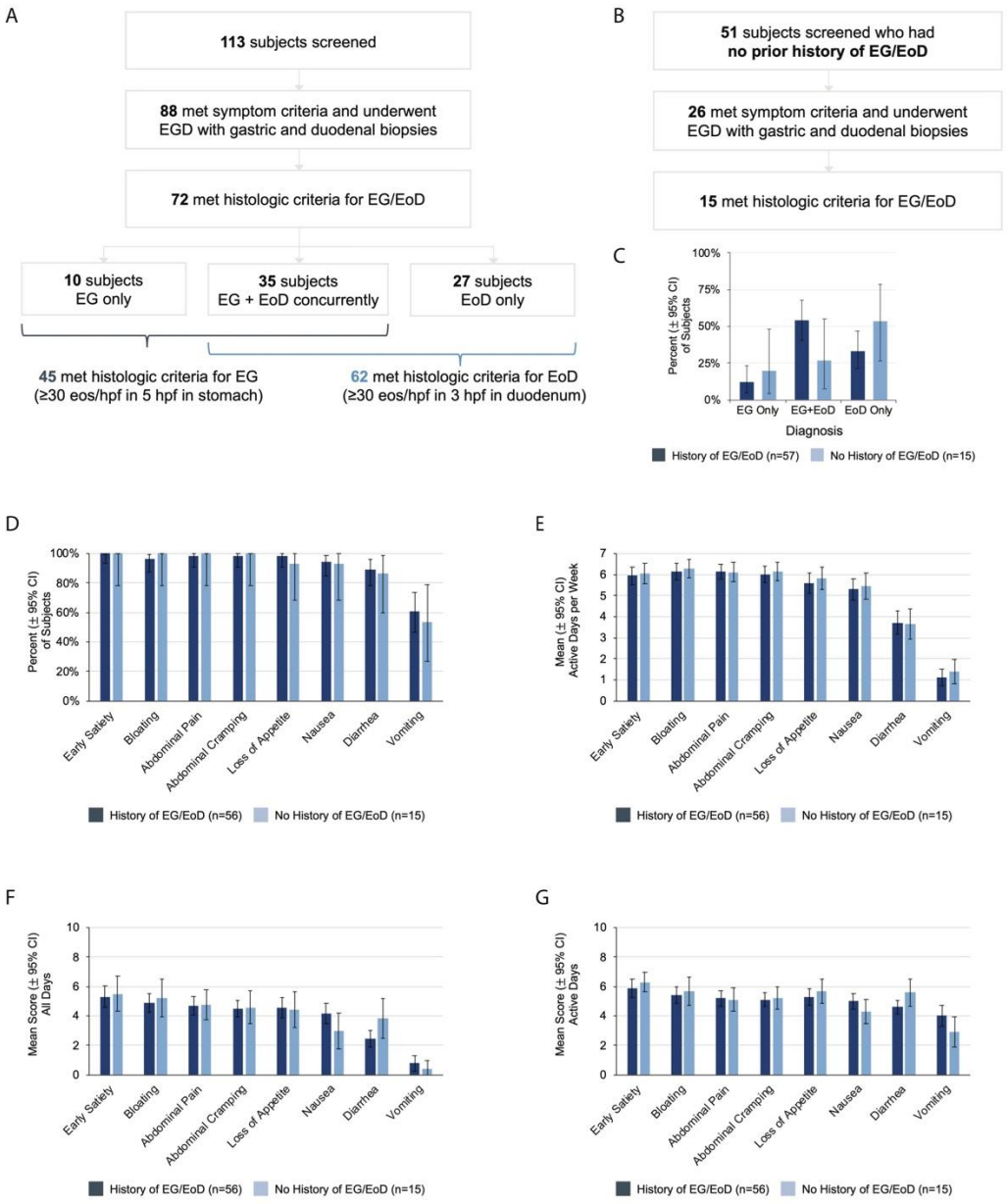


B.



(A-B) Representative images of hematoxylin- and eosin-stained, 5 µm-thick biopsy specimens from (A) gastric and (B) duodenal mucosae. Black circles indicate the 5 non-overlapping hpfs (area, 0.237 mm^2) selected from areas of greatest eosinophil density used for counting of eosinophils.

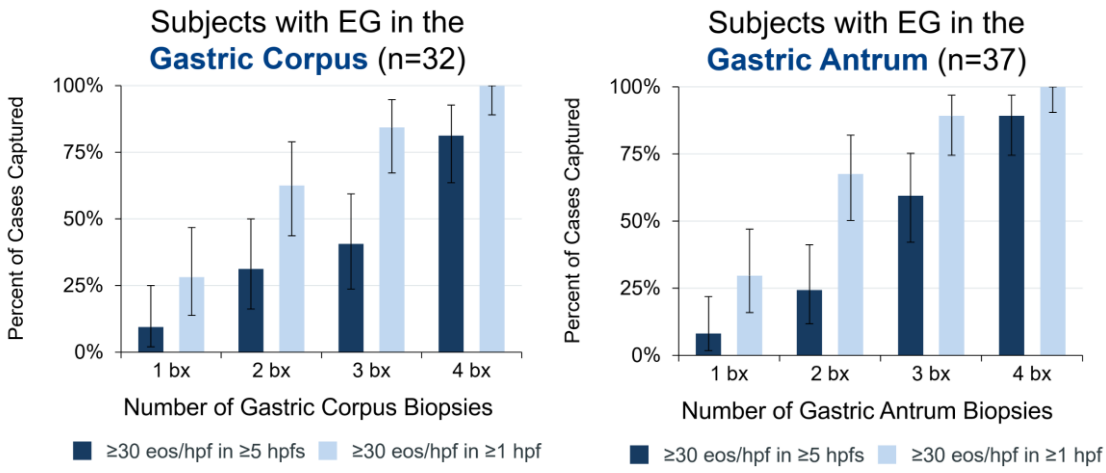
Figure S2. Study Screening and Biopsy Results and Symptom Data



(A) Study screening results. Subjects were enrolled from July 2018 through February 2019. (B) Diagnostic yield in subjects with no history of EG/EoD. (C) Distribution of subjects with EG only, EG+EoD, or EoD only in the group of subjects with prior history vs the group without prior history of EG/EoD. (D–G)Symptom presentation of subjects by prior history of EG/EoD. One subject did not complete the PRO symptomatic assessment during screening but was deemed to have moderate-to-severe symptoms, per investigator, underwent EGD with biopsy and was found to meet histologic criteria for EG and EoD. (D) Percent of subjects with each symptom at any point during screening. (E) Mean days per week each symptom was documented. (F) Mean score of each symptom calculated across subjects for all days, including days in which the symptom was not present (score=0). (G) Mean score of each symptom calculated across subjects for only the days in which the symptom was present (score>0).

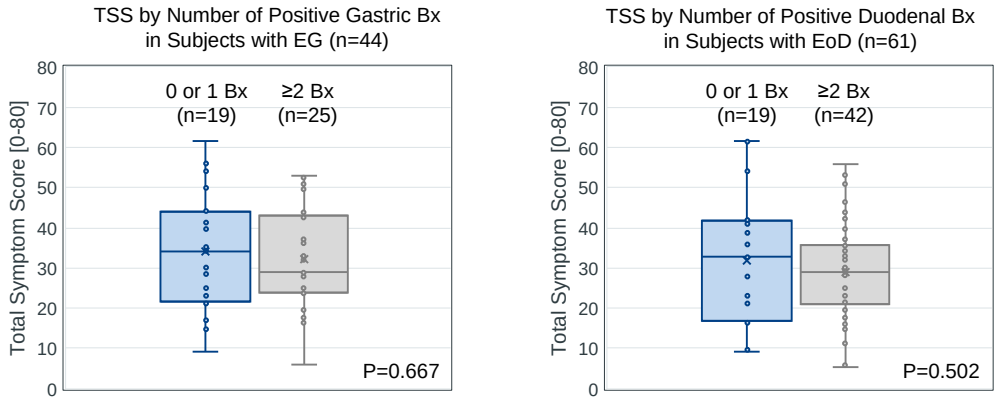
Figure S3. Number of Gastric Corpus and Antrum Biopsies Required for Diagnosis

A. **B.**



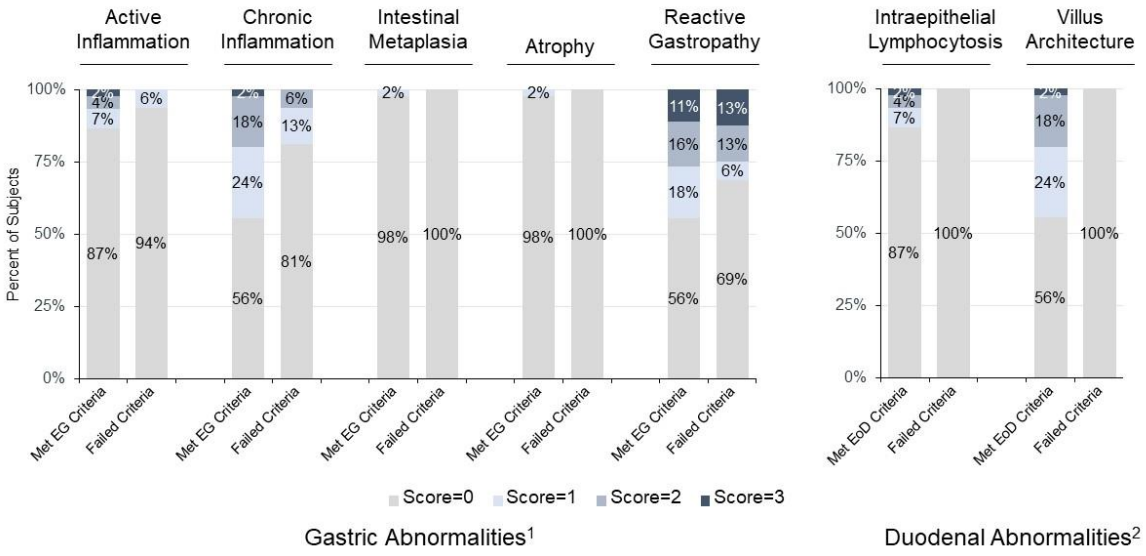
(A, B) Cumulative percent of (A) EG cases involving the gastric corpus and (B) EG cases involving the gastric antrum that would be captured by minimum number of biopsies out of a total of 4 corpus and 4 antrum biopsies collected per subject; data shown for 2 different thresholds: ≥30 eos/hpf in ≥5 or ≥3 hpfs (dark blue) and ≥30 eos/hpf in ≥1 hpf (light blue). Error bars indicate 95% CIs.

Figure S4. Total Symptom Score by Number of Positive Biopsies



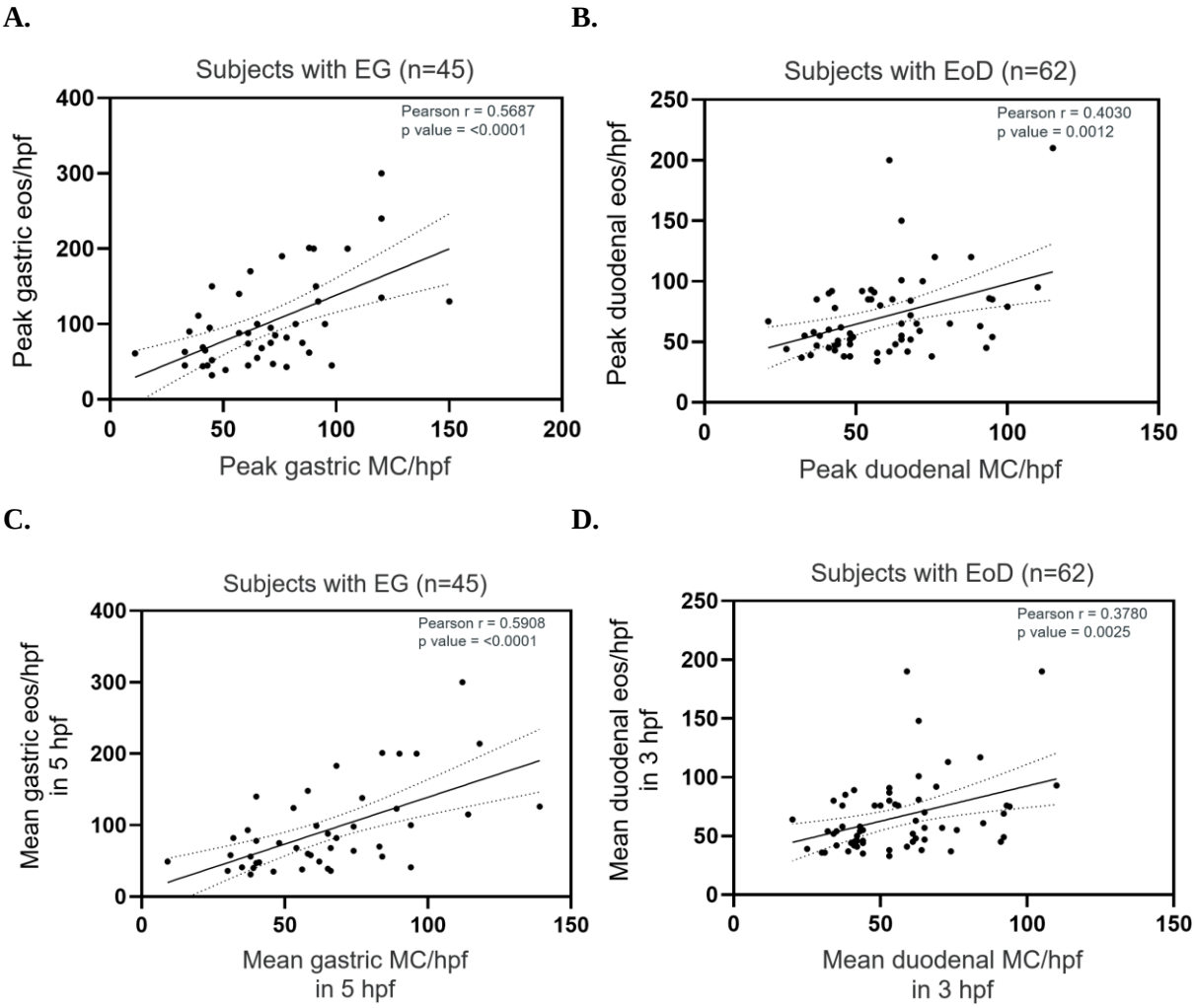
Box and whisker plots (Tukey method) comparing total symptom scores in EG/EoD subjects with 0 or 1 positive biopsies (blue icons) vs those with ≥2 positive biopsies (gray icons); a positive biopsy was defined as ≥30 eos/hpf in ≥5 hpfs in a single gastric biopsy for EG (*left*) and as ≥30 eos/hpf in ≥3 hpfs in a single duodenal biopsy for EoD (*right*). *P* values were calculated using the 2-sample *t* test.
Bx, biopsy; TSS, total symptom score

Figure S5. Gastric and Duodenal Histologic and Morphologic Abnormalities



Percentages of EG and EoD subjects with gastric and duodenal morphologic abnormalities in histopathologic evaluation (score >0 for active inflammation, intestinal metaplasia, atrophy, intraepithelial lymphocytosis, or villus architecture or if score >1 for chronic inflammation or reactive gastropathy).

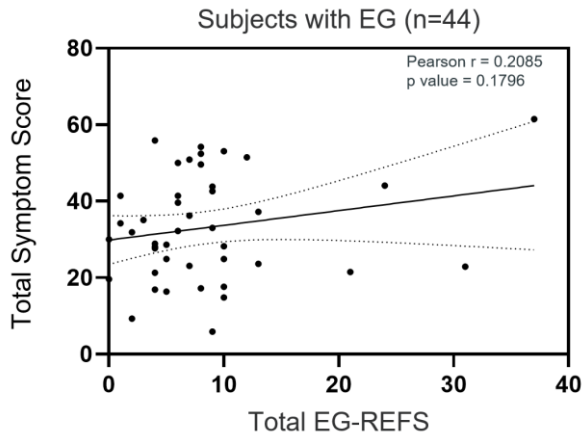
Figure S6. Analysis of Correlation Between Tissue Eosinophil and Mast Cell Counts



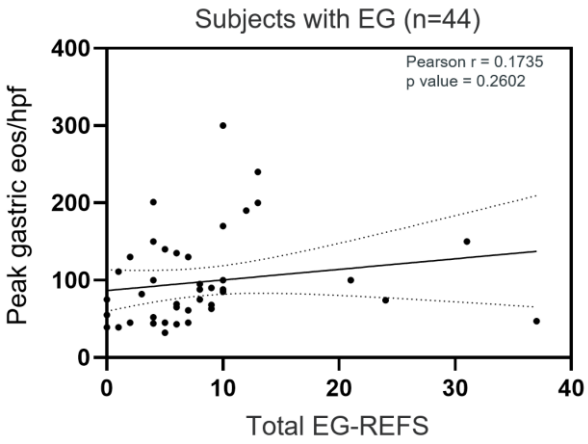
(A–D) Analyses of correlation between tissue eosinophils and mast cells (MC). (A, B) Peak eos/hpf and peak MC/hpf in (A) stomach of subjects with EG and (B) duodenum of subjects with EoD. (C, D) Mean eos/hpf and mean MC/hpf in the (C) stomach in subjects with EG and (D) duodenum in subjects with EoD. Pearson correlation coefficient (r) and *P* values are shown. Simple linear regression (solid line) with 95% CIs (dashed lines).

Figure S7. EG-REFS Correlation Analyses

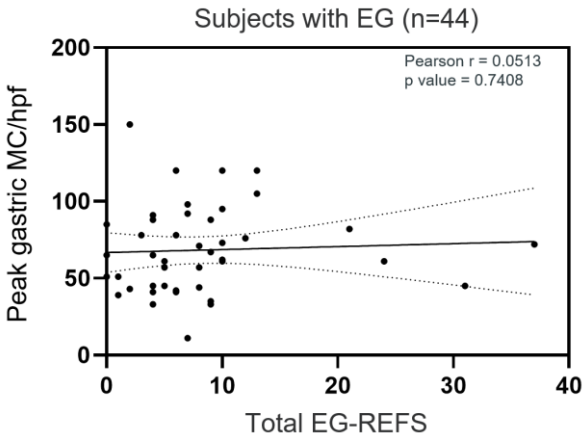
A.



B.

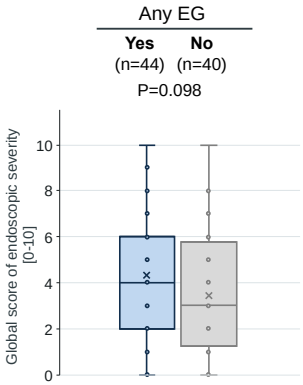


C.



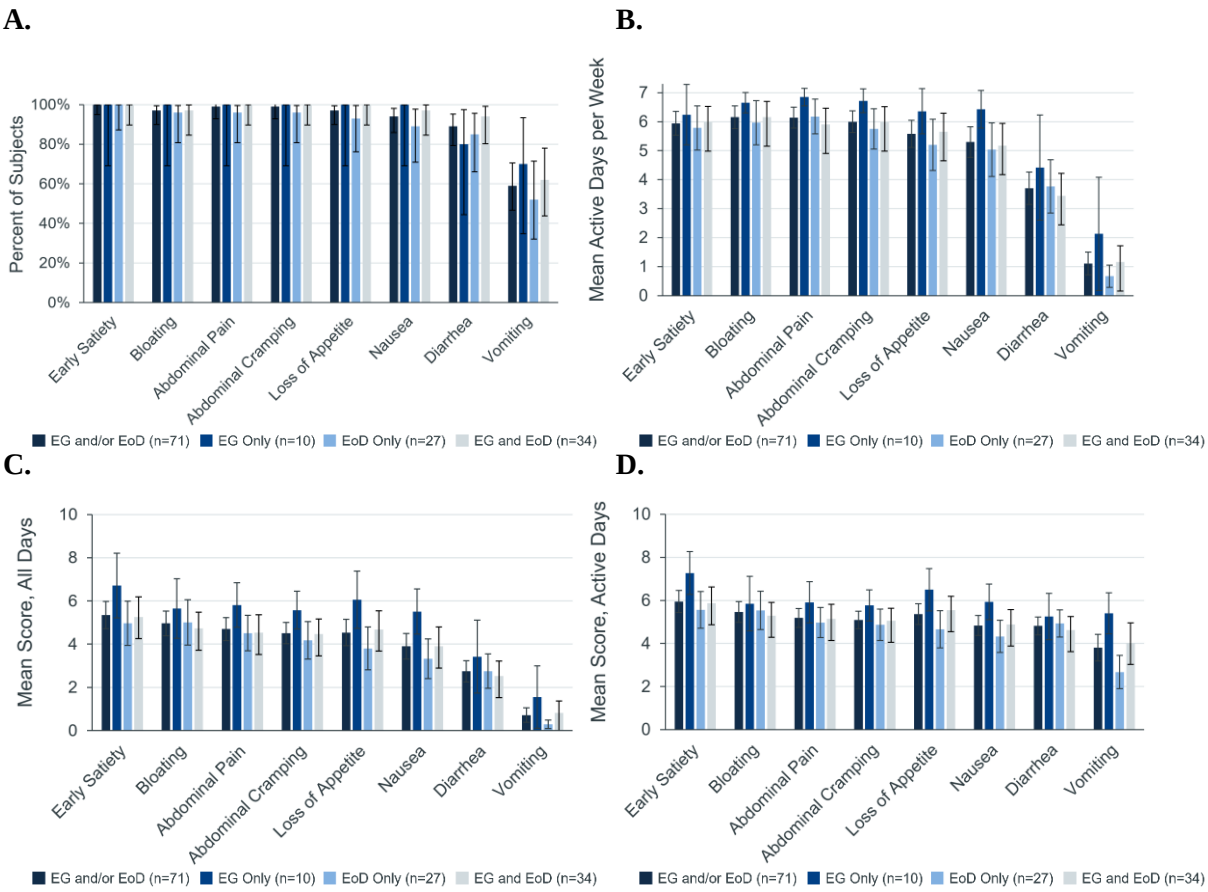
(A–C) In the subset of subjects with EG, analyses of the correlation between total EG-REFS score and (A) total symptom score, (B) peak gastric eos/hpf, and (C) peak gastric mast cells (MC)/hpf. Pearson correlation coefficient (r) and *P* values are shown. Simple linear regression (solid line) with 95% CIs (dashed lines).

Figure S8. Global Endoscopic Severity Scores in Subjects With and Without EG



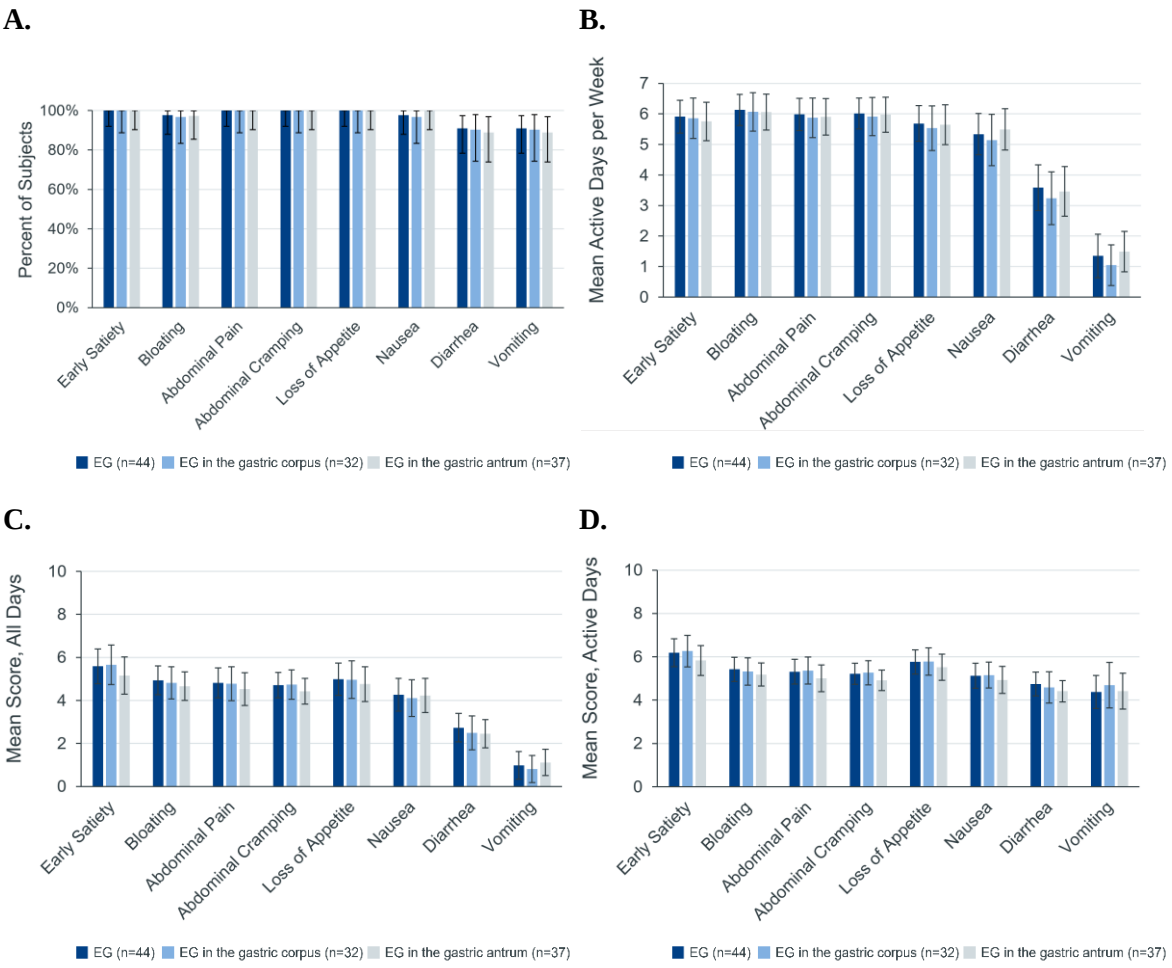
Box and whisker plots (Tukey method) comparing global score of endoscopic severity of the stomach in subjects with EG vs subjects with moderate-to-severe gastrointestinal symptoms but without EG.
P value was calculated using the 2-sample *t* test.

Figure S9. Symptoms in Mutually Exclusive Groups



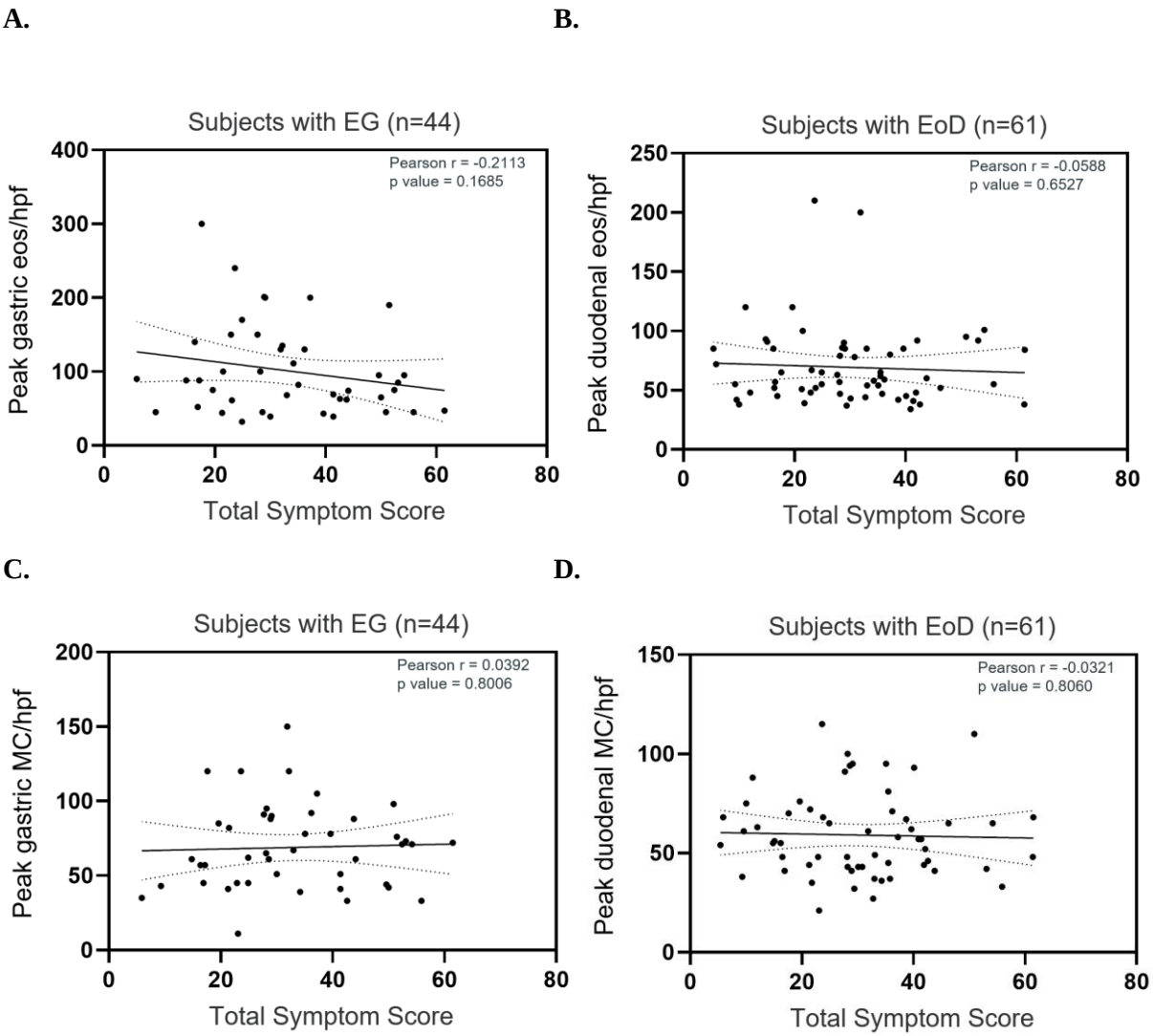
(A–D) Symptoms of mutually exclusive groups of subjects who met criteria for EG/EoD. Symptoms were assessed daily during the study screening period by PRO questionnaire. Black bars indicate all subjects with EG and/or EoD (n=71), dark blue bars indicate subjects with only EG (n=10), light-blue bars indicate subjects with only EoD only (n=27), and gray bars indicate subjects with EG plus EoD (n=34). One subject who did not complete the PRO assessment during screening but was deemed to have moderate-to-severe symptoms, per the investigator, underwent EGD with biopsy and was found to meet histologic criteria for EG and EoD. Error bars indicate 95% CIs. (A) Percent of subjects with each symptom at any point during screening. (B) Mean days per week each symptom was documented. (C) Mean score of each symptom calculated among subjects for all days, including days in which the symptom was not present (i.e., those with a score of 0). (D) Mean score of each symptom calculated among subjects for only the days in which the symptom was present (i.e., a score greater than 0).

Figure S10. Symptoms of Subjects with Gastric Corpus vs Antrum Involvement



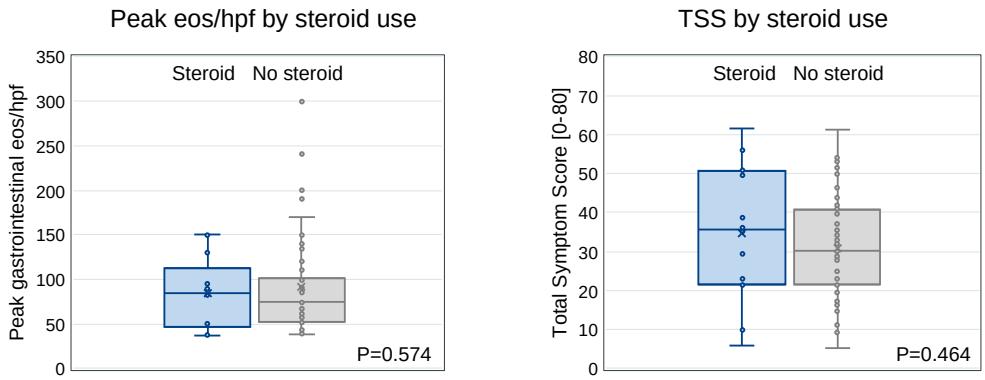
(A–D) Symptoms of subjects who met criteria for EG, assessed daily during the study screening period by the PRO questionnaire. Dark blue bars indicate all subjects with EG (n=44), light-blue bars indicate subjects with EG in the gastric corpus (n=32), and gray bars indicate subjects with EG in the gastric antrum (n=37). One subject who did not complete the PRO assessment during screening but was deemed to have moderate-to-severe symptoms, per the investigator, underwent EGD with biopsy and was found to meet the histologic criteria for EG. Error bars show 95% CIs. (A) Percent of subjects with each symptom at any point during screening. (B) Mean days per week each symptom was documented. (C) Mean score of each symptom calculated among subjects for all days, including days in which the symptom was not present (i.e., those with a score of 0). (D) Mean score of each symptom calculated among subjects for only the days in which the symptom was present (i.e., a score greater than 0).

Figure S11. Total Symptom Score Correlation Analyses



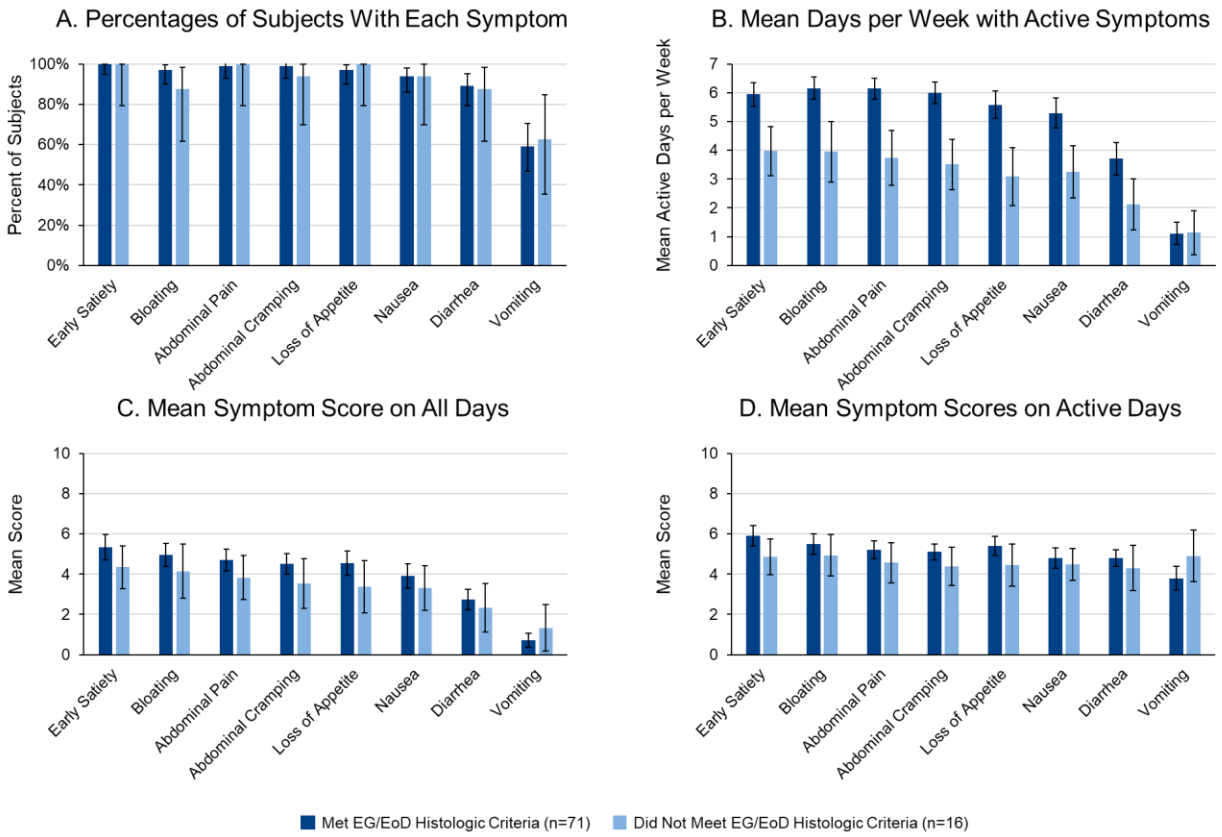
(A–C) Analyses of the correlation between total symptom score and (A) peak gastric eos/hpf in subjects with EG, (B) peak duodenal eos/hpf in subjects with EoD, (C) peak gastric mast cells (MC)/hpf in subjects with EG, and (D) peak duodenal MC/hpf in subjects with EoD. Pearson correlation coefficient (r) and *P* values are shown. Simple linear regression (solid line) with 95% CIs (dashed lines).

Figure S12. Peak Eosinophils and Total Symptom Score by Steroid Use



Box and whisker plots (Tukey method) comparing EG/EoD subjects with (blue icons) and without (gray icons) background steroid use in terms of peak gastrointestinal eos/hpf (defined as the highest count from either stomach or duodenum) (*left*) and total symptom score (TSS) (*right*). Background steroid use was defined as prednisone ≤10 mg daily, topical steroid (budesonide) capsules, or equivalent as a pre-existing regimen and taken throughout the study. *P* values were calculated using the 2-sample *t* test.

Figure S13. Symptoms in Subjects who did vs did not Meet Histologic Criteria for EG/EoD



Graphs showing percentages of subjects with each symptom (A), mean days per week with active symptoms (B), mean symptom scores for all days (C), and mean symptom scores on days with active symptoms (D) for patients who met the histologic criteria for EG/EOD (dark blue) and patients who did not met histologic criteria (light blue). Error bars indicate 95% CIs.

What You Need to Know:

BACKGROUND: Eosinophilic gastritis (EG) and eosinophilic duodenitis (EoD) are underdiagnosed. We analyzed biopsies from subjects with gastrointestinal symptoms in a randomized trial to determine rates of EG and EoD and the number of biopsies required to optimize detection.

FINDINGS: More than half of subjects (58%) with moderate–severe GI symptoms were found to have EG/EoD. Counting of eosinophils in at least 8 gastric and 4 duodenal biopsies was required to identify patients with EG/EoD.

IMPLICATIONS FOR PATIENT CARE: EG and EoD are underdiagnosed—it is important to count eosinophils in multiple gastric and duodenal biopsies from patients with moderate-to-severe gastrointestinal symptoms to identify those with EG/EoD, so they can receive appropriate treatment.