Determination of Optimal Eosinophil Thresholds for Diagnosis of Eosinophilic Gastritis and Duodenitis: A Pooled Analysis of 4 Prospective Adult Studies

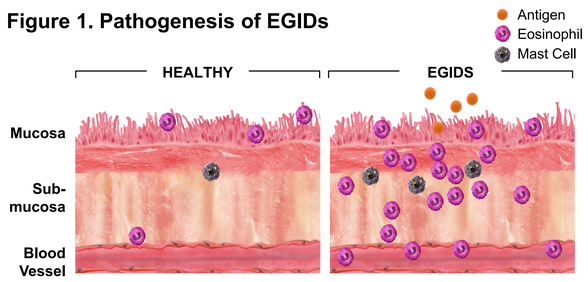
Evan S. Dellon MD MPH[1], Enoch Bortey PhD[2], Alan T. Chang[2], Craig A. Paterson MD[2], Kevin Turner DO[3], Robert M. Genta MD[4]

[1]University of North Carolina, Chapel Hill, NC; [2]Allakos Inc., San Carlos, CA; [3]UT Southwestern, Dallas, TX; [4]Baylor College of Medicine, Houston, TX

BACKGROUND

- Eosinophilic gastrointestinal disorders (EGIDs) are diagnosed based on a combination of symptoms and elevated numbers of eosinophils (eos) observed in biopsy specimens from esophagus (eosinophilic esophagitis [EoE]), stomach (eosinophilic gastritis [EoG]), and/or upper intestine (eosinophilic duodenitis [EoD])[1]

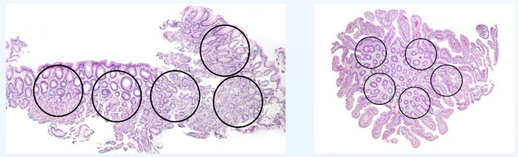
Figure 1. Pathogenesis of EGIDs



- Different eosinophil thresholds have been set for diagnosis of EoG and EoD, but there has been no consensus on the criteria[2,3,4,5,6,7]
- Eos threshold requirements for regulatory approval of therapeutics may not necessarily reflect appropriate eos thresholds in high-power fields (hpfs) used in clinical practice[8]
  - EoE example: diagnosis at 15 eos/hpf, FDA uses ≤6 eos/hpf as remission for drug approval but 0 is pathogenically normal
- Eosinophil enumeration in gastric and duodenal biopsies in some studies have required counts of eos in multiple hpfs, specifically 5 hpfs for EoG and 3 hpfs for EoD[9,10]
  - Thresholds requiring multiple hpfs may not be practical for routine clinical use

Figure 2. Representative Images of 5 Non-Overlapping Fields per Specimen as an approach to assessing counts

Gastric Mucosae **Duodenal Mucosae**



- Representative images of hematoxylin- and eosin-stained, 5 µm-thick biopsy specimens
- 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

- An evidence-based approach to establish appropriate eos thresholds for EoG and/or EoD is needed

OBJECTIVES

- To investigate optimal cutoff values for accurate detection of EoG or EoD using data from 4 prospective studies
- To explore a single peak hpf threshold indictive of EoG and EoD

METHODS

Patients

- Individual patient level data were obtained from four prospective studies: Phase 2 ENIGMA1[4], Phase 3 ENIGMA2, Prevalence Study, and one on asymptomatic controls
- The symptom entry criteria from Phase 3 ENIGMA2 was applied to identify participants from the ENIGMA1 and prevalence studies; patients were *H pylori*-negative with total symptom scores[4] (TSS6) ≥10 (weekly average referred to as symptom burden) and either abdominal pain, diarrhea, and/or nausea scores ≥3 at baseline. Controls has TSS6≤1
- Resulting patient population:
  - ENIGMA1 (n=74), ENIGMA2 (n=324), Prevalence (n=309); total N=707
  - Asymptomatic Controls (n=33)
- Biopsy results were evaluated from all participants and eos were counted[11], EoG defined as ≥30 eos/hpf in ≥5 hpfs, EoD defined as ≥30 eos/hpf in ≥3 hpfs)

Figure 3. Screening Protocol

Symptom PRO

- Patients with prior diagnosis or suspected EoG /EoD entered screening
- Patients with TSS6 ≥10 and either abdominal pain, diarrhea, or nausea ≥3 on a PRO questionnaire (Fig. 4) qualified for an upper endoscopy (EGD) with biopsy

EGD with Biopsy

- Multiple biopsies (≥12) were taken from each symptomatic patient according to a standardized protocol:
  - 8-10 gastric biopsies
  - 4-6 duodenal biopsies
  - 4-6 esophageal biopsies (only if patient had a history of EoE or if EoE features were observed during EGD)

Histologic Criteria

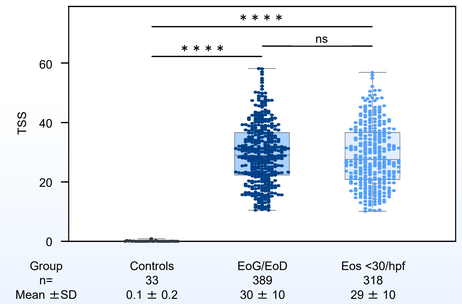
- Single pathologist evaluated stained biopsy samples and counted eosinophils (Fig. 5)
- Entry criteria
  - **≥30 eos/hpf in 5 hpfs (stomach)** and/or **≥30 eos/hpf in 3 hpfs (duodenum)**
  - No other known cause for GI symptoms or tissue eosinophilia

Statistical Analyses

- **Area under the receiver operating characteristic (AUROC)** curve analysis was performed to identify the best eos cutoff for detection of EoG and EoD
  - **ROC curves**: evaluate the predictive accuracy of a diagnostic test by calculating its sensitivity and specificity
  - **Sensitivity**: proportion of subjects who are correctly categorized as having disease (e.g., EoG) among those who truly have the disease
  - **Specificity**: proportion of subjects who are correctly categorized as not having the disease among all subjects who truly don't have the disease
- **Area under the ROC curve (AUC)** of 1.0 indicates that an instrument can discriminate perfectly between active disease and none, whereas a value of 0.5 indicates that an instrument has no discriminating power
- Two approaches were used to determine for the eos cutoff value analysis relating symptom burden to hpf(s)
  - **Estimate of Youden Index** for different threshold points
    - Measures biomarker effectiveness and enables the selection of an optimal threshold value (cutoff point) for the marker[12]
    - Youden Index = Maximum (Sensitivity + Specificity -1)
    - Index =1 indicates no false negative or false positive rate
  - **Estimate of ABS** (Sensitivity-Specificity) for different threshold points
    - Measures the absolute difference between sensitivity and specificity
    - Sensitivity, Specificity equality when ABS (Sensitivity-Specificity) = 0

RESULTS

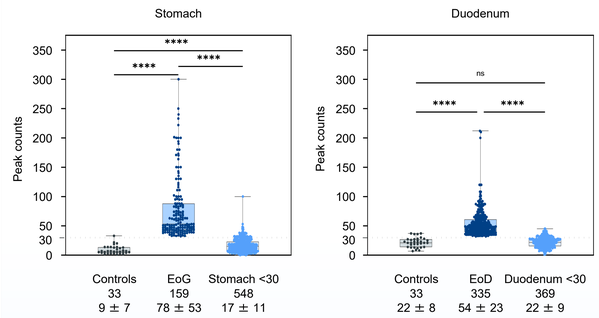
Figure 4A. Symptoms Across 3 Prospective EoG/EoD Trials



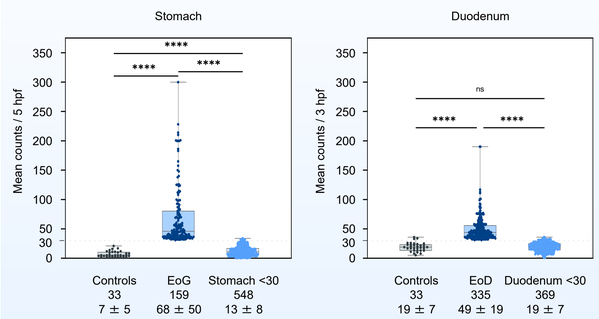
≥30 eos/hpf in 5 hpfs appear to be under-calling symptomatic patients

Figure 4B. Peak and Mean Eosinophil Counts Across 3 Prospective EoG/EoD Trials

Peak Tissue Eosinophil Counts



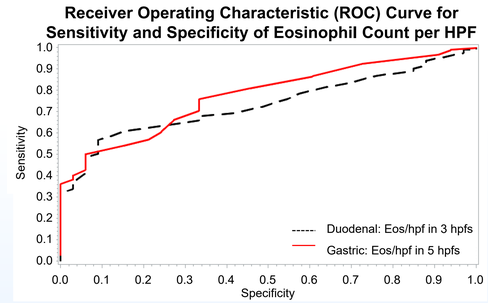
Mean Tissue Eosinophil Counts



≥30 eos/hpf in 5 hpfs appear to be under-calling symptomatic patients with gastric eos above asymptomatic control gastric counts

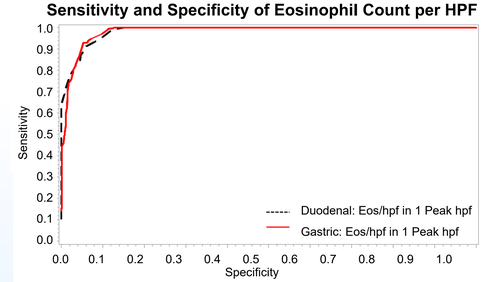
Patients meeting ENIGMA2 symptom criteria and negative for *H pylori*; two patients did not have duodenal biopsy samples
Caps, min and max; boxes, 25th and 75th percentiles; center line, median; Welch's unpaired t-test, * P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001; ns, not significant

Figure 5A. ROC Curves for 5 Gastric HPFs and 3 Duodenal HPFs



- The ROC curve areas relating symptom burden with eosinophil counts per 5 gastric hpfs and 3 duodenal hpfs were 0.78 and 0.74, respectively
- Utilizing AUC curves and two separate cutoff value analyses, optimal eos threshold is **20 eos/hpf in 5 gastric hpfs for EoG and 33 eos/hpf in 3 duodenal hpfs for EoD**

Figure 5B. ROC Curves for Single Gastric and Single Duodenal HPF



- The ROC curve areas relating disease characteristics with eosinophil counts per 1 gastric hpf and 1 duodenal hpf were 0.94 and 0.94, respectively
- Utilizing AUC curves and two separate cutoff value analyses, the optimal eos threshold for a single hpf is is **33 eos/hpf in 1 gastric hpf for EoG and 37 eos/hpf in 1 duodenal hpf for EoD**

CONCLUSIONS/DISCUSSION

- Using multiple hpfs, we identified optimal eos counts that correlate with qualifying symptom burden:
  - **EoG gastric thresholds: 20 eos/5 hpfs**
  - **EoD duodenal thresholds: 33 eos/3 hpfs**
- However, thresholds requiring counts in multiple hpfs are not practical for routine use. As an alternative, we are suggesting tissue eos thresholds in a single hpf, which revealed excellent sensitivity and specificity:
  - **EoG gastric thresholds: 33 eos/1 hpf**
  - **EoD duodenal thresholds: 37 eos/1 hpf**
- These thresholds for EoG and EoD could be used to help develop future practical histopathologic diagnostic guidelines

Reference: (1) Gonsalves, et al. Clin Rev Allergy Immuno 2019; (2) Collins, Gastroenterol Clin N Am 2014; (3) Brenner, et al. Gastro 2020; (4) Dellon, et al. NEJM 2020; (5) Reed, et al. Clin Gastroenterol Hepatol 2020; (6) Shoda, et al. J Allergy Clin Immunol 2020; (7) Dellon, et al. Clin Gastroenterol Hepatol 2021; (8) Hirano, et al. AGA EoE Guidelines. Gastro 2020; (9) Benralizumab trials: EudraCT 2021-000084-14, NCT03473977; (10) Dupilumab trial: NCT0367854; (11) Turner, et al, Am J Surg Pathol 2022; (12) Fluss, et al. Biom J 2005.

Presented at the Digestive Disease Week (DDW) Annual Meeting May 21-24, 2022