Persistent Pain in Eosinophilic Esophagitis Exhibits a Distinct Clinical and Molecular Phenotype

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Introduction: Eosinophilic Esophagitis (EoE) is a chronic, antigen-driven allergic disease of increasing prevalence that causes severe gastrointestinal pain. Many EoE patients report refractory pain despite the use of eosinophil-depleting treatment. This suggests that there is a more complex mechanism driving the symptoms of EoE that extend beyond the presence of eosinophils in the mucosa. We suggest the hypothesis that EoE patients with and without persistent pain symptoms will exhibit distinct differences in their medical records, including comorbid conditions, treatment regimens, biopsy data, and gene expression profiles.

Methods: We conducted a retrospective case-control study comparing medical records of two pediatric cohorts: a "pain positive" group (n=47) with at least two mentions of pain per year (EoE+), and a "pain negative" group (n=47) with no mentions of EoE-specific pain (EoE-). These cohorts were selected from Cincinnati Children's Hospital Medical Center electronic medical records utilizing a Named Entity Recognition (NER) algorithm. We extracted data related to patient demographics, treatment regimen, comorbidities, and biopsy findings. Results were compared between groups using the Chi-squared test and Fisher's exact test. Immunofluorescence microscopy was used to label neurons with □III-tubulin staining in available patient biopsies from the same cohorts, allowing comparison of nerve densities in each group. Sub-cohorts of 15 patients from both the EoE+ and EoE- groups with available esophageal biopsy RNA were chosen for gene expression analysis. Gene expression data was analyzed via quantitative PCR, focusing on genes known to play roles in EoE pathophysiology or neuroimmune signaling, including *Alox15, CCL26, TAC1,* and *TRPV1*. The Mann-Whitney t-test was used for statistical analysis.

Results: On average, EoE+ patients were first evaluated for EoE at an older age (p=0.0122). There were no differences between groups based on sex, length of care, race, or ethnicity. Biopsy evaluation showed no differences between groups in maximum number of eosinophils on first or most recent endoscopy but revealed that 55% of the EoE+ patients were in histological remission (<15 eosinophils/hpf). EoE+ patients exhibited a higher percentage of multimodal treatment, requiring pharmacologic and food interventions (p=0.0069), and more commonly had their treatment regimen changed (p=0.0269). EoE+ patients exhibited increased levels of co-morbid conditions, including asthma (p=0.0217) and hypermobility (p=0.0271). Immunofluorescent analysis revealed higher nerve density in EoE+ biopsies (p=0.0315). EoE+ patients showed lower expression of the inflammatory mediator *Alox15* (p=0.002881), even in remission (p=0.0001).

Conclusion: EoE patients who experience refractory pain symptoms are first evaluated at an older age, receive more complex treatment regimens, exhibit higher frequency of comorbid diseases, display an increased nerve density in the esophageal epithelium, and express lower

levels of *Alox15*, a mediator of inflammatory pain. These findings highlight that persistent pain in pediatric EoE patients is associated with distinct clinical and molecular features beyond eosinophilic inflammation alone.

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