Utility of 15(S)-HETE as a Serological Marker for Eosinophilic Esophagitis

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The pathogenesis of eosinophilic esophagitis (EoE) involves Th2-mediated eosinophil recruitment and degranulation into the esophagus. However, measuring serum Th2 cytokines, eosinophils, and eosinophil-derived products does not reliably distinguish EoE from control populations. Non-invasive methods to diagnose EoE are lacking. We evaluated the diagnostic value of a novel candidate biomarker of EoE: 15(S)-hydroxyeicosatetraenoic acid (HETE). We used immunoassay to measure 15(S)-HETE and cytokine profiles in patients undergoing endoscopy with known or suspected EoE. 31 subjects were enrolled, 16 with EoE, and 15 with an alternate diagnosis. 15(S)-HETE was elevated in the EoE group compared to non-EoE group. The sensitivity and specificity of 15(S)-HETE to be used as a non-invasive marker is 50% and 80%, respectively. 15(S)-HETE may aid in the diagnosis of EoE.

Eosinophilic esophagitis (EoE) is a chronic, allergen-mediated, inflammatory condition affecting the esophagus1. Its prevalence has been increasing, and has been recently estimated at 56.7/100,0002. In teenagers and adults, EoE typically presents with dysphagia, heartburn, or food impaction, whereas toddlers and young children tend to present with nonspecific symptoms such as feeding intolerance, growth failure, or abdominal pain.

Diagnosis of EoE in all age groups is complicated by clinical and histologic overlap with other gastrointestinal conditions, particularly gastroesophageal reflux disease (GERD). Distinguishing these disorders is invasive, expensive, and time-consuming. Once a diagnosis of EoE is established, monitoring treatment efficacy poses similar difficulties. There is little correlation between symptom severity and esophageal inflammation, so endoscopy is required3. Patients may benefit from reliable and non-invasive methods to diagnosis and monitor EoE.

Efforts have usually focused on identifying peripheral blood markers of EoE. A subset of EoE patients have increased peripheral absolute eosinophilia counts (AEC), compared to controls3,4, though AEC alone does not reliably differentiate these populations. Eosinophil-specific mediators, such as eosinophil-derived neurotoxin and eotaxin-3 are increased in the plasma of EoE subjects with active disease, and correlate with eosinophil infiltration into the esophagus4. EoE is recognized as a Th2-predominant systemic inflammatory process. Th2 cytokines such as IL-5 and IL-13 are increased in the plasma of EoE subjects compared to controls5–8. While these studies provide details about the pathogenesis of EoE, none have identified a simple blood test which can reliably diagnose EoE. Endoscopy with biopsy remains the gold standard for diagnosing and monitoring EoE disease activity.

Recent evidence suggested a role for arachidonate 15-lipoxygenase (ALOX15) in EoE. ALOX15 is up-regulated in esophageal mucosal biopsies of EoE patients compared to GERD patients and healthy controls9–11. Similarly, esophageal squamous epithelial cells of EoE patients, but not controls, are diffusely positive on immunostaining for ALOX159. Staining for ALOX15 may be helpful in equivocal cases of esophageal eosinophilia, where the diagnosis of EoE is unclear12. ALOX15 is mainly expressed in cytosol and plasma membrane in squamous cells of EoE mucosa10 and bronchial epithelial cells after IL-13 exposure11. So far, there is no evidence demonstrating that ALOX15 can be secreted into circulation. However, 15(S)-hydroxyeicosatetraenoic acid (15(S)-HETE), a metabolite of ALOX15 and detectable in peripheral blood14, 15, could be a good indicator of EoE presence and/or severity. Detectable 15(S)-HETE have been shown in the urine and its diagnostic utility was proposed for patients with Zellweger syndrome15. 15(S)-HETE appeared to be involved in asthma, an atopic...
condition like EoE\(^a\). Severe asthmatics had higher levels of 15(S)-HETE in bronchoalveolar lavage samples compared to mild or moderate asthmatics and healthy controls\(^b\).

We hypothesized that (1) 15(S)-HETE would be elevated in the sera of EoE patients compared to those without EoE; (2) that 15(S)-HETE, in combination with peripheral eosinophil count and Th2 cytokines typically found in EoE, reliably distinguish EoE from other conditions.

Materials and Methods
The study design was reviewed and approved by Investigational Review Board (IRB) of Lifespan/Rhode Island Hospital (Providence, RI). All methods were performed in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all participants and/or their legal guardians.

Subject selection. Subjects were recruited from the Division of Pediatric Gastroenterology at Hasbro Children’s Hospital, Warren Alpert Medical school of Brown University (Providence, RI), a tertiary care center located in an urban setting. Over a 12-month period, patients undergoing upper endoscopy with known or suspected EoE were screened.

Inclusion criteria included undiagnosed children with symptoms and medical history concerning for EoE. Symptoms considered suspicious for EoE included epigastric abdominal pain, heartburn, dysphagia, food impaction, vomiting, or failure to thrive. We also included known EoE patients who had not been treated with topical or systemic steroids during the 8 weeks prior to endoscopy, and those on dietary-elimination regimens for whom food re-introduction endoscopy was being performed. Exclusion criteria included patients with comorbid gastrointestinal or autoimmune diseases such as inflammatory bowel disease, celiac disease, Helicobacter gastritis, and thyroid disease.

We enrolled 31 participants. Patients or their legal guardians provided written informed consent. Pertinent medical information including demographics, concomitant medications, family and participant medical history was collected.

Participants were categorized into two groups: (1) EoE or (2) non-EoE. Diagnosis of EoE was made according to 2013 census guidelines\(^c\). This included at least 8 weeks of proton-pump-inhibitor (PPI) administration prior to a diagnostic endoscopy to exclude PPI-responsiveness. In instances in which PPI responsiveness was not evaluated (N = 5), EoE diagnosis was supported by the presence of the clinical and pathologic findings typical for this condition.

Peripheral Absolute Eosinophil Counts (AECs) and Cytokine and 15(S)-HETE immunoassays. Peripheral blood was drawn from each participant just prior to endoscopy. A routine hematology complete blood count (CBC) was performed on each sample. The absolute eosinophil count (AEC) was extracted from the CBC report. The remaining plasma was immediately aliquoted and kept in –70°C for batch immunoassays. Assays were performed in triplicate and a standard curve was generated for each analyte. Cytokine assays were carried out on BioPlex 200 (Luminex, Austin TX), a multiplex bead-based immunoassay testing platform using a custom made 11-plex sandwich capture assay kit developed previously\(^d\). IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, gamma interferon (IFN-\(\gamma\)), and tumor necrosis factor alpha (TNF-\(\alpha\)) were the cytokines tested.

15(S)-HETE assay was carried out on a Synergy\(^\text{TM}\) H4 Hybrid Microplate Reader (BioTek, Winooski, VT) using an 15(S)-HETE EIA Kit (Item No. 534721) from Cayman Chemical Company (Ann Arbor, MI) following manufacturer protocol. Included in the Kits were plates pre-coated with mouse anti-rabbit IgG, a rabbit polyclonal antibody specific to 15(s)-HETE and a 500 ng/ml standard based upon which standard curves were generated to quantitate individual sample. The detect limit was approximately 170 pg/ml. Linear range was from 170–5000 pg/ml. Samples whose original concentrations falling out of the higher end of the linear range was diluted and re-assayed. The wavelength to detect the absorbance was set at 410 nm.

Endoscopic Examination and Histological Evaluation of Endoscopic Biopsies. A standard upper endoscopy on was performed on each patient. At minimum, two biopsies from the proximal and distal esophagus were obtained for the study. Per routine practice, biopsies were also obtained from duodenum and stomach to exclude other gastrointestinal pathology. Histological assessments were conducted by the study pathologists (SL, MR) to determine eosinophil counts. The maximal eosinophil count in one high power field from proximal and distal biopsies was used in this study for each patient.

Statistical analysis. Data were analyzed with JMP Pro 13.0 (SAS Institute, Cary, NC). Student’s t-test was used to compare continuous variables (two-tail). Chi-square test was used to compare categorical variables and when the cell numbers were small, Fisher’s exact test was used. Receiver operating characteristic (ROC) analysis was performed based on logistic regression modeling. Cut-off value for each parameter was based on the threshold level in logistic regression when the sum of sensitivity and specificity was the largest. Spearman correlation was used to evaluate association between two continuous variables. Results were considered significant for P values less than 0.05.

Results
Subject characteristics. A total of 16 EoE patients and 15 non-EoE patients were enrolled in the study. Patient characteristics and their correlations with EoE status were summarized in Table 1. The non-EoE patients included 10 cases of functional dyspepsia, 3 cases of GERD, and 2 cases of globus. There was no age difference between EoE and non-EoE patients. There was a male predominance in EoE patients (73.68%). Symptomatically, food impaction was reported by EoE patients more often than non-EoE patients (25% vs 0%, \(p = 0.1012\)), while non-EoE patients reported more heartburn (53.3% vs 25%, \(p = 0.1030\)). Endoscopically, presence of furrows was significantly more frequently observed in EoE patients than in non-EoE patients (93.8% vs 26.7%, \(p = 0.0002\))
and overall abnormal findings were significantly higher in EoE patients than in non-EoE patients (93.8% vs 40%; p = 0.0021). EoE patients reported significantly more atopy than non-EoE patients (93.8% vs 60.0%, p = 0.0373).

Microscopically, biopsies from EoE patients had significantly higher intraepithelial eosinophil densities than those from non-EoE patients (49.8/hpf vs 2.6/hpf, p < 0.0001). EoE biopsies more commonly had basal cell hyperplasia and subepithelial fibrosis (p = 0.0373 and 0.0068, respectively).

Peripheral 15(S)-HETE, absolute eosinophil count, and cytokines in EoE and non-EoE subjects.

A 2.4-fold elevation of peripheral 15(S)-HETE level was observed in EoE patients (p = 0.2131; Table 1 and Fig. 1A). There were significant increases of peripheral absolute eosinophil count in EoE patients compared to non-EoE patients (3.1-fold; p = 0.0291; Table 1 and Fig. 1A). However, 9 out of 16 EoE patients and 15 out of 15 non-EoE patients had normal AEC based on a reference range of 0.0–0.5 K/µl.

| Clinical characteristics and analytes | EoE (n = 16) | Non-EoE (n = 15)* | P-value** |
|--------------------------------------|-------------|------------------|----------|
| Mean age at biopsy (±SEM)            | 11.2 ± 1.3  | 12.1 ± 1.3       | 0.6641   |
| Male subjects (n, %)                 | 14 (73.68)  | 5 (26.32%)       | 0.0014   |
| Symptoms                             |             |                  |          |
| Vomiting (n, %)                      | 5 (31.3)    | 6 (40.0)         | 0.6107   |
| Dysphagia (n, %)                     | 10 (62.5)   | 8 (53.3)         | 0.3076   |
| Abdominal pain (n, %)                | 7 (43.8)    | 11 (73.3)        | 0.0921   |
| Food impaction (n, %)                | 4 (25.0)    | 0 (0.0)          | 0.1012   |
| Heartburn (n, %)                     | 4 (25.0)    | 8 (53.3)         | 0.1030   |
| Failure to thrive (n, %)             | 2 (12.5)    | 3 (20.0)         | 0.5698   |
| Abnormal EGD findings (n, %)         | 15 (93.8)   | 6 (40.0)         | 0.0021   |
| Esophageal erythema (n, %)           | 4 (24.0)    | 3 (20.0)         | 0.7389   |
| Rings (n, %)                         | 2 (12.5)    | 0 (0.0)          | 0.4839   |
| Ridging (n, %)                       | 3 (18.8)    | 1 (6.7)          | 0.3052   |
| Furrows (n, %)                       | 15 (93.8)   | 4 (26.7)         | 0.0002   |
| White exudates (n, %)                | 7 (43.8)    | 2 (13.3)         | 0.0564   |
| Erosions (n, %)                      | 5 (31.3)    | 2 (13.3)         | 0.2265   |
| Presence of any atopy (n, %)         | 15 (93.8)   | 9 (60.0)         | 0.0373   |
| Rhinitis (n, %)                      | 11 (68.8)   | 7 (46.7)         | 0.2113   |
| Dermatitis (n, %)                    | 6 (37.5)    | 4 (26.7)         | 0.5179   |
| Food allergy (n, %)                  | 9 (56.3)    | 3 (20.0)         | 0.0659   |
| Asthma (n, %)                        | 8 (50.0)    | 4 (26.7)         | 0.1794   |

| Histological findings               |             |                  |          |
|--------------------------------------|-------------|------------------|----------|
| Max eosinophil count*** (±SEM)       | 49.8 ± 6.4  | 0.8 ± 0.5        | <0.0001  |
| Basal cell hyperplasia (n, %)        | 7 (43.8)    | 1 (6.7)          | 0.0373   |
| Submucosal fibrosis (n, %)           | 7 (43.8)    | 0 (0.0)          | 0.0068   |
| HETE (pg/ml, ±SEM)                   | 1378.2 ± 451| 5755.9 ± 915     | 0.2131   |
| AEC (K/µl, ±SEM)                     | 0.52 ± 0.15 | 0.17 ± 0.032     | 0.0291   |

| Th2 cytokines (pg/ml, ±SEM)          |             |                  |          |
|--------------------------------------|-------------|------------------|----------|
| IL-4                                 | 632.5 ± 213.9| 229.4 ± 74.8     | 0.0967   |
| IL-5                                 | 59.1 ± 17.7 | 17.4 ± 8.7       | 0.0583   |
| IL-6                                 | 218.1 ± 76.8| 53.4 ± 24.7      | 0.0587   |
| IL-10                                | 230.8 ± 62.8| 67.4 ± 28.1      | 0.0311   |
| IL-13                                | 523.3 ± 156.6| 218.4 ± 65.3    | 0.1047   |

| Other cytokines (pg/ml, ±SEM)        |             |                  |          |
|--------------------------------------|-------------|------------------|----------|
| IL-1                                 | 523.0 ± 142.2| 270.6 ± 89.1     | 0.2430   |
| IL-2                                 | 63.2 ± 37.5 | 19.3 ± 16.4      | 0.2666   |
| IL-8                                 | 7.7 ± 3.7   | 3.2 ± 0.84       | 0.3684   |
| IL-12                                | 523.6 ± 249.3| 113.9 ± 49.5    | 0.1586   |
| TNF-α                                | 297.9 ± 79.8| 136.0 ± 42.4     | 0.1982   |
| IFN-γ                                | 2017.3 ± 696.2| 1060.0 ± 386.4 | 0.2165   |

Table 1. Patient characteristics and serological analyte levels. EoE: eosinophilic esophagitis; SEM: standard error of mean; EGD: esophagogastroduodenoscopy. *Non-EoE patients include 10 cases of functional dyspepsia, 4 cases of GERD, and 2 cases of globus. **P-value: 2-tail t-test for continuous variables; “W” indicates Wilcoxon nonparametric test if the data distribution is not Gaussian; chi-square for categorical variables; “F” indicates 2-tail Fisher’s exact test when the cell number is too small for chi-square. ***Maximal eosinophil count was determined from the high-power field with the highest count.
Peripheral Th2-related cytokine elevations (2.4–4.1-fold; \( p = 0.0311–0.1047 \)) were observed in EoE patients compared to non-EoE patients (Fig. 1B) including IL-10 significantly elevated in EoE patients (3.4-fold; \( p = 0.0311 \)). Of the cytokines not directly involving Th2 activation, the fold changes ranged from 1.9 to 4.6 (\( p = 0.1586–0.3584 \); Table 1 and Fig. 1C).

While no significant association existed between 15(S)-HETE and peripheral AEC (Spearman \( \rho = 0.3306; p = 0.744 \)), AEC was significantly associated with all the Th2 cytokines, IL-12 and TNF-\( \alpha \) (Table 2). All cytokines were not significantly associated with 15(S)-HETE (Table 2).

Peripheral 15(S)-HETE levels and tissue histology in EoE and non-EoE patients. We assessed the relationship between peripheral 15(S)-HETE levels and histologic characteristics of esophageal biopsies. The correlation between 15(S)-HETE levels and peak eosinophil count was significant (Spearman \( \rho = 0.3635; p = 0.0444 \); Table 2). There was significant difference in 15(S)-HETE levels among participants with esophageal fibrosis (\( P = 0.0074 \)) while no difference was seen with respect to basal cell hyperplasia (\( P = 0.6569 \)).

Utilities of 15(S)-HETE, absolute eosinophil count and cytokines in EoE diagnosis. Receiver operating characteristic (ROC) curves were constructed to investigate the use of peripheral 15(S)-HETE, AEC and cytokines. 15(S)-HETE alone yielded an area under curve (AUC) of 0.633 for the diagnosis of EoE with sensitivity of 50%, specificity of 80% and accuracy rate of 64.5% in this cohort when the cut-off value was set at 7500 pg/ml (Table 3 and Fig. 2). AEC alone yielded an AUC of 0.773 for diagnosis of EoE with sensitivity of 53.3%, specificity of 93.3% and accuracy rate of 71% with cut-off value set at 0.4 k/µl. Individual cytokines related to Th2 activation yielded AUC values ranging from 0.671 to 0.7292 with sensitivities from 43.8% to 62.5%, specificities from 80% to 93.3%, and accuracy rates from 64.5% to 74.2% (Table 3). When 15(S)-HETE and AEC were combined in one model, the AUC increased to 0.7822. When AEC and Th2 cytokines were combined, the AUC became 0.8489. Adding 15(S)-HETE to AEC and Th2 cytokines improved the AUC to 0.8622 (Fig. 2A,B). When AEC and all the cytokines were combined, the AUC was 0.9511; addition of 15(S)-HETE improved the AUC to 0.9556 (Fig. 2A,B).

Discussion

Diagnosis and management of EoE is complicated by a lack of reliable peripheral biomarkers of disease activity. Our goal was to determine if plasma 15(S)-HETE, a downstream metabolite of ALOX15, can be used as non-invasive markers for EoE. We hypothesized that 15(S)-HETE, in combination with Th2 cytokines, may distinguish EoE from non-EoE patients.

We found that 15(S)-HETE levels were increased 2.4-fold in the plasma of the EoE group compared to the non-EoE controls. To our knowledge, this finding has not been previously reported in the literature. Its significance is however unclear, although we speculate that 15(S)-HETE may play a role in the pathogenesis of EoE. There is conflicting evidence on whether 15(S)-HETE exhibits pro- or anti-inflammatory properties. 15(S)-HETE expression of ALOX15 in mast cells, monocytes, and epithelial cells, it is likely that these contribute as well5,24–26.

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In our study, Th2 cytokines IL-10 correlated with levels (\( p = 0.0311 \)). IL-5 and IL-6, also members of Th2 cytokine, had weak correlation with 15(S)-HETE (\( p = 0.0583 \) and 0.0587, respectively). The relationship between these proteins is unclear, as it pertains to the pathogenesis of EoE. Th2 cytokines have been shown to stimulate ALOX15 activity16,19. In EoE, we suspect that Th2 cytokines mobilize ALOX15-expressing cells, which in turn generate 15(S)-HETE. We found a significant correlation between AEC and 15(S)-HETE. As such, we presume eosinophils are primarily responsible for the pool of circulating 15(S)-HETE. Since Th2 cytokines also induce expression of ALOX15 in mast cells, monocytes, and epithelial cells, it is likely that these contribute as well5,24–26.

Serum IL-1, IL2, IL-8, IL-12, TNF-\( \alpha \) and IFN-\( \gamma \), typically associated with Th1 activation, were markedly elevated in the EoE group although statistical significance was not obtained. Th1 activation in esophageal biopsies of EoE subjects has been shown in previous studies5,27,28. However, our finding of these TH1 cytokines in the serum of EoE patients is novel. In mice, HETE proteins have been shown to stimulate macrophage production of TNF-\( \alpha \). Similarly, human studies demonstrate increased mononuclear cell TNF-\( \alpha \) production among...
participants with variations in HETE-related genes\(^{30}\). Together, these findings suggest a relationship among 15(S)-HETE and mononuclear cell production of Th1 cytokines in the pathogenesis of EoE. The participation of Th1 pathway in EoE is consistent with our AUC study where AUC values increased if all the cytokine data were incorporated in the analysis compared with Th2 cytokines alone.

There are limitations in our study. Since there is no gold standard to diagnose EoE, it is possible that we misclassified some of our enrollees. PPI-responsiveness in 5 of the 15 subjects in the EoE group was not assessed.

**Figure 1.** (A) Peripheral 15(S)-HETE level and absolute eosinophil count (AEC) in EoE and non-EoE patients. (B) Peripheral Th2 cytokine levels in EoE and non-EoE patients. (C) Peripheral non-Th2 cytokine levels in EoE and non-EoE patients. Horizontal line indicates level of mean. Y-axis is in log-scale if a normal distribution is achieved after log-transformation.
Table 2. Spearman correlation of 15(S)-HETE and peripheral AEC with cytokines. *Cytokines involving Th2 pathway.

| Correlation       | 15(S)-HETE | Peripheral AEC |
|-------------------|------------|----------------|
|                   | Spearman p | P-value        |
|                   | Spearman p | P-value        |
| 15(S)-HETE        | N.A.       | 0.3306         |
|                   |            | 0.0744         |
| Peak eosinophil  | 0.3635     | 0.0444         |
| count in esophagus| 0.6272     | 0.0002         |
| IL-4*             | 0.2725     | 0.1381         |
|                   | 0.4495     | 0.0127         |
| IL-5*             | 0.2492     | 0.1763         |
|                   | 0.4304     | 0.0176         |
| IL-6*             | 0.1916     | 0.3019         |
|                   | 0.4507     | 0.0124         |
| IL-10*            | 0.276      | 0.1328         |
|                   | 0.4135     | 0.0231         |
| IL-13*            | 0.2452     | 0.1836         |
|                   | 0.4       | 0.0285         |
| IL-1              | 0.1958     | 0.2911         |
|                   | 0.3487     | 0.0589         |
| IL-2              | 0.3035     | 0.097          |
|                   | 0.3436     | 0.063          |
| IL-8              | 0.1098     | 0.5564         |
|                   | 0.2388     | 0.2038         |
| IL-12             | 0.2903     | 0.1132         |
|                   | 0.4592     | 0.0107         |
| TNF-α             | 0.2562     | 0.1641         |
|                   | 0.4439     | 0.014          |
| IFN-γ             | 0.2341     | 0.205          |
|                   | 0.2991     | 0.1083         |

Table 3. Areas under curve (AUC), cut-off values, specificities and sensitivities of assays to diagnose EoE. *The unit of analytes is pg/ml except AEC which is K/µl. **Accuracy is the summation of true positive and true negative. ***Whole Model Test in logistic Fit analysis.

|                | AUC      | Cut-off value* | Sensitivity | Specificity | Accuracy ** | P-value *** |
|----------------|----------|----------------|-------------|-------------|-------------|-------------|
| 15(S)-HETE     | 0.633    | ≥7500          | 50.0%       | 80.0%       | 64.5%       | 0.0340      |
| AEC            | 0.773    | ≥0.4 K/µl      | 53.3%       | 93.3%       | 71.0%       | 0.0024      |
| IL-4           | 0.675    | ≥270           | 56.3%       | 80%         | 67.7%       | 0.0527      |
| IL-5           | 0.709    | ≥22            | 43.8%       | 93.3%       | 67.7%       | 0.0338      |
| IL-6           | 0.6917   | ≥90            | 50%         | 80%         | 64.5%       | 0.0295      |
| IL-10          | 0.7292   | ≥96            | 62.5%       | 86.7%       | 74.2%       | 0.0159      |
| IL-13          | 0.671    | ≥230           | 56.3%       | 80%         | 67.7%       | 0.0518      |
| IL-12          | 0.648    | ≥210           | 43.8%       | 86.7%       | 64.5%       | 0.0600      |
| TNF-α          | 0.635    | ≥280           | 37.5%       | 86.7%       | 64.5%       | 0.0707      |

Figure 2. Receiver operating characteristic (ROC) curves for diagnosis of EoE. (A) The area under curve (AUC) was calculated for 4 conditions: 15(S)-HETE alone; combination of 15(S)-HETE, and peripheral AEC; combination of 15(S)-HETE, AEC and Th2 cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13); combination of 15(S)-HETE, AEC, and all the cytokines. (B) The AUC was calculated for 3 conditions: Peripheral AEC alone; combination of AEC and Th2 cytokines; combination of AEC and all the cytokines.
endoscopically due to insufficient follow-up or parent preference. Furthermore, majority of the patients in both groups were taking PPI at the time of endoscopy, generally at a dose of at least 1 mg/kg/day for younger patients or 30–40 mg/day for older patients. Given recent evidence suggesting anti-inflammatory properties of PPIs, independent of an acid suppressive mechanism, it is unclear how the concurrent administration of this medication may influence our findings.\textsuperscript{31} Nevertheless, we found no correlation between 15(S)-HETE levels and PPI exposure at the time of blood sampling (Supplemental Table 1).

Testing peripheral 15(S)-HETE levels should be limited to the patients who are clinically suspicious for EoE. 15(S)-HETE levels were not statistically different in patients with or without specific symptoms listed in Table 1 (data not shown). Therefore, 15(S)-HETE should not be used to triage patients with general symptoms such as abdominal pain. Utilities of the assay in diagnosing diseases that have similar symptoms such as celiac disease and inflammatory bowel disease are to be investigated in future studies.

Previous work has shown atopic status as an independent predictor of serum Th2 cytokine levels, regardless of participant group (i.e. EoE vs non-EoE control)\textsuperscript{34}. In contrast, in our study, Th2 cytokine profiles and 15(S)-HETE levels corresponded to diagnosis and not to atopic status (Supplemental Table 2). Finally, the diagnosis of atopy was based on patient/parent reporting and/or supporting physician documentation. We did not employ IgE levels, radioallergosorbent test (RAST), skin, or patch testing to confirm. Thus, concern remains for atopy as a confounding variable, particularly since EoE and atopic conditions are frequently coincident.

In the current study, peak eosinophil count (PEC) was used as an index to quantitate the severity of the EoE pathology and it was significantly associated with 15(S)-HETE and peripheral AEC (Table 2). Recently studies have shown that a comprehensive EoE histology scoring system (EoEHSS) was better than PEC for disease diagnosis and monitoring\textsuperscript{33,34}. With EoEHSS gaining more acceptance, further EoE studies are likely to adopt EoEHSS as a standard histological evaluation.

In conclusion, 15(S)-HETE may participate in the dysregulated immune response which characterizes EoE. Further studies are needed to clarify its role in this process. 15(S)-HETE alone is not better than AEC and Th2 cytokines as a noninvasive means to distinguish EoE from other GI conditions. However, there may be role for this novel marker in combination with other peripheral markers, such as eosinophil-derived neurotoxin (EDN), eotaxin-3, and IL-13, in the diagnosis and management of EoE.

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