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A MULTICENTER LONG-TERM COHORT STUDY OF EOSINOPHILIC ESOPHAGITIS VARIANTS AND THEIR PROGRESSION TO EOE OVER TIME

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ABSTRACT

BACKGROUND: Eosinophilic esophagitis (EoE) variants have been recently characterized as conditions with symptoms of esophageal dysfunction resembling EoE, but absence of significant esophageal eosinophilia. Their disease course and severity have yet to be determined.

METHODS: Patients from six EoE-centers with symptoms of esophageal dysfunction, but peak eosinophil counts of <15/hpf in esophageal biopsies and absence of gastro-esophageal reflux disease with at least one follow-up visit were included. Clinical, (immuno)-histological and molecular features were determined and compared with EoE and healthy controls.

RESULTS: We included 54 patients with EoE variants (*EoE-like esophagitis 53.7%*; *lymphocytic esophagitis* 13.0%; *non-specific esophagitis 33.3%*). In 8 EoE-like esophagitis patients, EoE developed after a median of 14 months (IQR 3.6-37.6). Such progression increased over time (17.6% year 1, 32.0% year 3, 62.2% year 6). Sequential RNA sequencing analyses revealed only seven genes associated with this progression (with TSG6 and ALOX15 among the top three upregulated genes) with upregulation of a previously attenuated Th2 pathway. Immunostaining confirmed the involvement of eosinophil-associated proteins (TSG6, ALOX15) and revealed a significantly increased number of GATA3-positive cells during progression indicating a Th1/Th2 switch. Transition from one EoE variant (baseline) to another variant (during follow-up) was seen in 35.2% (median observation time of 17.3 months).

CONCLUSION: Transition of EoE variants to EoE suggests the presence of a disease spectrum. Few genes appear to be associated with the progression to EoE with upregulation of a previously attenuated Th2 signal. These genes, including GATA3 as a Th1/Th2 switch regulator, may represent potential therapeutic targets in early disease pathogenesis.

Keywords: Dysphagia; esophageal eosinophilia; lymphocytic esophagitis; next generation RNA sequencing.

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic inflammatory disorder of the esophagus that is defined clinically by symptoms of esophageal dysfunction and histologically by an eosinophil-predominant infiltration of the esophageal squamous epithelium with at least 15 eosinophils in at least one high power field [hpf]. (1) EoE is considered an antigen-mediated allergic inflammation, where food-born antigens trigger a Th2 response with upregulation of cytokines such as IL-5 and IL-13, consecutively promoting eosinophil infiltration to the site of inflammation. (2) Long-term follow-up has revealed EoE's potential to progress to a fibrostenotic phenotype with development of esophageal strictures. (3, 4) Swallowed topical corticosteroids are the most widely used therapeutic modality with proven efficacy in both short- and long-term management. (5, 6) In addition, the anti IL4/IL13 antibody Dupilumab has been approved by the FDA and EMA. (7) However, alternative treatment options are needed given the limited anti-fibrotic efficacy of available therapies and a loss of response over time. (6, 8)

EoE variants have been recently characterized as conditions with symptoms of esophageal dysfunction resembling EoE, but either absence of esophageal eosinophilia or eosinophil counts not fulfilling the diagnostic cutoff of 15 eos/hpf. (9) They appear to be a heterogenous group, including at least three different subtypes, namely EoE-like esophagitis, lymphocytic esophagitis and non-specific esophagitis. Mechanistically, EoE variants stand out for epithelial barrier dysfunction, absence of an EoE-typical Th2 response, but molecular fingerprints that partially overlap with classical EoE. (9) They can therefore be considered as non-eosinophilic subgroups of a larger disease spectrum, where EoE represents the most extreme phenotype. However, it remains unknown whether or not one or several EoE variants can progress to EoE over time. In addition, disease course and severity of these variants with regards to their stricturing potential – a common feature of classic EoE – have yet to be determined. As the role of eosinophils as the main driver of symptoms and inflammation in EoE has been questioned lately, (10-13) follow-up analyses of EoE variants (with progression to EoE over time) will also provide potential insights in the (non-eosinophil) pathogenesis of EoE paving the road for alternative therapeutic agents.

Here, we present a multicenter long-term cohort study of patients with EoE variants with clinical, histological and molecular data, elucidating the potential to progress to EoE over time with a particular focus on modulator genes that are upregulated in this progression process.

METHODS

Study design

In this multi-center study including patients from 6 EoE referral centers, we analyzed clinical, histological, and molecular follow-up data of patients with EoE variants. The study was approved by the local ethics committee of each of the participating centers.

Patients and data collection

Inclusion and exclusion criteria for this cohort have been previously published. (9) Briefly, patients were included if they had typical EoE symptoms, but proven absence of esophageal eosinophilia of 15 eosinophils (eos) per hpf despite no anti-eosinophil treatment with the availability of at least 6 esophageal biopsies following a structured biopsy protocol (3 from the distal and 3 from the proximal esophagus). Patients were excluded for other diseases associated with eosinophil infiltration of the esophageal mucosa such as gastroesophageal reflux disease (GERD) and eosinophilic gastroenteritis. GERD was excluded as previously described. (9) The structured data collection was performed by means of a standardized spreadsheet. All data were anonymized. For details see **Supplementary Material**.

Histological re-examination

All eight individual components of the validated EoE histological scoring system (EoE-HSS), in addition to peak eosinophil count per hpf, as well as lymphocytic infiltration and presence of acute inflammatory cells were assessed by two EoE reference pathologists (MC, CB) at baseline and during follow-up visits. (14) As previously published, EoE variants were classified into EoE-like esophagitis (<15 eos/hpf, but otherwise typical histological EoE features), lymphocytic esophagitis (lymphocyte-predominant inflammation with high numbers of intraepithelial lymphocytes (≥30 per hpf) and typical peripapillar infiltration), and non-specific esophagitis (histological infiltration of lymphocytes or neutrophils not fulfilling the numerical and distributional criteria of lymphocytic esophagitis). (9)

Immunostaining

Formalin-fixed, paraffin-embedded esophageal biopsies were shipped at room temperature according to a material transfer agreement from each participating center to the Swiss EoE Clinic. The samples were sectioned, and slides were subsequently processed for immunofluorescent analyses as previously described. (9) For details on determined proteins and analyses see **Supplementary Material.**

RNA Isolation and RNA sequencing studies

Esophageal biopsies from a subset of patients with EoE-like esophagitis and progression to EoE in the follow-up (5 patients, time point 0=diagnosis of EoE-like esophagitis, time point 1=diagnosis of EoE) were processed for next generation RNA sequencing (RNA-seq) and RNA-seq libraries were prepared as previously described. (9) Active EoE (n=10) and esophagus-healthy individuals (n=7) already included in our cohort and analyzed by RNA-seq in a previous publication (9) served as controls (**Supplementary Material**).

Statistical analyses

For statistical analyses, GraphPad Prism software version 8.3.0 and R version 3.6.0 were used. Quantitative data are shown as mean with standard deviation (SD) or median with interquartile range (IQR). Categorical data were compared using χ^2 test or Fisher's exact test; Quantitative data were compared using two-samples t-test or Wilcoxon rank sum test (depending on whether or not data was normally distributed); for time to progression to EoE during follow-up, Kaplan Meier curves were computed. For analysis of immunostaining data (at baseline and at follow-up), one-way ANOVA was used to analyze quantitative data for statistical significance. For the purpose of this study, p-value of < 0.05 was considered statistically significant.

RESULTS

Patient demographics

From our previous characterization study including 69 patients with EoE variants, we identified a total of 54 with available follow-up data (**Figure 1a**). Median age at diagnosis of these patients was 47.9 years (IQR 31.9-63.0) with a median duration of symptoms of 26.5 months (IQR 11.9-74.9). 28 were females (51.9%) and 50 subjects were of Caucasian descent (92.6%). Data from three visits per patient (IQR 3-4, range 2-10) were available for analysis with a median follow-up time of 17.3 months (IQR 6.6-33.4, range 0.4-89.6). Based on H&E histology at baseline (reviewed by an expert EoE pathologist), 29 patients were diagnosed with EoE-like esophagitis (53.7%), 7 with lymphocytic esophagitis (13.0%), and 18 with non-specific esophagitis (33.3%). For details see **Table 1**.

Disease course during follow-up

Endoscopic dilation was needed in 16 subjects: in 7 patients with EoE-like esophagitis (24.1%), in 5 with lymphocytic esophagitis (71.4%) and in 4 subjects with non-specific esophagitis (22.2%). For details see **Table 1**.

Progression to EoE at follow-up visit

In 8 patients, EoE developed during the follow-up period with a median time to EoE of 14 months (IQR 3.6-37.6). Such progression was only seen in patients with EoE-like esophagitis at baseline: Progression to EoE increased over time, from 17.6% at 1 year, to 32.0% at 3 years and 62.2% at 6 years (**Figure 1b**), with a considerable and significant increase in peak eosinophil counts in these patients (median fold increase of 6.9, see **Supplementary Figure 1**). Seven of these patients had detectable, but little esophageal eosinophil infiltration at baseline endoscopy visit (median 4 eos/hpf, IQR 3.5-8.6, range 3-11), while one patient neither had epithelial nor subepithelial eosinophil infiltration (0 eos/hpf). None of the patients had significant subepithelial eosinophil levels. Compared to the 21 EoE-like esophagitis patients without progression, peak eosinophil counts at baseline were significantly higher (median 3.9 vs 1.0 eos/hpf, p=0.027). However, there was no such difference when looking at histological disease activity beyond eosinophilia (EoE-HSS stage and grade), presence of basal zone hyperplasia, or endoscopic disease activity graded by the EREFS score. In addition, there were no differences between the two groups with regards to family history for EoE, presence of allergies, need for dilatation, response to steroid treatment, and diagnostic confirmation time. There was a trend towards a lower proportion

of females (12.5% vs. 47.6%, p=0.081) and a lower age at diagnosis in patients with progression to EoE over time (33.5y vs. 47.9y, p=0.093). For details about the comparison of EoE-like esophagitis patients with vs without progression to EoE, see **Table 2**.

Progression from one variant to another at follow-up visit

Transition from one EoE variant (baseline) to another variant (during follow-up) was seen in 19 patients (35.2%, **Figure 1c**) based on conventional H&E histology: 6 patients from EoE-like to non-specific esophagitis; 6 patients from non-specific to EoE-like esophagitis; 3 from EoE-like to lymphocytic esophagitis; 2 from lymphocytic to EoE-like esophagitis; 1 from lymphocytic to non-specific esophagitis; and 1 from non-specific esophagitis to lymphocytic esophagitis. 6 of these 19 patients with transition from one variant to another showed further transition or progression with one patient transitioning from EoE-like to non-specific esophagitis and then EoE (**Figure 1d**).

Sequential RNA-seq analyses

In 5 of the 8 patients who progressed from an EoE variant (EoE-like esophagitis at baseline) to EoE at follow-up, tissue for sequential RNA-seq analyses (time point 0 diagnosis of EoE-like esophagitis = baseline, time point 1 diagnosis of EoE = follow-up) was available. For details on these patients, see **Supplementary Table 1 (Supplementary Material)**. RNA-seq analyses revealed upregulation of the same top pathways (at the time of EoE diagnosis during follow-up visit) as in classical EoE (compared to esophagus healthy controls) with the typical involvement of the Th2 pathway and upregulation of *IL5*, *IL13* or the eotaxin 3 receptor *CCR3* (**Figure 2a**). For a heatmap, see **Figure 2b**. Indeed, comparative analyses demonstrated strongly overlapping pathways and upstream regulators in these newly developed EoE cases (follow-up visit) compared to classic EoE (**Supplementary Figure 1a, Supplementary Material**). Nonetheless, when comparing them directly with classical EoE (without prior EoE-like esophagitis), a total of 173 differentially expressed genes were identified (**Supplementary Table 2**). For a volcano plot, see **Supplementary Figure 1b (Supplementary Material)**.

Surprisingly, only seven genes were significantly upregulated during progression from EoE-like esophagitis to EoE (baseline vs follow-up visit, **Figure 2c**), the most upregulated of them were associated with eosinophil recruitment to the esophageal mucosa such as *TSG6*, *ALOX15* and *SLC26A4*. Looking at disease pathways, mRNA profiles at the time of EoE-like esophagitis diagnosis (baseline) and at the time of progression to EoE (follow-up visit)

showed considerable overlaps with one exception: an attenuated Th2 signal at baseline, with activation during progression to EoE (**Figure 2d**).

Immunostaining

To confirm the involvement of above-mentioned proteins in the progression from EoE-like esophagitis (baseline) to EoE (at follow-up visit), we performed immunostaining for two of the top differentially expressed genes (TSG6, ALOX15), and EPX as positive control. Immunostaining for ALOX15 and TSG6 showed increases in their protein expression during progression from baseline to EoE (follow-up visit), comparable to patients with already established EoE, and paralleling an increase in EPX. Two patients with considerable EPX expression at baseline (which was not appreciated as eosinophil infiltration on conventional histology) were excluded from analysis. Given significant upregulation of a previously attenuated Th2 pathway, we next looked into known switch regulators for Th1 and Th2 lineages such as *GATA3* and *T-bet*. Immunostaining revealed a significantly increased number of both *GATA3* and *T-bet* positive cells during progression from EoE-like esophagitis (baseline) to EoE (follow-up visit), similarly to what is seen in classical EoE. A significant increase in the *GATA3* to *T-bet* ratio (4.7-fold) during progression from EoE-like esophagitis (baseline) to EoE (follow-up visit) potentially indicates a switch from a Th1 to a Th2 dominant inflammatory response associated with this progression and EoE in general (**Figure 4**).

DISCUSSION

EoE variants have been recently identified as non-EoE subgroups of a larger disease spectrum. (9) Still, as of yet, it remains unknown whether one or several EoE variants can progress to EoE over time and whether or not these variants can present features of fibrotic disease. Based on our analysis, including follow-up data of 54 patients over 17 months, our main findings are: 1) Dilatation for stricturing disease is frequently needed in EoE variants; 2) Transition from one variant to another variant or progression to EoE occurs in 50% of patients; 3) Few genes appear to be associated with progression to EoE with upregulation of a previously attenuated Th2 signal.

Dilatation is needed in almost a third of the patients diagnosed with an EoE variant, particularly patients with lymphocytic esophagitis. However, both EoE-like esophagitis and even non-specific esophagitis patients show a stricturing potential. This finding corresponds

with our previous results indicating a severe clinical presentation with frequent bolus impactions in EoE variants, some patients even necessitating endoscopic bolus removal. (9) It also goes in line with previous RNA-seq data indicating involvement of pro-fibrotic pathways. (9) Thus, follow-up endoscopies in patients with EoE variants should be considered, particularly those with worsening symptoms, in order to assess for and treat esophageal strictures. Given the limited follow-up, the need for repetitive dilatations could not be assessed in the current study. Thus, it remains unclear, if underlying disease needs to be treated as in EoE, or if (one-time) dilatation could be sufficient. However, data on transition and progression indicate ongoing underlying disease activity. Longer follow-up will finally help to answer this open question. In addition, it remains to be determined whether or not treatment with steroids could interfere with the stricturing potential. At least, its positive impact on clinical disease severity has been suggested in our previous publication. (9) Nonetheless, data on steroids' anti-fibrotic efficacy in the treatment of EoE have been limited. (15-17)

Transition from one variant to another variant occurs frequently in the follow-up of EoE variant patients. Indeed, more than a third of the studied patients showed a change in histological features over time (baseline vs follow-up visit). This is particularly noteworthy, as the histological presentation based on H&E coloration were distinct at baseline and diagnostic criteria for each variant mutually exclusive. Thus, sampling error, or difference in (histological) disease presentation at various levels in the esophagus do not appear to be the case. The assessment of at least 6 biopsies (3 from the distal and 3 from the proximal esophagus) further limits such bias. Furthermore, it is intriguing to see that even lymphocytic esophagitis patients can change its histological presentation over time as lymphocytic esophagitis has been considered an entity distinct from EoE in the past and also shows some considerable differences with regards to age, atopic comorbidities and family history. (18, 19) Our data, in contrast, suggest a disease spectrum, including all the three variants EoE-like esophagitis, non-specific esophagitis and lymphocytic esophagitis. Of note, 6 patients (thus one third of all patients with transitioning disease) showed more than 1 transition over time, suggesting a considerable flux in disease presentation. Nevertheless, these observations based on H&E histology need further confirmation by sequential RNA-seq data.

Progression to EoE was seen in 8 patients over time. All of them presented with EoE-like esophagitis at baseline (with one of them showing transition to non-specific esophagitis before progressing to EoE). Probability of progression to EoE increased in EoE-like esophagitis patients over time, with 17.6% at 1 year, to 32.0% at 3 years and 62.2% at 6 years. These data indicate that longer follow-up could actually result in a higher detection of EoE. Such longer follow-up with a larger sample size will eventually answer the question whether all of these patients end up with being diagnosed with EoE and if not, which baseline factors are predictive for such progression. It would be particularly intriguing to look at deeper tissue since subepithelial changes might be responsible for some inflammatory changes not captured within the esophageal mucosa. However, the few patients with subepithelial tissue available in this study did not reveal any important findings. More deeper tissue might lead to clearer insights, but subepithelial tissue is unfortunately not captured very often with a standard biopsy forceps. (20) The current analysis highlights the presence of a potential disease spectrum, where EoE only represents the most prominent and severe phenotype. Nevertheless, while one patient transitioned to non-specific esophagitis before the progression to EoE, no such progression was seen in patients with lymphocytic esophagitis. Longer follow-up data will finally answer the question whether or not such progression can occur over time in this subgroup. However, given the possibility of lymphocytic esophagitis transitioning to the two other variants, a progression to EoE at least has to be considered warranting close follow-up with repetitive biopsies in these patients.

Intriguingly, longitudinal RNA-seq data revealed that only few genes are upregulated during progression from EoE-like esophagitis (baseline) to EoE (follow-up visit). Both eosinophil and non-eosinophil associated genes have been identified. Tertiary analyses further revealed the upregulation of a previously attenuated Th2 pathway. Immunostaining confirmed the involvement of the top upregulated genes (TSG6, ALOX15) during progression to EoE, and also their involvement in classical EoE (without previously detected EoE-like esophagitis). In addition, the switch from a Th1 to a Th2 inflammatory response has been confirmed by an increase of the GATA3 to T-bet ratio. ALOX15 and TSG6 (also known as TNFAIP6) have been previously shown to be increased in active EoE and IL-13-treated epithelial cells, without any further experimental exploration. (21, 22) Thus, their exact role in EoE remains elusive, but both genes have been previously implicated in other atopic

diseases. (23-26) In-depth exploration of these genes and GATA3 as a key Th1/Th2 switch regulator should be considered, particularly given their potential role in early disease pathophysiology. However, further studies are needed to prove whether or not the newly developed EoE is exactly the same as classical EoE without previous EoE-like disease. While our data indicate progression to EoE, these patients still show some features of persisting EoE-like esophagitis (such as a Th1 signal), which however might be lost over time.

Our study has several strengths and limitations. It is the first follow-up study of patients with EoE variants, further supporting the concept of a disease spectrum. The inclusion of sequential RNA-seq data and immunofluorescence experiments confirming the RNA-seq results make our data more robust. Limitations of our cohort have been previously discussed in detail. (9) One major limitation is the short follow-up of only 17 months in the median. For some patients only one or two follow-up visits were available. Therefore, it remains unclear whether or not some patients may have progressed to EoE after a longer follow-up time, particularly patients diagnosed with lymphocytic esophagitis. Longitudinal RNA-seq was only performed in patients with progression to EoE, thus no data are available for patients transitioning form one variant to another variant. However, in-depth description of these variants including RNA-seq has been previously published by our group. (9) We cannot exclude the possibility of a referral bias, particularly in light of a relatively high percentage of patients with a positive family history. Thus, our findings are not applicable 1:1 to non-expert centers. It is difficult to know whether some of our patients actually had burned-out EoE at baseline. However, historical data on long-term follow-up of patients with long-lasting untreated EoE show ongoing inflammation together with the development of fibrosis, thus burned-out EoE – although theoretically logical – has not been described in the literature. (3) While the patients included in this study showed increased EREFS scores, only 3 patients had moderate rings and 3 had severe rings. Thus, the presence of burned-out (fibrotic-only) disease at baseline is unlikely and if it occurs, it would have been observed in no more than 11%. Finally, as treatment was not assessed in a systematic manner in the follow-up, possible confounding of our results cannot be excluded. However, a chart re-review of our EoE-like esophagitis patients with progression did not reveal a lower percentage of patients receiving PPI or steroid treatment, compared to non-progressors. In fact, the opposite was seen (75% vs vs 48% (p=0.2378). Thus, non-progression was not biased by an eventual over-treatment with STC. On the other side, one could assume that at

least some of the patients that were treated with STC, but did not progress to EoE, might have in the absence of steroid treatment.

In conclusion, frequent transition from one EoE variant to another and progression to classic EoE over time suggest the presence of a disease spectrum. Disease monitoring can potentially detect such progression to EoE over time. Few genes appear to be associated with such progression to EoE during follow-up with upregulation of a previously attenuated Th2 signal. These genes, including GATA3 as a key Th1/Th2 switch regulator, may represent potential therapeutic targets. Further studies are needed to identify risk factors for progression to EoE over time and to characterize the role of the identified genes in more detail.

Supplementary Material - http://links.lww.com/CTG/B87

Supplementary Table 2 - http://links.lww.com/CTG/B88

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TABLE AND FIGURE LEGENDS

Table 1: Demographics and disease characteristics in all EoE variants combined and stratified by each variant.

	EoE variants n=54	**EoE-like esophagitis n=29**	**Non-specific esophagitis n=18**	**Lymphocytic esophagitis n=7**
Baseline				
Female Gender	28 (51.9%)	11 (37.9%)	13 (72.2%)	4 (57.1%)
Age at onset, years	44.5 (IQR 25.9-59.2)	38.4 (IQR 18.5-51.5)	46.1 (IQR 26.8-53.1)	62.9 (IQR 56.5-66.9)
Age at diagnosis, years	47.9 (IQR 31.9-63.0)	44.9 (IQR 30.6-55.4)	49.1 (IQR 32.9-61.9)	65.5 (IQR 50.4-69.9)
Atopic comorbidities	24 (44.4%)	15 (51.7%)	7 (38.9%)	2 (28.6%)
Ancestry - Caucasians - African Americans - NA	 50 (92.6%) 2 (3.7%) 2 (3.7%)	 27 (93.1%) 2 (6.9%) 0 (0%)	 16 (88.9%) 0 (0%) 2 (11.1%)	 7 (100%) 0 (0%) 0 (0%)
Family history for EoE	16 (29.6%)	10 (34.5%)	6 (33.3%)	0 (0%)
Previous PPI	33 (61.1%)	17 (58.6%)	12 (66.7%)	4 (57.1%)
Steroids	0 (%)	0 (0%)	0 (0%)	0 (0%)
Diagnostic confirmation time, months	26.5 (IQR 11.9-74.9)	24.7 (IQR 11.9-81.3)	39.3 (IQR 12.4-112.6)	24.4 (IQR 12.6-55.3)
Follow-up				
Number of follow-up visits	3 (IQR 3-4)	3 (IQR 3-4)	4 (IQR 3-5)	3 (IQR 2.5-3)
Time of follow-up, months	17.3 (IQR 6.6-33.4)	12.4 (IQR 3.9-37.4)	20.2 (IQR 8.8-33.4)	18.2 (IQR 12.9-24.0)
Development of EoE	8 (14.8%)	8 (27.6%)	0 (0%)	0 (0%)
Endoscopic dilation	16 (29.6%)	7 (24.1%)	4 (22.2%)	5 (71.4%)

Table 2: Comparison of EoE-like esophagitis patients with vs without development of EoE during follow-up.

	EoE-like esophagitis with development of EoE (n=8)	EoE-like esophagitis without development of EoE (n=21)	p-value
Demographics			
Female Gender	1 (12.5%)	10 (47.6%)	0.081
Age at onset, years	21.1 (12.0-46.1)	40.9 (29.6-56.1)	0.108
Age at diagnosis, years	33.5 (16.8-47.1)	47.9 (35.8-57.4)	0.093
Atopic comorbidities	5 (62.5%)	10 (47.6%)	0.474
Family history for EoE	3 (37.5%)	7 (33.3%)	0.833
Previous PPI	4 (50.0%)	13 (61.9%)	0.561
Diagnostic confirmation time, months	24.4 (13.3-47.3)	27.6 (10.0-99.0)	0.980
Disease activity			
EREFS Score	0.5 (0-2)	0 (0-2)	0.840
Peak eosinophil count, eos/hpf	3.9 (3.3-7.7)	1.0 (0.0-3.3)	**0.027**
EoE-HSS Grade	0.2 (0.1-0.2)	0.1 (0.0-0.3)	0.305
EoE-HSS Stage	0.2 (0.1-0.2)	0.1 (0.0-0.3)	0.353
Subepithelial eosinophil count (available for 17), eos/hpf	1.0 (1.0-4.8), n=5	1.5 (0.0-3.8), n=12	0.476
Detetectable subepithelial eosinophilia (available for 17**)**	4/5 (80.0%)	7/12 (58.3%)	0.394
Follow-up			
Number of follow-up visits	4 (3-4)	3 (3-4)	0.602
Time of follow-up, months	24 (4-38)	10.5 (5-37)	0.933

Figure 1: A) Flow chart of study patients. B) Kaplan Meier analysis showing progression of EoE-like esophagitis to EoE over time. C) Transition from one variant to another variant during the follow-up period with numbers between pies indicating the number of patients (and percentage) transitioning from one variant to another variant. D) Patients with more than one additional variant during follow-up (n=5) and one additional patient with transition from EoE-like esophagitis to non-specific esophagitis and then progression to EoE. Blue color indicates EoE-like esophagitis, orange color indicates lymphocytic esophagitis, yellow color indicates non-specific esophagitis, and green color indicates EoE.

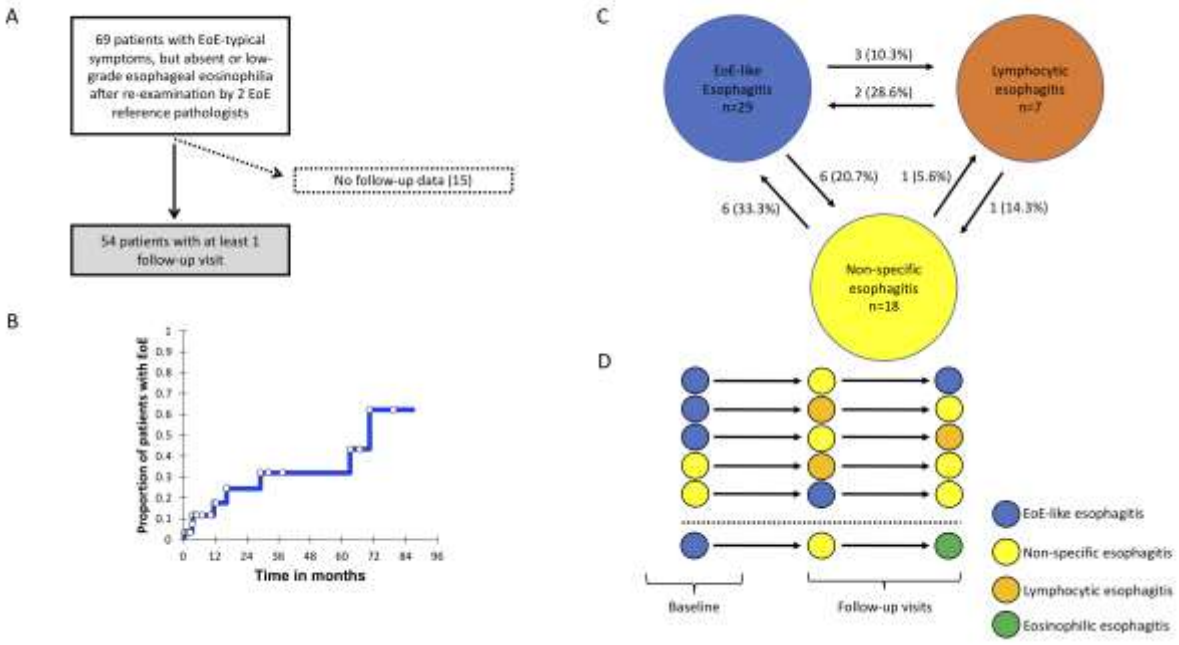

Figure 2: A) IPA pathway analysis of patients with newly developed EoE during the follow-up, with the top pathways ranked by -log10 p-value. Boxes on the right show the most upregulated genes in the three top pathways (ranked by log2 fold change). B) Heatmap of the mRNA profile of newly developed EoE vs classical EoE. Red color indicates upregulation, blue color indicates downregulation. C) Vulcano plot for differentially expressed genes during progression from EoE-like esophagitis to newly developed EoE. Red colors indicate a significant change during progression defined by a log2 fold change of at least 2 and an FDR of <0.05. D) Comparative pathway analysis in patients with progression from EoE-like esophagitis (on the left) to newly developed EoE (on the right). Orange color indicates upregulation, blue color indicates downregulation.

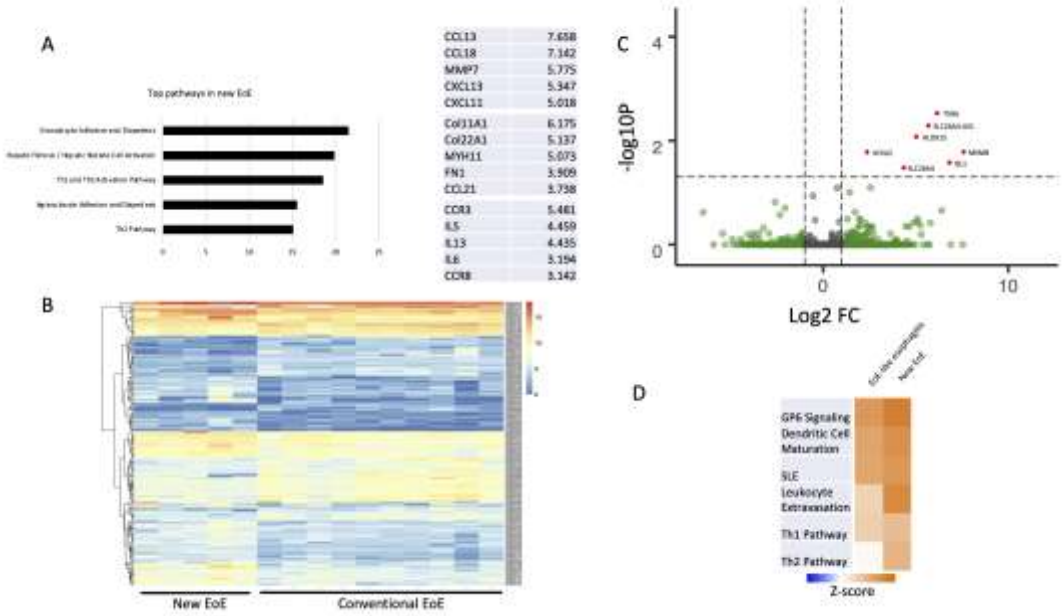

Figure 3: Esophageal expression of TSG6 (green) and EPX (red) in the upper line, and ALOX15 (green) and EPX (red) in the lower line, as assessed by immunofluorescence, in patients with EoE-like esophagitis at baseline (A), time of newly developed EoE (B), esophagus healthy controls (C) and classic EoE (D). Hoechst H3570 was used for nuclear staining. Panels on the right show quantification in epithelial cells for TSG6 (E), ALOX15 (F) and EPX (G).

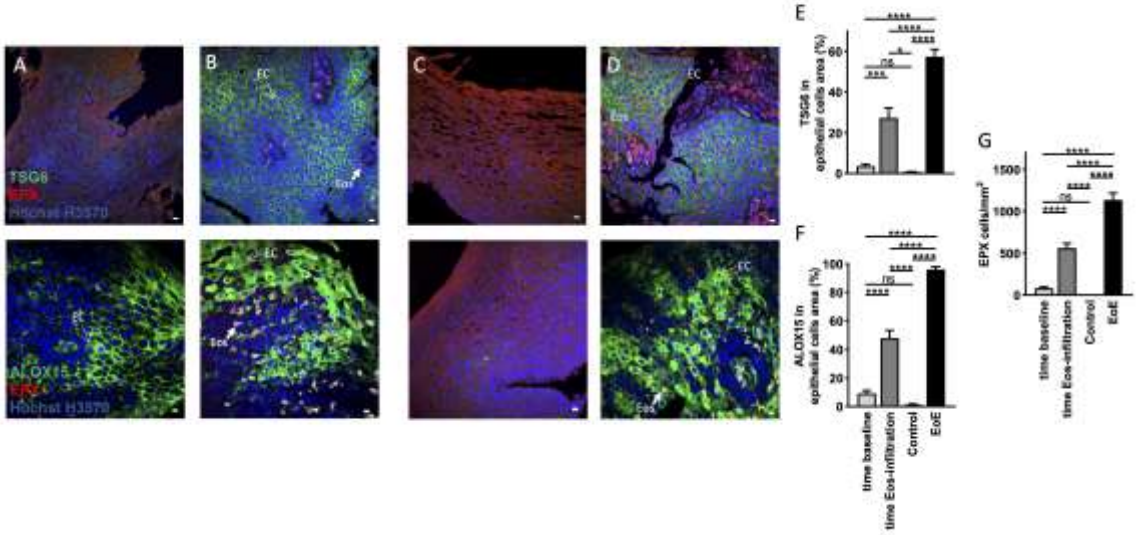

Figure 4: Esophageal T-bet and GATA-3 expression as assessed by immunofluorescence analysis in patients with EoE-like esophagitis at baseline (A), time of newly developed EoE (B), esophagus healthy controls (C) and classic EoE (D). Hoechst H3570 was used for nuclear staining. Panels on the right show quantification of T-bet (E), GATA3 (F), and the GATA3 to T-bet ratio in EoE-like esophagitis at baseline vs at the time of eosinophil infiltration (G). AU arbitrary units.