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New insights into an emerging disease

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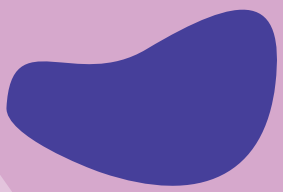

Willemijn E. de Rooij

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Eosinophilic Esophagitis: New insights into an emerging disease
Thesis, University of Amsterdam, The Netherlands

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Eosinophilic Esophagitis

New insights into an emerging disease

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ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
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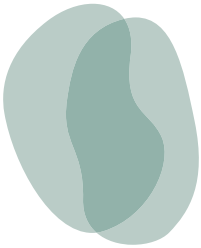

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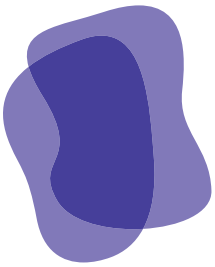

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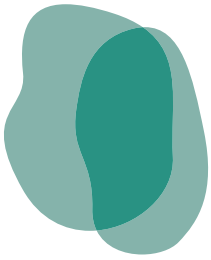

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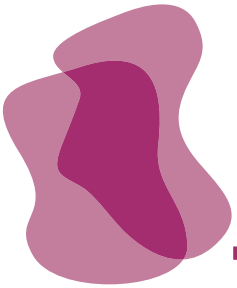

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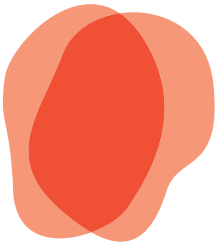

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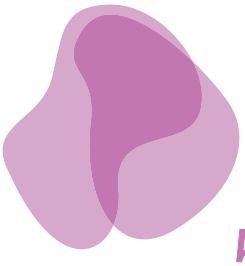

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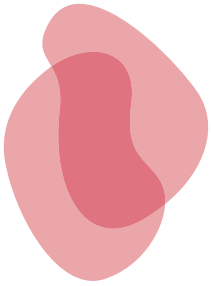

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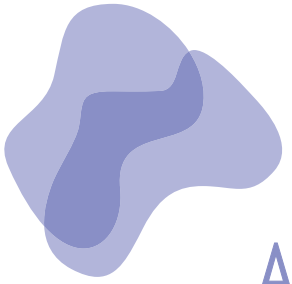

A

CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

GENERAL INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic immune-mediated disease characterized by symptoms of esophageal dysfunction and infiltration of the esophageal mucosa with eosinophils.[1,2,3] After a few case reports in the late 70s, the disease - as it is recognized today - was described in 2 case series in the early 90s.[4,5] Since then, EoE has gone from a rare condition to a widely recognized substantial cause of upper gastrointestinal morbidity in children and adults. The 'relatively new' field of EoE-research has rapidly evolved over the past 25-years, with advanced understanding of its natural disease course, its pathogenesis and more insights into diagnostics and efficacy of various treatments. Yet, much remains unknown on this emerging chronic disease.

EPIDEMIOLOGY

In the past 2 decades, EoE has moved from descriptions in rare case reports to recent prevalence estimates of 34.4 per 100.000 inhabitants in the United States (US).[6] The peak incidence of EoE is between the ages of 20 and 40 years, with a 3:1 male-to-female ratio in every age group.[7] Even though clinicians are becoming more familiar with this relatively new disease, the worldwide expansion of EoE over successive years cannot be simply attributed to awareness alone and seems to be outpacing any increase in diagnosis or detection.[6,8,9] The epidemiology is rapidly evolving and genetic predisposition has been indicated. However, the rise of new EoE cases paralleling other increasing Western diseases (e.g., atopic morbidities and inflammatory Bowel Disease (IBD)), which suggests that (non-) allergic environmental disparities may also be critical in disease manifestation.[10-13] At present, EoE incidence reports are inconsistent due to mixed study designs (e.g., case series vs. register based), different diagnostic criteria and diversified reporting. The Dutch register-based pathology database (PALGA) contains data from all 46 pathology laboratories, with nationwide coverage. This offers a unique opportunity to present an update of accurate annual EoE incidence rates within the entire population in the Netherlands over the past 25-years as described in **Chapter 2**.

PATHOPHYSIOLOGY

Overall, the evolution of EoE is thought to be a multifactorial interplay of genetics, environment and host immune system factors that are involved in multiple pathways.[14-16] First-degree relatives of EoE patients are more prone to develop EoE compared to the general population.[17] Candidate and genome-wide association studies have identified EoE to have a complex model of inheritance, with 31 candidate genes (e.g., EMSY, Calpain-14)

that are associated with the development of EoE.[18-21] However, the overall dramatic rise of EoE frequency especially in the Western world indicates - aside from these genetic factors - a pivotal role for the environment, in particular factors in early life (e.g., Cesarean section and antibiotic exposure).[17]

Food allergens have been suggested to play a causal role in EoE pathogenesis after primary reports of disease remission in children during treatment with an amino-acid based formula (AAF).[22] In predisposed individuals, culprits, being ubiquitous in daily foods (e.g., milk), are associated with infiltration of the esophageal mucosa with a mixed granulocyte population (mast cells, eosinophils and basophils).[23,24] The proposed immunological mechanism is illustrated by an immune response that is primarily regulated by T-helper type 2 (Th2) cells against food- (and aero) allergens. Esophageal allergen exposure triggers release of alarmins: Interleukin (IL)-33 and Thymic stromal lymphopoietin (TSLP) - both potent enhancers of Th2 immunity - with subsequent secretion of pro-inflammatory cytokines such as: IL-4, IL5 and IL-13 and pro-fibrotic mediators.[14,25]

The vigorous inflammatory state of the esophagus leads to epithelial barrier dysfunction, eosinophilic inflammation and eventually tissue remodeling and fibrosis. Transforming Growth Factor (TGF)-β has a central role in EoE, as it is designated to the five main areas of disease pathogenesis (i.e., epithelial remodeling, smooth muscle dysfunction, collagen deposition and angiogenesis).[26,27] Esophageal fibrosis is defined as excessive extracellular matrix deposition, particularly collagen fibers, in the lamina propria of the mucosa.[28] Yet, a significant gap remains in the understanding of the biological processes and pro-fibrotic signaling pathways involved in EoE-related fibrosis.[28,29]

DIAGNOSTICS

The paradigm of diagnosing EoE consists of symptoms of esophageal dysfunction (i.e., dysphagia and food impaction) and eosinophilic inflammation with ≥ 15 eosinophils per microscopic high-power-field (*eos/hpf*) at esophageal biopsy.[1,2,31] Non-specific symptoms are often seen in children (i.e., abdominal pain, failure to thrive and feeding disorders), whereas adults typically present with dysphagia and food impaction.[1,2,10] Endoscopic signs of disease activity are detected in almost ~ 90% of the symptomatic patients.[32] While edema, linear furrows and white exudates suggesting active inflammation are usually seen in children, both inflammatory and fibrotic endoscopic signs (i.e., rings and strictures) are often manifest in adults (**Figure 1**).[32]

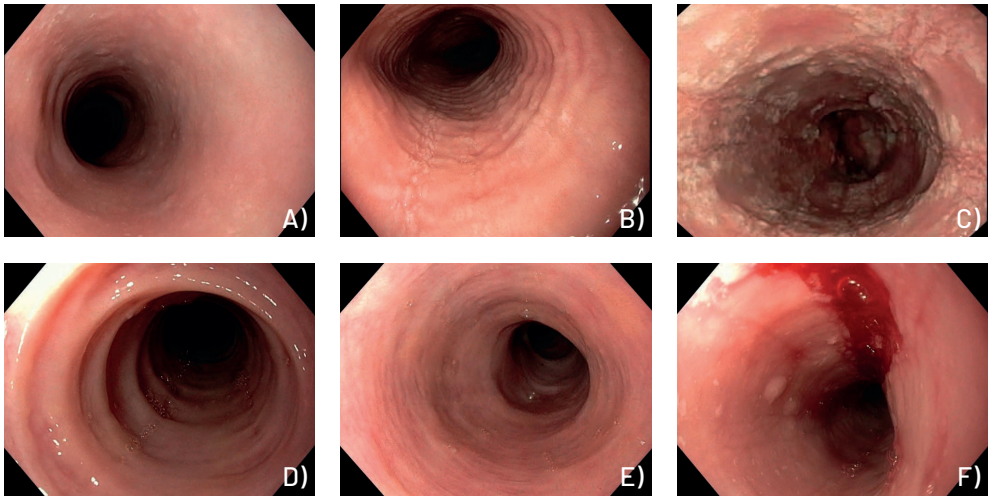

FIGURE 1. | Endoscopic appearance of esophagus tissue in eosinophilic esophagitis (EoE) patients. Endoscopic features (A) Mucosal pallor representing edema; (B) Linear furrows; (C) White exudates; (D) Concentric rings (i.e., Trachealization); (E) Stricture; (F) Crêpe paper like appearence of the esophagus. Endosopic images courtesy of Willemijn E. de Rooij, MD, Department of Gastroenterology & Hepatology, Amsterdam University Medical Center, Amsterdam.

The association between symptoms and biological disease activity (i.e., endoscopic- and histological features) is only modest in EoE patients.[33] As such, invasive upper endoscopy procedures with biopsies are required for diagnosis and disease monitoring. Six biopsies sampled from ≥ 2 different levels of the esophagus provides sufficient diagnostic accuracy for this 'patchy disease', since eosinophils are unevenly distributed throughout the mucosa.[31] Pathological assessment (hematoxylin and eosin) of EoE mucosa also demonstrates other abnormalities such as: eosinophil micro abscesses, hyperplasia of the basal cell layer, epithelial spongiosis (i.e., dilation of intracellular spaces) and increased lamina propria fibrosis (**Figure 2**).

For the last decade, the diagnosis of EoE required the presence of ≥ 15 *eos/hpf* at esophageal biopsy after a minimal 8-week course of high-dose proton pump inhibitor (PPI) treatment. However, in the international consensus guidelines (2018), it is agreed that PPIs are first line therapy for EoE instead of a diagnostic criteria. Hence, a PPI-trial was removed from the diagnostic algorithm.[31] Current guidelines differ in their recommendations for sampling biopsies of the stomach and/or duodenum, in order to rule out other relevant generalized or eosinophilic gastrointestinal (GI) disorders (e.g., parasitic infection, celiac

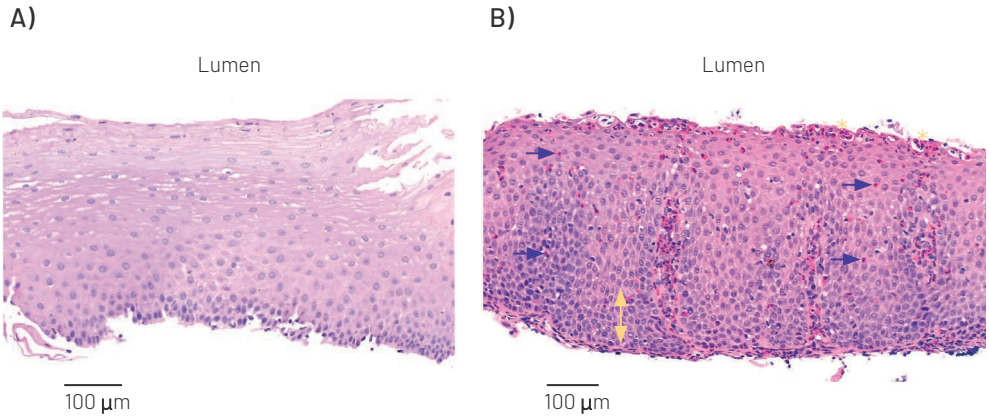

FIGURE 2. | Histologic appearance of normal esophagus tissue and eosinophilic esophagitis (EoE). Histologic features Hematoxylin and eosin; A) Normal esophagus B) EoE mucosa representing Basal zone hyperplasia (yellow arrow); Eosinophils (purple arrows) and Eosinophil abscess (yellow asterisks). Histological image courtesy of Aart Mookhoek, MD, PhD, Institute of Pathology, University of Bern.

disease, non-EoE eosinophilic gastrointestinal disorders (EGIDs)).[1-3,31,34] Most recently, the updated international guidelines advocated that additional biopsy specimens from the stomach and/or duodenum should also be sampled if i) indicated by clinical symptoms (i.e., dyspepsia, abdominal pain, vomiting/nausea or diarrhea) or ii) endoscopic gross abnormalities suggestive for the presence of other gastric or small intestinal conditions.[31] However, evidence is lacking on the diagnostic value of these additional biopsies sampled from the stomach and duodenum. In **Chapter 3** we have determined the diagnostic utility of these additional gastric and duodenal biopsies within a large cohort of adult EoE patients, so future evidence-based statements may be established for daily practice.

MANAGEMENT

The management of EoE has originally consisted of the "3D-approach": diet, drugs and dilation. Anti-inflammatory therapy includes dietary elimination of culprit foods and chronic use of medication (i.e., PPIs and swallowed topic steroids), which should be combined with endoscopic dilation in case of strictures. At present, treatment objectives in EoE are i) to improve symptoms ii) to reduce eosinophilic inflammation to prevent persistent histological activity and the risk of esophageal remodeling and fibrotic complications.

In **Chapter 4** we provide an overview of established EoE pharmacotherapies that have been evaluated as treatments, as well as other promising therapeutics that are in the drug development pipeline for EoE. A critical question in the development of therapeutics remains whether targeting the esophageal inflammation with medication (PPIs and swallowed topical steroids) can achieve this treatment goal, or if anti-fibrotic agents capable of modifying the natural course of EoE are warranted. Its heterogeneous presentation also suggests that future treatment requires a more personalized approach, with strategies depending on EoE-endotypes being more or less fibrotic. Similar to asthma, EoE may be clustered into different 'endotypes' using clinical and molecular features. In this thesis, we also explored whether changes of surrogate disease markers, such as EoE transcripts and their clinical correlates (i.e., eosinophils, symptoms and endoscopic signs) measured before and after dietary intervention, could provide initial insights into potential genetic determinants of different EoE-endotypes (**Chapter 6**).

The management of EoE needs an integrated approach, with a fundamental role for identification and elimination of culprit foods. Diets target the adaptive immune system (i.e., dampen the antigen-driven T-cell response by removal of culprit foods), with no modification of signaling pathways or inflammatory cell-apoptosis as mostly follows after steroids or biological targets. However, this has not been empirically studied in adult EoE. In **Chapter 6** we evaluated the effect on the changes of 10 indicated gene expression markers after treatment with an elimination diet for 6 weeks. Elemental diets (i.e., AAF as sole source of nutrition) have proven to be highly effective (85% - 95% disease remission rates) in EoE patients of all ages.[22,35,36] However, adherence is challenged by its poor palatability and impact on social life. Empiric removal of culprits (i.e., elimination of four-foods (FFED) or six-foods (SFED)) has been the most widely used diet for EoE patients in clinical practice.[37] Generally, the more rigorous the empiric elimination is, the more effective, but the more difficult it is to implement in daily life. For that reason, there has been a considerable interest in more efficient empiric diets to induce disease remission and lower costs as well as quality-of-life (QOL) burdens of treatment. Apart from the hypoallergenic effects of AAF - which may decrease the risk of dietary errors (i.e., allergen cross contamination), it was suggested to have immune-modulating properties itself. In **Chapter 5**, the efficacy of a new 'combined diet strategy' of empiric elimination of causative foods with AAF added to the diet is compared to a standard FFED (milk, wheat/gluten, egg and soy) in a randomized controlled trial.

IMPACT ON PATIENTS' DAILY LIFE

Paralleling the emerging prevalence of EoE, also an increasing number of studies reported EoE being associated with a substantial disease burden that affects patients' Health-Related Quality Of Life (HRQOL), healthcare systems and society in general.[38] From the patients' perspective, being diagnosed with this 'relatively new' disease with need for life-long treatment, disturbing symptoms of dysphagia and food impaction as well as invasive procedures for disease monitoring may be of specific concern.[39-41] The (long) road to an EoE diagnosis and onwards can be a difficult journey, which also impacts on patients' mental and social health. The EoE-research field yields a significant gap on this essential topic, with only a few studies available that are mainly focused on the expanded risk of anxiety and depressive symptoms measured within the construct of disease specific HRQOL (EoE-QOL-A).[42,43] In general - despite of their clinical and public health importance - the presence of psychological disorders seems to be often underdiagnosed and undertreated, especially when coexisting with physical illness.[44] At present, it remains even unknown whether EoE patients also receive mental care if they feel distressed. However, provision of sufficient mental care first needs more insights into the presence of mental distress among patients with EoE and its determinants (e.g., clinical and socio-demographic factors). This important topic will be further addressed in **Chapter 7**.

HRQOL is essentially a multi-dimensional concept that is driven by patients' physical, psychological and social status, as well as attitudes, concerns and behaviors in response to having a chronic disease.[45] Most of EoE patients adapted their eating behaviors (e.g., avoid highly textured foods, taking smaller bites, drinking more water during meals) or use dietary restrictions (avoidance of culprit foods) to manage symptoms and, in particular avert from food impactions.[46] Although not always being recognized, EoE patients generally display avoidance behaviors of eating (alone or with others) in daily social-life, as a result of swallowing anxiety or fear of giving others the impression of a state of illness.[47,48] How individuals cope with the physical, social and mental burden that is linked to stressful life events (e.g., having a chronic illness) determines patients' QOL, and is therefore an important outcome in multiple other chronic disease populations e.g., IBD and rheumatoid arthritis.[47,49,50] Given that currently no studies are available within the EoE-research field on this topic, we determined coping strategies and the degree to which different coping styles are related to (disease specific) HRQOL within a large cohort of adults with EoE (**Chapter 8**). Taken together, understanding of the psychosocial burden of 'living with EoE' is important to deliver adequate patient-centered (mental) care and covers a key objective of this thesis.

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CHAPTER 2

EMERGING INCIDENCE TRENDS OF EOSINOPHILIC ESOPHAGITIS OVER 25-YEARS: RESULTS OF A NATIONWIDE REGISTER-BASED PATHOLOGY COHORT

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ABSTRACT

RATIONALE

Eosinophilic esophagitis (EoE) has emerged from a case-reportable illness in the early 1990s to a distinct clinicopathological entity. Increasing worldwide incidences have been observed, although due to various study designs estimates are inconsistent.

AIM

To determine population-based annual incidence rates over a time period of 25 years.

METHODS

A nationwide register-based pathology (PALGA) search was performed to identify reports describing esophageal eosinophilia between 1995 and 2019. EoE was identified if the diagnosis was confirmed by the pathologist. Crude incidence rates were estimated by the number of new EoE cases per year and matched with population data.

RESULTS

Between 1995 and 2019, 7361 unique patients' reports mentioned esophageal eosinophilia, of these 4061 were classified as EoE (71% male, mean age 37.9 ± 18.4 years). In total, 639 (16%) children (< 18 years) were diagnosed. The incidence increased from 0.01 in 1995 (95% CI 0.0 - 0.04) to 3.16 (95% CI 2.90 - 3.44) per 100.000 inhabitants in 2019. EoE was significantly more prevalent in males (OR 2.48 | 95% CI 2.32 - 2.65; vs. females $p < 0.001$) and adults (OR 1.42 | 95% CI 1.31 - 1.55; vs. children $p < 0.001$). Highest incidences were observed in 2019, being 4.37 (95% CI 3.94 - 4.84) vs. 1.97 (95% CI 1.68 - 2.29) per 100.000 males and females, respectively ($p < 0.001$). No seasonal variation was observed.

CONCLUSION

Over the past quarter century, the annual rates of newly diagnosed EoE patients raised dramatically and this increase has not reached a deceleration yet.

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic immune-mediated disease characterized by symptoms of esophageal dysfunction and infiltration of the mucosa with eosinophils.[1,2] Dysphagia and food impaction are the most typical complaints in adults, whereas gastroesophageal reflux disease (GERD) - symptoms and failure to thrive, feeding disorders as well as abdominal pain predominates in children.[1-3] EoE is diagnosed per consensus guideline if: i) there are symptoms of esophageal dysfunction and ii) ≥ 15 eosinophils (eos) per high power field (hpf) under routine light microscopy after hematoxylin and eosin staining are present in at least one esophageal biopsy.[4] Over the past years, EoE has emerged from a case-reportable illness in the early 1990s to a distinct clinicopathological entity.[5,6] Although clinicians are becoming more familiar with this relatively new disease, the expanded EoE frequency cannot be simply attributed to raised awareness alone and is outpacing any increase in diagnosis or detection.[7,8] The EoE epidemiology is rapidly evolving and while genetic predisposition has been indicated, the increasing number of new EoE cases strongly suggests that (non-) allergic environmental disparities may also be critical in disease manifestation.[3,9] A worldwide tendency of rising EoE incidences have been reported, though current estimates are inconsistent due to variety in study designs (e.g., register based or insurance database vs. hospital based case series), heterogeneous reporting as well as modified diagnostic criteria.[10-12] Over successive years, the frequency of EoE in the Netherlands has also increased tremendously and it remains unclear whether this still continues to rise.[13,14] Hence the Dutch register-based pathology database (PALGA) offers a unique opportunity to present an update of accurate EoE incidence rates with nationwide coverage.[11,12,15] Therefore, we aimed to 1) asses the annual EoE incidence rates within the entire population in the Netherlands over the past 25-years 2) to identify demographic trends (i.e., gender, age and date of diagnosis) over time.

METHODS

DATA COLLECTION

This cross-sectional study was conducted by using results from the nationwide network and registry from cyto- and histopathology in the Netherlands (PALGA).[15] This archive contains data from all 46 pathology laboratories and has national coverage since 1991. Summarized histology reports are collected and encoded by pathologists based on the Systemized Nomenclature of Medicine (SNOMED) issued by the College of American Pathologists. By the end of 2017, more than 72 million pathology reports from over 12

million patients in the Netherlands have been archived in this database. All reports are encoded and comprise information on type of sample, macroscopic and microscopic features as well as a final conclusion of the pathologist. Of note, our study was reviewed by the Medical Ethics Committee of our institution, the Amsterdam University Medical Centre (UMC)(W19_392 # 19.457).

DIAGNOSTIC CRITERIA AND CASE FINDING STRATEGY

In this follow-up study, a similar diagnostic framework for case identification was used as was previously published by our research group.[13,14] In addition to the previous search (1995 - 2015), the national database PALGA completed a comprehensive search to retrieve all pathology reports, matching the terms "esophagus" in combination with "eosinophilic inflammation", "eosinophilic hyperplasia", "eosinophilia", "eosinophi", or "allerg" between the first of January 2016 and the 31st of December 2019. All reports including primary carcinomas or describing eosinophilia in other regions of the Gastrointestinal (GI) tract were excluded. After the first search, all duplicates were removed. All patients that were included in one of our previous search strategies between the years 1995 and 2015, without confirmation of diagnosis were re-reviewed and included if: i) EoE was diagnosed based on a new pathology report and/or ii) EoE was suspected in retrospect based on previous reports and additional information with regards to the indication of performed esophagogastroduodenoscopy (EGD).[13,14] All cases were classified as EoE if: 1) the diagnosis was confirmed by the pathologist and/or 2) the degree of esophageal eosinophilia in one biopsy sample (taken from ≥ 2 levels of the esophagus) was described as "markedly" (or words of comparable meaning), which was interpreted as similar to ≥ 15 *eos/hpf* by the reviewers (BDvR, MJW, WEdR, MEB). All reports describing "mild" (i.e., moderate or words of similar meaning) esophageal eosinophilia without mentioning a peak eosinophil count of ≥ 15 *eos/hpf* were excluded. A manual review of all reports was performed by the first reviewer and a second reviewer was asked in case of uncertainty to reach consensus. After the first manual review, additional information with regards to the indication of the performed EGD with biopsies was requested in case of uncertainty. A comprehensive evaluation of these reports including additional data was done by the reviewers and cases were excluded if an EGD was performed due to suspicion of other potential causes of esophageal eosinophilia, such as: Drugs, Parasitic infection, Crohn's disease or GERD. Clinical information that was considered to further support the diagnosis of EoE included, e.g., symptoms of dysphagia or food impaction, typical endoscopic signs of EoE (e.g., furrows, rings). Of note, in several cases the diagnosis of concomitant GERD

was made if suggestive clinical signs were mentioned in the requested information with regards to the indication of the EGD performed. Endoscopic signs of reflux esophagitis or typical reflux-related symptoms were interpreted as being suggestive for the diagnosis or GERD. After re-review, all reports classified as EoE were included for final analysis. Furthermore, demographic data (gender, age and date of diagnosis) as well as relevant histological features (spongiosis, micro-abscesses, basal zone hyperplasia and sub-epithelial fibrosis) were derived from our database.

ENDOSCOPY WITH ESOPHAGEAL BIOPSY SAMPLING

To estimate the number of EGDs with esophageal biopsy sampling performed between 1995 and 2019, the PALGA database was queried. Search criteria were 'esophagus' and 'biopsy'. Of note, outcomes of this search yields an estimation of the total number of unique endoscopies with biopsies performed, considering that the search was not manually reviewed.

STATISTICAL ANALYSIS

Statistical analysis was performed by using IBM SPSS Statistics (version 25.0) (SPSS, Chicago, USA). To calculate the annual incidence rates between 1995 and 2019 the total Dutch population was considered to be at risk for developing EoE. Crude incidence values were calculated by using the total number of newly diagnosed EoE patients and matched with Dutch population data (https://www.cbs.nl). Incidence rates were calculated for the entire population and stratified for gender and age. Descriptive statistics were used to assess demographic characteristics, presented as mean (± SD) for normally distributed continuous data and percentages (%) for categorical data. Groups were compared with chi-square statistics and unpaired t-test, as appropriate. A p-value of 0.05 was considered to be statistically significant. Odds Ratio (OR) and 95% Confidence Intervals (CI) were calculated by using MedCalc Software Ltd (Ostend, Belgium).

RESULTS

CASE IDENTIFICATION

The database search between the first of January 1995 and the 31st of December 2019 included a total of 14.963 reports, of which 5298 reports were excluded due to non-existing esophageal eosinophilia or the presence of eosinophils in the GI-tract. In addition, another 598 reports, including revisions, incorrect reports or duplicates (i.e., double reports or

previous EoE diagnosis between 1995 - 2016) were removed. In total, 7361 unique patients, which covered a total of 9068 reports with esophageal eosinophilia, were considered to be eligible for final inclusion. After the first review, 5076 unique reports were classified as 'suspected for the diagnosis of EoE'. In total, 4061 unique patients were identified with a confirmed diagnosis of EoE following a second critical appraisal based on requested clinical information with regards to the indication of performed EGD (n = 3509). Of note, 31 patients being already included in our previous search (1995 - 2016) with no diagnosis of EoE were re-reviewed, of which EoE was confirmed in 27 patients. In total, 3974 (98%) reports were diagnosed with EoE at the first EGD. Furthermore, 2110 patients were diagnosed with different disease entities other than EoE. A flow chart of case identification is presented in **Figure 1**.

PATIENT CHARACTERISTICS

A male predominance (71%) was confirmed in our cohort, with a mean age at diagnosis of 37.9 ± 18.4 years. In total, 639 (16%) children (< 18 years) and 3422 (84%) adults were diagnosed. Of all identified adult EoE patients, 2419 (71%) were male and 1003 (29%) female. The mean age at diagnosis in adults was 42.9 ± 15.4 years, with a significant higher age in females compared to males (44.5 ± 16.5 years vs. 42.2 ± 14.8 years; $p < 0.001$). In children, the mean age at diagnoses was 10.9 ± 5.3 years, of which no difference between males and females (11.1 ± 5.2 vs. 10.4 ± 5.5; $p = 0.138$) was observed. EoE was diagnosed at all ages (3:1 male-to-female ratio), with patients between the ages of 20 and 49 years being mostly affected. An overview of all newly identified EoE patients within the years of 1995 and 2019, stratified by gender and age is presented in **Figure 2**.

Furthermore, 108 patients were determined as EoE with a concomitant esophageal disease based on the conclusion of the pathologist. In total, 18 (17%) patients were identified with EoE and coexisting GERD, 64 (59%) patients with EoE and Barrett's esophagus as well as 26 (24%) patients with EoE and esophageal candidiasis. In addition, no seasonal variations in the diagnosis of EoE were observed within the entire study timeframe (**Figure 3**).

HISTOLOGICAL FEATURES

The degree of esophageal eosinophilia in all identified EoE patients was mentioned in 1608 (40%) unique reports, of which 1473 (36%) were classified as marked (i.e., pronounced) and 135 (3%) as mild (i.e., moderate). Of note, in all 135 reports describing 'mild' esophageal eosinophilia, the diagnosis EoE was confirmed and/or ≥ 15 *eos/hpf* were described by the

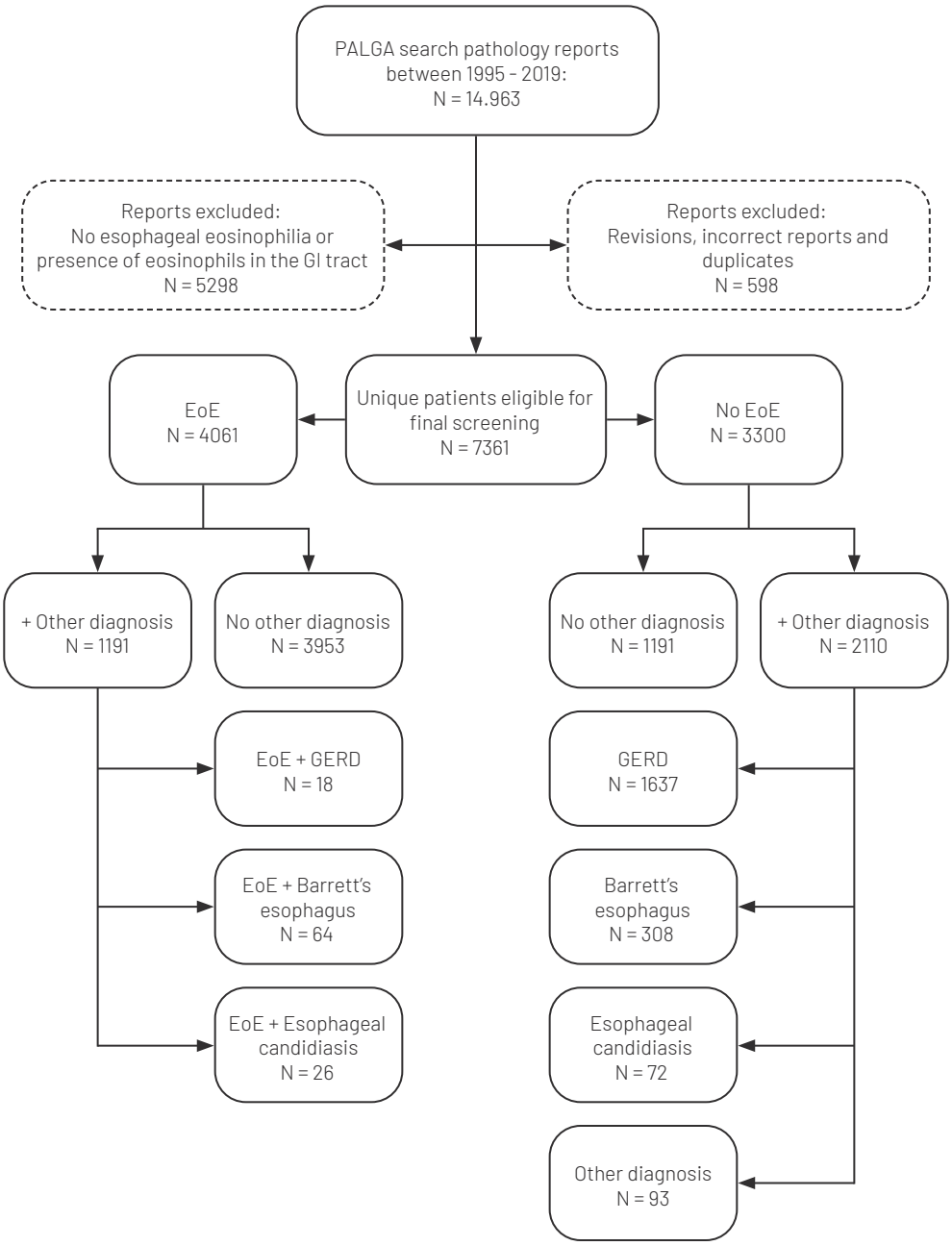

FIGURE 1. | Flowchart of case identification.
After the initial PALGA search, revisions, incorrect reports, and duplicates, as well as reports with absence of esophageal eosinophilia or presence of eosinophils in the gastrointestinal (GI) tract were excluded. A total of 7361 unique patients were eligible for review, of which 4061 cases were identified as eosinophilic esophagitis (EoE) in accordance with the conclusion of the pathologist

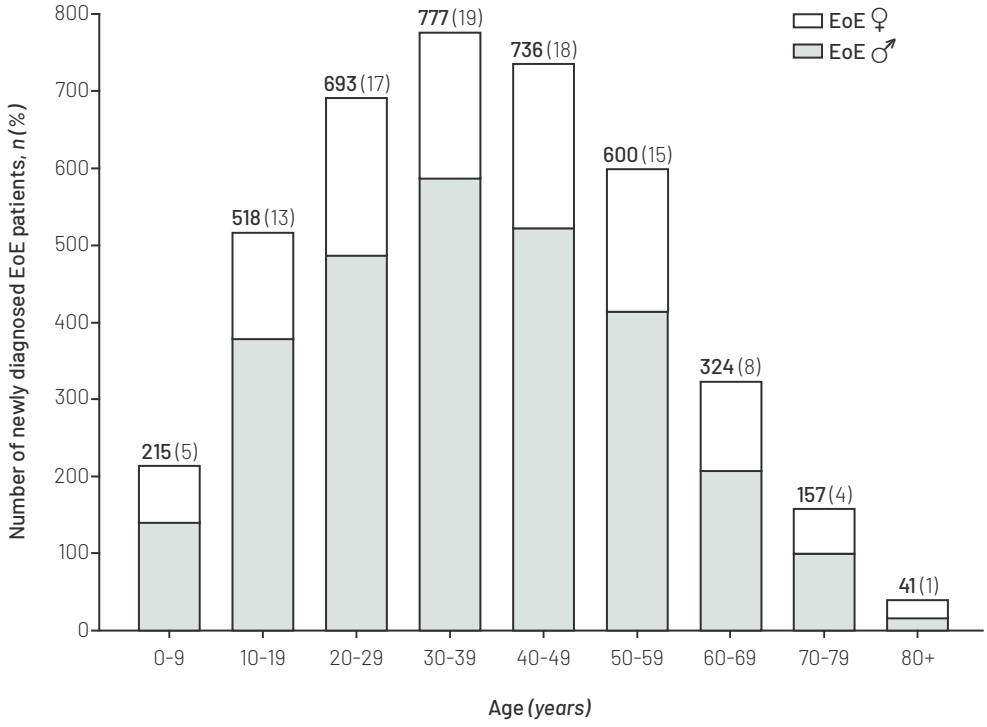

FIGURE 2. | Distribution of age at diagnosis in male and female patients with eosinophilic esophagitis (EoE), presented in 10 years strata.

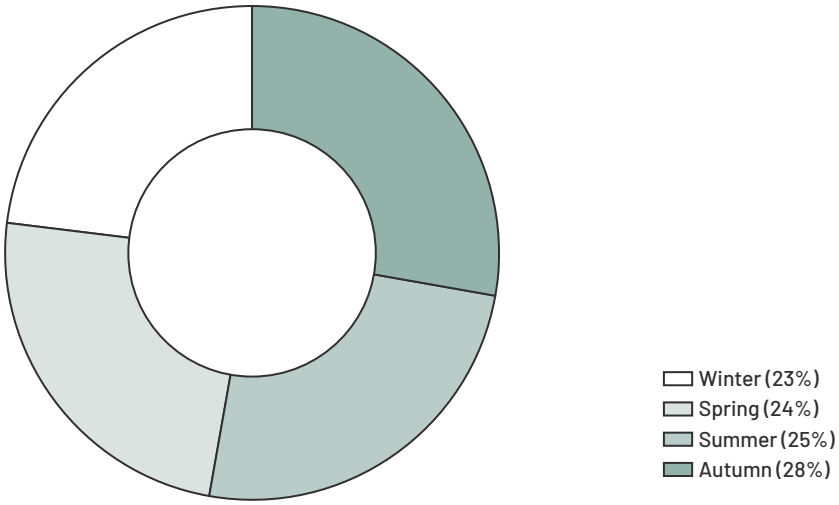

FIGURE 3. | Seasonal distribution of newly diagnosed eosinophilic esophagitis (EoE) patients between 1995 - 2019 in the Netherlands.

pathologist. In only 588 (15%) reports the esophageal eosinophilia was quantified as ≥ 15 *eos/hpf* within the entire study timeframe. Between 1995 - 2009, quantification of the esophageal eosinophilia (i.e., ≥ 15 *eos/hpf*) was not stated in any of the reports. Between 2010 - 2014 and 2015 - 2019 the esophageal eosinophilia was described as ≥ 5 *eos/hpf* in 124 (3%) and 464 (12%), respectively pathology reports. Additional histologic features such as spongiosis, micro-abscesses, basal zone hyperplasia and sub-epithelial fibrosis were described in less than 2% of all pathology reports.

INCIDENCE RATES OF EOE

The entire Dutch population comprised a total of 15.424.122 inhabitants in the year of 1995 and 17.282.163 inhabitants in the year of 2019 in accordance with the Central Bureau of Statistics (CBS). The average annual incidence of new cases per year during this time period is estimated to be 0.99 (95% (CI) 0.84 - 1.15) per 100.000 inhabitants, based on a population of 16.390.837 citizens. Between the years of 1995 and 2004, the incidence rates increased slightly from 0.006 (95% CI 0.002 - 0.036) new cases per 100.000 inhabitants in 1995 to 0.08 (95% CI 0.04 - 0.14) new cases per 100.000 inhabitants in 2004. From then on, an impressive rise of yearly new EoE diagnosis was observed between 2005 and 2019, comprising rates of 0.14 (95% CI 0.09 - 0.21) to 3.16 (95% CI 2.90 - 3.44) new cases per 100.000 inhabitants. During the time period between 1995 - 2019, males were significantly more at risk for the presence of EoE compared to females ((OR) 2.48 | 95% CI 2.32 - 2.65; $p < 0.001$). Annual incidence rates of males and females between the years of 1995 and 2019 are presented in **Figure 4**.

Over the past 25 years, adults were significantly more affected compared to children, with estimated average rates of 1.1 (95% CI 0.89 - 1.3) and 0.7 (95% CI 0.48 - 1.1), respectively new cases per 100.000 inhabitants (OR 1.46 | 95% CI 1.34 - 1.59; $p < 0.001$). Trends of incidence rates in children and adults are demonstrated in **Figure 5**.

The highest disease occurrence was observed in the final year of our analysis, with incidence rates in males and females of 4.37 (95% CI 3.94 - 4.84) vs. 1.97 (95% CI 1.68 - 2.29) per 100.000 inhabitants ($p < 0.001$). In 2019, EoE was mostly diagnosed in patients between the ages of 20 and 29, with significantly higher rates in males compared to females of 1.83 (95% CI 1.45 - 2.28) vs. 0.90 (95% CI 0.64 - 1.24 new cases per 100.000 inhabitants), respectively ($p < 0.001$). The majority of EoE patients (55%) were identified between the years of 2015 and 2019, with an estimated annual incidence over this time

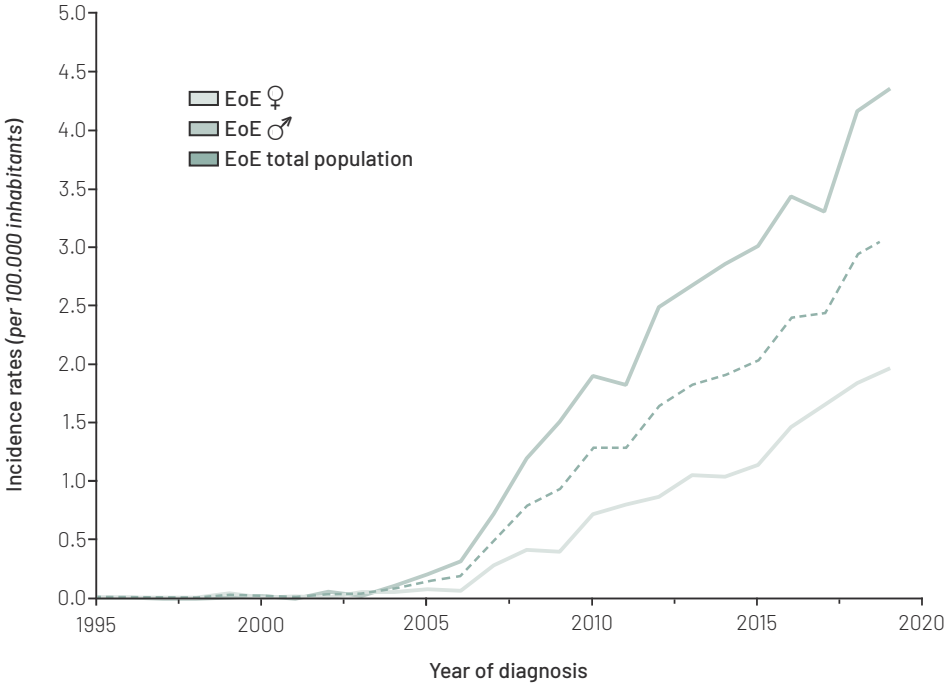

FIGURE 4. | Incidence rates of eosinophilic esophagitis (EoE) in males and females between 1995 - 2019 in the Netherlands.

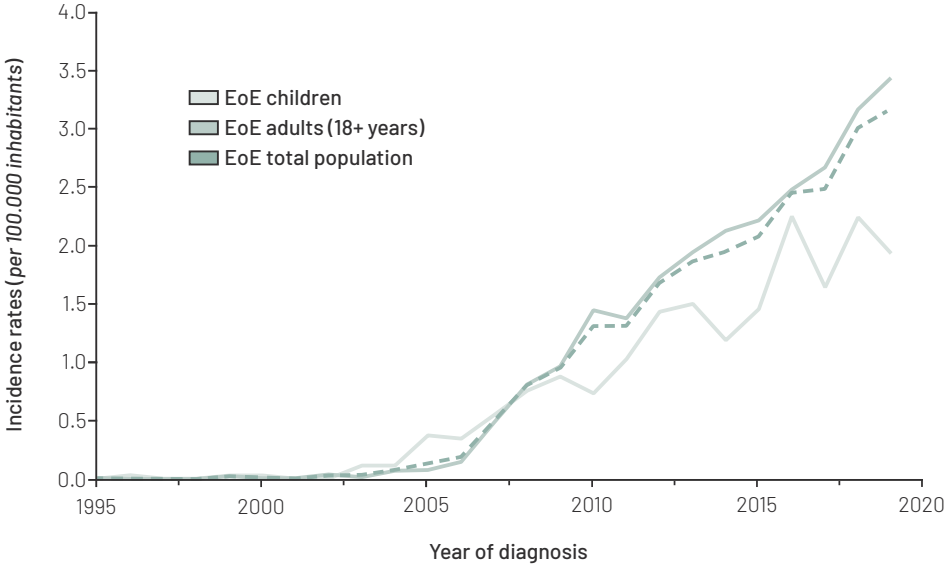

FIGURE 5. | Incidence rates of eosinophilic esophagitis (EoE) in children and adults (18+ years) between 1995 - 2019 in the Netherlands.

period of 2.63 (95% CI 2.4 - 2.9) new EoE cases per 100.000 inhabitants. Distribution of year of diagnosis in 5 years' strata for male and female patients is presented in **Figure 6**.

ENDOSCOPY WITH ESOPHAGEAL BIOPSY SAMPLING

Within the study time frame, a 2.6-fold increase of endoscopy with esophageal biopsy sampling was observed. The number of EGDs with biopsies performed per year increased from 8217 in 1995 per 100.000 inhabitants to 21.605 per 100.000 inhabitants in 2019 (**Figure 7**).

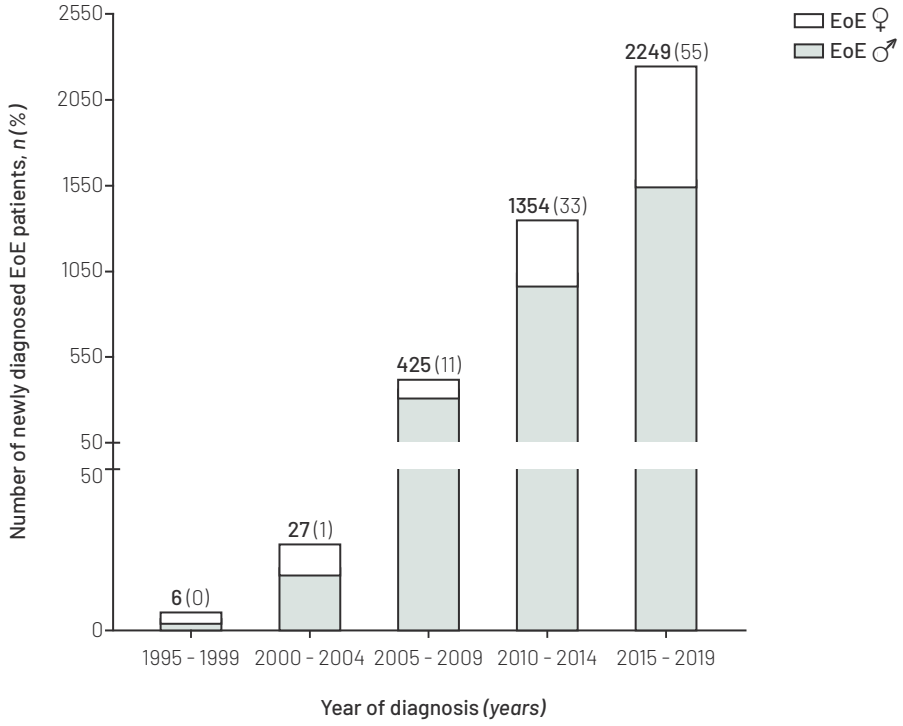

FIGURE 6. | Distribution of year of diagnosis in male and female patients with eosinophilic esophagitis (EoE), presented in 5 years strata.

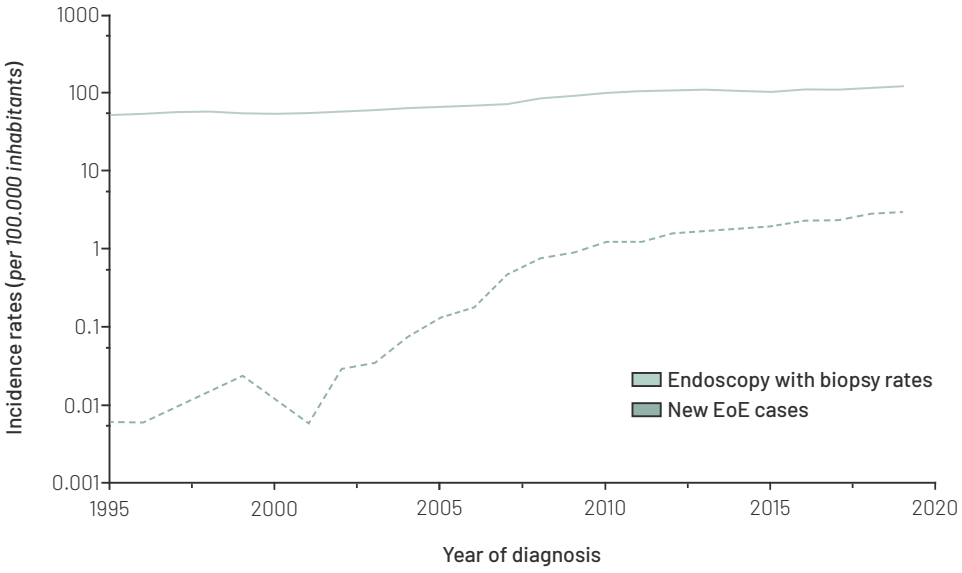

FIGURE 7. | New EoE cases per 100.000 inhabitants per year and the estimated number of yearly performed endoscopies with esophageal biopsy sampling per 100.000 inhabitants, between the years of 1995 to 2019. Logarithmic Y-axes are used to visualize outcomes of different orders of magnitude in one graph.

DISCUSSION

Observations on emerging incidence trends of EoE in the Netherlands are presented in this nationwide register-based study. Over a quarter century, the incidence of EoE has expanded a 316-fold and is still continuing to increase. A male predominance (3:1 ratio) was confirmed in this large cohort and patients between the ages of 29 to 49 years were most often affected. Within the entire study period, the EoE incidence was significantly higher in adults compared to children. These findings are consistent with previous literature and the natural course of this chronic progressive disease.[7,10,16,17] Given the rise in EoE frequency and its non-fatal nature, the prevalence in the Netherlands is estimated to be nearly 23.5 (95% CI 22.8 - 24.2) EoE cases per 100.000 inhabitants in 2019 and has doubled again within the past 5-years. Although the estimated prevalence suggests that EoE is still a relatively rare disease by absolute numbers, the increasing and still ongoing frequency of new cases underscores the real magnitude of this emergent disease that is nearly approaching those of Crohn's disease in European countries.[18-20] Moreover, the number of annual new EoE diagnosis increased from 0.006 new cases per 100.000 inhabitants in 1995 to 3.6 new cases per 100.000 inhabitants in 2019. These observations are similar

to a register-based study from Denmark reporting incidence rates of 0.13 to 2.6 new EoE cases per 100.000 inhabitants between the years of 1997 to 2012.[21] Of note, other European population-based EoE incidence rates were remarkably higher within this time frame compared to our observations.[22-24] With regards to a meta-analysis of Navarro *et al*., the pooled annual incidence rates between the years 1989 to 2017 in North-America and Europe were 7.1 and 2.7 cases per 100.000 inhabitants, respectively.[10] However, our results are in the lower spectrum of previous reported findings, with an estimated annual average incidence rate of 0.99 cases per 100.000 inhabitants. Nevertheless, these discrepancies in EoE incidence rates among studies are more likely explained by heterogeneous case definition and study designs rather than geographic variation.

The ongoing rise of EoE incidences in the Netherlands are consistent with previous findings on EoE being an increasingly common disorder in developed countries.[10] Several explanations have been suggested for the observed rise in prevalence in recent years. At first, increased detection bias following more widespread use of EGD with biopsies in general practice was considered a potential causative factor. However, we demonstrated only a 2.6-fold expansion of endoscopies with esophageal biopsy sampling performed within the study window, whereas the incidence of EoE raised a 316-fold. Moreover, multiple other studies have also confirmed that the increase of newly diagnosed patients far outpaces any expansion in EGD with biopsy.[8,14,23] The overall dramatic rise of EoE frequency is paralleling other increasing Western diseases (e.g., atopic morbidities and Crohn's disease), thereby suggesting a pivotal role for the environment in EoE pathogenesis.[25,26] Early childhood is known to be important for immune maturation, hence developmental susceptibility might be influenced by early-life experiences.[27] It was therefore hypothesized that modern hygienic conditions may result in less exposure to microbes during infancy, subsequently causing a defect in immune tolerance and increased sensitivity to allergic diseases.[28,29] Moreover, early life events (e.g., Cesarean section and antibiotic exposure) are considered to cause alterations of the composition and diversity of the microbiome, potentiating a T-helper type 2 (Th2) immune mediated response in certain sensitive individuals.[30-32] In addition, changes in environmental factors (e.g., genetic modification, food additives as well as water- and/or air pollution) and a Western dietary pattern (i.e., diet low in fibers and high in saturated fat) are also associated with microbial dysbiosis.[33-36] Moreover, the decline in frequency of *Helicobacter Pylori* infections and increasing prevalence of GERD in developed countries over the past several decades are both considered to contribute to the rapid rise of EoE.[37,38]

Interestingly, also the emerging EoE frequency closely coincides with increased acid-suppressant medication use, by that linking the rising use of proton-pump-inhibitors (PPIs) as a potentiating factor (i.e., prevention of peptic digestion of food allergens and microbial dysbiosis) to the development of EoE.[39-45] Taken all together, several mechanisms explaining the increase in EoE incidence have been suggested but none of these seems to offer a complete clarification. Although there is little evidence linking aeroallergen exposure to disease onset and flares, the exact role of allergic environmental factors in the pathogenesis remains unclear.[46,47] However, within the timeframe of our study no seasonal variations in EoE diagnosis was observed.

Some methodological challenges were encountered during this study. No data were available on patients' characteristics (e.g., symptoms and medical history) due to the use of encoded PALGA-pathology reports. Therefore, the majority of EoE diagnosis (52%) were exclusively based on histological information. Moreover, in only 588 (15%) reports the esophageal eosinophilia was quantified as ≥ 15 *eos/hpf* by the pathologist. These limitations of our diagnostic framework may have resulted in an underestimation of the observed EoE incidences. Nevertheless, 3509 (48%) reports were re-reviewed by using additional information with regards to the indication of performed EGD in order to expand the reliability of our case finding strategy. Of note, a former medical chart review (i.e., clinical, endoscopic and histological data) was performed by our research group, by that affirming a clinicopathological EoE diagnosis in 721 (33%) randomly selected cases between 1995 - 2015.[48] Moreover, all PALGA reports were consistently registered, hence no further histological pathognomonic features were included in our diagnostic strategy. Despite these limitations our study design has multiple strengths as well. At present, this is the largest population-based study providing most recent data on EoE incidence rates within a 25-year time period. Also, the risk of selection bias was reduced by the consistent use of one similar diagnostic framework with nationwide coverage of histological data.[13,14] Regarding our diagnostic strategy, we consider these results to reflect a valid and consistent overview of EoE incidence rates over the past quarter century.

In conclusion, we present observations on escalating EoE incidences over a considerable timeframe of 25-years in the Netherlands. From these results, it is clear that EoE incidence has not stabilized yet and continues to rise. These findings underscore the need to further investigate the mechanisms underlying its pathogenesis and which dynamic environmental components could lead to such an expansion of EoE cases.

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CHAPTER 3

UTILITY OF GASTRIC AND DUODENAL BIOPSY SAMPLING IN ADULT EOSINOPHILIC ESOPHAGITIS PATIENTS TO RULE OUT OTHER GASTROINTESTINAL DISORDERS

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ABSTRACT

RATIONALE

According to consensus Guidelines, if eosinophilic esophagitis (EoE) is suspected, not only esophageal but also gastric and duodenal biopsy specimens should be sampled in order to exclude other generalized or eosinophilic gastrointestinal (GI) disorders, such as eosinophilic gastroenteritis or celiac disease. However, the diagnostic yield for this remains unclear.

AIM

To assess the diagnostic yield of biopsy sampling from the stomach and duodenum in adult EoE patients to rule out generalized or eosinophilic GI disorders.

METHODS

A retrospective chart-review was conducted in untreated adult EoE patients that underwent upper endoscopy with biopsies sampled from the esophagus, stomach and duodenum. Standardized (electronic) case-report forms were used to extract clinical, endoscopic and histologic data.

RESULTS

In total, 93 adults (71% males, age 36.4 (interquartile range 28.4 - 49.1) years) with untreated EoE (≥ 15 eosinophils/high-power-field) were included. Symptoms of dysphagia and food impaction were reported in 93% and 58%, respectively of the patients. Typical endoscopic EoE-features were present in 77 (85%) patients. The yield of routinely sampled gastric and duodenal biopsy specimens in our cohort was 3.6% (95% Confidence Interval: 2.6% - 4.8%)(n/N = 1/93) for a relevant other generalized or eosinophilic GI diagnosis and 30% for other histological diagnosis such as non-specific or *Helicobacter Pylori* gastritis. In total, 62 (67%) patients presented with other GI symptoms and/or endoscopic abnormalities of the stomach and/or duodenum - which both may be suggestive for other relevant GI conditions. The diagnostic yield for a relevant generalized or eosinophilic GI disorder in this subgroup was, 4.8% (95% CI 3.4% - 6.7%)(n/N = 1/62).

CONCLUSION

Gastric and duodenal biopsy specimens seem to have limited diagnostic value for the exclusion of generalized or eosinophilic GI disorders in adults with EoE.

INTRODUCTION

Eosinophilic esophagitis (EoE) is an allergen/immune-mediated esophageal disorder.[1,2] At present, the paradigm for EoE diagnosis requires the presence of symptoms of esophageal dysfunction and eosinophilic inflammation with ≥ 15 eosinophils per high-power-field (*eos/hpf*) at esophageal biopsy.[3] The frequency of EoE, as it is recognized today, has increased dramatically after its first description as a case series in the early 1990s.[4-7] Since then, EoE has gone from being considered a relatively new disease to becoming the most frequent cause of dysphagia and food impaction.[8,9] The association between symptoms and biological disease activity (i.e., endoscopic- and histological features) is typically moderate in EoE patients.[10] Hence invasive procedures such as upper endoscopy with biopsies are required for diagnosis and monitoring of disease activity. In total, six biopsies sampled from ≥ 2 different levels of the esophagus supports sufficient accuracy for the diagnosis of EoE according to consensus guidelines.[11] Additional biopsy specimens from the stomach and/or duodenum should also be sampled if i) indicated by clinical symptoms (i.e., dyspepsia, abdominal pain, vomiting/nausea or diarrhea) or ii) endoscopic gross abnormalities suggestive for the presence of other gastric or small intestinal conditions.[3] However, evidence is lacking on the diagnostic value of these additional biopsies to rule out other relevant generalized or eosinophilic gastrointestinal (GI) disorders (e.g., celiac disease, parasitic infection, non-EoE eosinophilic gastrointestinal disorders (EGIDs). More importantly, these characterized alternate symptom etiologies or causes of esophageal eosinophilia such as non-EoE EGIDS are mostly rare, or its true relationship remains controversial as illustrated by the previous suggested association between celiac disease and EoE in adults.[12-16] For that reason, the diagnostic value of biopsy specimens sampled from the stomach and duodenum needs to be determined within a large cohort of adult EoE patients, so future evidence-based statements on its utility in daily practice may be established. Therefore, the main objective of this study was to assess the diagnostic yield of biopsy sampling from the stomach and duodenum in adult EoE patients to rule out other generalized or eosinophilic GI disorders.

METHODS

STUDY DESIGN AND PATIENTS

In this observational study, a retrospective medical chart review was conducted from our EoE cohort of patients who attended the outpatient clinic of the Amsterdam University Medical Centre (UMC). All adult patients (aged 18 and over) with untreated EoE at the time, in whom

biopsy specimens from the stomach and duodenum were sampled during upper endoscopy were included in this study. The Electronic Data Capture Castor was used to safely collect and store all study data. Our study was reviewed by the Medical Ethics Committee of our institution and formal evaluation was waived according to Dutch law (W20_509 # 20.562).

DATA COLLECTION

Demographics and clinical data

Electronic medical records of consecutive patients that qualified for the study were extensively reviewed by using a standardized electronic form to extract clinical, endoscopic and histologic data. Demographics (i.e., gender, age during upper endoscopy) and clinical data (i.e., medical history, atopic constitution and medication use) were obtained from all patients. Specifically, patients were screened for the presence of other relevant generalized or eosinophilic GI disorders, including celiac disease, inflammatory bowel disease (IBD), hypereosinophlic syndrome (HES), connective tissue disease (CTD) (e.g., scleroderma, vasculitis) or parasitic infection. Moreover, atopic comorbidities such as: allergic rhinitis, asthma, food allergy or atopic dermatitis were reported. Previous medication and relevant medication being used during upper endoscopy, specifically - if related to potential drug hypersensitivity reactions - were also documented.

Symptoms, endoscopic signs and histological features

The presence of clinical symptoms, such as: dysphagia, food impaction, dyspepsia (e.g., heartburn, bloating, belching, hiccups), retrosternal pain, abdominal pain, diarrhea or vomiting/nausea were evaluated. Typical esophageal endoscopic EoE features including inflammatory signs (i.e., edema, exudates and furrows) and fibrotic signs (i.e., rings and strictures) as reported by the endoscopist by using a standardized entry system (Endobase software system, Olympus, Winter & Ibe GmbH, Hamburg, Germany) were collected in our database. These features were classified according to the subcomponents of the EoE Endoscopic Reference Score (EREFS).[17] All subcomponents were presented as the percentage (%) of patients having these typical EoE-related signs at upper endoscopy. Documented endoscopic abnormalities of the stomach and/or duodenum were further classified as: erythema, edema, gastritis/duodenitis, mucosal defects (i.e., erosions, ulcerations, friable-, nodular- or granular mucosa) and other abnormal findings. Furthermore, all associated histology reports were reviewed and esophageal peak eosinophil counts (PEC) were collected. Additionally, biopsy specimens sampled from the stomach (i.e., antrum and corpus) and duodenum (bulb and second part of the

duodenum) were reviewed. All gastric biopsies describing findings, such as: active/non-active (chronic)(i.e., chemical, reactive, *Helicobacter Pylori*) gastritis, fundic gland polyps or presence of intraepithelial eosinophils were classified as 'abnormal'. Patients with intraepithelial eosinophils of ≥ 30 *eos/hpf*, observed in at least 5 hpf in the stomach were further classified as being suspected for a non-EoE EGID.[18-20] Moreover, duodenal pathology reports describing histological findings, such as: active duodenitis (i.e., chemical, reactive, *Helicobacter Pylori*), intraepithelial eosinophils, signs of Celiac disease (i.e., intraepithelial lymphocytosis (IEL) > 30/100 epithelial cells, lamina propria inflammation, villous atrophy or description of the Marsh classification) and positive test for Gardia Lambia were marked as 'abnormal'. Patients presenting with intraepithelial eosinophils of ≥ 30 *eos/hpf*, in at least 3 or more hpf in the duodenum were classified as being suspected for a non-EoE EGID diagnosis.[21]

Diagnostic yield of gastric and duodenal biopsy sampling and clinical subgroup identification

The total yield of routine gastric and duodenal biopsy sampling included all histological changes that met the criteria for a histological diagnosis (e.g., active gastritis, non-EoE EGIDs). However, we considered only the diagnostic yield of histological changes, such as: signs of celiac disease, parasitic diseases and non-EoE EGIDs being relevant to rule out other generalized or eosinophilic GI disorders. Furthermore, subgroups were identified according to consensus guidelines, with the yield of a relevant other generalized or eosinophilic GI disorder being determined in each subgroup.[3] Patients presenting with additional GI symptoms, such as: dyspepsia, abdominal pain, vomiting/nausea and diarrhea that may be suggestive for a relevant other generalized or eosinophilic GI disorder were classified as being 'clinically suspected'. The presence of endoscopic abnormalities (e.g., erythema, gastritis/duodenitis) in patients were classified as the subgroup being 'endoscopically suspected' for a relevant other generalized or eosinophilic GI disorder. Patients that had additional GI symptoms and/or endoscopic abnormalities were classified as the subgroup being 'clinically and/or endoscopically' suspected.

STATISTICAL ANALYSIS

Statistical analysis was performed by using IBM SPSS Statistics (version 25.0) (SPSS, Chicago, USA). Descriptive statistics were used to assess demographic characteristics, presented as median with interquartile range (IQR) for non-normally distributed continuous data and percentages for categorical data. The total diagnostic yield was defined as

the proportion of patients in which biopsies sampled from the stomach and duodenum provided a relevant other generalized or eosinophilic GI diagnosis out of the total number of patients that were analyzed. Sensitivity, specificity, positive and negative predictive values for relevant other generalized or eosinophilic GI disorders in clinical subgroups were calculated and presented with 95% confidence interval (95% CI). Overall diagnostic accuracy, expressed as a proportion of correctly classified patients (true positives and true negatives) among all patients was calculated. Diagnostic evaluation was performed by using MedCalc Software Ltd (Ostend, Belgium).

RESULTS

PATIENT CHARACTERISTIC

In total, 93 adult patients with active EoE (≥ 15 *eos/hpf*) were included for analysis. The median age was 36.4 (IQR 28.4 - 49.1) years and a male pre-dominance (71%) was confirmed (Table 1). Atopic diathesis was noted in 64 (69%) patients, of which the majority (58%) had been diagnosed with allergic rhino-conjunctivitis. Previous therapeutic treatment for EoE was documented in 54 (58%) patients. No medication use that may be related to potential drug hypersensitivity was reported at the time of endoscopy with biopsies. None of the patients had a medical history of other relevant generalized or eosinophilic GI diseases. More details on patients' characteristics are presented in **Table 1**.

SYMPTOMS

Dysphagia (93%) and food impaction (58%) were the most frequently reported symptoms. Other less documented symptoms were dyspepsia (35%), retrosternal pain (24%), abdominal pain (22%), diarrhea (8%) and nausea/vomiting (5%) (**Figure 1**).

ENDOSCOPIC SIGNS

Typical endoscopic EoE features were present in 77 (85%) patients. Inflammatory signs, including furrows, edema and exudates were reported in 57%, 53% and 42%, respectively of these patients. Endoscopic characteristics related to fibrosis e.g., rings and strictures were observed in 51% and 14%, respectively of patients (**Figure 2**).

Abnormal endoscopic findings of the stomach were reported in 30 (33%) patients, of which the presence of erythema (70%) was most frequently documented. Other abnormal findings were: gastritis (23%), edema (17%), mucosal defects (17%) and other remarkable anomality's (10%) e.g., hematin spots (**Figure 3**). Moreover, abnormal endoscopic findings of

TABLE 1. | Baseline characteristics of the study population (n = 93)

Characteristics	
Male gender, *n (%)*	66 (71)
Age, years, *median (IQR)*	36.4 (28.4 - 49.1)
History of allergic disease, *n (%)*	64 (69)
Allergic rhinitis	50 (54)
Food allergy	19 (21)
Atopic dermatitis	18 (19)
Asthma	17 (18)
Previous medication use, *n (%)*	54 (58)
PPIs, *n (%)*	39 (42)
Topical steroids, *n (%)*	3 (3)
PPIs + topical steroids, *n (%)*	4 (4)
Other *, *n (%)*	8 (9)
Medical history of other ** relevant (eosinophil-related) diseases, yes, *n (%)*	0 (0)

* Montelukast, 6-Thioguanine (6-TG), Antihistamines, Histamine-2 blockers.
** Celiac disease, IBD, HES, Connective tissue disease (e.g., scleroderma, vasculitis) or parasitic infection.
PPIs = Proton Pump Inhibitors, CD = Celiac Disease, IBD = Inflammatory Bowel Disease. HES = Hypereosinophilic syndrome.

the duodenum were observed in 19 (21%) patients. Documented findings were: duodenitis (32%), erythema (26%), edema (16%) and mucosal defects (5%). Other endoscopic abnormalities of the duodenum were noted in 6 (32%) patients e.g., endoscopic signs of villous atrophy or Brunner's glands (**Figure 4**).

HISTOLOGICAL FEATURES

The presence of eosinophil predominant inflammation of the esophagus (i.e., ≥ 15 *eos/hpf*) was confirmed by the pathologist in all patients. The median PEC in the esophageal biopsies was 50 (IQR 31.3 - 75) (**Table 2**).

Remarkable histological findings of the gastric specimens were observed in 34 (37%) patients. Active gastritis was reported in 18 of these 34 patients (53%), with the presence of *Helicobacter Pylori* being confirmed in 7 (39%) reports. Non-active (chronic) gastritis was observed in 10 (29%) patients. The presence of intraepithelial eosinophils was noted in 4 (12%) patients, with a median PEC of 15 (IQR 12 - 25). Other remarkable findings (18%) were reactive changes (**Table 2**).

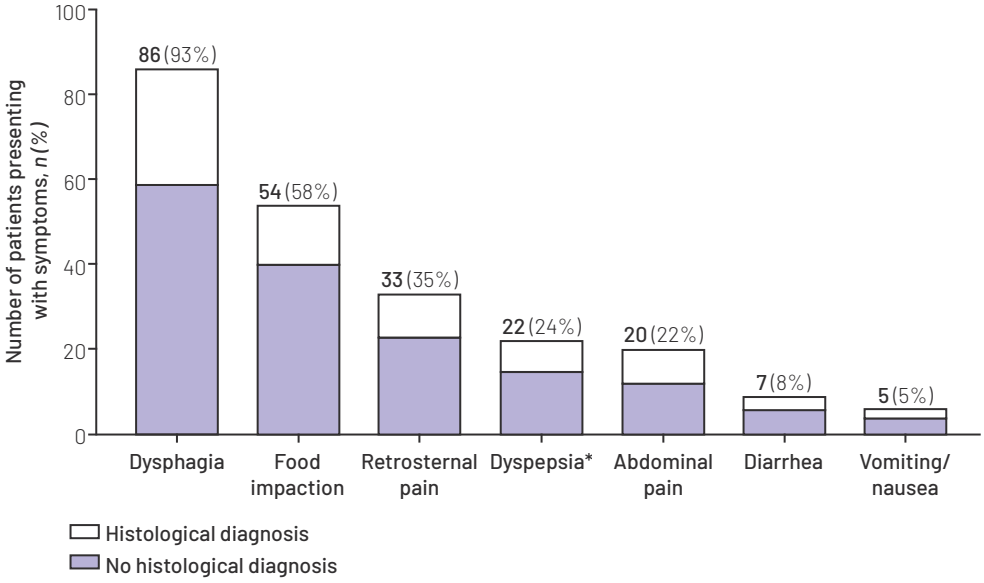

FIGURE 1. | Number n (%) of patients presenting with symptoms during upper endoscopy with biopsy in the study population, stratified for histological diagnosis or no histological diagnosis (n = 93).
* i.e., symptoms of heartburn, bloating, belching, hiccups.

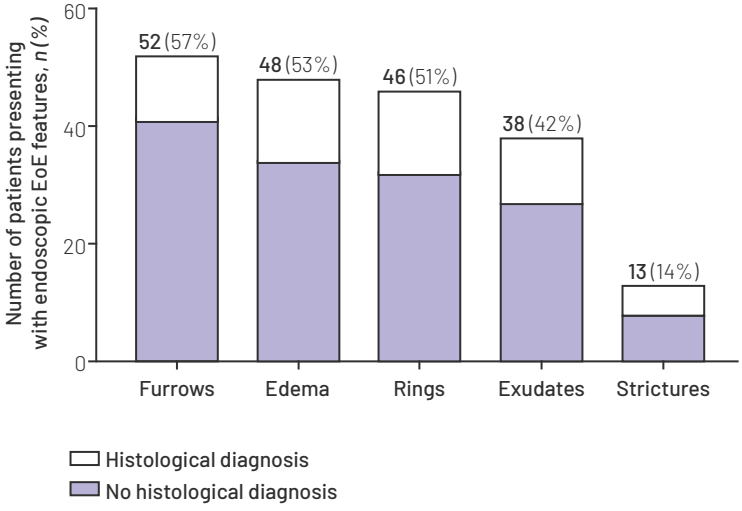

FIGURE 2. | Number n (%) of patients presenting with inflammatory (i.e., furrows, edema, exudates) and fibrotic (rings, strictures) signs during upper endoscopy with biopsy in the study population, stratified for histological diagnosis or no histological diagnosis (n = 93).

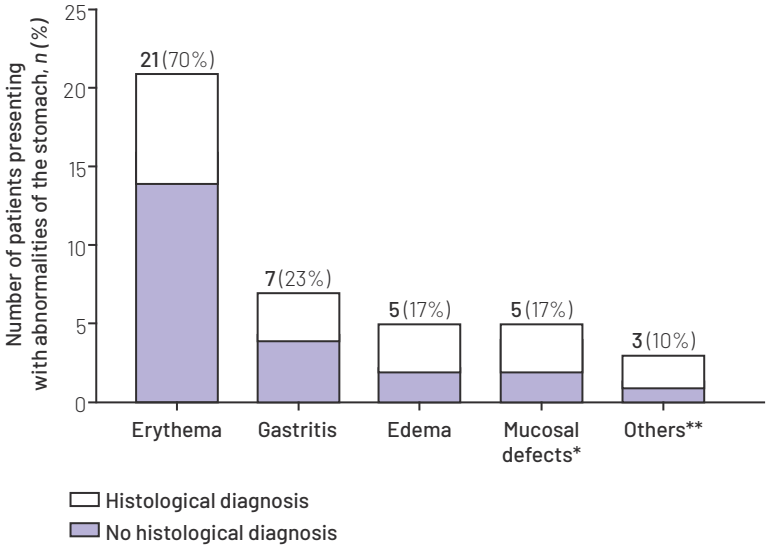

FIGURE 3. | Nuamber n (%) of patients with endoscopic abnormalities of the stomach during upper endoscopy with biopsy in the study population, stratified for histological diagnosis or no histological diagnosis (n = 93).
* i.e., Erosions, ulcerations, friable-, nodular- or granular mucosa.
** e.g., Hematin spots.

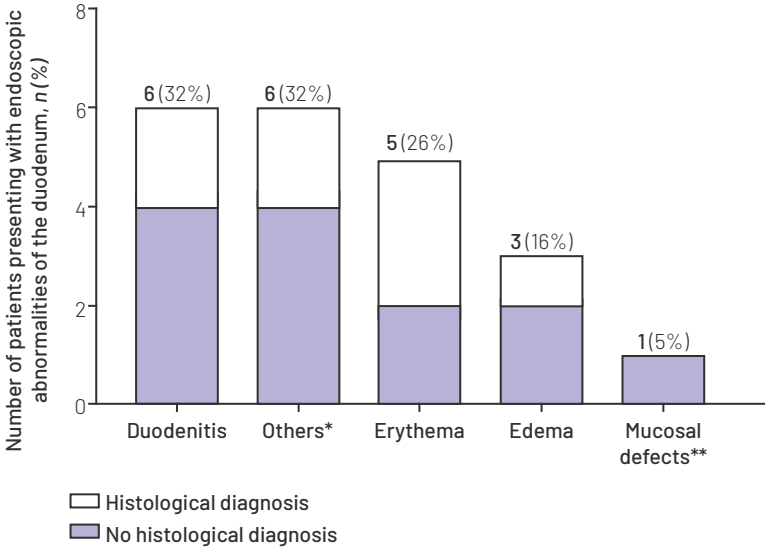

FIGURE 4. | Number n (%) of patients with endoscopic abnormalities of the duodenum during upper endoscopy with biopsy in the study population, stratified for histological diagnosis or no histological diagnosis (n = 93).
* e.g., Endoscopic signs of villous atrophy or Brunner's glands.
** i.e., Erosions, ulcerations, friable-, nodular- or granular mucosa.

Furthermore, remarkable histological findings of duodenum specimens were described in 11 (12%) pathology reports, including active (i.e., peptic, reactive) duodenitis in 4 (36%) patients and other histological features in 3 (27%) patients e.g., reactive changes. The presence of intraepithelial eosinophils (36%) was described in 4 (36%) reports, with a median PEC of 23.5 (IQR 16.8 - 36.3) (**Table 2**).

TABLE 2. | Histological findings in the study population *(n = 93)*

Histological features	**Total sample (n = 93)**
Esophagus	
Presence of esophageal eosinophilic inflammation, *n (%) (i.e., ≥ 15 eos/hpf)*	93 (100)
Peak eosinophil count quantified, *n (%)*	68 (73)
Peak eosinophil count, *median (IQR)*	50 (31.3 - 75)
Stomach	
Histological abnormalities, *yes, n (%)*	34 (37)
Non-active (chronic) gastritis, *n (%)*	10 (29)
Active gastritis (i.e., chemical, reactive, *Helicobacter Pylori), n (%)*	18 (53)
Presence of intraepithelial eosinophils, *n (%)*	4 (12)
Peak eosinophil count, *median (IQR)*	15 (12 - 25)
Fulfilling the histological criteria for the diagnosis of a non-EoE EGID, *yes, n (%) (i.e., presence of ≥ 30 eos/hpf (≥ 5 hpf)*	0 (0)
Fundic gland polyps, *n (%)*	0 (0)
Other remarkable findings, *n (%)*	6 (18)
Duodenum	
Histological abnormalities, yes, *n (%)*	11 (12)
Active duodenitis (i.e., chemical, reactive, *Helicobacter Pylori), n (%)*	4 (36)
Presence of intraepithelial eosinophils, *n (%)*	4 (36)
Peak eosinophil count, *median (IQR)*	23.5 (16.8 - 36.3)
Fulfilling the histological criteria for the diagnosis of a non-EoE EGID, *yes, n (%) (i.e., presence ≥ 30 eos/hpf (≥ 3 hpf)*	1 (25)
Signs of celiac disease*, *n (%)*	0 (0)
Gardia Lambia, positive test, *n (%)*	0 (0)
Other remarkable findings, *n (%)*	3 (27)

EoE = Eosinophilic esophagitis, PEC = Peak eosinophil count, non-EoE EGID = non-EoE Eosinophilic gastrointestinal disorder, IQR = Inter quartile range, eos = eosinophils, hpf = high power field.
* I.e., intra-epithelial lymphocytosis (IEL > 30/100 epithelial cells), lamina propria inflammation, villous atrophy or description of Marsh classification.

DIAGNOSTIC YIELD OF GASTRIC AND DUODENAL BIOPSY SPECIMENS IN CLINICAL SUBGROUPS

Routine sampling of gastric and duodenal biopsies

Routine sampling of biopsy specimens from the stomach and duodenum in the total study population (n = 93) showed histological changes in 28 (30%) patients, of which 23 (82%) patients were diagnosed with active/non-active (chronic) gastritis, 2 (7%) patients with active duodenitis, 2 (7%) patients with gastritis together with duodenitis and 1 (4%) patient met the histological criteria for a non-EoE EGID (**Table 2**). Of note, the diagnosis of eosinophilic gastroenteritis (EGE) in a single patient was suspected based on the presence of generalized GI eosinophilia, with a PEC ≥ 15 *eos/hpf* in the esophagus and ≥ 30 *eos/hpf* in at least 3 hpf in the duodenum.[21,22] In total, the yield of routine gastric and duodenal biopsy sampling for the proportion of patients with histological diagnosis such as gastritis was 30% (n/N = 28/93). However, only the single diagnosis of EGE was considered being a relevant other generalized or eosinophilic GI disorder within the context of a definitive diagnosis of EoE (diagnostic yield: 3.6% (95%CI 2.6% to 4.8%) (n/N = 1/93)) (**Supplementary Table 1**).

CLINICAL AND/OR ENDOSCOPIC INDICATION OF BIOPSY SAMPLING

Forty-seven (51%) patients in our study population presented with additional GI symptoms and were classified as being 'clinically suspected' for other relevant generalized or eosinophilic GI disorders. The diagnostic yield in this 'clinically suspected' subgroup was 7.1% (95% CI 4.6% - 10.9%), one diagnosis of EGE (n/N = 1/47). Other etiologies were diagnosed in 28% of this subgroup and included mainly gastritis (n/N = 13/47) (**Supplementary Table 1**).

Additionally, endoscopic abnormalities of the stomach or duodenum were observed in 36 (40%) patients, so therefore being classified as 'endoscopically suspected' for a relevant other generalized or eosinophilic GI disorder. A histological diagnosis was confirmed in 39% of this subgroup (n/N = 14/36), with the majority (64%) of diagnosis being acute or chronic gastritis. Still the diagnostic yield for a relevant other generalized or eosinophilic GI disorder in this 'endoscopically suspected' subgroup was 7.1% (95% CI 4.8% - 10.6%), being one diagnosis of EGE (n/N = 1/36) (**Supplementary Table 1)**.

In total, 62 (67%) patients were classified as being 'clinically and/or endoscopically' suspected for a relevant other generalized or eosinophilic GI disorder, with 21 (45%) of

these patients receiving a histological diagnosis (n/N = 21/62). However, this diagnosis was most often acute or chronic gastritis (76%). The diagnostic yield for a relevant other generalized or eosinophilic GI disorder in this subgroup was 4.8% (95% CI 3.4% - 6.7%) (n/N = 1/62)(**Supplementary Table 1**). No histological diagnosis was confirmed in patients (33%) that were not 'clinically and/or endoscopically' suspected for a relevant other generalized or eosinophilic GI disorder.

FOLLOW-UP AFTER EGE DIAGNOSIS

One (1%) patient with previously diagnosed EoE met the clinical and histological criteria for the diagnosis of EGE. Atopic constitution (i.e., allergic rhinitis, asthma and food allergy) was reported in this patient, with no medical history of other relevant generalized or eosinophilic GI disorders. No concomitant medication use was documented and the fecal sample test was negative for ova or parasites. Lab test results of complete blood count (CBC) with differential were within the reference values. Moreover, observations on serum albumin and Iron/ferritin levels were also normal. Endoscopic appearance of edema and a 'ringed' esophagus together with marked edema of the stomach and duodenum were observed at the time of diagnosis. Histological assessment after 6 weeks of diet intervention (i.e., 4-Food elimination diet) showed complete disappearance of eosinophilia in the upper GI-tract. Former symptoms were still present, though less apparent. Concentric rings were also noticed at week 6, however, no more signs of edema of the upper GI-tract were observed. Follow-up after 6 months dietary treatment (elimination and re-introduction of foods) showed deterioration of esophageal eosinophilia (≥ 15 *eos/hpf*). Interestingly, no more signs of eosinophilia in the stomach nor yet the duodenum were noted at histological evaluation.

DISCUSSION

This is the first study on the added value of standard gastric and duodenal biopsy specimens in a large cohort of adults with active EoE. As such, the added diagnostic value of routine sampling of gastric and duodenal biopsies yielded a histological diagnosis in 28 (30%) patients. However, most of these diagnoses were active- or chronic gastritis, with no relevance to the management and/or impact on the previous diagnosis of EoE. Only 1 (1%) patient was diagnosed with EGE on duodenal biopsies. Therefore, we considered the diagnostic yield of 3.6% for a relevant other generalized or eosinophilic disorder in our cohort to be generally very low.

When narrowing down the 47 (51%) patients presenting with significant GI symptoms and/or 36 (40%) patients with gastric and/or duodenal endoscopic abnormalities - which both may be suggestive for other relevant generalized or eosinophilic GI disorders - the overall group of patients that needs to be biopsied according to consensus guidelines is reduced to 62 (67%) patients.[3] However, with still only one patient being diagnosed with EGE in this subgroup, the diagnostic yield of 4.8% (n/N = 1/62) continues to be low. Consequently, biopsy specimens sampled from the stomach and duodenum, regardless of a potential clinical or endoscopic indication, seems to have limited utility in a large cohort of EoE patients. It thus seems essential to avoid extra sampling of gastric and duodenal biopsies in patients without a clear indication, considering a substantial economic burden of EoE is related to medical resource utilization costs (e.g., upper endoscopy with biopsy).[23,24] Dispose of irrelevant biopsy proceedings lowers health-care costs (i.e., reduced time and complication risk) and may also improve patients' tolerability of such invasive. Hence, additional biopsies should only be sampled in patients with endoscopic abnormalities and/or clinical symptoms compatible with a gastric or duodenal GI disorder, not only in clinical practice but also in trials. This is also in line with The Food and Drug Administration (FDA) Guidance for clinical research in EoE, suggesting that stomach and/or duodenum specimens should be sampled in adult EoE patients, if clinically indicated by symptoms or abnormal endoscopic findings.[25] Notwithstanding, in several clinical trials and in hospitals, biopsies from the stomach and duodenum are routinely sampled at least once in all EoE patients. However, based on our observations there does not seem to be a rationale for this.

A previous study by Vanstapel *et al*. suggests that systematic determination of PECs in gastric and duodenal biopsy specimens in adult EoE patients may have frequent added value with regard to detection of generalized eosinophilia.[26] Reported median PECs in 46 gastric and 27 duodenal biopsy specimens of patients with active EoE (≥ 15 *eos/hpf*) in that study were 3 (IQR 2 - 10) and 25 (IQR 16 - 44), respectively. The authors argue that these findings support taking gastric and duodenal biopsies in all patients with EoE, yet it may be questionable how clinically relevant these findings are. At present, no consensus recommendations for either clinical or pathological diagnosis of EGIDs except for EoE consists. Various cut-off values for eosinophils in the stomach (30 - 70 *eos/hpf*) and duodenum (26 - 52 *eos/hpf*) have been reported.[20-22,27] It might therefore be argued that the findings by Vanstapel *et al.*, are still within the normal spectrum.

Despite of its treatment being similar to EoE in the majority of cases, non-EoE-EGIDs may often be followed clinically with no treatment after diagnosis.[28] With regards to the patient being diagnosed with EGE in our cohort, 6 months follow-up showed relapse of esophageal eosinophilia (≥ 15 *eos/hpf*) at histological assessment after reintroduction of culprit foods. More importantly, no signs of generalized eosinophilia in the upper GI-tract were observed any more. This remarkable finding of spontaneous remission has previous been well-described within a large subgroup of patients presenting with a single disease flare.[28,29] Aside from non-EoE EGIDs being a rare condition, the relevance of disease detection with regards to the implications for practice seems to be even more at issue.[12]

Our study has several limitations, mainly related to its retrospective design. First, we did not used validated symptom outcome measures at the time of upper endoscopy. However, the use of standardized case report forms for data-extraction may have improved the validity of our results. Secondly, endoscopic signs were based on physician reports, thus subject to bias. Nevertheless, the use of a single documentation system with pre-defined entry options for the generation of endoscopic reports may have increased the validity of our endoscopic outcomes. Furthermore, in case of uncertainty endoscopic images were reviewed in order to support the final conclusion and by that also extending the reliability of our strategy. Therefore, despite these few limitations, our study adds to the existing literature first observations on the limited diagnostic value of biopsy specimens sampled from the stomach and duodenum within a large cohort of adult EoE patients. Particularly, the use of a comprehensive diagnostic framework, incorporating clinical (i.e., symptoms, medical history, medication use), endoscopic and histological data, certainly improved the validity of our study design. Additionally, the pathologist was specifically requested for signs of 'eosinophilia' (i.e., increased influx of intraepithelial eosinophils) or presence of non-EoE EGIDs in our cohort which may also have improved the reliability of our outcomes.

In summary, this is the first and largest study until now demonstrating that biopsy specimens routinely sampled from the stomach and duodenum have limited utility in adult EoE patients. Standard biopsies are not indicated in adult EoE patients without endoscopic abnormalities in the stomach and/or duodenum or suggestive symptoms and should be avoided in these cases.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE 1. | Diagnostic yield of biopsy specimens sampled from the stomach and duodenum for a relevant other generalized (eosinophilic) gastrointestinal disorder in the study population, stratified for clinical subgroups (n = 93).

Subgroup characteristics	n/N	Diagnostic yield				
		Sensitivity	Specificity	Accuracy	PPV	NPV
Routine biopsy sampling						
Histological diagnosis (n = 28)	1/93	100 (2.5 - 100)	70.7 (60.3 -79.7)	71 (60.6 - 80)	3.6 (2.6 - 4.8)	100
'Clinically' suspected *i.e., Dyspepsia, abdominal pain, vomiting/nausea and diarrhea*						
Histological diagnosis (n = 14)	1/47	100 (2.5 - 100)	71.7 (56.5 - 84)	72.3 (57.4 -84.4)	7.1 (4.6 - 10.9)	100
'Endoscopically' suspected *i.e., Erythema, edema, gastritis/duodenitis, erosions, ulcerations, friable-, nodular- or granular mucosa*						
Histological diagnosis (n = 14)	1/36	100 (2.5 - 100)	62.9 (44.9 -78.5)	63.9 (46.2 -79.2)	7.1 (4.8 - 10.6)	100
'Clinically and/or endoscopically' suspected						
Histological diagnosis (n = 21)	1/62	100 (2.5 - 100)	67.2 (54 - 78.7)	67.7 (54.7 -79.1)	4.8 (3.4 - 6.7)	100

Values are presented as % with 5% confidence interval.
n = number of diagnosis of relevant other generalized (eosinophilic) disorders, N = number of subgroup sample, CI = confidence interval, PPV = positive predictive value, NPV = negative predictive values.
Histological diagnosis = e.g., Active/non-active (chronic) gastritis or duodenitis (i.e., chemical, reactive, *Helicobacter Pylori*), non-EoE Eosinophilic gastrointestinal disorders (EGIDs).

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CHAPTER 4

PHARMACOTHERAPIES FOR THE TREATMENT OF EOSINOPHILIC ESOPHAGITIS: STATE OF THE ART REVIEW

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ABSTRACT

Eosinophilic esophagitis (EoE), a chronic allergic disorder of the esophagus, is characterized by symptoms of esophageal dysfunction and eosinophil-predominant inflammation. The incidence of EoE has increased substantially over the past two decades, coinciding with the "allergy epidemic". Current treatment options consist of dietary intervention, endoscopic dilatation, and pharmacotherapy, including proton pump inhibitors and swallowed topical corticosteroids. Given that EoE is a chronic progressive disease that is prone to relapse after cessation of therapy, these treatment options are suboptimal for long-term management. Persistent, uncontrolled esophageal inflammation is associated with esophageal remodeling and stricture formation; therefore, the need for alternative treatments is of paramount importance. The pathogenesis of EoE is currently under intense investigation, and recent insights concerning cellular and molecular etiology have led to the development of therapies that target specific pathophysiological pathways.

This article provides an overview of established EoE pharmacotherapies, which include proton-pump-inhibitors (PPIs) and swallowed topical steroids. Additionally, anti-allergic targets, immunosuppressives, and monoclonal antibodies (such as mepolizumab, reslizumab, QAX576, RPC4046, dupilumab, omalizumab, and infliximab) that have been evaluated as treatments for EoE are summarized. Finally, several promising therapeutic agents (e.g., sialic acid-binding Ig-like lectin 8 [Siglec-8] antibodies, the transforming growth factor β1 [TGFβ1] signal blocker losartan, CC chemokine receptor type 3 [CCR3] antagonists, thymic stromal lymphopoietin [TSLP] antibodies, antibodies targeting the α4β7 integrin, anti-interleukin-9 antibodies, and anti-interleukin-15 antibodies) that target specific molecules or cells implicated in the pathogenesis of EoE are proposed.

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic, progressive, T-helper type 2 (Th2) immune-mediated disorder characterized by symptoms of esophageal dysfunction and eosinophil-predominant inflammation.[1,2] The incidence and prevalence of EoE has risen considerably since it was first described as a unique disease entity in the early 1990s.[3-6] In industrialized countries, incidence and prevalence estimates are 5 to 10 cases per 100,000 persons and 0.5 to 1 per 1000 persons, respectively.[3,7-13] By comparison, these rates are similar to those of Crohn's disease.[14] The rise of EoE coincides with the global "allergy epidemic", and most EoE patients have atopic comorbidities such as allergic rhinitis, asthma, immunoglobulin E (IgE)-mediated food allergies and atopic dermatitis.[15,16] The peak incidence of EoE is between the ages of 20 to 40 years, with a 3:1 male-to-female ratio in every age group.[17] Children often present with non-specific symptoms of abdominal pain, failure to thrive and feeding disorder, whereas adults typically experience dysphagia and food impaction.[1,2,18]

When EoE is suspected on clinical grounds, an upper endoscopy with at least six biopsies taken from two levels of the esophagus is recommended.[19] Endoscopic disease activity is detected in approximately 90% of symptomatic patients. While edema, linear furrows and white exudates are common in pediatric EoE, both inflammatory and fibrotic features - including rings and strictures - frequently manifest in adults.[20] A diagnosis of EoE is confirmed if at least one esophageal biopsy shows a minimum of 15 eosinophils per high power field (*eos/hpf*) and other causes of esophageal eosinophilia are excluded.[21]

In clinical practice, the management of EoE has historically consisted of the "3D-approach": diet, drugs and dilation, with the choice of strategy depending on disease phenotype (inflammatory and/or fibrotic) and patient preferences. A therapeutic algorithm is proposed in the European EoE guidelines, as shown in **Figure 1**.[21] Patients with EoE are prone to relapse following initial response to therapy, and long-standing inflammation is associated with esophageal remodeling and consecutive stricture formation.[22-24] Although reducing or eliminating esophageal inflammation may prevent the fibrotic process, direct evidence to support this theory is lacking. Thus, the treatment objectives in EoE are to reduce symptoms of esophageal dysfunction and prevent long-term complications and esophageal damage by maintaining histologic remission.

This article summarizes contemporary pharmacological strategies for treating EoE, drugs currently under investigation, and therapeutic targets on the horizon.

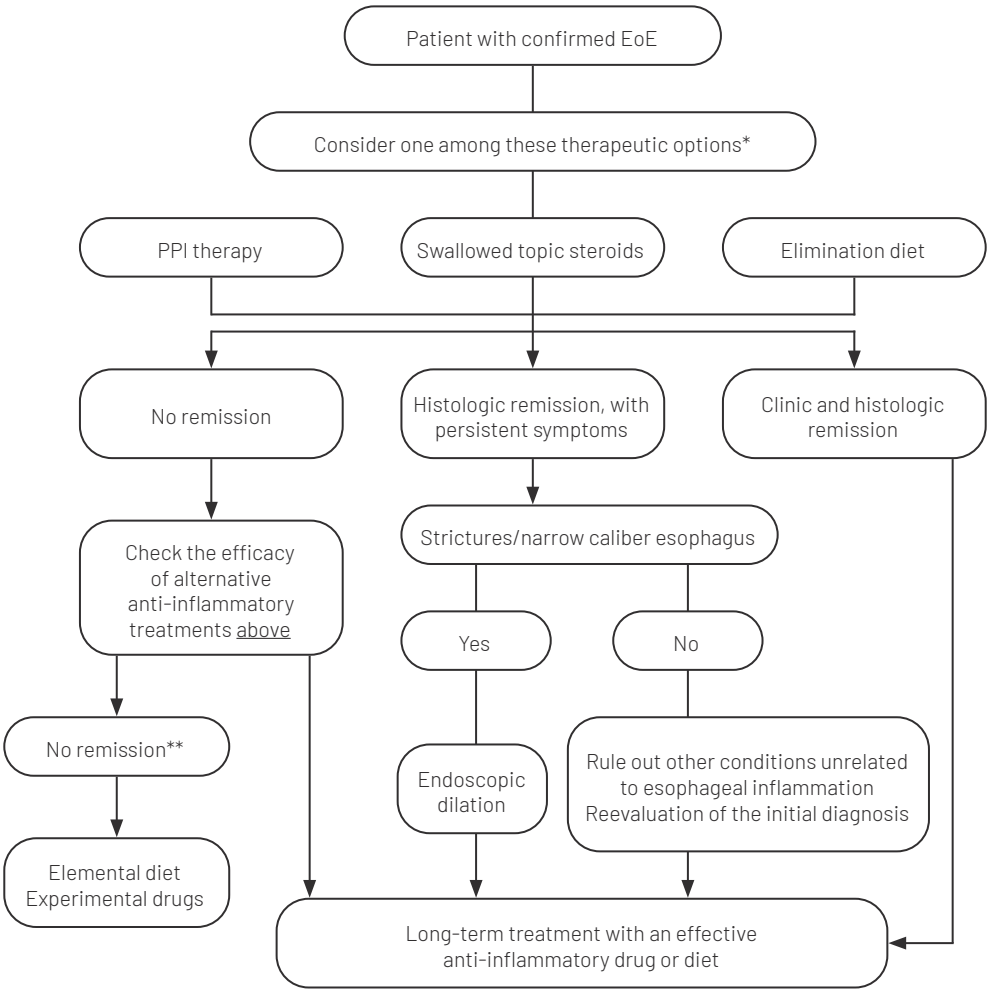

FIGURE 1. | Therapeutic algorithm proposed by Lucendo *et al*. for treating eosinophilic esophagitis in clinical practice.

* In patients with persistent symptoms under anti-inflammatory therapy, endoscopic dilation should be considered.

** Refer the patient to an EoE center.

GOALS OF THERAPY

Management of EoE requires an integrated approach with identification and avoidance of dietary antigens playing a fundamental role. The short-term goals of medical therapy include symptom resolution and attainment of histologic remission, defined as an eosinophil count of less than 15 *eos/hpf*, while growing body of evidence indicates that prevention of dysmotility and strictures is a long-term management goal.[22,24] Thus, medical treatments that prevent submucosal fibrosis and tissue re-modeling are of considerable interest. Whether targeted anti-inflammatory therapy can achieve this goal, or a need exists for antifibrotic agents capable of changing the natural course of disease, are critical questions for drug development.

INDUCTION AND MAINTENANCE THERAPY

After successful induction with corticosteroids EoE recurs almost uniformly with drug cessation. Accordingly, effective maintenance therapy is needed.[1,25,26] A growing body of evidence demonstrates the value of proton pump inhibitors (PPIs) as maintenance agents. Sustained one-year remission rates of approximately 70% to 80% have been reported for low-dose PPI maintenance therapy among children and adults.[27-29] Long-term PPI use is generally safe, although the lowest effective dose should be used to minimize potential complications.[30,31] Most efficacy data for swallowed topical corticosteroids comes from short-term induction studies, and as such, the efficacy and safety of maintenance therapy with these agents is poorly understood. Observational studies suggest that the benefits of corticosteroids diminish over time.[32,33] Only one placebo-controlled RCT has evaluated the effect of swallowed budesonide (0.25 mg BID). After one year of follow-up, 35.7% (5/14) of patients receiving budesonide achieved disease remission compared with 0% (0/14) of placebo patients.[34] In a prospective, open-label study of 54 children who received swallowed aerosolized fluticasone, a sustained remission rate of 63% was observed after two years of follow-up.[35]

Potential adverse events associated with long-term systemic corticosteroid exposure include oral and esophageal candidiasis infections, adrenal suppression, growth retardation, osteopenia, osteoporosis, glucose intolerance and cataract formation.[18,36,37] However, due to limited absorption and high first-pass metabolism by the liver, systemic effects of swallowed corticosteroids are minimal.[38] Due to the fibrosing nature of the EoE disease process and high rate of recurrence following cessation of induction therapy, prolonged treatment may be indicated. At present, the minimum dose of swallowed topical

corticosteroids required to effectively and safely maintain remission of EoE is unknown. Furthermore, the durability of this strategy is suspect based upon existing data.[39]

MANAGEMENT OF COMPLICATIONS AND TREATMENT ALGORITHMS

Esophageal rigidity with symptoms such as dysphagia and food impaction are consequences of the progressive, fibrostenotic course of EoE. It is estimated that each additional year of undiagnosed EoE increases the risk of stricture by 9%.[24] Therefore, prevention and reversal of structural remodeling and fibrosis are attractive therapeutic goals.[1] In EoE, the Th2-response is characterized by several pro-inflammatory cytokines that promote eosinophil activation and recruitment to the esophageal tissue as well as activation of basophils and mast cells.[40-42] Eosinophils express transforming growth factor beta (TGF-β), which induces tissue fibrosis and subsequent esophageal remodeling and stricture formation.[43] In addition, wall stiffness increases esophageal smooth muscle cell gene expression of phospholamban and collagen I by mechanical signals ("mechanosignaling"), which results in smooth muscle hypertrophy. This inflammation-independent mechanism implies that treatment strategies focussed on blocking the effects of inflammatory mediators may be effective in EoE management.[44]

In clinical practice, choice of treatment strategy depends on EoE phenotype (inflammatory and/or fibro-stenotic) and patient preferences.[21] Both dietary intervention and swallowed topical corticosteroids are efficacious in patients with an inflammatory phenotype,[45,46] while those with fibrostenosing disease may be less likely to respond to an elimination diet.[47] Limited evidence indicates that control of inflammation may decrease the need for subsequent esophageal dilation of fibrostenotic strictures in adult EoE patients, thereby suggesting that remission of eosinophilic inflammation reduces the process of tissue remodelling and fibrosis.[48] However, other studies have shown that resolution of superficial epithelial eosinophilia does not preclude sub-epithelial remodeling and progression to stricture formation.[47,49,50] The process of sub-epithelial remodelling and fibrosis requires further elucidation with a key question being whether this progression is reversible. Although age and disease duration may be critical factors for disease progression, little is known about other determinants. Better understanding of the molecular mechanisms of fibrosis in EoE are needed to inform clinical decision-making.

PROTON PUMP INHIBITORS

The role of PPIs in EoE management has evolved over the last two decades. Past guidelines recommended initiating eight weeks of high-dose PPI therapy in patients with a suspected diagnosis of EoE to rule out PPI-responsive esophageal eosinophilia (PPI-REE) - a designation used to describe patients with symptomatic, endoscopic and histologic evidence of EoE who do not present with gastroesophageal reflux disease (GERD) yet respond to PPI therapy.[1,21,51] More recent insights indicate that although PPI-REE is a sub-phenotype of EoE, EoE and GERD are not mutually exclusive.[52] It is hypothesized that i) increased acid exposure may be secondary to EoE since esophageal dysmotility contributes to reflux, and ii) patients with GERD are predisposed to develop EoE given that gastric acid damages the mucosal barrier.[53] For this reason, PPIs are now used as first-line or adjunctive therapy in both PPI-REE (though this term is being phased out) and EoE with co-existing GERD.[19]

Additional observations support the notion that a complex relationship exists between acid reflux and EoE. Patients with EoE are more sensitive to acid exposure compared to healthy controls, and PPIs are effective for reducing pain.[54] The efficacy of PPIs for reducing symptomatic esophageal eosinophilia has been reported in several case series and a small clinical trial that showed a 50% (5/10) rate of disease remission after 8 weeks of PPI therapy.[55-58] According to a systematic review and meta-analysis that included data from 619 patients (188 children and 431 adults) with symptomatic esophageal eosinophilia, 60.8% (376/619) of cases had clinical improvement and 50.5% (313/619) cases achieved histologic remission (defined as < 15 *eos/hpf*) after PPI treatment.[59] The mechanism by which PPIs reduce esophageal eosinophilia may be secondary to restoration of mucosal barrier integrity and reduced environmental allergen exposure.[60]

The potential role of acid suppression in EoE management is also supported by the observation that vonoprazan, a potassium-competitive acid blocker (P-CAB), induces histologic remission in PPI-non-responsive EoE patients.[61] However, PPIs are associated with several acid-independent anti-inflammatory effects which may reduce esophageal eosinophilia, including attenuation of Th2-cytokine-induced eotaxin-3 expression - a process relevant to reduced eosinophil activation and migration.[62-64] PPIs also inhibit acid-induced endothelial expression of adhesion molecules (including intracellular adhesion molecule 1 [ICAM-1] and vascular cell adhesion molecule 1 [VCAM-1]), which may decrease eosinophilic inflammation.[65] In contrast to the effect on epithelial cells,

PPIs do not appear to inhibit Th2-cytokine stimulated eotaxin-3 expression by esophageal fibroblast directly, suggesting that PPIs have limited impact on esophageal remodeling and formation of fibrosis.[66]

Although PPIs are endorsed in current EoE treatment guidelines, no specific recommendations exist regarding the role of PPIs as initial or combination therapy.[19] PPI use remains off-label as this drug class has not been formally registered by any regulatory agency as an EoE therapy.

TOPICAL CORTICOSTEROIDS

Swallowed topical corticosteroids are a mainstay EoE therapy that provide an anti-inflammatory effect by non-specifically inhibiting the Th2 immune response, with secondary improvement in esophageal barrier integrity and reduced esophageal remodeling and fibrosis.[67,68] An observational study of 20 pediatric patients who received methylprednisolone (1.5 mg/kg) twice daily for four weeks provided the first evidence that oral corticosteroids are effective in treating EoE. Clinical remission and clinical response were achieved in 65% (13/20) and 95% (19/20) of patients, respectively, with the average number (± standard deviation [SD]) of *eos/hpf* declining from 34.2 ± 9.6 to 1.5 ± 0.9.[69] Twelve months after treatment, 50% (10/20) of patients remained asymptomatic. In a subsequent randomized controlled trial (RCT) of 80 pediatric patients with EoE, there was no difference observed between the oral systemic corticosteroid prednisone (1 mg/kg BID) and oral topical corticosteroid fluticasone propionate (2 puffs [110 µg/puff] QID). After four weeks of therapy, 95% (30/32) of patients receiving prednisolone and 94% (34/36) of those assigned to fluticasone propionate attained combined clinical remission and histologic improvement.[26] Importantly, systemic adverse events (e.g., hyperphagia, weight gain and cushingoid features) were reported in 40% (16/40) of prednisolone-treated patients whereas none of the topically-treated patients experienced systematic, steroid-related adverse events. It should be noted that 15% (6/40) of fluticasone propionate-treated patients developed esophageal candidiasis compared to 0% (0/40) in the prednisolone group.

No corticosteroids are currently approved by the U.S. Food and Drug Administration (FDA) for the treatment of EoE. Nevertheless, oral aerosolized fluticasone propionate and oral viscous budesonide are frequently prescribed as off-label therapy. Recently,

these treatments were directly compared for initial treatment of EoE in a randomized, double-blind trial.[147] Patients were randomized to receive oral viscous budesonide slurry BID plus a placebo inhaler (n = 56) or a multi-dose fluticasone inhaler BID plus placebo slurry (n = 55). Between baseline and week 8, the mean peak eosinophil count decreased from 73 to 15 *eos/hpf* and 77 to 21 *eos/hpf* in the oral viscous budesonide and multi-dose fluticasone inhaler groups, respectively (p = 0.31). Similarly, there was no statistically significant between-group difference with respect to the change in the Dysphagia Symptom Questionnaire (DSQ) score: the mean DSQ-score decreased from 11 to 5 in the oral viscous budesonide group and from 8 to 4 in the multi-dose fluticasone inhaler group (p = 0.70). These findings suggest that both oral aerosolized fluticasone and oral viscous budesonide are acceptable EoE treatments.

In an RCT comparing eight weeks of viscous (n = 11) and nebulized topical budesonide (n = 11) therapy, the latter agent was demonstrated to be more effective than the former in reducing esophageal eosinophilia, likely due to better esophageal distribution and increased contact time.[70] This finding underscores the importance of bioavailability and has prompted the initiation of pharmacokinetic and dose finding studies of existing corticosteroids, in addition to the development of novel formulations.

Induction treatment with two budesonide formulations (effervescent tablet for orodispersible use [budesonide effervescent tablet, BET, 1 or 2 mg BID] and viscous suspension [budesonide viscous suspension, BVS, 5 ml BID, 0.4 mg/ml]) was evaluated in a placebo-controlled RCT that included 76 adult EoE patients.[71] Over 94% of patients in the BET, BVS and placebo groups achieved clinical response, defined as a decrease in the dysphagia score of at least 3 points from baseline after two weeks. Budesonide - irrespective of dose or formulation - was demonstrated to be statistically superior to placebo for induction of histologic remission (BET 1mg/d = 100% [19/19]; BET 2mg/d = 94.7% [18/19]; BVS = 94.7% [18/19]; placebo = 0% [0/19]). When asked which formulation was preferable, 80% of patients chose BET over BVS. Moreover, in a placebo-controlled, phase III RCT of adult EoE patients, 58% (34/59) of those who received BET 1 mg BID achieved the primary endpoint of complete remission (defined as a mean dysphagia and odynophagia severity score < 2 on a scale of 0-10 for each day during week 5 of the double-blind study and a peak eosinophil count < 5 *eos/hpf*) compared with none of the patients in the placebo arm (0/29) at week 6 (p < 0.0001).[72] In 2017, the European Medicines Agency (EMA) approved swallowed effervescent budesonide for use in adult EoE patients.[73]

Recently, a tablet formulation of fluticasone propionate that dissolves on the tongue, APT-1011, was evaluated in a phase I trial of 22 healthy subjects (NCT03191864). Overall, APT-1011 demonstrated low systemic absorption (< 200 pg/ml), consistent with acceptable esophageal contact time. The potential effect of extending fluticasone propionate absorption among EoE patients with APT-1011 is currently under further investigation in a phase II, placebo-controlled RCT (NCT03191864).

ANTI-ALLERGIC TARGETS

The mast cell stabilizing agent cromolyn sodium, which modifies chloride channels in mast cell membranes, was initially investigated as an EoE treatment in a pediatric cohort study (n = 381) that failed to show either clinical or histologic improvement.[36] Similarly, a recent eight-week RCT that enrolled pediatric EoE patients did not demonstrate a statistically significant reduction in either clinical symptoms or peak eosinophil counts in those assigned to viscous oral cromolyn sodium compared to placebo.[74] The use of mast cell stabilizers is therefore not recommended as either an induction or maintenance therapy in EoE.

It was previously speculated that decreasing eosinophil chemotaxis and cellular activity by use of eosinophil-targeted agents may be an effective treatment strategy for eosinophilic-related gastrointestinal disorders and asthma. Data from an in-vitro study and retrospective chart review suggested that montelukast, a leukotriene D4 receptor antagonist that inhibits eosinophil protease activity and subsequent eosinophil chemoattraction, reduced symptoms and maintained remission in EoE.[75,76] However, a subsequent placebo-controlled RCT and a prospective cohort study failed to demonstrate efficacy for maintenance of corticosteroid-induced remission.[77,78]

Other prostaglandins play critical roles in the eosinophilic inflammation cascade. Prostaglandin D2 (PGD2, also known as chemoattractant receptor-homologous molecule expressed on Th2 cells [CRTH2]) mediates chemotaxis of eosinophils and expression of Th2 cytokines.[79,80] In a small, placebo-controlled RCT performed in 26 adult patients with corticosteroid-dependent or corticosteroid-refractory EoE, treatment with the selective CRTH2 antagonist OC000459 resulted in a significant, although modest, reduction in clinical symptoms and peak eosinophil counts compared to placebo after eight weeks of treatment.[81] These results support further investigation of CRTH2 antagonists as potential corticosteroid-sparing agents.

IMMUNOSUPPRESSIVES

Evidence supporting the use of immunosuppressives in EoE is limited. The published literature consists of a single case series (n = 3) that found thiopurines to be effective for maintaining clinical and histologic remission in corticosteroid-dependent EoE patients. It is postulated that azathioprine and 6-mercaptopurine inhibit the recruitment and/or proliferation of T- and B-lymphocytes in the esophageal epithelium, thereby decreasing antigen processing and subsequent esophageal inflammation.[82] However, the use of thiopurines is not recommended in EoE considering their unfavorable safety profile and lack of controlled evidence to support efficacy.[83] Data evaluating other immunosuppressives including cyclosporine, tacrolimus and methotrexate are not currently available.

MONOCLONAL ANTIBODIES

The introduction of biologic therapy has revolutionized the management of refractory allergic diseases such as asthma, atopic dermatitis and nasal polyposis. In EoE, the Th2 cytokine signature indicates an allergic etiology. As such, research efforts have focused on both evaluation of therapies designed for other atopic conditions and development of antibodies directed against EoE-specific pathways. Investigational monoclonal antibodies that directly target cell signaling proteins implicated in Th2-predominant inflammation include interleukin (IL)-5, IL-4, IL-13 and IgE. Monoclonal antibodies directed towards T-helper type 1 cytokines including tumor necrosis factor-alpha (TNF-α) antagonists have also been evaluated (**Table 1**).

IL-5 ANTAGONISTS

IL-5 is a pro-inflammatory cytokine secreted by T-lymphocytes, mast cells and eosinophils that induces eosinophil production, primes eosinophils to respond to activation signals, and promotes eosinophil trafficking to the esophagus.[84-86] Transgenic IL-5 overexpression is associated with the development of an EoE-like disease in murine models and local IL-5 inducing Th-2 cell overexpression in EoE.[87] Targeting the IL-5 pathway with monoclonal antibodies was first explored in atopic conditions that feature tissue eosinophilia (i.e., asthma, nasal polyposis and atopic dermatitis).[88-90] In EoE, two monoclonal antibodies against IL-5 have been evaluated.

Mepolizumab, a humanized anti-IL-5 monoclonal IgG1 antibody was first assessed in an open-label study (n = 4) of adults with longstanding, symptomatic EoE. Following three

TABLE 1. | Clinical studies evaluating monoclonal antibodies for the treatment of EoE

Target	Monoclonal antibody	Author	Design	Population (N)
IL-5	Mepolizumab	Stein 2006[90]	Open label, phase I RCT	Adults (4)
		Straumann 2010[91]	Placebo-controlled, phase II RCT	Adults (11)
		Assa'ad 2011[92]	Placebo-controlled, phase II RCT	Children (59)
IL-5	Reslizumab	Spergel 2012[93]	Placebo-controlled RCT	Children (226)
		Markowitz 2018[94]	OLE of placebo-controlled RCT	Children(9)
IL-13	QAX576	Rothenberg, 2015[99]	Placebo-controlled, phase II RCT	Adults (23)
IL-13	RPC4046	Hirano 2018[100]	Placebo-controlled, phase II RCT	Adults (99)
IL-4/IL-13	Dupilumab	NCT02379052	Placebo-controlled, phase II RCT	Adults (47)
IgE	Omalizumab	Clayton 2014[107]	Placebo-controlled, phase II RCT	Adults (30)
		Loizou 2015[108]	Open label, non-randomized	Adults (15)
Anti-TNF	Infliximab	Straumann 2008[111]	Open label, non-randomized	Adults (3)

+ Statistically significant response.
- No statistically significant response.
AE = Adverse event, OLE = Open-label extension, RCT = Randomized controlled trial.

infusions of intravenous mepolizumab (10 mg/kg, max. 750 mg) all patients demonstrated clinical response at week 4.[91] Although a substantial decrease in esophageal eosinophilia was observed, peak eosinophil counts remained above 20 *eos/hpf*. In contrast, limited clinical improvement and a substantial reduction in mean *eos/hpf* count was reported in subsequent placebo-controlled, phase II RCT (n = 11) that compared four weeks of mepolizumab treatment (750 mg/week for two doses, followed by 1500 mg/week for two doses if remission was not achieved) to placebo.[92] These findings are similar to those of a phase II RCT performed in 59 pediatric patients who received three monthly infusions of mepolizumab 0.55 mg/kg (n = 19), 2.5 mg/kg (n = 20) or 10 mg/kg (n = 20).[93] When the dose groups were combined, 89.5% (51/59) of patients had a mean esophageal eosinophil count lower than 20 *eos/hpf*.

Reslizumab, a fully humanized, IgG4 antibody against IL-5, was evaluated in a controlled RCT of 226 pediatric EoE patients who were randomized to monthly infusions of 1, 2 or 3 mg/kg of intravenous reslizumab or placebo. After four months of treatment there was a statistically significant difference in the proportion of reslizumab patients with a reduced median peak eosinophil count compared to placebo, yet reslizumab was not found to

Dosage	Duration (months)	Clinical response	Histologic response	Safety and tolerability
10 mg/kg monthly, max.750 mg	3	+	+	Mild AEs
750 mg weekly for 2 doses + 1500 mg for 2 doses if not in remission	1	-	-	Mild AEs
0.55, 2.5, or 10 mg/kg monthly	3	-	-	No AEs
1, 2 or 3 mg/kg monthly	4	-	+	Mild AEs
2 mg/kg monthly	108	+	+	Well tolerated
6 mg/kg monthly	3	-	+	Well tolerated
180 or 360 mg weekly	4	-	+	Mild AEs
300 mg weekly	3	+	+	Well tolerated
0.016 mg/kg/IgE every 2-4 weeks	4	-	-	Well tolerated
1 mg/kg/IgE monthly	3	+	+	Well tolerated
5 mg/kg monthly for 2 infusions	1	-	-	Well tolerated

be effective for induction of clinical response.[94] Six patients from one site continued to receive reslizumab (2 mg/kg) in an open-label extension phase.[95] Additionally, four patients were treated with reslizumab on the grounds of compassionate use. After nine years of treatment, reslizumab was associated with substantial improvement in symptoms related to EoE including dysphagia, abdominal pain, heart burn, vomiting and reflux as well as reduced eosinophil counts.

Targeting the IL-5 pathway by administration of benralizumab, an antibody that blocks the IL-5Rα receptor, is a highly effective therapy for asthma that recently received FDA and EMA approval as add-on maintenance therapy for children (≥ 12 years) and adults with severe eosinophilic asthma.[96-98] Benralizumab has not yet been evaluated in EoE, however a placebo-controlled clinical trial (NCT03473977) is currently investigating the efficacy and safety of three monthly doses of 30 mg benralizumab for the treatment of eosinophilic gastritis/gastroenteritis in children (≥ 12 years) and adults.

IL-13 AND IL-4/IL-13 ANTAGONISTS

IL-13 secreted by Th2 cells and activated eosinophils plays a vital role in the pathogeneses

of EoE by increasing eotaxin-3 and promoting fibroblasts to produce periostin, which increases eosinophil chemotaxis.[42,99] IL-13 also affects epithelial barrier integrity, as it is implicated in the dysregulation of the important basement membrane proteins desmosomal cadherin desmoglein 1, filaggrin and involucrin.[100,101] In mouse models, administration of pharmacological doses of IL-13 induces pathology similar to human EoE and has been shown to cause esophageal tissue remodeling. In addition, IL-13 was found to be markedly overexpressed in the esophagus of EoE patients.[41,42,102] Similarly, IL-4, a cytokine that causes naïve T-helper cells to differentiate into Th2 cells and activates B-cell class switching to produce IgE is found in increased concentrations in EoE patients.[41] Furthermore, stimulation of epithelial cells by IL-4 leads to production of eotaxin-3 through STAT6 signaling and subsequent recruitment of eosinophils into tissue. Two monoclonal antibodies targeting IL-13 (QAX576 and RPC4046) and one monoclonal antibody targeting IL-4/IL-13 (dupilumab) have been evaluated in EoE.

QAX576 was first evaluated as an EoE therapy during a phase II trial of 23 adults who were randomized to three infusions of QAX576 (6 mg/kg) or placebo at weeks 0, 4 and 8.[103] Although the primary endpoint (histologic response, defined as ≥ 75% reduction in peak esophageal eosinophil count) was not met, the mean eosinophil count was reduced by 60% in the QAX576-treated group compared with a 23% increase in the placebo arm at 6 months ($p = 0.004$). No significant improvement in dysphagia was reported by patients assigned to active drug compared to those who received placebo. Development of QAX576 has since been discontinued.

RPC4046 is a monoclonal that blocks IL-13 from binding to both IL-13 receptor subunit alpha 1 (IL13RA1) and 2 (IL13RA2). In a recent phase II placebo-controlled RCT, 99 adult EoE patients were assigned to RPC4046 (180 mg or 360 mg) or placebo once weekly in a 1:1:1 ratio.[104] After 16 weeks of treatment, a statistically significant reduction in mean eosinophil count was observed in both RPC4046 groups (180 mg: 94.8 ± 67.3, $p < 0.0001$; 360 mg: 99.9 ± 79.5, $p < 0.0001$) compared to placebo (4.4 ± 59.9). Moreover, patients treated with RPC4046 were statistically more likely to achieve endoscopic and histologic disease improvement as measured by difference in endoscopic severity score and total histological grade and stage scores, as measured by a validated disease activity index (the EoE histologic scoring system [EoE-HSS]).[105] A numerical trend in favor of RPC4046 was reported with respect to symptom improvement, particularly dysphagia. Additionally, results from the open-label extension (OLE) study, in which

patients received RPC4046 360 mg once weekly, demonstrated sustained symptomatic and histologic improvement at week 52 following successful induction therapy.[106] Two other IL-13 monoclonal antibodies, lebrikizumab and tralokinumab, have been successfully studied in asthma and atopic dermatitis and may be effective for the treatment of EoE.[107-110]

Dupilumab, a monoclonal antibody targeting the shared alpha subunit of the IL-4 and IL-13 receptors, was studied in a phase II trial of 47 patients who received 300 mg subcutaneous dupilumab or placebo for 12 weeks (NCT02379052). Clinical response, as measured by the Straumann Dysphagia Index, was significantly improved after 10 weeks treatment compared to placebo (45 % vs 19 % p = 0.0304). In addition, the peak eosinophil count was significantly reduced at week 12 among patients treated with dupilumab as compared to placebo (92% vs 15% p < 0.0001). Total EoE-HSS grade and stage scores and distensibility plateau were improved at week 12 (all < 0.001 vs placebo). Considering these promising results, a phase III trial that is currently recruiting was initiated to determine the efficacy and safety of dupilumab in adult EoE patients (NCT03633617).

Other agents targeting inhibition of IL-4 and IL-13 may be effective in down-regulating the Th2 immune response in patients with EoE. A phase I safety trial of MEDI 9314, an anti-IL-4Rα antibody, has completed and at present this drug will be developed as a treatment for atopic dermatitis (NCT02669667).

MONOCLONAL ANTIBODIES TARGETING IG-E

It is generally accepted that mast-cell activation in EoE is IgE-dependent, analogous to asthma.[1,111] Moreover, the increased number of IgE-bearing mast cells, increased B-lymphocyte density, upregulation of genes involved in B-cell activation and B-cell class switching to produce local IgE support the notion that EoE is an IgE-mediated disease.[112,113] From an epidemiologic perspective, the observation that food- and aero-allergen IgE mediated hypersensitivity is more frequent in EoE patients than the general population further suggests this concept.[1]

The monoclonal anti-IgE antibody omalizumab was initially evaluated in several casestudies that reported clinical, but not histologic or endoscopic, improvement.[114,115] Subsequently, an RCT was conducted in 30 adult EoE patients who received either subcutaneous omalizumab (0.016 mg/kg/IgE) or placebo. There was no statistically significant reduction in clinical symptoms or tissue eosinophil counts at week 16 when the active and control

groups were compared at week 16.[116] In addition, an open-label single arm trial showed that 33% (5/15) adult patients treated with omalizumab achieved complete clinical and histologic remission after 12 weeks of therapy (three infusions of 1 mg/kg/IgE).[117] These findings suggest that IgE does not play an important role in the inflammatory process in EoE. No drug development program of anti-IgE therapy is currently active.

TUMOR NECROSIS FACTOR-ALPHA (TNF-α) ANTAGONISTS

High concentrations of TNF-α are found in the esophageal tissue of EoE patients. While classically thought of as a TH1 cytokine, TNF-α generates a synergistic effect on IL-4 increased eotaxin-3 production.[40,118] Targeting TNF-α with the IgG1 monoclonal antibody infliximab has been shown to be an effective treatment in chronic inflammatory diseases such as Crohn's disease.[119] Infliximab (two 5 mg/kg infusions) was evaluated in a prospective study of three adult patients with corticosteroid-dependent EoE.[120] Although well-tolerated, infliximab therapy did not induce a clinical response or reduce the number of esophageal eosinophils. This experience should be interpreted with caution because of the small number of patients evaluated, however, it has discouraged further evaluation of this class of agents in EoE.

OTHER POTENTIAL THERAPEUTIC TARGETS

Several drugs that target specific molecules and or cells implicated in the pathogenesis of EoE have been proposed as potential future therapeutic agents (**Table 2**).

SIALIC ACID-BINDING IG-LIKE LECTIN 8 (SIGLEC-8)

Sialic acid-binding Ig-like lectin 8 (Siglec-8) is a cell surface protein selectively expressed on human eosinophils and mast cells. Specific antibodies binding to Siglec-8 causes eosinophil apoptosis via caspase and mitochondrial-dependent pathways. In mast cells, only inhibition of mediator release was observed.[121] In a murine model of EoE, administration of a monoclonal antibody to Siglec-F (the murine isoform of Siglec-8) decreased esophageal basal zone hyperplasia, angiogenesis and deposition of fibronectin, which are important histologic features in EoE pathogenesis.[122] In another mouse study, administration of AK002, a non-fucosylated IgG1 monoclonal antibody targeting Siglec-8, resulted in selective depletion of tissue and blood eosinophils and reduction of mast cells.[123] A phase II, placebo-controlled trial of AK002 in adult patients with eosinophilic gastritis and/or gastro-enteritis is currently recruiting (NCT03496571).

TABLE 2. | Potential therapeutic targets for EoE.

Target	Drug	Role in disease pathogenesis	Available data
Siglec-8	Anti-Siglect-8 antibodies (AK001 and AK002)	Eosinophil apoptosis and inhibition of mast cells	Eosinophilic gastritis/ gastro-enteritis (ongoing); atopic keratoconjunctivitis (ongoing)
TGFβ1	Angiotensin-1 receptor antagonist (Losartan)	Tissue remodeling and development of fibrosis	Connective tissue disease; EoE (with or without connective tissue disease; ongoing)
CCR3 (Eotaxin-3 receptor)	Anti-CCR3	Recruitment of eosinophils	Asthma
TSLP	Anti-TSLP (Tezepelumab, AMG 157)	Promotion of Th2-type immune response	Asthma; atopic dermatitis
Integrin α4β7	Vedolizumab	Mediates adhesion to MAdCAM-1 (improves eosinophil survival)	Crohn's disease; ulcerative colitis
IL-4Rα*	Anti- IL-4Rα (MEDI 9314)	Activation and recruitment of eosinophils	Healthy subjects in atopic dermatitis (upcoming)
IL-5Rα	Anti- IL-5Rα (Benralizumab)	Activation and recruitment of eosinophils	Asthma; atopic dermatitis (ongoing); nasal polyposis (ongoing); eosinophilic gastritis/ gastro-enteritis (ongoing)
IL-13*	Anti-IL-13 (Tralokinumab, Lebrikizumab)	Eosinophil recruitment, barrier dysfunction and remodeling	Atopic dermatitis; asthma
IL-9	Anti-IL-9 (MEDI 528)	Epithelial barrier dysregulation by alteration of E-cadherin	Asthma
IL-15	Anti-IL-15 (CALY-002)	Controls Th2 and Natural Killer T-cell responses, promotes epithelial inflammation and prevents from eosinophil apoptosis	Celiac disease (upcoming); EoE (upcoming)

* Dupilumab (IL-4/IL-13 antagonist) and RPC4046 (IL-13 antagonist) have been previously studied in EoE (see *Table 1*).
EoE = Eosinophilic esophagitis, IL = Interleukin.

TRANSFORMING GROWTH FACTOR β1 (TGFβ1)

The role of transforming growth factor β1 (TGFβ1) in tissue remodeling and the development of fibrosis in EoE is well established.[33] Losartan, an angiotensin-1 receptor antagonist widely used for the treatment of hypertension, reduces signaling of TGFβ1.[124-128] Losartan may be an effective therapy in patients with a fibrotic EoE-phenotype who experience persistent symptoms. In support of this concept, losartan has been used to prevent vascular complications in patients with connective tissue disorders such as Marfan and Loeys-Dietz syndrome.[129] A single clinical trial is evaluating the effect of losartan in EoE patients with or without connective tissue disorders (NCT03029091).

CC CHEMOKINE RECEPTOR TYPE 3 (CCR-3)

The CC chemokine receptor type 3 (CCR-3), which is primarily expressed on eosinophils and basophils, has multiple ligands including CCL-11, -24, and -26 (eotaxins). Eotaxin-3 (CCL-26) is one of the most potent chemo-attractants in EoE. Notwithstanding that an oral CCR3 antagonist (GW766944) was not effective in patients with asthma and eosinophilic bronchitis, blocking this chemokine receptor by use of either an anti-CCR3 monoclonal or small molecule could be an effective therapy for EoE.[130] There are no clinical trials currently evaluating CCR3 antagonists in EoE.

THYMIC STROMAL LYMPHOPOIETIN (TSLP)

EoE is associated with polymorphisms in the gene that encodes thymic stromal lymphopoietin (TSLP), a cytokine that promotes Th2-type responses. It was previously demonstrated in a mouse model that the development of eosinophilic inflammation was TSLP-dependent and could be prevented by using antibodies to this cytokine.[131] Furthermore, treatment with fluticasone propionate reduces expression of multiple pro-inflammatory cytokines including TSLP.[68] A fully human monoclonal IgG2 antibody against TSLP, tezepelumab (AMG 157), was evaluated in patients with mild allergic asthma, and a reduction of both early and late asthmatic responses was observed.[132] More recently, a phase II trial completed in 113 adult patients with moderate-to-severe atopic dermatitis showed a statistically significant improvement in the Eczema Area and Severity Index score compared to placebo.[133] Overall, targeting of TSLP needs to be further studied, and tezepelumab could hold promise as a potential target agent in EoE.

INTEGRIN α4β7

The α4β7 integrin, which is expressed on both T-lymphocytes and eosinophils, mediates

adhesion to the vascular endothelial cells of the gut through interaction with its ligand, mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1). This mechanism facilitates the migration of these cells from the vasculature into inflamed tissue. It is also noteworthy that e-cadherin, a second ligand for α4β7, is highly expressed by epithelial cells in human allergic gastrointestinal tissue including EoE. It is believed that this interaction enhances the retention of inflammatory cells in mucosal tissue.[134] Vedolizumab, a monoclonal antibody that selectively blocks the α4β7 integrin interaction with MAdCAM-1, is FDA-approved for moderate-to-severe Crohn's disease and ulcerative colitis. Recently, vedolizumab therapy in a patient with Crohn's disease and concurrent EoE was reported to induce remission of both diseases.[135] Consistent with this observation, a retrospective series showed improvement of eosinophil-associated gastrointestinal disorders following vedolizumab therapy for IBD. However, these data are uncontrolled and were not adjusted for the potential influences of known confounders such as corticosteroid therapy.[136] A pre-clinical trial is currently underway to investigate the mechanistic role of the α4β7 integrin and MAdCAM-1 pathway in eosinophil recruitment in EoE (NCT02546219). Further research is needed to further elucidate the potential role of vedolizumab and other anti-integrins as treatment for EoE.

IL-9

An increased concentration of IL-9 has been detected in the eosinophils of patients with active EoE.[137] Moreover IL-9 expressing mast cells are important in food allergies, and EoE patients sensitized to food have significant increased mast cells in the esophageal epithelium.[138,139] The effect of an anti-IL-9 antibody, MEDI-528, was evaluated in adults with uncontrolled asthma without success.[140] However, recent data showed that IL-9 and its effect on E-cadherin is an important mediator of esophageal epithelial dysfunction in EoE. Therefore, this pathway may represent a new therapeutic target.[141]

IL-15

IL-15, a cytokine that is up-regulated in human EoE, controls Th2 and natural killer T-cell responses, promotes epithelial inflammation and prevents eosinophil apoptosis.[142-145] The effects of IL-15 influence multiple cells that are relevant to the EoE-pathway, thus, blockade of this mediator may be an effective treatment target. An intercepting humanized anti-IL-15 antibody with unique neutralization of IL-15 cis and trans signaling that could be relevant to EoE treatment, CALY-002, was recently discovered.[146]

CONCLUSION

EoE is a chronic immune-mediated disorder of the esophagus which can adversely impact quality of life. Characterized by eosinophilic inflammation, patients typically experience dysphagia and food impaction as a result of progressive esophageal remodeling and fibrosis. It is now recognized that the pathophysiology of EoE resembles certain aspects of other allergic diseases such as asthma and atopic dermatitis, which has prompted the evaluation of drugs used to treat these conditions within the context of EoE. Furthermore, advanced understanding of the pathological processes involved in EoE has led to the development of unique compounds and the recognition of novel treatment targets that may prove to be effective.

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CHAPTER 5

EFFECT OF AMINO ACID-BASED FORMULA ADDED TO FOUR-FOOD ELIMINATION IN ADULT EOSINOPHILIC ESOPHAGITIS PATIENTS: A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

RATIONALE

Elimination of key foods restricts dietary options in eosinophilic esophagitis (EoE) patients. Addition of amino acid-based formula (AAF) to an elimination diet might facilitate adherence and, therefore, enhance efficacy of dietary management.

AIM

To evaluate whether addition of AAF to a Four-Food-Elimination-Diet (FFED) is more effective than FFED alone in decreasing eosinophilia, endoscopic signs, and clinical outcomes.

METHODS

This randomized controlled trial enrolled 41 adult patients with active EoE (≥ 15 eosinophils (eos) per high-power-field (hpf)) at baseline biopsy. Subjects were randomized (1:1 ratio) to groups given a FFED or FFED with addition of AAF providing 30% of their daily energy needs (FFED + AAF). Histological disease activity, endoscopic signs, symptoms and disease-related quality of life (EoEQoL) were measured at baseline and after 6 weeks of intervention.

RESULTS

Patients (60% male, age 34.5 (interquartile range (IQR) 29 - 42.8 years) were randomized to FFED (n = 20) or FFED + AAF (n = 21), 40 participants completed the diet. Complete histological remission (< 15 *eos/hpf*) was achieved in 48% of FFED+AAF-subjects (n = 21) vs. 25% of FFED-subjects (n = 20), respectively (p = 0.204). Peak eosinophil counts (PEC) decreased significantly in both groups between baseline and week 6, but the change in PEC between groups was not different (p = 0.130). A significant but similar endoscopic and symptomatic reduction was observed in both groups (all; $p < 0.05$). Total EoEQoL-scores significantly improved in the FFED + AAF group between baseline and week 6 (p = 0.007), and not in the FFED group.

CONCLUSION

The addition of AAF to a FFED did not lead to a larger decrease in PEC between baseline and 6 weeks, but may result in a significant improvement of QoL in adult EoE patients NL6014(NTR6778).

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic T-helper type 2 (Th2) immune-mediated disorder of the esophagus characterized by symptoms of esophageal dysfunction (i.e., dysphagia and/or food impaction) and eosinophil-predominant inflammation.[1,2] After its first description in the early 1990s, the worldwide EoE incidence and prevalence have surged to rates that outpace increased disease detection.[3-6] Food allergens have been suggested to play a causal role in EoE pathogenesis after primary reports of documented disease remission in children being treated with amino-acid based formula (AAF).[7]

The current management of EoE involves targeting the esophageal inflammation with mdical therapy (i.e., proton pump inhibitors (PPIs) or swallowed topical steroids) and dietary elimination of culprit foods.[8] EoE generally flares after cessation of induction treatment.[9,10,11] Hence maintenance therapy is needed, since long-standing eosinophilic inflammation is associated with esophageal narrowing and stricture formation often requiring dilation.[12-15]

The rationale of non-pharmacological therapy is linked to EoE pathogenesis, with dietary treatment being indicated as a potential safe and drug-free solution for long-term.[16-19] Elemental diets (i.e., complete removal of food allergens by exclusive use of AAF) have proven to be highly efficacious (85% - 95% disease remission rates) in EoE patients of all ages.[7,20-25] However, adherence is challenged by its poor palatability (i.e., absence of solid foods, monotonous taste) and impaired socialization. Therefore, the six-food elimination diet (SFED) has become a more preferred approach with consistently reported remission rates of 70% after restriction of milk, wheat/gluten, egg, soy, peanut/tree nuts, fish, and seafood.[16,18,26] Efficacy of elimination diets parallels the number of excluded foods, yet rigorous diet restrictions with risk of inadequate food intake (e.g., nutritional deficiencies or low calories) and subsequent need for multiple re-endoscopies impedes patients' acceptability in daily life.[27,28] As such, there has been extensive interest in more efficient empiric diets to induce disease remission and lower diet costs as well as quality-of-life (QoL) burdens of treatment.[29,30] Elimination of four-foods including wheat/gluten, milk, egg and either soy or legumes (FFED) is less-restrictive, but also less effective with remission rates in children and adults between 54% - 64%.[24,31] Patients' motivation and acceptance yields a key factor of successful dietary treatment in order to increase adherence and minimize impact on QoL. Aside from the hypoallergenic properties of AAF, which may decrease the risk of diet errors (i.e., mistakes of food label reading or

allergen cross-contamination), recent insights suggested it to have immune-modulating effects itself.[32-35] Hence, a combined strategy of empiric elimination of causative foods with AAF added to the diet may thus improve patients' adherence and acceptance along with efficacy of dietary management.

The aim of this study was, therefore to determine whether AAF added to a FFED is more effective than a standard FFED in decreasing esophageal eosinophilia, improving endoscopic signs, clinical- and nutritional outcomes in adult EoE patients.

METHODS

STUDY PATIENTS

In this single-centrer, open-label, randomized controlled trial, all patients were included from the outpatient clinic of the Amsterdam UMC motility center between December 2017 and January 2020. Adult patients were eligible for enrollment if EoE was diagnosed per consensus guidelines, defined as having symptoms of esophageal dysfunction (Straumann Dysphagia Instrument (SDI) score of ≥ 1) and ≥ 15 eosinophils (eos) per microscopic high-power field (hpf) on baseline biopsy.[9] Exclusion criteria were severe comorbidity scored as the American Society of Anesthesiologists (ASA) Physical Classification System class IV or higher, a recent history of gastrointestinal (GI) cancer or major GI surgery and the inability to stop anti-inflammatory drugs (i.e., topical or systemic steroids, leukotriene inhibitors or monoclonal antibodies). The study protocol was approved by the Medical Ethics Committee of our institution and prospectively registered in the Dutch trial registry NL6014(NTR6778). All participants provided written informed consent before taking part and were given a unique study-ID to ensure anonymity.

STUDY DESIGN

After signed informed consent at visit 1, patients consulted a dietician specialized in allergies for extensive nutritional evaluation. To guarantee sufficient intake and to improve diet adherence, patients subsequently received personalized nutritional advice with restriction of gluten, milk, soy and eggs (FFED). The amount of prescribed AAF added to the FFED in the intervention group was 30% of patients' daily caloric requirements, based on body-mass index and weekly physical activity. The AAF was consumed over 3 moments per day. After confirmation of eligibility by baseline upper endoscopy, patients were randomized in a 1:1 fashion to the treatment arms (FFED or FFED + AAF) using a

blocked randomization protocol with sealed envelopes. All participants underwent an esophagogastroduodenoscopy (EGD) at baseline and after 6 weeks of dietary intervention. Histologic-, endoscopic- and clinical- outcomes as well as nutritional outcomes were evaluated between week 1 and week 6. Side effects, patients' adherence, weight loss and AAF-intake were carefully monitored by a dietician and a physician during the 6 weeks of intervention. The study design overview is presented in **Supplementary Table 1**.

Study product

An amino acid-based, hypoallergenic powdered formula (Neocate Junior, Nutricia, Utrecht, the Netherlands) unflavored, strawberry- and vanilla flavor was used in this clinical trial. This formula was selected by the study team because of its relatively good taste compared to similar formulas. To increase adherence to the prescribed AAF-intake, patients were able to taste all three formulas during a test round to indicate their preferred flavor(s).

STUDY ENDPOINTS AND PROCEDURES

Primary endpoint

The primary outcome of this trial was the change in peak eosinophil count (PEC), measured as the maximum number of *eos/hpf*.

Secondary endpoints

In addition, the difference between groups in complete histological remission rates were evaluated, which was achieved if the reduction of absolute number of *eos/hpf* decreased to < 15. Other secondary pre-specified endpoints were endoscopic signs, clinical- and nutritional outcomes, including diet feasibility and adherence, as well as weight loss.

Histological outcomes

Six biopsies taken from the distal, mid and proximal esophagus were directly fixed in formalin and subsequently embedded in paraffin. After 24 hours the biopsies were sectioned at 5 µm thickness and stained with haematoxylin and eosin and tryptase. To determine eligibility for enrolment all biopsies were directly analyzed in the Amsterdam UMC pathology department to determine PEC as per standardized protocol. In a low-power view setting the area of most densely populated eosinophilia in the esophageal biopsy specimen was identified. A x400 magnification was used in order to determine the PEC per hpf (an area of 0.24 mm^2).

Endoscopic outcomes

During EGD, images of the esophagus were recorded for evaluation of endoscopic signs and were incorporated in a slideshow (Microsoft PowerPoint 2016; Microsoft Inc., Redmond, WA, USA). All images were blinded and scored according to the Endoscopic Reference Score (EREFS) by a single gastroenterologist with expertise in EoE to minimize the risk of inter-observer bias.[36] All endoscopic features were sub-classified as inflammatory (white exudates, edema and linear furrows) and fibrotic (rings and strictures) signs.[13]

Clinical outcomes

Symptoms of dysphagia were evaluated by means of the Straumann Dysphagia Instrument (SDI) measure.[37] This measure evaluates dysphagia frequency and intensity.[38] Furthermore, diet restrictions are known to impact QoL in EoE patients.[39] Therefore, disease specific QoL was assessed by the validated Adult Eosinophilic Esophagitis Quality of Life (EoEQoL) survey. Overall scores range from 0 to 96, with higher scores indicating better QoL. The total EoEQoL index score includes the weighted average of all QoL subscales.

Nutritional outcomes

To evaluate the effort needed to maintain the diet (i.e., feasibility), participants were asked to respond to the statement: "The diet is difficult to maintain for me" (0 - 4 = strongly agree - disagree). Participants were also asked to rate their diet adherence on a 10-point scale (0 - 10 = low - high) at week 6. In addition, at weeks 2 and 4, diet adherence was monitored via telephone and/or e-mail contact by the dietician/physician. Body Mass Index (BMI), nutritional intake (i.e., three-day food diaries), diet adherence and energy intake were evaluated at baseline and after 6 weeks of dietary intervention. The total consumption of AAF for each participant was calculated by the amount of returned empty and full study product cans at week 6. AAF consumption was also monitored via telephone and/or e-mail contact during the study period. Individual adherence to the prescribed intake of AAF (i.e., AAF adherence rate (%)) was defined as the total amount of consumed AAF (kilograms (kg)) as percentage of the total prescribed AAF (kg) over the period of 6 weeks.

SAMPLE SIZE CALCULATION

A single arm study of Molina Infante *et al.*, showed that a cohort of 52 adult EoE patients was sufficient to demonstrate a significant effect of a standard FFED.[40] A decrease of mean PEC per hpf from 55 with an estimated standard deviation (SD) of 30 to 24 (with a difference of 31) was observed. The SD after treatment was not reported in this study.

We used the reported mean PEC (*eos/hpf*) after treatment for responders (< 15 *eos/hpf*) 2 (0-8) and non-responders 45 (26-141) to estimate a SD of 1.96 (responders) and 29.64 (non-responders), respectively. The estimated pooled SD after treatment with a standard FFED was 20. Since no data was available on this new approach, the estimated improvement was partly based on efficacy rates of the elemental dietary treatment. We based the SD of our FFED + AAF-group on a study of Peterson *et al.*, evaluating the effect of an exclusive elemental diet treatment on EoE.[20] In this study the PEC (eos/hpf) after treatment decreased from 54 (SD32) to 10 (SD12) (with a difference of 44). Since the SD of the standard FFED-group and FFED + AAF-group were based on different populations, we assumed that an estimated SD of 15 would be appropriate.

Therefore, a sample size of 20 patients per treatment arm was calculated to provide 80% power to detect a clinically meaningful treatment effect, with an expected difference of 13 in mean change in PEC after treatment between the standard FFED-group and FFED + AAF-group, and with 5% significance and an assumed SD of 15.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM SPSS Statistics (version 25.0) (SPSS, Chicago, USA). Descriptive statistics were used to summarize all characteristics of the study groups. Categorical variables are described as percentages and continuous variables are expressed as mean (± standard deviation (SD)) or median (inter-quartile-range (IQR)). Change in PEC was analyzed by fitting a linear least squares model with treatment group and baseline PEC value as covariates. Categorical analyses between or within treatment groups were performed on secondary end points by using the Fisher's Exact test and McNemar's test. Comparisons of additional endpoints between groups and between pre- and post-treatment were performed by using the Wilcoxon signed rank test and Mann-Whitney U-test, as appropriate, in case of continuous data. The primary and secondary outcomes were evaluated in both the intention-to-treat (ITT) and per-protocol (PP) data sets. A two-sided p-value of < 0.05 was considered significant.

RESULTS

INCLUSIONS AND PATIENT CHARACTERISTICS

Fifty-three EoE patients with clinical active disease were invited for an intake visit at the outpatient clinic. A total of 52 patients were eligible for screening and underwent an

EGD with biopsies at baseline, after which 11 patients were excluded due to the absence of active disease at histological assessment (< 15 *eos/hpf*). Eventually, 41 patients m*et a*ll eligibility requirements and were randomized to the FFED (n = 20) group and FFED + AAF (n = 21) group and were analyzed according to ITT. A protocol violation was reported in 1 participant as a result of non-adherence to the diet at week 1 in the FFED-group with subsequent disqualification of the trial. In addition, a protocol violation was reported

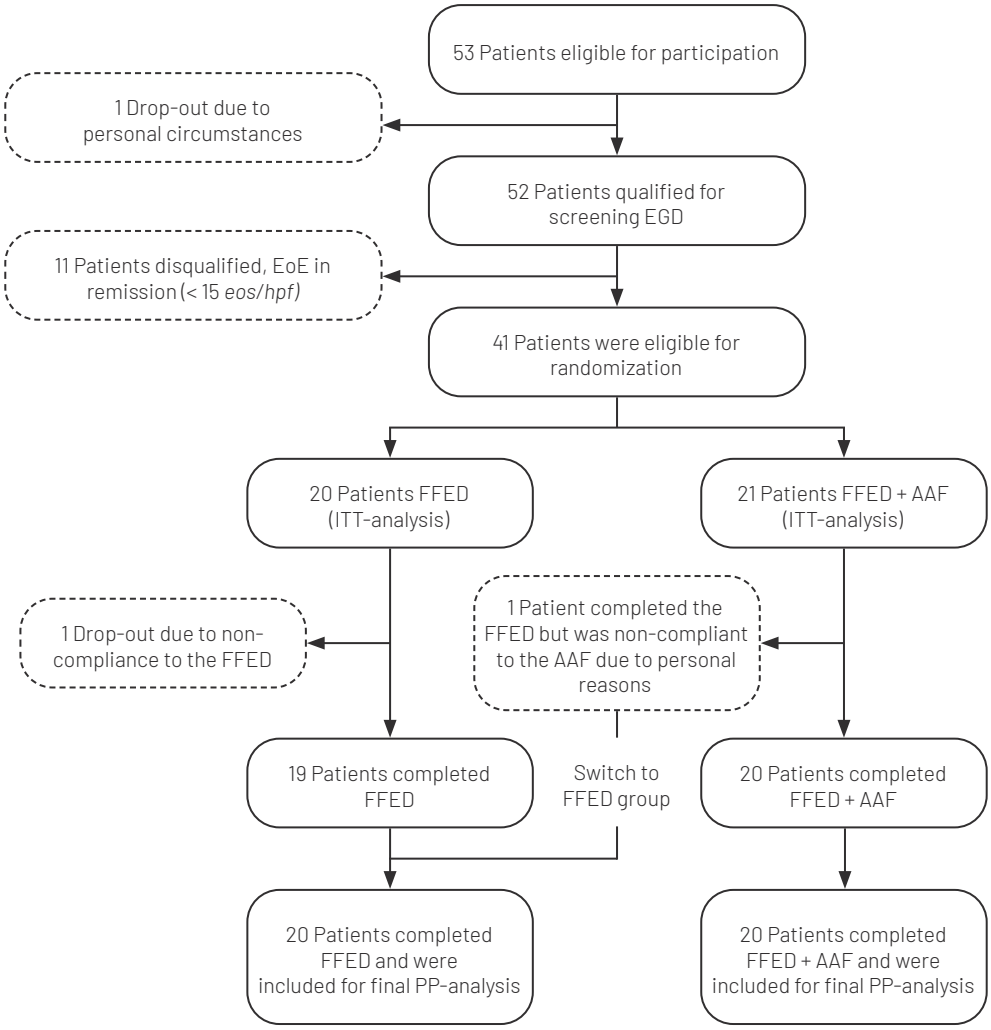

FIGURE 1. | Flow chart demonstrating the number of patients that were eligible for participation, randomization and ITT-analysis. In addition, all patients who discontinued the trial or were switched to the FFED group for final PP-analyses are presented.

due to non-adherence (0% intake) to the AAF-intake in one patient between baseline and week 6 in the FFED + AAF-group. Considering no other protocol deviations or violations were reported, this patient was switched to the FFED-group in the PP-analysis. In total, 40/40 EoE participants (FFED (n = 20) and FFED + AAF (n = 20) completed the diet after 6 weeks according to the protocol and were entered for final PP-analysis (**Figure 1**).

Baseline characteristics of the ITT-cohort were well balanced, with a male predominance in both groups. No significant differences were found on gender, age, race, presence of atopy, previous use of PPI's and BMI between both treatment groups. Most of the participants had ≥ 2 additional atopic comorbidities (**Table 1**). Observations were similar in the PP-cohort and these patient characteristics are presented in **Supplementary Table 2**.

TABLE 1. | Baseline characteristics of all patients *(n = 41)* that were eligible for randomization (ITT-cohort)

	ITT-cohort	
Characteristics	**FFED** ***(n = 20)***	**FFED + AAF** ***(n = 21)***
Male gender, *n (%)*	12 (60)	13 (62)
Age, *years, median (IQR)*	32 (27.5 - 43)	36 (29 - 42)
Race, *Caucasian, n (%)*	19 (95)	20 (95)
History of allergic disease, *n (%)*	17 (85)	18 (86)
Allergic rhinitis	14 (70)	14 (67)
Asthma	5 (25)	7 (33)
Atopic dermatitis	5 (25)	8 (38)
Food allergy	7 (35)	6 (29)
Angioedema	1 (5)	2(10)
Oral Allergy Syndrome	6 (30)	8 (38)
PPIs at baseline, *n (%)*	8 (40)	9 (43)
Prior use of topical steroids, *n (%)*	10 (50)	9 (43)
Esophageal stricture dilation, *n (%)*	1 (5)	2 (10)
Previous endoscopic intervention with food bolus extraction, *n (%)*	8 (40)	10 (48)
Diagnostic delay*, *median (IQR)*	5 (1 - 8.8)	2 (1- 9.5)
BMI (kg/m^2), *median (IQR)*	24.1 (22.4 - 28.4)	23.7 (22.2 - 26.6)

* Time interval between first reported EoE symptoms and year of diagnosis.
FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, ITT= Intention-to-treat, PPIs = Proton Pump Inhibitors, BMI = Body Mass Index.

HISTOLOGICAL OUTCOMES

Primary endpoint: Peak eosinophil count

A significant decrease of the median PEC from baseline to 6 weeks was observed in both groups of ITT-population (FFED + AAF: from 50 (IQR 45 - 100) to 22 (IQR 3.5 - 38); p = 0.001) and (FFED: from 56.5 (IQR 41.3 - 78.8) to 25 (IQR 12 - 50); p = 0.011), respectively (**Table 2A, Figure 2A, Supplementary Figure 1AB**). Primary endpoint analysis showed no difference in the change of the median PEC from baseline to 6 weeks between the two groups, FFED + AAF and FFED (-41.5 (SD 37) vs. -26.9 (SD 39)), respectively (p = 0.127) (**Table 2A, Figure 2A**). Comparing FFED + AAF vs. FFED at week 6 showed lower peak eosinophil levels in the participants treated with the combination of FFED + AAF (22 (IQR 3.5 - 38) vs. 25 (IQR 12 - 50), respectively (p = 0.158) (**Table 2A, Figure 2A**). Similar results were observed in the PP-cohort (**Supplementary Table 3A, Supplementary Figure 2AB**).

TABLE 2A. | Histological features before and after treatment in both groups

	ITT-cohort				
Histological outcomes	**FFED (n = 20)**	**FFED + AAF (n = 21)**	**P-value**	**B**	**SE B**
Peak Eosinophil Count					
Baseline, *eos/hpf, median (IQR)*	56.5 (41.3 - 78.8)	50 (45 - 100)	0.969[a]		
Post-treatment, *eos/hpf, median (IQR)*	25 (12 - 50)	22 (3.5 - 38)	0.158[a]		
P-value (paired pre/post treatment)	0.011[b] *	0.001[b] *	0.130[c]		
Absolute change in peak eosinophil count from baseline to wk 6, *eos/hpf, mean (SD)*	-26.2 (39.9)	-40 (36)		-16	10.3

FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, ITT = Intention-to-treat, SD = Standard Deviation, B = Unstandardized beta, SE B = Standard Error for the unstandardized beta.

[a] P-value FFED vs. FFED + AAF (Mann-Whitney U-test).

[b] P-value baseline vs. after-treatment (Wilcoxon signed rank test).

[c] Unadjusted p-value for the effect between treatment groups from linear least squares model with treatment group and baseline peak eosinophil count value as covariates.

* P-value (two-sided) of < 0.05, indicating a significant outcome.

Histological remission rates

Forty-eight percent of the FFED + AAF-subjects in PP-population showed complete histological remission (< 15 *eos/hpf*) at week 6 vs. 26% of the FFED-subjects (p = 0.204). Partial histological remission (≥ 50% reduction of pre-treatment PEC) was achieved in 24% of the FFED + AAF-subjects vs. 25% of the FFED-subjects after 6 weeks (p = 1.000) (**Table 2B, Figure 2B**). In addition, the proportions of FFED + AAF-subjects with peak eosinophil levels of ≤10 *eos/hpf* and ≤ 5 eos/hpf compared to the FFED-subjects at week 6 were 43% vs. 20% (p = 0.186) and 43% vs. 10% (p = 0.034), respectively. In FFED + AAF-subjects, 14% had PEC of ≤1 *eos/hpf* at week 6 vs. 0% of the FFED-subjects (p = 0.233) (**Table 2B**). Similar results were observed in the PP-cohort (**Supplementary Table 3B**).

TABLE 2B. | Histological features before and after treatment in both groups.

	ITT-cohort		
Histological outcomes	**FFED (n = 20)**	**FFED + AAF (n = 21)**	**P-value**
Histological remission rates			
Rate of patients with complete histological remission at wk 6 (< 15 *eos/hpf*), *n (%)*	5 (25)	10 (48)	0.204[a]
Rate of patients with partial histological remission at wk 6 (≥ 50% reduction of pre-treatment *eos/hpf*), *n (%)*	5 (25)	5 (24)	1.000[a]
Rate of patients with no histological remission at wk 6, *n (%)*	9 (45)	6 (29)	0.328[a]
Histological response thresholds			
Rate of patients with histological remission at wk 6 (≤ 10 *eos/hpf*), *n (%)*	4 (20)	9 (43)	0.186[a]
Rate of patients with histological remission at wk 6 (≤ 5 *eos/hpf*), *n (%)*	2 (10)	9 (43)	0.034[a]*
Rate of patients with histological remission at wk 6 (≤ 1 *eos/hpf*), *n (%)*	0 (0)	3 (14)	0.233[a]

FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, ITT = Intention-to-treat.
[a] P-value FFED vs. FFED + AAF (Fisher's exact test).
* P-value (two-sided) of < 0.05, indicating a significant outcome.

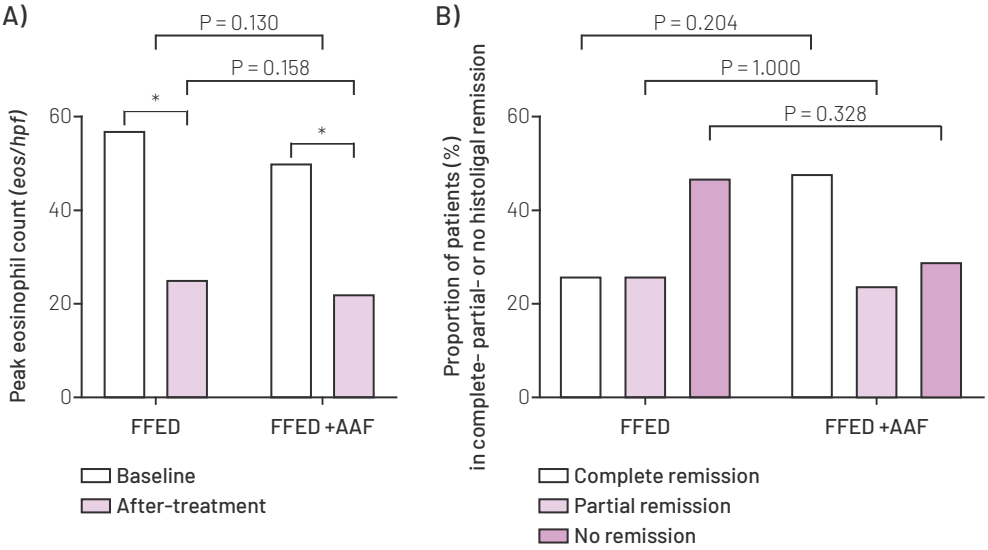

FIGURE 2. | Histological outcome measures (ITT-cohort) A) Peak eosinophil count (*eos/hpf*) Pre/post treatment and between groups B) Post treatment proportion (%) of patients in complete- partial- or no histological remission between groups.
Complete histological remission = Rate of patients with complete histological remission at week 6 (< 15 eos/hpf), partial histological remission = Rate of patients with partial histological remission at week 6 (≥ 50% reduction of pre-treatment eos/hpf), no histological remission = Rate of patients with no histological remission at week 6.
ITT = Intention-to-treat, eos = eosinophils, hpf = high-power-field.

ENDOSCOPIC OUTCOMES

The total EREFS-score significantly changed in both groups of the ITT-population after the diet (FFED + AAF: from 4 (IQR 3 - 5) to 3 (IQR 1.5 - 4); p = 0.002) and (FFED: from 4 (IQR 3.3 - 5) to 4 (IQR 1 - 4); p = 0.026), respectively. No difference in the change of the total EREFS-score from baseline to 6 weeks was observed between the FFED + AAF-group and FFED-group (-1 (IQR -2 - 0) vs. -1 (IQR -2 - 0)), respectively (p = 0.687) (**Table 3, Figure 3A**). In addition, inter-group ITT-analysis showed a significant improvement of the inflammatory sub-scores in both groups after intervention, whereas the fibrotic sub-score only significantly improved in the FFED + AAF-subjects (p = 0.013) and not in the FFED-subjects (p = 0.109) (**Table 3**). Results of the PP-analysis are presented in **Supplementary Table 4**. All individual components of the EREFS classification improved after treatment and pre/post treatment outcomes in the ITT-population were similar between both groups (all; p > 0.05). Similar observations were seen in the PP-population.

TABLE 3. | Endoscopic features before and after treatment in both groups.

Endoscopic outcomes	ITT-cohort		
	FFED (n = 20)	FFED + AAF (n = 21)	P-value
EREFS			
Total EREFS score			
Baseline, *median (IQR)*	4 (3 - 5)	4 (3 - 5)	0.685[a]
Post-treatment, *median (IQR)*	4 (1 - 4)	3 (1.5 - 4)	0.689[a]
P-value (paired pre/post treatment)	0.026[b] *	0.002[b] *	
Change in total EREFS score from baseline to wk 6, *median (IQR)*	-1 (-2 - 0)	-1 (-2 - 0)	0.687[a]
Inflammatory score			
Baseline, *median (IQR)*	2 (2 - 3)	3 (2 - 3)	0.469[a]
Post-treatment, *median (IQR)*	2 (1 - 2)	2 (1 - 2)	0.567[a]
P-value (paired pre/post treatment)	0.07[b] *	0.017[b] *	
Change in inflammatory score from baseline to wk 6, *median (IQR)*	0 (-1.75 - 0)	-1 (-1 - 0)	0.779[a]
Fibrostenotic score			
Baseline, *median (IQR)*	2 (0.25 - 3)	2 (1 - 2)	0.547[a]
Post-treatment, *median (IQR)*	1 (1 - 2)	1 (1 - 2)	0.933[a]
P-value (paired pre/post treatment)	0.109[b]	0.013[b] *	
Change in fibrostenotic score from baseline to wk 6, *median (IQR)*	0 (-1 - 0)	0 (-1 - 0)	0.341[a]

Endoscopic features are scored according to the EREFS classification and sub-classified as i) inflammatory signs including white exudates, edema and linear furrows ii) fibrostenotic signs including rings and strictures.
FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, ITT = Intention-to-treat.
[a] P-value FFED vs. FFED + AAF (Mann-Whitney U-test),[b] P-value baseline vs. after-treatment (Wilcoxon signed rank test).
* P-value (two-sided) of < 0.05, indicating a significant outcome.

SYMPTOM OUTCOMES

Dysphagia

The SDI-score decreased significantly from baseline to week 6 in both groups of the ITT-population (FFED + AAF: from 5 (IQR 3.5 - 6) to 3 (IQR 0.5 - 3.5); p = 0.001) and (FFED: from 5 (IQR 3.8 -7) to 2 (IQR 0 - 4); p = 0.001). No difference in the change of the total SDI-score from baseline to 6 weeks was observed between the FFED + AAF-group and FFED-group (-2 (IQR -4 - -2) vs. -2.5 (IQR -4.3 - -1)), respectively (p = 0.829) (**Table 4, Figure 3B**). Similar results were observed in the PP-population (**Supplementary Table 5**).

Disease specific Quality of Life

ITT-analysis showed that the disease specific QoL (EoEQoL) scores only significantly improved in the FFED+AAF-group (3 (IQR 2.4 - 3.2) - 3 (IQR 2.6 - 3.4); p = 0.007), whereas no significant improvement was observed in the FFED-group after 6 weeks treatment

(IQR 2.5 (1.8 - 3.3) to 2.8 (IQR 2.2 - 3.5); p = 0.378). No difference in the change of the total EoEQoL-score from baseline to 6 weeks was observed between the FFED + AAF-group and FFED-group (0.1 (IQR 0.04 - 0.56) vs. 0 (IQR -0.08 - 0.40)), respectively (p = 0.298) (**Table 4, Figure 3C**). Similar observations were seen in the PP-population (**Supplementary Table 5**).

Comparison of the EoEQoL-subscores in the ITT-population showed only a significant improvement of the change of the social impact score at week 6 in the FFED + AAF-subjects and not in the FFED-subjects (0.3 (IQR 0.1 - 1) vs. 0 (IQR -0.3 - 0.3)), respectively (p = 0.012) (**Supplementary Table 6**). The change of the EoEQoL-subscores; eating/diet impact, emotional impact, disease anxiety and swallowing anxiety after 6 weeks of intervention were similar between both groups (**Supplementary Table 6**). In addition, improvements from baseline to 6 weeks in the total EoEQoL-score and subscores of social impact, disease anxiety and swallowing anxiety were significant in the FFED + AAF-group (all; p < 0.05), whereas no significant improvements of the total EoEQoL-score and subscores were noted in the FFED-group (**Supplementary Table 6**). Furthermore, post-treatment EoEQoL eating/diet impact sub scores (4-items and 10-items) did not differ significantly between the FFED + AAF-group and FFED-group (4-items: 2.3 (IQR 2.0 - 2.8) vs. 2 (0.8 - 2.8)), respectively (p = 0.544) and (10-items: 2.5 (IQR 1.6 - 2.8) vs. 2.2 (1.4 - 2.7)), respectively (p = 0.361) (**Supplementary Table 6**). Similar results were observed in the PP-population.

NUTRITIONAL OUTCOMES

Weight loss, diet feasibility, diet adherence and AAF-intake

The median BMI (kg/m^2) in the ITT-population decreased significantly from 24 (IQR 22.3 - 26.7) to 23.8 (IQR 21.5 - 26) after FFED + AAF (p = 0.001) and from 24 (IQR 22.4 - 28.2) to 23.3 (IQR 22 -27.3) after FFED (p < 0.001). No difference in the change of BMI (kg/m^2) from baseline to 6 weeks was observed between the FFED + AAF-group and the FFED-group (-0.6(IQR -1.2 - -0.1) vs. -0.8 (IQR -1.5 - -0.3), respectively (p = 0.472) (**Table 5**) Furthermore, secondary endpoints on self-reported feasibility of and adherence to the dietary intervention were similar between groups (**Table 5**). Results were similar in the PP-population. No additional protocol deviations were reported by the dietician or physician regarding patients' adherence to the diet at week 6 in both groups. The median adherence rate of AAF-intake at week 6 was 84% (IQR 69 - 97) in the ITT-population and 87% (IQR 72 - 98) in the PP-population (**Supplementary Table 7, Supplementary Figure 3**).

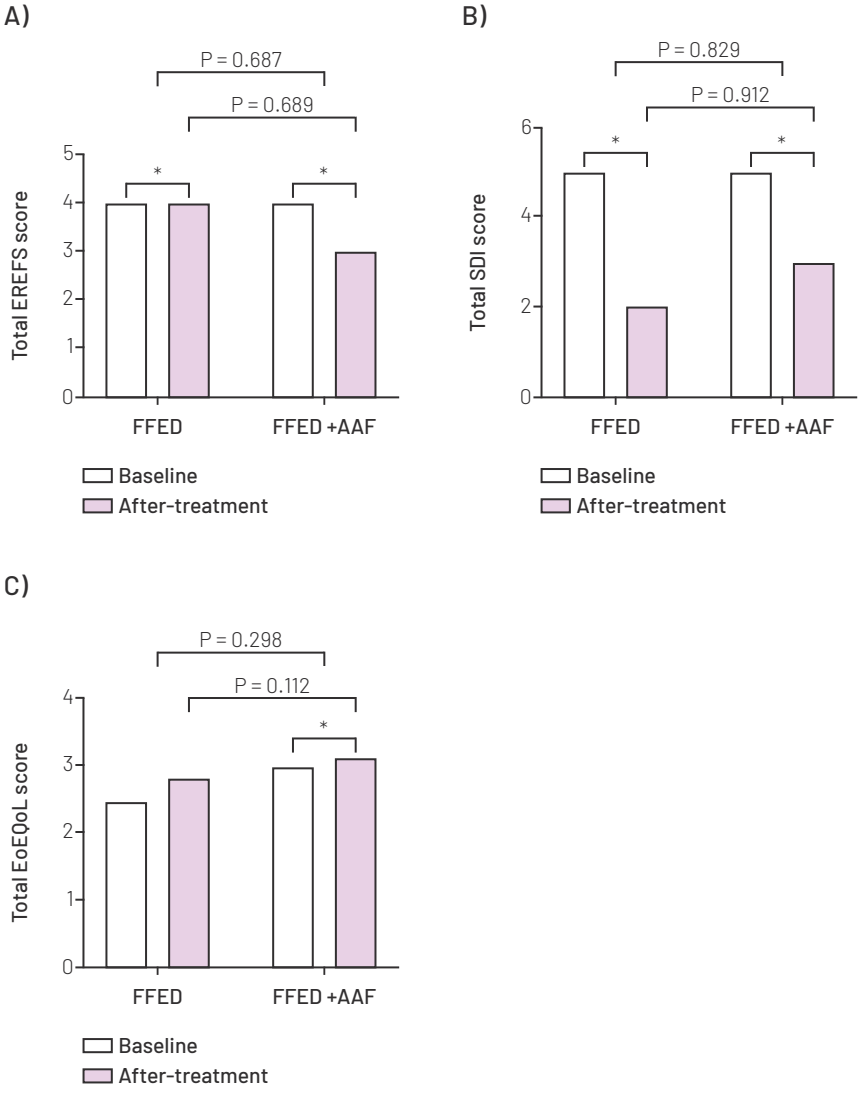

FIGURE 3. | Endoscopic and Symptom outcome measures (ITT-cohort) A) EREFS pre/post treatment and between groups B) SDI-PRO measure score pre/post treatment and between groups C) EoEQoL pre/post treatment and between groups.
ITT = Intention-to-treat, EREFS = Endoscopic Reference score, SDI = Straumann Dysphagia Instrument.

TABLE 4. | Symptoms and disease related Quality of life before and after treatment in both groups.

Symptom outcomes	ITT-cohort		
	FFED (n = 20)	FFED + AAF (n = 21)	P-value
Dysphagia symptoms			
SDI score			
Baseline, *median (IQR)*	5 (3.75 - 7)	5 (3.5 - 6)	0.343[a]
Post-treatment, *median (IQR)*	2 (0 - 4)	3 (0.5 - 3.5)	0.912[a]
P-value (paired pre/post treatment)	0.001[b] *	0.001[b] *	
Change in total SDI score from baseline to wk 6, *median (IQR)*	-2.5 (-4.25 - -1)	-2 (-4 - -2)	0.829[a]
Disease specific Quality of Life			
Total EoEQoL score			
Baseline, *median (IQR)*	2.46 (1.82 - 3.29)	2.96 (2.42 - 3.15)	0.345[a]
Post-treatment, *median (IQR)*	2.79 (2.21 - 3.5)	3.1 (2.6 - 3.44)	0.112[a]
P-value (paired pre/post treatment)	0.378[b]	0.007[b] *	
Change in total EoE-QoL score from baseline to wk 6, *median (IQR)*	0 (-0.08 - 0.4)	0.1 (0.04 - 0.56)	0.298[a]

SDI = Straumann Dysphagia Instrument, RDQ = Reflux Disease Questionnaire. RDQ score includes heartburn and regurgitation, EoEQoL = Adult Eosinophilic Esophagitis Quality of Life survey (24 items, weighted average), FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, ITT = Intention-to-treat.

[a] P-value FFED vs. FFED + AAF (Mann-Whitney U-test).

[b] P-value baseline vs. after-treatment (Wilcoxon signed rank test).

* P-value (two-sided) of < 0.05, indicating a significant outcome.

TABLE 5. | Weight monitoring, diet feasibility and adherence in both groups.

Nutritional outcomes	ITT-cohort		
	FFED (n = 20)	FFED + AAF (n = 21)	P-value
Weight loss			
BMI (kg/m^2)			
Baseline, *median (IQR)*	24 (22.4 - 28.3)	23.7 (22.2 - 26.7)	0.540[a]
Post-treatment, *median (IQR)*	23.6 (22 - 27.5)	23.4 (21.5 - 26)	0.645[a]
P-value (paired pre/post treatment)	< 0.001[b] *	0.001[b] *	
Change in BMI (kg/m^2), *median (IQR)*	-0.9 (-1.48 - -0.3)	-0.58 (-1.2 - 0)	0.248[a]
Weight loss (kg), *median (IQR)*	3 (1 - 5)	2 (0 - 4)	0.255[a]
Feasibility score			
Post-treatment, *median (IQR)*	3 (1 - 3)	3 (1.3 - 3)	0.872[a]
Self-reported adherence rate (%)			
Post-treatment, *median (IQR)*	90 (90 - 100)	90 (90 - 100)	0.867[a]

FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, ITT = Intention-to-treat, BMI = Body Mass Index.

[a] P-value FFED vs. FFED + AAF (Mann-Whitney U-test).

[b] P-value baseline vs. after-treatment (Wilcoxon signed rank test).

* P-value (two-sided) of < 0.05, indicating a significant outcome.

ADVERSE EVENTS

No serious adverse events occurred during the study period. One adverse event was reported in the FFED + AAF-group (i.e., emergency room visit due to severe abdominal pain after eating a kiwi), but was not related to the intervention or study product.

DISCUSSION

In this randomized controlled trial, we determined whether AAF added to a FFED was more effective than FFED alone in dietary treatment of adult EoE patients. Although our primary outcome was not significantly different between the two groups, lower peak eosinophil levels were seen in the FFED + AAF-subjects compared to the FFED-subjects (PP-population) after 6 weeks of treatment (17 (IQR 3.3 - 35.5) vs. 26.5 (IQR 14 - 48.8); p = 0.098). Moreover, a higher proportion of PP-subjects in the FFED + AAF-group achieved complete histological remission at week 6 compared to the FFED-group (50% vs. 25%; p = 0.191). Disease related QoL-scores significantly improved between baseline and week 6 in subjects treated with the FFED + AAF and not in the FFED-group. These findings could suggest that a combined approach of FFED and AAF may have benefits above FFED alone.

Significant intra-group improvements of histological, endoscopic and symptomatic outcomes were seen in both the FFED + AAF-group and FFED-group, which affirms previous reports on FFED efficacy in adult EoE patients.[24] Improvements in the intervention group were not statistically superior to those seen in the FFED-group between baseline and week 6, yet this trial was not powered to show differences between groups in these pre-specified secondary outcomes.

Considering the levels of post-treatment eosinophilia and other histological endpoints, it is possible that the absence of a significantly different primary outcome might have resulted from a low power (type II error). Since no data is available on this combined dietary approach, the estimated improvement of the intervention group was based on a study of Peterson *et al.*, in which AAF-intake comprised 100% of patients' caloric intake.[20] Therefore, expected post-treatment differences between FFED + AAF and FFED-subjects used in our power calculations may have been overestimated (large effect size) resulting in a sample size with too low power.

The overall observed (complete-)remission rate of 38% (PP-cohort) is remarkably lower compared to a study by Molina Infante *et al.*, in which complete histological remission (< 15 *eos/hpf*) was reported in 54% EoE patients after 6 weeks FFED.[40] The use of a more extensive food elimination approach in the study of Molina Infante *et al.*, including gluten, milk, egg and all kind of legumes (e.g., soy, lentil, peanut) alternatively to only soy, may explain the observed differences in remission rates.[41,28,18] Although a prospective approach was used in the study by Molina Infante *et al.*, our randomized controlled design with comprehensive monitoring of participants may have resulted in a lower risk of selection bias. There are more data of lower-than-expected results in a recent large multicenter trial in both pediatric and adult EoE, suggesting a potential bias in previous cohort studies as one of the explanations for the observed discrepancies in results. Comparison of 1FED (milk) to FFED in children showed similar histologic improvements and remission rates (~40%) to our study.[42] In adults, 1FED (milk) to SFED showed that histological response (< 15 *eos/hpf*) was similar between groups (34% vs. 40%).[43]

With regards to the overall high proportion of participants (25%) in partial histological remission (≥ 50% reduction of pre-treatment PEC) at week 6, it could also be argued that the intervention period was too short to determine efficacy of the diet. In addition to this, considering that both treatment arms eliminated the same potential food triggers, there is still the conceptual issue that both groups had the same probability of having culprit foods in the diet that were not eliminated in the FFED. This may be also a reason for the absence of a more evident response in the FFED + AAF-group.

For dietary treatment to be effective, patients should adhere to it as much as possible; therefore, their motivation and acceptance of the impact of a diet is key. During the study a significant improvement of the EoEQoL-score was observed in the FFED + AAF-group, whereas the FFED-group showed no change in this score.[21,44,39] Also intra-group comparison showed a significant improvement of the 'social impact' domain (e.g., 'I feel frustrated when people think I cause my own choking episodes by eating too fast or taking too big bites') in patients treated with the FFED + AAF combination. It could be hypothesized that the option of using AAF to reach the required daily intake, facilitated participation of patients in normal social life, instead of it being perceived as a limitation. Several participants stated to have benefited the AAF, since they felt it was feasible to decrease their daily solid food intake while still maintaining adequate nutrient intake and a healthy body weight. Also, they considered the AAF as feasible snack while underway from

home. Self-reported diet feasibility scores were similar between groups, indicating that this combined diet (i.e., palatability of the AAF included) is acceptable and well-tolerated.

Aside from its hypoallergenic properties, the specially designed AAF includes multiple macro- and micronutrients. Hence the risk of potential nutritional deficiencies that are common when eliminating key foods might be reduced. Vitamin B1, B2, B6, Folic acid, and Vitamin D intake was significantly higher in the FFED + AAF-group compared to the FFED-group (data not shown). In addition, the beneficial effects of this combined approach may be further supported by AAF itself, which is suggested to have immune modulating properties.[32-52]

Taken together, it seems that this combined dietary approach of AAF added to a FFED is acceptable for patients and keeps them motivated. Hence this may increase diet adherence and thus long-term efficacy of the strategy. These observations provide also future directions for a 'combined dietary approach' as long-term therapy, which has also been suggested as maintenance approach for Inflammatory Bowel Disease patients.[53]

Our study design has a few methodological limitations. Firstly, we did not adjust for adherence of AAF-intake which may have affected our results. However, based on exploratory subgroup analysis of individual adherence rates we judged the overall AAF-intake of 84% at group level sufficiently high (**Supplementary Table 7, Supplementary Figure 3A, 3B**). In addition, some patients in a normal setting will also not adhere to the prescribed AAF-intake, so therefore our results provide a more 'real-life' estimate of the effect size. Secondly, we did not include a placebo formula in this trial, so we were not able to determine whether the potential benefit of AAF is related to the lack of placebo, potential immune-modulating properties and/or increased diet adherence. However, it was previously observed that the addition of a placebo does not affect esophageal eosinophilic inflammation in EoE.[54,55] Finally, histological assessment (i.e., determination of PEC) was performed per standardized protocol by multiple blinded pathologists (Amsterdam UMC pathology department) instead of central reading, which may have increased the risk of observer bias. The risk of observer bias on endoscopic outcomes was reduced by our blinded endoscopic scoring strategy and the use of the validated EREFS. Despite these limitations, our study adds to the existing literature being the first adult EoE combination-dietary intervention trial with a randomized controlled study design. Another strength of our study lies in the extensive patient monitoring within the study timeframe, thereby

increasing diet adherence (e.g., less risk of diet errors and improved patients' motivation) as well as adherence to AAF-intake. Another strength is the use of multiple outcome measures (i.e., endoscopic, symptoms, QoL and nutrition related).

In summary, the addition of AAF to a FFED did not lead to a larger decrease in PEC between baseline and 6 weeks, but may result in a significant improvement of QoL in adult patients with EoE. Thus further investigation within a larger sample seems warranted.

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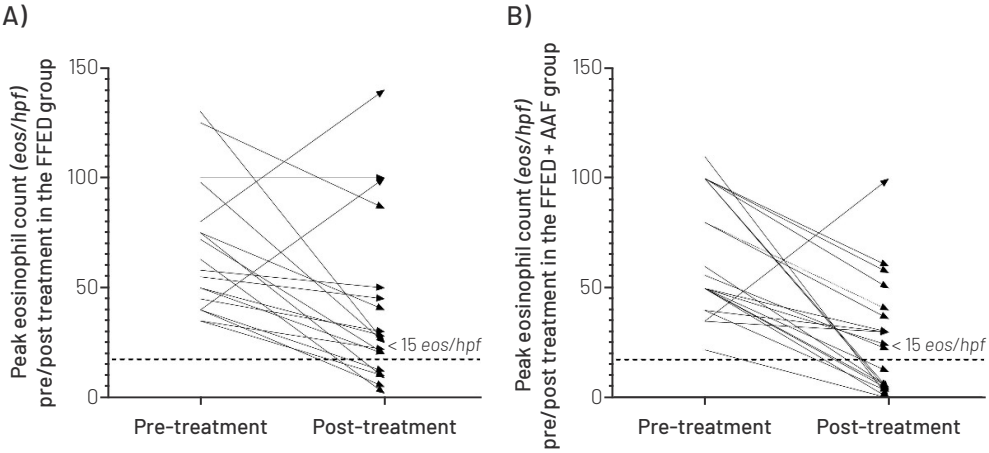
SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE 1. | Study overview.

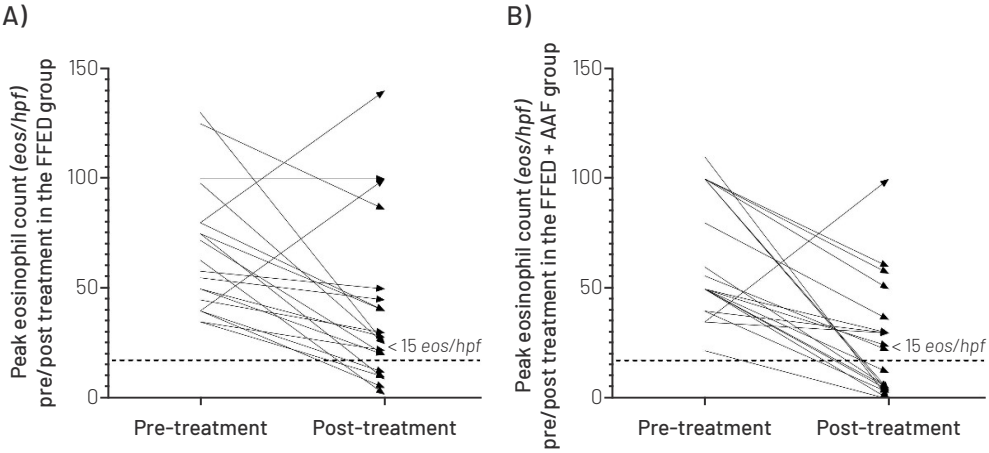
	Case identification	Baseline	Start of diet treatment			End of diet treatment
Time line (weeks)	-2	-0.5	0	2	4	6
Visits	Visit 1	Visit 2	Telephone / e-mail visit 1	Telephone/ e-mail visit 2	Telephone/ e-mail visit 3	Visit 3
Procedures						
Informed consent	x					
Demographics / and medical history	x					
Nutritional consultation	x					
EGD		x				x
Review of eligibility requirements		x				
Randomization			x			
Histological outcomes		x				x
Endoscopic outcomes		x				x
PRO measures		x				x
Nutritional outcomes		x		x	x	x
Adherence to AAF consumption				x	x	x
Adverse events		x				x

EGD = Esophagogastroduodenoscopy, AAF = amino acid-based formula, PRO measures = Patient Reported Outcome measures.



SUPPLEMENTARY FIGURE 1. | ITT-cohort: decrease in peak eosinophil count (*eos/hpf*) after treatment in; A) FFED group B) FFED + AAF group.

ITT = Intention-to-treat, eos = eosinophils, hpf = high-power-field, FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula.



SUPPLEMENTARY FIGURE 2. | ITT-cohort: decrease in peak eosinophil count (*eos/hpf*) after treatment in; A) FFED group B) FFED + AAF group.

ITT = Intention-to-treat, eos = eosinophils, hpf = high-power-field, FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula

SUPPLEMENTARY TABLE 2. | Baseline characteristics of all patients who completed the trial according to the protocol (n = 40) in both groups (PP-cohort).

Characteristics	PP-cohort	
	FFED (n = 20)	**FFED + AAF (n = 20)**
Male gender, *n (%)*	12 (60)	12 (60)
Age, years, *median (IQR)*	32 (27.5 - 43)	36.5 (29.25 - 42)
Race, Caucasian, *n (%)*	19 (95)	19 (95)
History of allergic disease, *n (%)*	17 (85)	17 (85)
Allergic rhinitis	14 (70)	13 (65)
Asthma	6 (30)	6 (30)
Atopic dermatitis	5 (25)	7 (35)
Food allergy	6 (30)	5 (25)
Angioedema	1 (5)	2(10)
Oral Allergy Syndrome	7 (35)	7 (35)
PPIs at baseline, *n (%)*	7 (35)	9 (45)
Prior use of topical steroids, *n (%)*	10 (50)	8 (40)
Esophageal stricture dilation, *n (%)*	1 (5)	2 (10)
Previous endoscopic intervention with food bolus extraction, *n (%)*	7 (35)	10 (50)
Diagnostic delay *, *median (IQR)*	4.5 (1 - 7.5)	3 (1- 9.75)
BMI (kg/m^2), *median (IQR)*	24.1 (22.4 - 28.4)	24.0 (22.3 - 26.7)

FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, PP = Per-protocol, PPIs = Proton Pump Inhibitors, BMI = Body Mass Index.
* Time interval between first reported EoE symptoms and year of diagnosis.

SUPPLEMENTARY TABLE 3A. | Histological features before and after treatment in both groups.

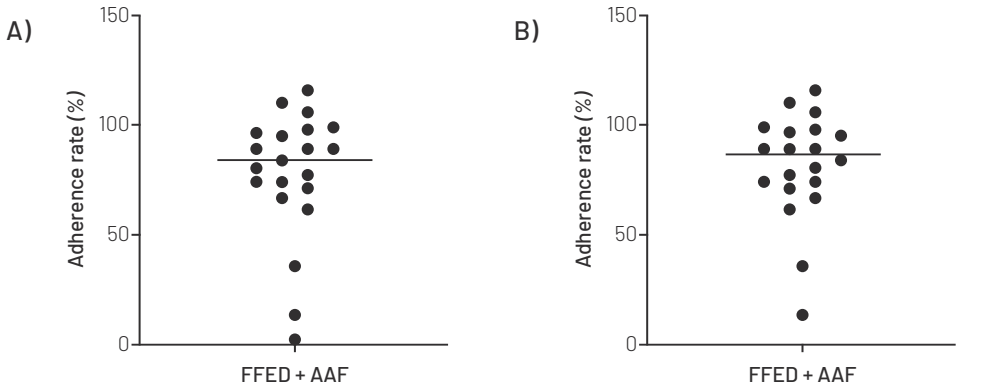
	PP-cohort				
Histological outcomes	**FFED (n = 20)**	**FFED + AAF (n = 20)**	**P-value**	**B**	**SE B**
Peak Eosinophil Count					
Baseline, *eos/hpf, median (IQR)*	60.5 (41.25 - 80)	50 (42.5 - 100)	0.796[a]		
Post-treatment, *eos/hpf, median (IQR)*	26.5 (14 - 48.75)	17 (3.25 - 35.5)	0.098[a]		
P-value (paired pre/post treatment)	0.008[b] *	0.001[b] *			
Absolute change in peak eosinophil count from baseline to wk 6, *eos/hpf, mean (SD)*	-26.9 (39)	-41.5 (37)	0.127[c]	-16.9	10.2

FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, SD = Standard Deviation, B = Unstandardized beta, SE B = Standard Error for the unstandardized beta, PP = Per-protocol.
[a] P-value FFED vs. FFED + AAF (Mann-Whitney U-test).
[b] P-value baseline vs. after-treatment (Wilcoxon signed rank test).
[c] Unadjusted p-value for the effect between treatment groups from linear least squares model with treatment group and baseline peak eosinophil count value as covariates.
* P-value (two-sided) of < 0.05, indicating a significant outcome.

SUPPLEMENTARY TABLE 3B. | Histological features before and after treatment in both groups.

	PP-cohort		
Histological outcomes	**FFED (n = 20)**	**FFED + AAF (n = 20)**	**P-value**
Histological remission rates			
Rate of patients with complete histological remission at wk 6 (< 15 *eos/hpf*), *n (%)*	5 (25)	10 (50)	0.191[a]
Rate of patients with partial histological remission at wk 6 (≥ 50% reduction of pre-treatment *eos/hpf*), *n (%)*	6 (30)	4 (20)	0.716[a]
Rate of patients with no histological remission at wk 6, *n (%)*	9 (45)	6 (30)	0.514[a]
Histological response thresholds			
Rate of patients with histological remission at wk 6 (≤ 10 *eos/hpf*), *n (%)*	4 (20)	9 (45)	0.176[a]
Rate of patients with histological remission at wk 6 (≤ 5 *eos/hpf*), *n (%)*	2 (10)	9 (45)	0.031[a]*
Rate of patients with histological remission at wk 6 (≤ 1 *eos/hpf*), *n (%)*	0 (0)	3 (15)	0.231[a]

FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, PP = Per-protocol.
[a] P-value FFED vs. FFED + AAF (Fisher's exact test).
* P-value (two-sided) of < 0.05, indicating a significant outcome.



SUPPLEMENTARY FIGURE 3. | Distribution of individual AAF adherence rates (%) at group level.
A) Intention-to-treat cohort B) Per-protocol cohort.
AAF = amino acid-based formula .

SUPPLEMENTARY TABLE 4. | Endoscopic features before and after treatment in both groups.

	PP-cohort		
Endoscopic outcomes	**FFED (n = 20)**	**FFED + AAF (n = 20)**	**P-value**
EREFS			
Total EREFS score			
Baseline, *median (IQR)*	4 (3.25 - 5)	4.5 (3 - 5)	0.685[a]
Post-treatment, *median (IQR)*	4 (1.25 - 4)	3 (1.25 - 4)	0.689[a]
P-value (paired pre/post treatment)	0.016[b] *	0.004[b] *	
Change in total EREFS score from baseline to wk 6, *median (IQR)*	-1 (-2 - 0)	-1 (-2 - 0)	0.687[a]
Inflammatory score			
Baseline, *median (IQR)*	2 (2 - 3)	3 (2 - 3)	0.469[a]
Post-treatment, *median (IQR)*	2 (1 - 2)	2 (1 - 2)	0.567[a]
P-value (paired pre/post treatment)	0.038[b] *	0.029b *	
Change in inflammatory score from baseline to wk 6, *median (IQR)*	0 (-1.75 - 0)	-1 (-1 - 0)	0.779[a]
Fibrostenotic score			
Baseline, *median (IQR)*	2 (0.25 - 3)	2 (1 - 2)	0.547[a]
Post-treatment, *median (IQR)*	1 (1 - 2)	1 (1 - 2)	0.933[a]
P-value (paired pre/post treatment)	0.197[b]	0.005[b] *	
Change in fibrostenotic score from baseline to wk 6, *median (IQR)*	0 (-1 - 0)	0 (-1 - 0)	0.341[a]

Endoscopic features are scored according to the EREFS classification and sub-classified as i) inflammatory signs including white exudates, edema and linear furrows ii) fibrostenotic signs including rings and strictures.
FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, PP = Per-protocol, IQR = Interquartile range.
[a] P-value FFED vs. FFED + AAF (Mann-Whitney U-test).
[b] P-value baseline vs. after-treatment (Wilcoxon signed rank test).
* P-value (two-sided) of < 0.05, indicating a significant outcome.

SUPPLEMENTARY TABLE 5. | Symptoms and disease related Quality of life before and after treatment in both groups.

	PP-cohort		
Symptom outcomes	**FFED (n = 20)**	**FFED + AAF (n = 20)**	**P-value**
Dysphagia symptoms			
SDI score			
Baseline, *median (IQR)*	5 (4 - 7)	5 (3.25 - 6)	0.359[a]
Post-treatment, *median (IQR)*	2 (0 - 4)	3 (0.25 - 3.75)	0.751[a]
P-value (paired pre/post treatment)	< 0.001[b] *	0.002[b] *	
Change in total SDI score from baseline to wk 6, *median (IQR)*	-3 (-4 - -1)	-2 (-4 - -2)	0.616[a]
Disease specific Quality of Life			
Total EoEQoL score			
Baseline, *median (IQR)*	2.46 (1.83 - 3.5)	2.96 (2.40 - 3.12)	0.564[a]
Post-treatment, *median (IQR)*	2.79 (2.22 - 3.45)	3 (2.58 - 3.46)	0.164[a]
P-value (paired pre/post treatment)	0.491[b]	0.003[b] *	
Change in total EoE-QoL score from baseline to wk 6, *median (IQR)*	0 (-0.08 - 0.29)	0.13 (0.13 - 0.63)	0.128[a]

SDI = Straumann Dysphagia Instrument, RDQ = Reflux Disease Questionnaire. RDQ score includes heartburn and regurgitation, EoEQoL = Adult Eosinophilic Esophagitis Quality of Life survey (24 items, weighted average), FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, PP = Per-protocol.

[a] P-value FFED vs. FFED + AAF (Mann-Whitney U-test).

[b] P-value baseline vs. after-treatment (Wilcoxon signed rank test).

* P-value (two-sided) of < 0.05, indicating a significant outcome.

SUPPLEMENTARY TABLE 6. | Disease specific Quality of Life subscores before and after treatment in both groups.

	ITT-cohort		
Disease specific Quality of Life outcomes	**FFED (n = 20)**	**FFED + AAF (n = 21)**	**P-value**
EoE-QoL-A survey			
Total EoE-QoL score *(24 items, weighted average)*			
Baseline, *median (IQR)*	2.46 (1.82 - 3.29)	2.96 (2.42 - 3.15)	0.345[a]
Post-treatment, *median (IQR)*	2.79 (2.21 - 3.5)	3.1 (2.6 - 3.44)	0.112[a]
P-value (paired pre/post treatment)	0.378[b]	0.007[b] *	
Change in total EoE-QoL score from baseline to wk 6, *median (IQR)*	0 (-0.08 - 0.4)	0.1 (0.04 - 0.56)	0.298[a]
Eating/diet impact *(4 items, weighted average)*			
Baseline, *median (IQR)*	2.0 (1.25 - 3.8)	2.75 (1.88 - 3.25)	0.188[a]
Post-treatment, *median (IQR)*	2.0 (0.75 - 2.75)	2.25 (2.0 - 2.75)	0.544[a]
P-value (paired pre/post treatment)	0.492[b]	0.117[b]	
Change in eating/diet impact score from baseline to wk 6, *median (IQR)*	0 (-1.25 - 0.5)	-0.5 (-0.5 - 0.5)	0.929[a]
Eating/diet impact *(10 items, weighted average)*			
Post-treatment, *median (IQR)*	2.2 (1.4 - 2.7)	2.5 (1.6 - 2.8)	0.361[a]
Social impact *(4 items, weighted average)*			
Baseline, *median (IQR)*	3.0 (2.4 - 3.8)	2.5 (2.25 - 3.0)	0.225[a]
Post-treatment, *median (IQR)*	3.0 (2.25 - 4.0)	3.25 (2.5 - 4.0)	0.796[a]
P-value (paired pre/post treatment)	0.569[b]	0.002[b] *	
Change in social impact score from baseline to wk 6, *median (IQR)*	0 (-0.25 - 0.31)	0.25 (0.06 - 0.9)	0.012[a] *
Emotional impact *(8 items, weighted average)*			
Baseline, *median (IQR)*	2.9 (2.1 - 3.5)	3.25 (2.88 - 3.5)	0.204[a]
Post-treatment, *median (IQR)*	3. (2.1 - 3.5)	3.4 (2.9 - 3.8)	0.199[a]
P-value (paired pre/post treatment)	0.181[b]	0.068[b]	
Change in emotional impact score from baseline to wk 6, *median (IQR)*	0.13 (0 - 0.38)	0.06 (0 - 0.25)	0.906[a]
Disease anxiety *(5 items, weighted average)*			
Baseline, *median (IQR)*	2 (1.2 - 3.4)	2.6 (1.9 - 3)	0.582a
Post-treatment, *median (IQR)*	2.4 (1.6 - 3.4)	3 (2.6 - 3.7)	0.06a
P-value (paired pre/post treatment)	0.057b	<0.001b *	0.068a
Change in disease anxiety score from baseline to wk 6, median (IQR)	0 (-0.3 - 0.8)	0.4 (0.2 - 0.8)	

Table continues on next page

SUPPLEMENTARY TABLE 6. | continued.

	ITT-cohort		
Disease specific Quality of Life outcomes	**FFED (n = 20)**	**FFED + AAF (n = 21)**	**P-value**
EoE-QoL-A survey			
Swallowing anxiety *(3 items, weighted average)*			
Baseline, *median (IQR)*	2.67 (2 - 4)	3.33 (2.42 - 3.67)	0.854[a]
Post-treatment, *median (IQR)*	3.33 (2.33 - 4)	3.67 (3.33 - 4.0)	0.138[a]
P-value (paired pre/post treatment)	0.081[b]	0.005[b] *	
Change in swallowing anxiety score from baseline to wk 6, *median (IQR)*	0 (-0.1 - 0.75)	0.33 (0 - 0.67)	0.274[a]

EoE-QoL-A = Adult Eosinophilic Esophagitis Quality of Life survey, FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, ITT = Intention-to-treat.
[a] P-value FFED vs. FFED + AAF (Mann-Whitney U-test), [b] P-value baseline vs. after-treatment (Wilcoxon signed rank test).

SUPPLEMENTARY TABLE 7. | Adherence to AAF intake in the intervention group.

	ITT-cohort	PP-cohort
AAF consumption at group level	**FFED + AAF (n = 21)**	**FFED + AAF (n = 20)**
Total prescribed AAF (kg), *median IQR*	6.18 (5.38 - 6.72)	6.18 (5.38 - 6.72)
Total consumed AAF (kg), *median IQR*	5 (4 - 5.6)	5.1 (4.4 - 5.8)
Total adherence rate (%)*, *median IQR*	84 % (69 - 97)	87 % (72 - 98)
Degree of AAF adherence in subgroups		
High (≥ 75% adherence rate), *n (%)*	13 (62)	13 (65)
Medium (50% - 75% adherence rate), *n (%)*	5 (24)	5 (25)
Low (< 50 % adherence rate), *n (%)*	3 (14)	2 (10)

FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, IQR = Interquartile range, Kg = Kilograms, ITT = Intention-to-treat, PP = Per-protocol.
*Adherence rate (%) per patient = Consumed AAF as percentage of the total amount of prescribed AAF for 6 weeks in each individual patient.

OF NOTE The amount of prescribed daily AAF consumption included 30% of patients' calorie intake based on body mass index and weekly physical activity.

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CHAPTER 6

GENE EXPRESSION AND CLINICAL OUTCOMES AFTER DIETARY TREATMENT FOR EOSINOPHILIC ESOPHAGITIS: A PROSPECTIVE STUDY

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ABSTRACT

RATIONALE

EoE is an allergen-mediated disease and elimination diets have proven to be effective to obtain clinical and histological remission. However, the effect of elimination diets on specific EoE transcripts and their clinical correlates is relatively unknown.

AIM

To evaluate the effect of dietary treatment (four food elimination diet (FFED) with or without addition of amino acid-based formula (AAF) on a variety of pro-/anti-inflammatory, epithelial/barrier function and remodeling/fibrosis related markers of disease activity and clinical correlates (eosinophils, symptoms, endoscopic signs) in adult EoE patients.

METHODS

We conducted an analysis of biopsy samples and data collected during a randomized controlled trial with an elimination diet in adult patients with active EoE (≥ 15 eosinophils (eos) per high-power-field (hpf)). Demographics, symptoms (SDI-score), endoscopic signs (EREFS) and peak eosinophil counts/hpf were recorded at baseline and after 6 weeks of treatment. Transcripts of 10 indicated genes were measured (qPCR) and compared to clinical correlates at baseline and after treatment.

RESULTS

Forty patients (pooled FFED + FFED + AAF) (60% male, age 34.5 (interquartile range (IQR) 29 - 42.8 years) completed the diet. Peak eosinophil counts/hpf, symptoms and endoscopic signs were significantly decreased after 6-week dietary treatment. DSG-1 levels were significantly upregulated from baseline to week 6, whereas IL-13, CAPN-14, IL-5, IL-10, CCL-26, POSTN, TSLP, CPA-3 and TGF-β were significantly downregulated after 6 weeks of diet (all; < 0.01). Prior to treatment, upregulation of CAPN-14 and lower levels of DSG-1 were associated with clinical fibrotic phenotypes, whereas upregulation of IL-10 was linked to food impaction phenotypes.

CONCLUSION

These findings strongly suggest that elimination diets, besides a clinical and histological response, are associated with a broad transcriptional response at the level of the esophageal epithelium.

INTRODUCTION

Eosinophilic esophagitis (EoE) is an allergen/immune-mediated esophageal disorder, characterized by symptoms of esophageal dysfunction (i.e., dysphagia and food impaction) and eosinophilic infiltration of the esophageal epithelium.[1,2] There has been a growing understanding of EoE pathogenesis following its first description in the early 1990s.[3,4] Overall, the evolution of EoE is a multifactorial interplay of genetics, environmental and host immune system factors that are involved in multiple pathways.[5,6] The proposed immunological mechanism is illustrated by an immune response that is primarily regulated by T-helper type 2 cells (Th2) against food- (and aero) allergens. Thymic stromal lymphopoietin (TSLP) is released by activated esophageal epithelial cells after allergen exposure and has an important role in promoting Th2 differentiation.[5] Activated dendritic cells initiate T-cell polarization to Th2 cells, that serve as a source of pro-inflammatory cytokines, such as: Interleukin (IL)-5 and IL-13 or products induced by these cytokines (IL-13-induced eotaxin-3 (CCL-26)).[7] Genes specific to mast cells, such as those that encode carboxypeptidase 3A (CPA-3), were also found to be highly expressed in the EoE Transcriptome.[8] Locally activated eosinophils and mast-cells produce Transforming Growth Factor (TGF)-β, a key cytokine for epithelial cell transformation and fibrosis.[9] Moreover, IL-13 induced Calpain (CAPN)-14 - which is specifically found to be overexpressed in EoE patients - downregulates desmoglein (DSG)-1, a barrier protein, by that disrupting the esophageal epithelial barrier.[10] Loss of DSG-1 may also potentiate allergic inflammation through the induction of pro-inflammatory mediators, such as periostin (POSTN).[11,12] Finally, the potent anti-inflammatory cytokine IL-10 seems to be of interest since it was found to be upregulated in pediatric EoE patients compared to controls, by that linking this pleiotropic immunoregulatory cytokine to EoE pathogenesis.[13]

Diets have proven to be effective in EoE and target the adaptive immune system (i.e., suppression of antigen-driven T-cell response by elimination of culprit foods) with no modification of signaling pathways or inflammatory cell-apoptosis as often occurs after steroids or biological targets.[14-17] There is a relative scarcity of data evaluating the effect of dietary treatment on gene expression patterns in adult EoE, in particular in the context of clinical features.[5,7,18] Considering its heterogeneous disease presentation and the clinical impact of fibrotic complications, personalized treatment strategies based on EoE-endotypes being more or less fibrotic may be needed. Therefore, we aimed to investigate the effect of a Four-Food Elimination diet (FFED) (i.e., exclusion of gluten, milk, soy and eggs) on multiple pro-/anti-inflammatory (IL-5, IL-13, TSLP, POSTN CPA-3,

CCL-26 and IL-10), epithelial/barrier function (DSG-1, CAPN-14) and remodeling/fibrosis (TGF-β) related markers of disease activity and clinical correlates (eosinophils, symptoms, endoscopic signs) in adult EoE patients.

METHODS

STUDY DESIGN AND PATIENTS

We conducted an analysis of biopsy samples and data collected during a randomized controlled trial of adult EoE patients. The parent study, of which details have been described previously,[19] included patients from the outpatient clinic of the Amsterdam UMC motility center between December 2017 and January 2020.[19] Adult patients (≥ 18 years) were eligible for study inclusion if EoE was diagnosed per consensus guidelines (i.e., presence of symptoms related to esophageal dysfunction and ≥ 15 eosinophils (eos) per microscopic high-power field (hpf) at baseline biopsy).[20] Patients were excluded if they had severe comorbidity scored as American Society of Anesthesiologists (ASA) Physical Classification System class IV or higher, the inability to stop anti-inflammatory drugs (i.e., topical or systemic steroids, leukotriene inhibitors or monoclonal antibodies), a recent history of gastrointestinal cancer or major Gastrointestinal surgery. This study was approved by the Medical Ethics Committee of the Amsterdam UMC and prospectively registered in the Dutch trial registry NL6014(NTR6778). Written informed consent was obtained from all participants before taking part and a unique study ID was given to ensure anonymity.

Study protocol

After informed consent was obtained, participants underwent an upper endoscopy with biopsy sampling at baseline and after 6 weeks of dietary treatment. Histologic features, endoscopic signs and symptoms were evaluated at baseline and at week 6. If consent was obtained and eligibility was confirmed after baseline upper endoscopy, patients were randomized (1:1 fashion) to either a Four-Food Elimination Diet (FFED) (i.e., exclusion of gluten, milk, soy and eggs) or a FFED with the addition of an amino-acid based formula (AAF) providing 30% of patients' daily energy needs (FFED + AAF) by using a blocked randomization protocol (i.e., sealed envelopes). Comparison of FFED + AAF vs. FFED in the parent study did not show a significant difference between both groups on clinical, endoscopic and histological outcomes. To evaluate the general effect of an elimination diet on gene expression in a large sample of EoE patients, data of both groups were pooled in this follow-up study. In our trial, trends towards lower histological disease activity in

patients treated with the FFED + AAF compared to those treated with FFED alone were observed.[19] Therefore, a subgroup analysis was performed on the treatment effect of the AAF added to the FFED on gene expression levels.

Biopsies that were sampled prior and after 6 weeks of dietary treatment were used to measure gene expression related to disease activity (i.e., eosinophils, symptoms, endoscopic signs).

STUDY PROCEDURES

Clinical data, sample collection and clinical subgroup definition

Demographics, symptoms and endoscopic data were recorded prospectively by using standardized case report forms. Symptoms of dysphagia were evaluated by means of the Straumann Dysphagia Instrument (SDI) measure.[21] This measure ranges from 0 - 9 and consists of 2-items (dysphagia frequency (0 - 4) and dysphagia intensity (0 - 5)). A 'clinical response' was defined as a reduction of ≥ 3 points of the after-treatment SDI-score compared to baseline.

Upper endoscopy was performed and endoscopic features of EoE were classified according to the modified Endoscopic Reference Score (EREFS) grading system.[22] Endoscopic features were sub-classified (EREFS) as inflammatory (white exudates, edema and linear furrows) and fibrotic (rings and strictures) signs.

During upper endoscopy, six biopsies were taken from the distal, mid and proximal esophagus per standardized protocol. A x400 magnification was used in order to determine the peak eosinophil count (PEC) per hpf (an area of 0.24 mm^2). 'Histological remission' after induction treatment was defined as patients achieving a PEC of < 15 *eos/hpf* at histological assessment after diet treatment.

Clinical findings were further defined by means of clinical phenotype definition, which has been previously described by Dellon *et al.*,[23] Patients presenting with symptoms of food impaction (i.e., SDI measure, item 2; dysphagia intensity of ≥ 3) were defined (yes or no) as 'food-impaction' phenotypes (vs. 'non-food impaction' phenotypes). Patients were defined (yes or no) as having a 'fibrotic' phenotype, if endoscopically 'rings' and/or 'strictures' were present (i.e., EREFS fibrotic sub score ≥ 1)(vs. 'non-fibrotic' phenotype). Gene expression levels were compared at 2 time points (i.e., baseline and after 6 weeks) between patients with these pre-defined clinical subgroups.

GENE EXPRESSION DETERMINATION

In addition to the biopsies for histology, three more biopsies were taken from the mid esophagus during upper endoscopy at baseline and after treatment. Gene expression was measured in these esophageal samples to define overall expression levels of the indicated genes (IL-5, IL-13, TSLP, POSTN CPA-3, CCL-26, IL-10, DSG-1, CAPN-14 and TGF-β). These three additional biopsies were immediately immersed in RNA stabilisation reagent (RNA-later, Invitrogen/Thermo Fisher Scientific, Baltics UAB, Vilnius). First, the biopsies were stored for 24h at 4 °C, with subsequent storage at - 80 °C. The mid-esophageal biopsies in RNA-later (-80 °C storage) were sent on dry ice for processing and gene expression testing to Utrecht University. Biopsies in RLT lysis buffer (Qiagen mRNeasy kit) containing 10% β-mercaptoethanol were homogenized by using the Precellys homogenisator (Bertin, France). RNA extraction was performed on homogenized specimens using the RNeasy mini kit (Qiagen, Basel, Germany) according to the manufacturer's' instructions. The concentration of RNA was measured by using NanoDrop One spectrophotometry (Isogen Life Sciences, Utrecht, The Netherlands) and subsequently 500 ng RNA was used for cDNA synthesis by using the iScript cDNA synthesis kit (Biorad, Veenendaal, The Netherlands). Quantitative real-time (RT) PCR was performed on a CFX96 Touch quantitative real-time (q) PCR device (Biorad, Veenendaal, The Netherlands) to determine the gene expression levels measured as threshold cycles (Ct). Commercially available primers for IL-5, IL-10, IL-13, CPA-3, CAPN-14, DSG-1, CCL-26, POSTN and TSLP were obtained (all from Biorad). RPL13A was used as a reference gene for normalization of all genes of interest (Biolegio, 5'CATAGGAAGCTGGGAGCAAG3' and 5'GCCCTCCAATCAGTCTTCTG 3') and was used to calculate normalized mRNA expression. The mRNA level was calculated with CFX manager software and corrected for the expression of RPL13A with 100x2^(RPL13A-gene of interest. Relative values of the gene of interest were calculated by extracting after treatment values by the genes of interest prior to treatment.

STATISTICAL ANALYSIS

Statistical analysis was performed by using IBM SPSS Statistics (version 25.0) (SPSS, Chicago, USA). Descriptive statistics were used to summarize all characteristics of the study sample. Categorical variables are described as percentages and continuous variables are expressed as mean with standard deviation (SD) or median with interquartile ranges (IQR). Baseline and after treatment values within the total sample (n = 40) or subgroups (FFED, n = 20 and FFED + AAF, n = 20) were compared by using the Wilcoxon signed rank test for ordinal data and McNemar's test for categorical data. Normally and non-normally distributed

continuous data between (clinical)(sub)groups were compared by using a t-test or Mann-Whitney U-test, if appropriate. A p-value of < 0.05 was considered statistically significant.

RESULTS

PATIENTS' CHARACTERISTICS

Fifty-two patients were eligible for inclusion. After baseline endoscopy, 11 patients were excluded due to non-active disease (< 15 *eos/hpf*) at histological evaluation. Forty out of the 41 patients who started the diet treatment (FFED group (n = 20) and FFED + AAF group (n = 21)), completed the trial according to the protocol guidelines. A male predominance (60%) was confirmed with a median age of 34.5 (IQR 29 - 42.8) years. The majority of patients (63%) had ≥ 2 additional atopic comorbidities. Details on baseline characteristics of all included EoE patients who completed the 6 weeks dietary treatment are listed in **Table 1**.

DIETARY TREATMENT EFFECT ON HISTOLOGICAL, ENDOSCOPIC AND SYMPTOMATIC OUTCOMES AND GENE EXPRESSION

Treatment effect on esophageal eosinophilia, symptoms, endoscopic signs

Six weeks of dietary treatment (data pooled of FFED and FFED + AAF) reduced the median peak eosinophil count (PEC) significantly from 55.5 (IQR 41.3 - 93.5) to 24.5 (IQR 5 - 43.8) after 6 weeks (p < 0.001) (**Table 2**). Fifteen patients out the 40 (38%) had esophageal peak eosinophil counts of < 15 *eos/hpf* (i.e., histological remission) after 6 weeks of dietary treatment. Symptom severity, measured by means of the SDI-score, significantly decreased from 5 (IQR 4 - 6) to 2 (IQR 0 - 4) at week 6 (p < 0.001) (**Table 2**). A clinical response (i.e., reduction of ≥ 3 points of the SDI-score compared to baseline) was observed in 20 patients (50%) after 6 weeks of dietary treatment (**Table 2**). Additionally, the total EREFS score significantly decreased from 4 (IQR 3 - 5) to 3 (IQR 1.25 - 4) after 6 weeks of dietary treatment (p < 0.001). Also, significant reductions of both the inflammatory- and fibrotic subscores were observed from baseline to week 6: 2 (IQR 2 - 3) - 2 (IQR 1 - 2); p = 0.003)) and 2 (IQR 1 - 3) - 1 (IQR 1 - 2); p < 0.001), respectively (**Table 2**). More details on symptoms, endoscopic and histological features before and after treatment are presented in **Table 2**.

Gene expression baseline/after treatment

Evaluation of gene expression in esophageal biopsy specimens at baseline and after treatment (n = 40, both groups pooled) showed significantly upregulated levels of DSG1 (p = 0.001) (**Figure 1A**). This increase in DSG-1 coexisted with a decrease in IL-13 and CAPN-14

TABLE 1. | Baseline characteristics of all patients who completed the diet intervention (n = 40).

Characteristics	
Male gender, *n (%)*	24 (60)
Age, years, *median (IQR)*	34.5 (29 - 42.8)
Race, Caucasian, *n (%)*	38 (95)
History of allergic disease, *n (%)*	34 (85)
Allergic rhinitis	27 (68)
Asthma	12 (30)
Atopic dermatitis	12 (30)
Food allergy	11 (28)
Food impaction' phenotype, yes, *n (%)*	23 (58)
Fibrotic phenotype, *n (%)*	32 (80)
Esophageal stricture dilation, *n (%)*	3 (8)
Previous endoscopic intervention with food bolus extraction, *n (%)*	17 (43)
Diagnostic delay *, *median (IQR)*	4 (1 - 9)

EoE = Eosinophilic esophagitis, 'Food impaction' phenotype = patients presenting with symptoms of food impaction, 'Inflammatory-only phenotype' = patients presenting with exudates, edema and/or furrows with no endoscopic signs of fibrotic features (i.e., rings, strictures), 'Fibrotic' phenotype = presence of 'rings' and/or 'strictures' at upper endoscopy.
* Time interval between first reported EoE symptoms and year of diagnosis.

TABLE 2. | Clinical, histological and endoscopic signs before and after treatment.

	Baseline (n = 40)	Post-treatment (n = 40)	P-value
Histology			
Peak eosinophil counts, median (IQR)	55.5 (41.3 - 93.5)	24.5 (5 - 43.8)	< 0.001a *
Histological remission [i], *yes, n (%)*		15 (38)	
Endoscopic signs			
EREFS score (total), *median (IQR)*	4 (3 - 5)	3 (1.25 - 4)	< 0.001 a *
EREFS Inflammatory score, *median (IQR)*	2 (2 - 3)	2 (1 - 2)	0.003 a *
EREFS Fibrotic score, *median (IQR)*	2 (1 - 3)	1 (1 - 2)	< 0.001 a *
Symptoms			
SDI-score, *median (IQR)*	5 (4 - 6)	2 (0 - 4)	< 0.001 a *
Clinical response [ii], *median (IQR)*		20 (50)	

EoE = Eosinophilic esophagitis, EREFS = Endoscopic features are scored according to the EREFS classification and sub-classified as i) inflammatory signs including white exudates, edema and linear furrows ii) fibrotic signs including rings and strictures, SDI = Straumann Dysphagia Instrument, IQR = Inter quartile range,
[i] Histological remission = patients with a peak eosinophil count of < 15 eosinophils (eos) per high power field (hpf) after intervention.
[ii] Clinical response = reduction of ≥ 3 points of the post-treatment SDI-score compared to baseline.
[a] P-value baseline vs. post-treatment (Wilcoxon signed rank test).
[b] P-value baseline vs. post-treatment (McNemar test).
* P-value (two-sided) of < 0.05, indicating a significant outcome.

(all; $p < 0.001$), which are both also involved in epithelial barrier function. In addition, the genes encoding for IL-5, IL-10, CCL-26, POSTN, TSLP, CPA-3 and TGF-β were significantly downregulated after treatment compared to baseline (all; < 0.01)(**Figure 1B-J**).

Esophageal eosinophilia and gene expression baseline/after treatment

Spearman's correlation analysis demonstrated a mild positive correlation for the PEC levels after treatment and mRNA expression levels of IL-5 ($r = 0.32$; $p = 0.061$) and a strong positive correlation for levels of CCL-26 ($r = 0.41$; $p = 0.008$), IL-13 ($r = 0.5$; $p = 0.002$) and CPA-3 ($r = 0.4$; $p = 0.01$) at week 6. Moreover, a significant negative correlation between peak eosinophil counts and mRNA expression levels of DSG-1 ($r = -0.39$; $p = 0.014$) at week 6 was observed. The expression levels of CAPN-14, IL-10, TSLP, TGF-β and POSTN at week 6 did not correlate with the PEC after the diet (all; $p > 0.05$). In addition, a significant positive correlation was observed between the absolute change in PEC from baseline to week 6 and the relative gene expression of CPA-3 after the diet ($r = 0.337$; $p = 0.038$). However, no correlations were found between the absolute change in PEC (baseline/after treatment) and the relative gene expression for the other 9 genes of the EoE-panel (all; $p > 0.05$)

Clinical phenotypes and mRNA expression

Significantly higher baseline mRNA expression levels of IL-10 were shown in 23 patients (58%) who were identified as 'food impaction' phenotypes (vs. 'non-food impaction' phenotypes; $p = 0.01$) (**Table 2, Figure 2A**) indicating a role for IL-10 in this phenotype. Additionally, significantly higher baseline transcript levels of CAPN-14 and lower levels of DSG-1 were observed in 32 patients (80%) with a 'fibrotic' phenotype (vs. 'non-fibrotic' phenotype; $p = 0.002$ and $p = 0.0018$), respectively (**Table 2, Figure 2B, 2C**). In addition, no differences in gene expression levels of all 10 genes of the EoE panel associated with clinical phenotypes were observed after treatment.

Clinical and histological response and gene expression after treatment

The relative mRNA expression of genes encoding for IL-13 after treatment was significantly lower in 20 patients (50%) presenting with a clinical response after the diet (vs. no clinical response; $p = 0.006$)(**Figure 3C**). Moreover, the relative mRNA expression levels of genes encoding for IL-13 ($p = 0.02$) and IL-5 ($p = 0.02$) were significantly lower in the 15 patients (38%) achieving histological remission after the diet compared to those remaining with active disease (**Table 2, Figure 3A, 3B**).

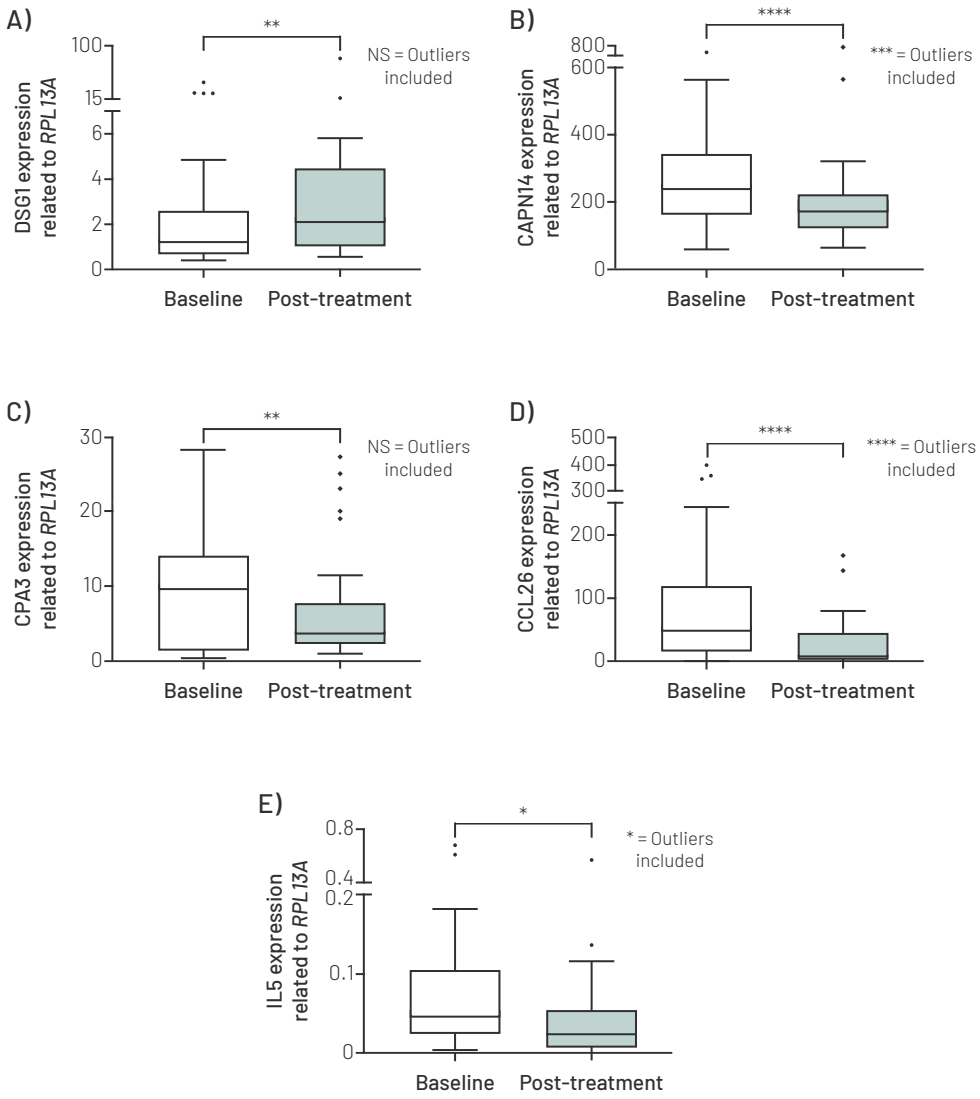
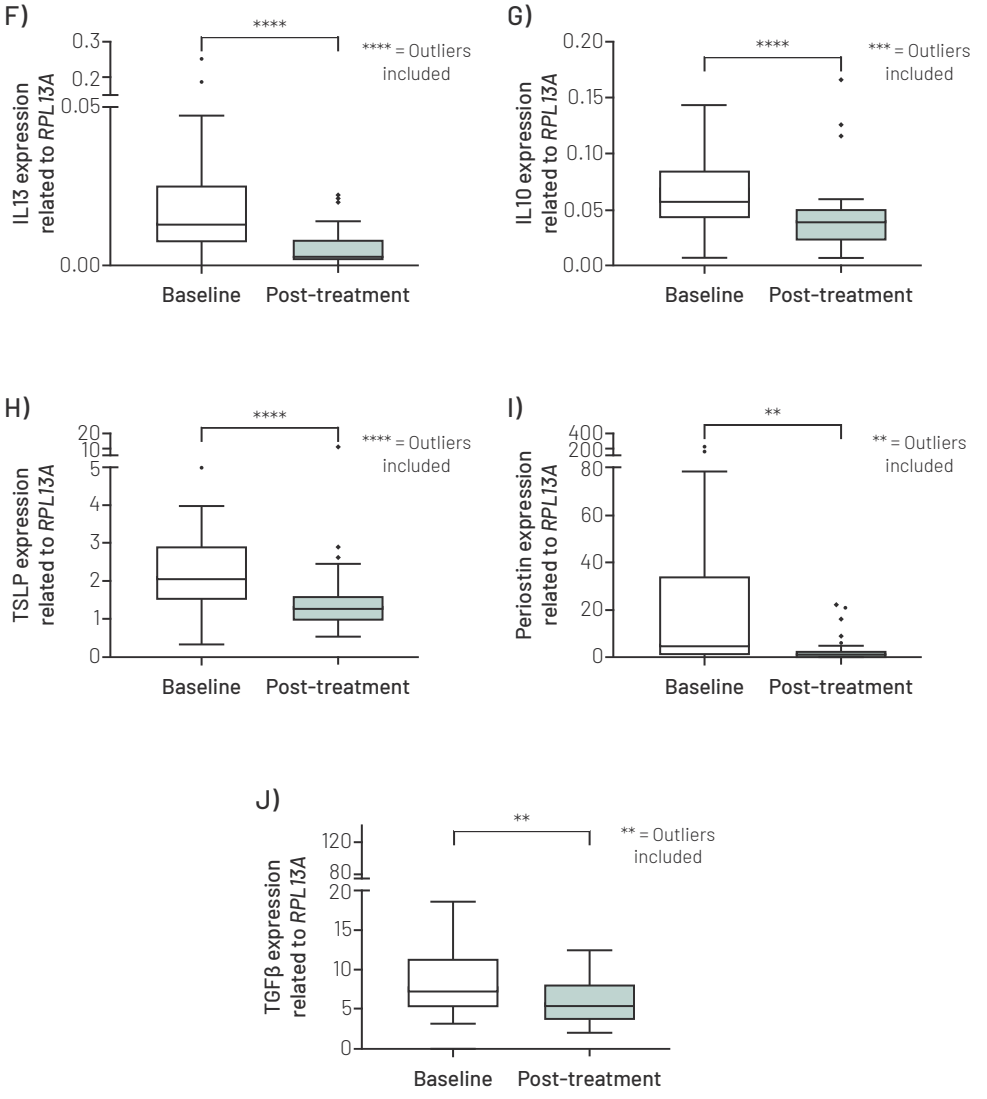


FIGURE 1. | Effect of an elimination diet on the expression of genes encoding for; A) Desmoglein (DSG)1 B) Calpain (CAPN)14 C) Carboxypeptidase(CP)A3 D) Chemokine-ligand (CCL)26 E) Interleukin (IL)5 F) Interleukin (IL)13. G) Interleuking (IL)10. H) Thymic stromal lymphopoietin (TSLP) I) Periostin and J) Transforming growth factor (TGF)β pre- and post-treatment in the entire EoE sample (n = 40, both groups pooled).
The statistical difference between gene expression levels from baseline vs. post-treatment was calculated by means of Wilcoxon Signed Rank test.



The statistical difference between gene expression levels from baseline vs. post-treatment was calculated by means of Wilcoxon Signed Rank test.
EoE = Eosinophilic esophagitis, NS = No significant outcome.
* P-value (two-sided) of < 0.05, indicating a significant outcome.
** P-value (two-sided) of < 0.01.
*** P-value (two-sided) of < 0.001.
**** P-value (two-sided) of < 0.0001.

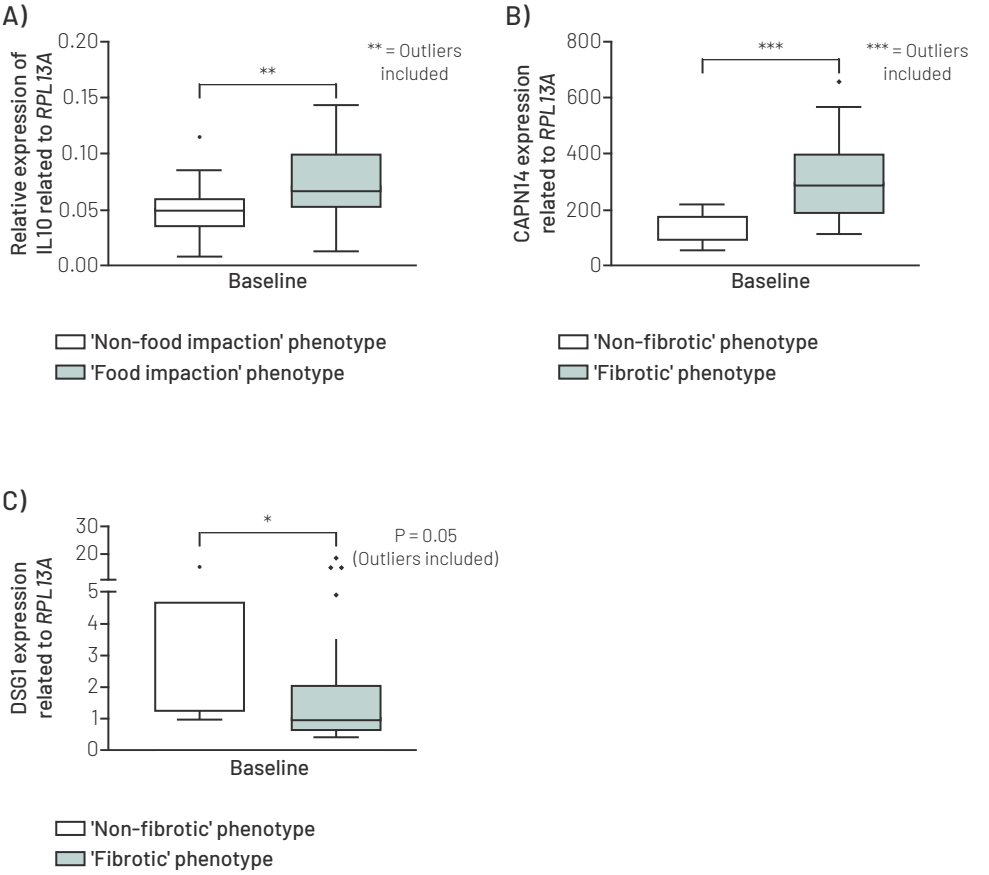


FIGURE 2. | Expression levels of genes of interest in EoE patients (n = 40) with different clinical phenotypes before diet intervention A) Interleukin(IL)10 levels in 'food impaction 'phenotypes vs. 'non-food impaction' phenotypes B) Calpain (CAPN)14 levels in 'fibrotic' phenotypes vs. 'non-fibrotic' phenotypes C) Desmoglein (DSG)1 levels in 'fibrotic' phenotypes vs. 'non-fibrotic' phenotypes.
EoE = Eosinophilic esophagitis, 'Food impaction' phenotype = patients presenting with symptoms of food impaction, 'Fibrotic' phenotype = presence of 'rings' and/or 'strictures' at upper endoscopy.
The statistical difference of gene expression levels at baseline between clinical subgroups was calculated using a t-test or Mann-Whitney U test, as appropriate.
* P-value (two-sided) of < 0.05, indicating a significant outcome.
** P-value (two-sided) of < 0.01.
*** P-value (two-sided) of < 0.001.
**** P-value (two-sided) of < 0.0001.

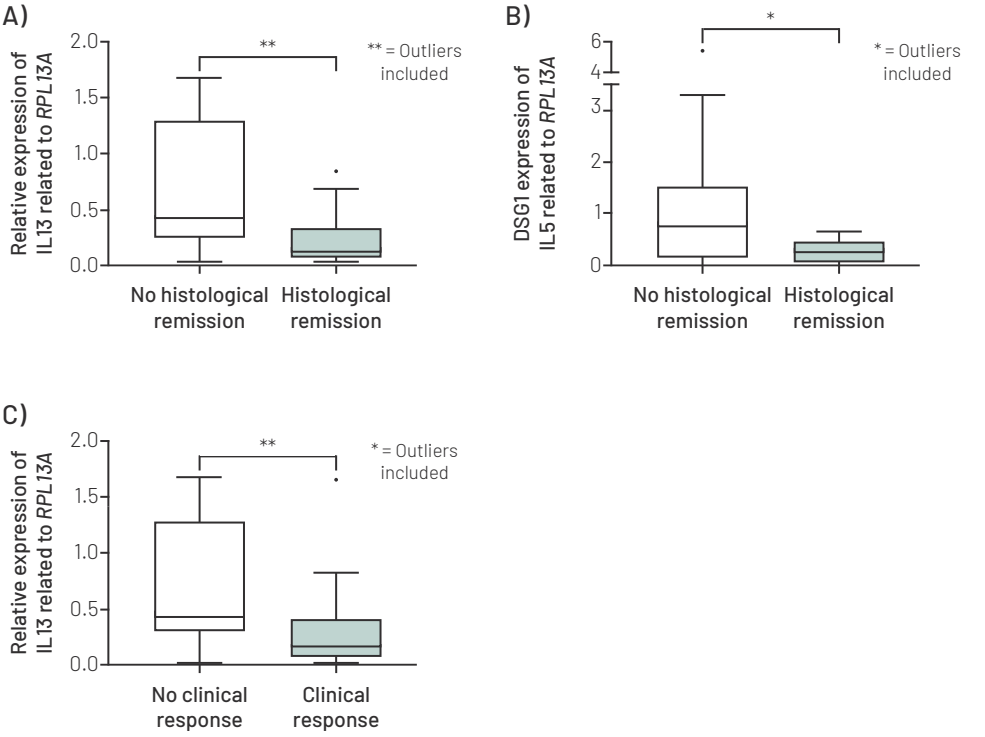


FIGURE 3. | Relative expression of genes of interest in EoE patients (n = 40) achieving histological remission vs. no histological remission; A) Interleukin(IL)13, B) Interleukin(IL)5 and in EoE patients (n = 40) showing a clinical response vs. no clinical response; C) Interleukin(IL)13 after diet intervention.
EoE = Eosinophilic esophagitis, Histological remission = <15 eosinophils (eos) per high power field (hpf) after intervention at histological assessment.
Clinical response = reduction of ≥ 3 points of the Straumann Dysphagia Instrument (SDI) score at week 6 compared to baseline.
The statistical difference of gene expression levels after treatment between clinical subgroups was calculated by using a t-test or Mann-Whitney U test, as appropriate.
* P-value (two-sided) of < 0.05, indicating a significant outcome.
** P-value (two-sided) of < 0.01.
*** P-value (two-sided) of < 0.001.
**** P-value (two-sided) of < 0.0001.

SUBGROUP ANALYSIS: TREATMENT EFFECT OF AAF ADDED TO A FFED ON GENE EXPRESSION

Subsequently, the patients being treated for 6 weeks with FFED (n = 20) were compared with those treated with FFED + AAF (n = 20) for gene expression in esophageal biopsy specimens. At baseline, inter-group comparison between patients treated with FFED or FFED + AAF showed no significant differences for transcripts of all 10 genes of our EoE-panel (all; $p > 0.05$)(**Figure 4A-1J**). The relative change in gene expression of DSG-1 in FFED + AAF treated patients from baseline to after treatment was significantly higher compared to the relative change in FFED treated patients after treatment ($p = 0.04$) (**Figure 4A**). Also the relative gene expression of CPA-3 in FFED + AAF treated patients was significantly more downregulated compared to FFED treated patients after treatment ($p = 0.003$) (**Figure 4C**). The relative change in expression levels from baseline to week 6 for the other 8 genes of the EoE-panel was similar between both groups (all; $p > 0.05$) (**Figure 4B, 4D-J**).

Within group comparison showed a significant upregulation of mRNA expression levels of DSG-1 from baseline to week 6 in patients treated with FFED + AAF ($p = 0.001$)(**Figure 4A**). In addition, a significant reduction of transcripts for CAPN-14, DSG-1, CPA-3, CCL-26, IL-5, IL-13, IL-10, TSLP, POSTN and TGF-β was observed after treatment with FFED + AAF (all; $p < 0.05$)(**Figure 4B-J**). Moreover, comparison from baseline to after treatment in patients treated with FFED alone showed significantly decreased mRNA expression levels of CAPN-14, CCL-26, IL-13 and IL-10 after 6 weeks (all; $p < 0.05$)(**Figure 4B, 4D, 4F, 4G**), whereas no differences in transcripts of DSG-1, CPA-3, IL-5, TSLP, POSTN and TGF-β were observed after treatment (all; $p > 0.05$) (**Figure 4A-C, 4E, 4G, 4H-J**).

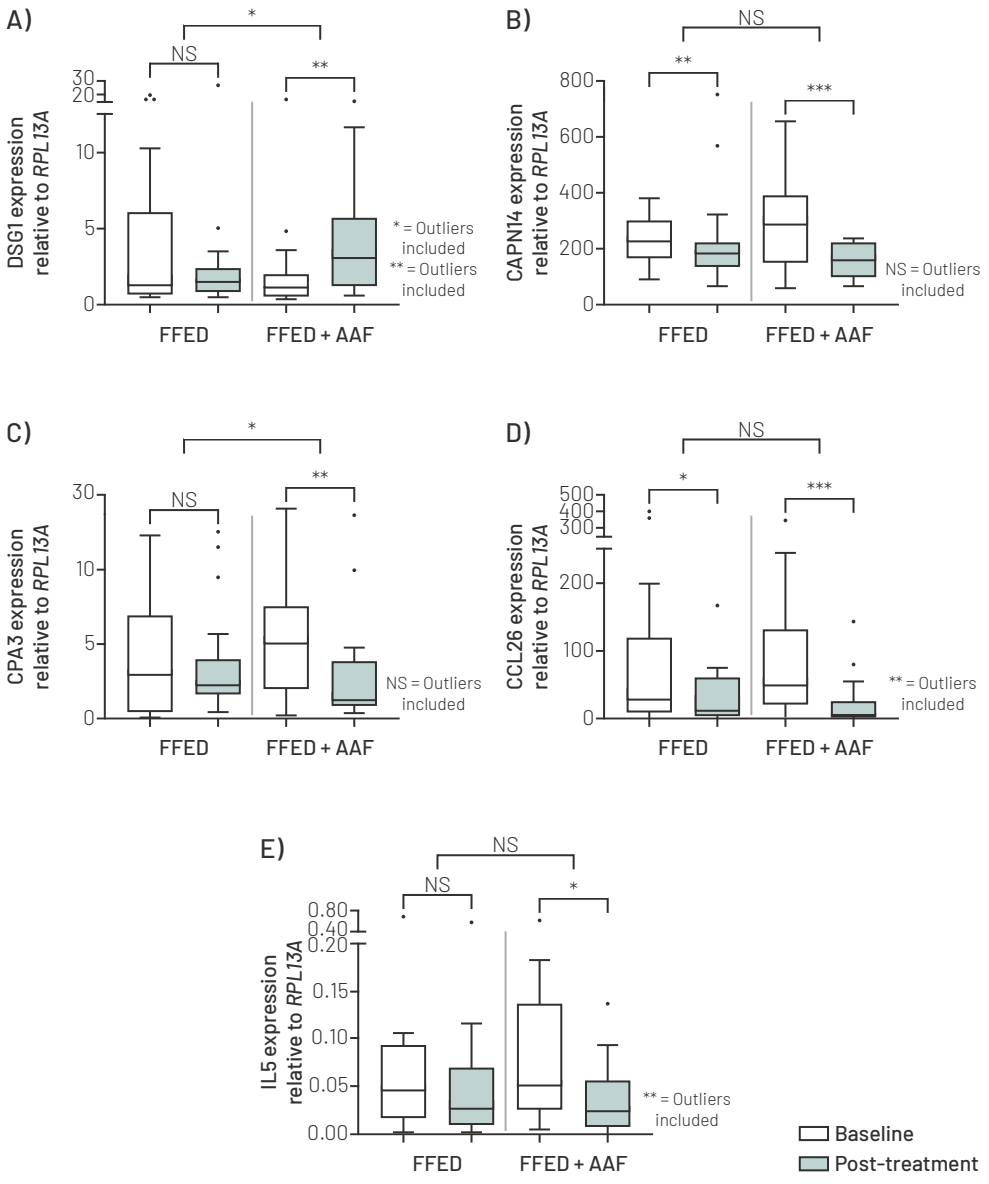


FIGURE 4. | Intra-group comparison of the expression of genes encoding for; A) Desmoglein (DSG)1 B) Calpain (CAPN)14 C) Carboxypeptidase(CP)A3 D) Chemokine-ligand (CCL)26 E) Interleukin (IL)5.
EoE = Eosinophilic esophagitis, FFED = Four Food Elimination Diet, FFED + AAF = Four Food Elimination Diet with addition of amino acid-based formula, NS = Non significant outcome.
The statistical difference between gene expression levels from baseline vs. post-treatment within subgroups was calculated by means of Wilcoxon Signed Rank test. Inter-group comparison of the relative mRNA expression levels of the 10 genes of the EoE-panel from baseline to 6 weeks between FFED and FFED + AAF was calculated by using a t-test or Mann-Whitney U test, as appropriate.

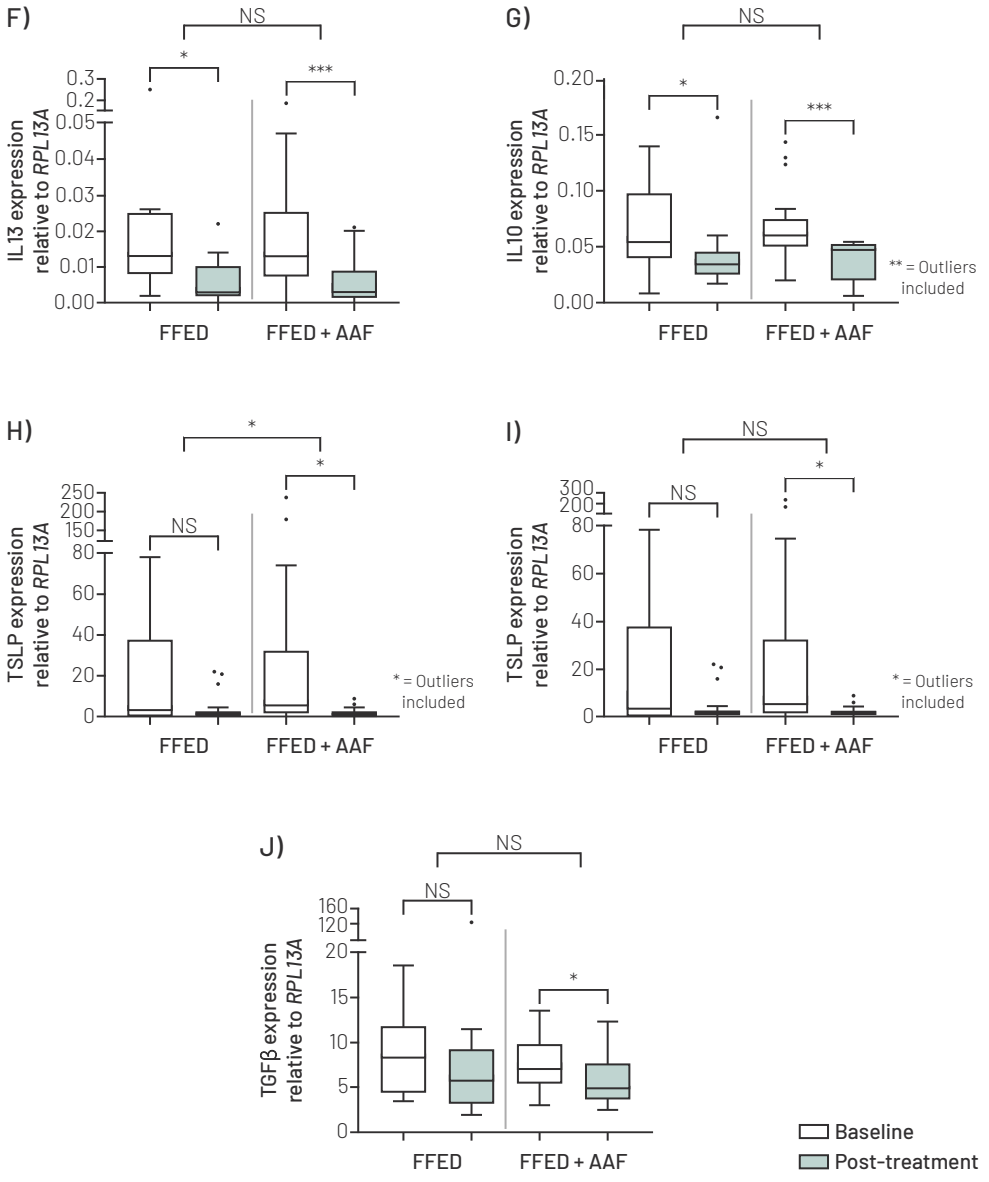


FIGURE 4 continued. | Intra-group comparison of the expression of genes encoding for; F) Interleukin (IL)13. G) Interleuking (IL)10. H) Thymic stromal lymphopoietin (TSLP) I) Periostin and J) Transforming growth factor (TGF)β pre- and post-treatment. In addition, inter-group comparison of the relative mRNA expression levels of the 10 genes of the EoE-panel from baseline to 6 weeks is presented.

* P-value (two-sided) of < 0.05, indicating a significant outcome.

** P-value (two-sided) of < 0.01.

*** P-value (two-sided) of < 0.001.

**** P-value (two-sided) of < 0.0001.

DISCUSSION

This is the first prospective study evaluating the effect of (2 types of) dietary treatment on the changes of 10 indicated gene expression markers related to disease activity and clinical outcomes (eosinophils, symptoms and endoscopic signs) in adult EoE patients. Our study shows a broad transcriptional response on the esophageal epithelium, targeting multiple key pathways that are leading to this common disease state. We observed that transcript levels of proteins associated with epithelial/barrier function, such as DSG-1 were significantly upregulated after 6 weeks of dietary treatment. Moreover, transcripts of multiple pro-inflammatory (IL-5, IL-13, TSLP, POSTN CPA-3, CCL-26), the pleiotropic cytokine IL-10 as well as markers related to epithelial/barrier function (CAPN-14) and remodeling/fibrosis (TGF-β) were significantly downregulated after treatment.

Given the paucity of data in the literature on the effect of dietary treatment on gene expression profiles in EoE, our findings are not directly comparable to previous studies. Warner's *et al*. reported a similar significant reduction of mRNA expression levels of Th2 cytokines (IL-5, IL-13) and pro-inflammatory mediators such as TSLP and POSTN in adult EoE patients after 4-weeks of an exclusively elemental diet.[24,25]

Additionally, in our study, significant lower transcript levels of Th2 cytokines (IL-5 and IL-13) were seen in patients achieving histological remission (i.e., < 15 *eos/hpf*) compared to those with no histological remission after 6 weeks of diet. Moreover, gene expression levels of IL-5, IL-13, CCL-26 and CPA-3 after the diet showed positive correlations with peak eosinophil counts. These effects of the dietary treatment are in line with previously reported elements of EoE pathogenesis.[6-12,26] The major effector cytokine IL-13 stimulates epithelial production of eotaxin-3 (CCL-26), a potent chemoattractant for eosinophils and basophils and promotes tissue eosinophilia.[27-30] In addition to this, IL-5 is secreted by Th2 cells, eosinophils and mast cells and promotes eosinophil activation and trafficking to the esophagus.[10,27,31] Both trials with anti-IL-5 and anti-IL-13 treatment in pediatric and adult EoE have demonstrated a reduction of esophageal eosinophilia.[32-36] Additionally, CPA-3 showed also a significant positive correlation between the absolute change in peak eosinophil counts at week 6 and the relative gene expression of CPA-3 after the diet. As such, a direct relationship between the density of eosinophils and mast cell markers (CPA-3) has been demonstrated both in our study and in previous reports.[8,37] These findings further support an important role for mast cell activation in EoE pathogenesis and in the mechanism of dietary treatment in reversing mast cell activity. Moreover,

IL-13 also induces tissue remodeling (e.g., promoting collagen deposition), which leads to esophageal rigidity and fibrostenosis, resulting in clinical symptoms of dysphagia and food impactions.[38-40] This working mechanism may support our finding that IL-13 is expressed in significantly lower levels in patients with a clinical response after 6 weeks of elimination diet.

A significantly higher expression level of CAPN-14 and lower levels of DSG-1 was observed in patients with a 'fibrotic' phenotype (vs. 'non-fibrotic' phenotype) at baseline. Some data in literature provide additional context for our findings. Increased expression of CAPN-14 is induced by IL-13, which leads to disruptive effects on the esophageal epithelium by impairment of barrier integrity in association with loss of DSG-1 expression.[5,41-43] A retrospective study by Lyle *et al*. recently suggested CAPN-14 genetic variants being associated with earlier disease onset in pediatric EoE.[44] In addition to this, longstanding eosinophilic inflammation is associated with esophageal remodeling and stricture formation.[45] CAPN-14 was found to be dynamically upregulated as a function of disease activity in previous studies.[46] Our findings of CAPN-14 being significantly more upregulated in 'fibrotic' phenotypes, suggests that CAPN-14 may be linked to EoE patients with a more severe disease phenotype. In general, TGB-β signaling pathway is considered as the central mediator of fibrosis in EoE.[9,47] Although visual changes of the esophagus may be seen on endoscopy as rings and strictures, identification of sub epithelial fibrosis requires deep esophageal biopsies. This may be an explanation for the absence of a significant difference between transcripts of TGB-β in these phenotypes.

Furthermore, only IL-10 (an anti-inflammatory cytokine) was expressed in significantly higher levels in patients presenting with a 'food impaction' phenotype compared to the 'non-food impaction' phenotypes prior to treatment. However, the reason for this remains unclear. Although data remains scarce on the role of IL-10 in EoE, higher levels of IL-10 expression between EoE and controls have been observed in a pediatric sample.[13] Since gene expression of IL-10 was significantly downregulated after the diet, the role of this anti-inflammatory cytokine may thus be related to an immunoregulatory response instead. In a pediatric EoE study by Rosenberg *et al.*, it was observed that esophageal Immunoglobulin (Ig)G4 levels correlated with eosinophils and levels of IL-10.[48] Excess pro-inflammatory Th2 responses, as seen in clinical settings involving chronic antigen exposure (e.g., beekeepers) are known to induce regulatory T cells, which secrete high levels of IL-10, inducing class switching to IgG4. It has been suggested in previous literature that IgG4 production may be a compensatory mechanism to dampen the ongoing Th2

inflammatory response in EoE. Thus, our observations on IL-10 being significantly downregulated after the diet may be related to a reduction of food antigen exposure in the esophagus and a reduction of Th2 activation.

A few limitations of this study need to be acknowledged. First, this was a single center study of a small sample of adults only, so it is difficult to compare results directly to prior gene expression studies that have been primarily performed in pediatric EoE populations. In addition, the small sample size is limiting its statistical power. Secondly, we did not include healthy individuals without EoE. We were therefore not able to assess whether expression levels normalized after diet treatment. Third, the gene expression analysis was limited to 10 selected genes, so it is possible that additional differences might be observed after broader RNA sequencing. However, there are also multiple strengths that lend validity to the results. This is the first study evaluating the effect of an elimination diet on the expression levels of pro-inflammatory and epithelial/barrier function related genes that were previously suggested to play an important role in EoE pathogenesis. Moreover, specimens were handled and stored uniformly, and extensive prospectively collected clinical data were available to allow full clinical, endoscopic, and histologic characterization of all EoE patients. Another strength is the use of different clinical outcome measures (i.e., symptoms, endoscopic), and avoidance of observer bias by our blinded endoscopic scoring strategy.

In summary, this study suggests that elimination diets, in addition to a clinical and histological response, are associated with a broad transcriptional response at the level of the esophageal epithelium in EoE patients. Multiple pathways that are leading to this common disease state are affected after dietary treatment, with significant changes of gene expression markers related to inflammation (IL-5, IL-13, TSLP, POSTN CPA-3, CCL-26 and IL-10), epithelial/barrier function (DSG-1, CAPN-14) and remodeling/fibrosis (TGF-β). In particular, upregulation of CAPN-14 and lower levels of DSG-1 were associated with 'fibrotic' phenotypes, whereas upregulation of IL-10 was linked to 'food impaction' phenotypes. These results provide initial insight into genetic determinants of different presentations of EoE and provide a foundation for future mechanistic studies.

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CHAPTER 7

MENTAL DISTRESS AMONG ADULT PATIENTS WITH EOSINOPHILIC ESOPHAGITIS

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ABSTRACT

RATIONALE

Data on the prevalence of mental distress among adult eosinophilic esophagitis (EoE) patients are scarce. Also, a significant gap remains in the understanding of which determinants are related to significant psychological symptoms and whether distressed patients require and receive mental care.

METHODS

Adult EoE patients were invited to complete standardized measures on anxiety/depressive symptoms (HADS) and general psychopathology (SCL-90-R). All scores were compared to general population norms. Socio-demographic and clinical factors were assessed.

RESULTS

In total, 147 adult EoE patients (61% males, age 43 (IQR 29 - 52) years were included (response rate 71%). No difference with general population values was found for total anxiety and depressive symptoms (7.8 ± 6.6 vs. 8.4 ± 6.3; p = 0.31). A total of 38/147 (26%) patients reported high levels of anxiety and/or depressive symptoms (HADS-A ≥ 8: 35/147 (24%) and HADS-D≥ 8: 14/147 (10%)), indicative of a possible psychiatric disorder. In a multivariate analysis, age between 18 - 35 years was independently associated with high levels of anxiety (HADS-A ≥ 8) (OR 3.0 | 95% CI 1.3 -6.9; p = 0.01). The SCL-90-R Global Severity Index (GSI) was significantly higher compared to the general population (p < 0.001). Significant signs of general mental distress (GSI≥ 80th percentile) were observed in 51 (36%) EoE patients, of which 29 (57%) patients denied having any mental problems and only 8 (16%) patients received mental care.

CONCLUSION

A considerable proportion of adult EoE patients suffers from mental distress, with a 3-fold risk of significant anxiety in those patients younger than 35-years. Therefore, population-based studies are required and a pro-active approach in the screening for and treatment of these psychological symptoms in EoE practice seems essential.

INTRODUCTION

EoE is a chronic immune-mediated disorder of the esophagus triggered by food allergens, with an Worldwide increasing prevalence with rates almost comparable to inflammatory bowel disease (IBD).[1,2] EoE is characterized by mucosal eosinophilic infiltration and subsequent esophageal dysfunction, which manifests in symptoms of dysphagia for solid foods and food impaction.[3] EoE affects all ages (3:1 male-to-female ratio), with a peak incidence between the ages of 20 and 40 years.[4] At present, the management of EoE involves targeting the esophageal eosinophilic inflammation with drugs or elimination of food allergens. EoE is associated with a substantial disease burden that affects patients' health-related quality-of-life (HRQOL), healthcare systems and society in general.[5] Multiple aspects such as, disturbing symptoms of dysphagia and food impaction as well as the need for life-long treatment are associated with impaired HRQOL.[6-8] A recent medical record review observed a prevalence of psychiatric health comorbidities in almost one-third of EoE patients, in which older age, female gender and longer symptom duration were found to be associated with the presence of a mental health disorder.[9] Current research has mainly focused on increased risk of developing anxiety and depressive symptoms, measured within the construct of disease specific HRQOL (EoE-QOL-A).[8,10,11] This validated measure consists of 5 domains that evaluates important disease related topics (e.g., issues related to having a chronic disease or swallowing anxiety) and has been widely used in the EoE-research field.[5,11,12] Still a significant gap remains in our understanding of the impact on mental health and its determinants in this chronic disease as well as if distressed EoE patients receive mental treatment. Notwithstanding, insufficient treatment of psychiatric comorbidities in patients with a chronic physical illness (e.g., IBD and rheumatoid arthritis) has been associated with more severe symptoms and disease flares, therapeutic non-adherence and subsequent increased health-care costs.[13-15] However, provision of sufficient mental care in adult EoE patients first requires more insights into the presence of mental distress and its determinants (e.g., clinical and demographic factors). Therefore, we aimed to evaluate in this study: i) The presence of mental distress among adult EoE patients ii) the degree to which clinical and socio-demographic factors are related to significant levels of mental distress and iii) if EoE patients with severe symptoms of general mental distress receive mental care.

METHODS

STUDY DESIGN AND POPULATION

An observational cross-sectional study design was used to assess mental distress among adult EoE patients. Consecutive patients from our EoE cohort (i.e., patients who attended the outpatient clinical of the Amsterdam UMC Motility Center between 2011 - 2020) were invited to participate in this study between July 2019 and February 2020 (i.e., recruitment period). An informed consent letter including self-reported questionnaires was sent to the EoE cohort and distributed at the outpatient clinic during this recruitment period. Patients with a documented diagnosis of EoE according to the consensus guidelines (i.e., ≥ 15 eosinophils per high-power-field), aged 18 and over, with a sufficient command of written Dutch to complete a self-reported survey were considered eligible for inclusion.[16] Once consented, all patients completed a paper or digital version of the questionnaires. All data was safely collected and stored by using the Electronic Data Capture Castor. A flowchart of patient inclusion and participation rate is presented in **Supplementary Figure 1**.

DATA COLLECTION

Socio-demographics and clinical outcomes

A self-designed (standard fixed choice) questionnaire was used to elicit details concerning socio-demographic and clinical information. Socio-demographic variables, such as: gender and education level (low: primary or secondary school and high: College or University) as well as specific information on the year of symptom onset and diagnosis of EoE, history of endoscopic interventions and previous dilations, EoE treatment (medical or dietary treatment) and concomitant atopic diseases were included. In addition, patients were asked if they felt to have current mental health problems and whether they received mental care. Clinical symptoms of dysphagia and food impaction (i.e., clinical disease activity) were evaluated by means of the Straumann Dysphagia Instrument (SDI).[17] Severe clinical disease activity was defined as current symptoms of daily dysphagia and food impaction.

STUDY QUESTIONNAIRES AND REFERENCE POPULATION

Anxiety and depression

Anxiety and depressive symptoms were measured with the standardized and validated Hospital Anxiety and Depression Scale (HADS). This 14-item self-assessment scale was developed to screen for depression and anxiety symptoms (recall period of 7-days). The HADS consists of 7 anxiety- and 7 depression-items, of which the total scores range from

0 (no complaints) to 21 (maximum complaints). A score of ≥ 8 on either subscale signifies a symptom severity indicative for a possible anxiety and/or depressive disorder.[18] Anxiety and depression symptom scores of all EoE patients were compared to a subgroup of 199 patients, which was derived from 3492 respondents of the general Dutch population.[19]

General mental distress

Symptoms of general mental distress were evaluated by means of the validated Symptom Checklist-90-Revised (SCL-90-R). This questionnaire consists of 90-items to assess for general self-reported psychological symptoms over the past 7 days. The SCL-90-R-items represent 8 domains, including agoraphobia, anxiety, depression, somatization, sensitivity, insufficiency of thinking and acting, hostility and sleep disturbance.[20] Each item is rated on a 5-point scale of distress, ranging from 1 (none) to 5 (extreme). The total SCL-90-R score (Global-Severity-Index (GSI)) is calculated by substitution of all subdomain scores and ranges from 0 to 450, with higher scores indicative for mental distress. SCL-90-R-scores of our EoE sample were compared to a reference cohort of 2368 respondents (norm group II) of the Dutch general population.[21] In addition, cut-off scores were used to identify patients with severe symptoms of general mental distress, indicated as GSI scores of 'above normal' and 'high' (corresponding to the 80th percentile of the norm group II), that are clinically relevant and may be indicative of a mental disorder.[21]

STATISTICAL ANALYSIS

Statistical analysis was performed by using IBM SPSS Statistics (version 25.0) (SPSS, Chicago, USA). Descriptive statistics was used to assess socio-demographic and clinical characteristics. Data are presented as mean (± Standard Deviation (SD)) or median (Inter-Quartile-Range (IQR)). To characterize our sample, levels of the validated Patient Reported Outcome (PRO) measures (HADS/SCL-90-R) were compared to previously published general population norms.[19,20] Independent sample t-tests were used to compare mean scores of the HADS and SCL-90-R in EoE patients to the general population norms. Univariate logistic regression analyses were performed to identify (clinically relevant) factors associated with high levels of anxiety (HADS-A ≥ 8). Demographic variables with a p-value of < 0.20 were subsequently entered into multivariate logistic regression analysis with backward selection. Associations between clinical disease activity (SDI-scores) and HADS-A and HADS-D as well as all subscales of the SCL-90-R were assessed by Pearson's or Spearman's rank correlations coefficients, as appropriate. A p-value of < 0.05 was considered to be statistically significant.

ETHICAL CONSIDERATIONS

This cross-sectional study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO). An exemption to seek formal approval was provided by the Medical Ethics Committee of the Amsterdam UMC at 25-03-2019 (W19_103#19.136). All participants provided informed consent before taking part and were given a unique study-ID to ensure anonymity.

RESULTS

PATIENT CHARACTERISTICS

In total, 147 adult EoE patients were included (61% males, median age 43 (IQR 29 - 52) years), representing a response rate of 71%. Atopic constitution was observed in 119 (81%) patients. The median disease duration in our cohort was 3 (IQR 1 - 6) years, with 49 (33%) patients diagnosed within the prior year. Diagnostic delay, measured as time interval between first reported EoE symptoms and year of diagnosis was 5 (IQR 2 - 14) years. In total, 21 (14%) patients had prior esophageal dilation and multiple endoscopic interventions with food bolus extraction were reported in 62 (42%) patients (**Table 1**).

ANXIETY AND DEPRESSIVE SYMPTOMS

Evaluation of anxiety and/or depressive symptoms (HADS) showed no difference in the total HADS score in our EoE sample compared to the general population (7.8 ± 6.6 vs. 8.4 ± 6.3; p = 0.31)(**Figure 1**). Anxiety (HADS-A) and depression (HADS-D) symptom scores in EoE patients were also both similar compared to the general population (HADS-A: 4.8 ± 4.2 vs. 5.1 ± 3.6; p = 0.47 and HADS-D: 3 ± 3 vs. 3.4 ± 3.3; p = 0.1), respectively (**Figure 1A**). Additionally, no differences were observed for the HADS-total, HADS-A and HADS-D mean scores in female EoE patients compared to the general population (all; $p > 0.05$). Moreover, male EoE patients showed significantly lower HADS-total, HADS-A and HADS-D scores compared to the general population (all; $p < 0.05$). In our EoE sample, significantly higher levels of the HADS-total score in females were observed compared to males (9.4 ± 7.9 vs. 6.8 ± 5.5; p = 0.02) (**Figure 1B**). Furthermore, significant higher levels of the HADS-A were detected in female EoE patients compared to males (6.1 ± 4.9 vs. 4.1 ± 3.6; p = 0.005), whereas HADS-D scores between male and female patients were similar (2.8 ± 2.5 vs. 3.4 ± 3.6; p = 0.226)(**Figure 1B**).

In our cohort, high levels of anxiety (HADS-A ≥ 8; indicative of an anxiety disorder) were observed in 35 (24%) patients, with no gender difference (male vs. female; p = 0.11). High levels of depression (HADS-D ≥ 8; indicative of a depressive disorder) were reported in

14 (10%) patients, whereas females were significantly more affected compared to males (6% vs.16%; p = 0.048). Furthermore, 14 (10%) patients had high levels of both anxiety and depression (HADS-A ≥ 8 and HADS-D≥ 8; indicative of both psychiatric disorders), of which the proportion of females was significantly higher (male 3% vs. female 14%; p = 0.023). Hence, a total of 38 (26%) patients (no difference between male vs. female; p = 0.123) scored high levels of anxiety and/or depressive symptoms; indicative of at least one of these psychiatric disorders.

TABLE 1. | Socio-demographic and clinical characteristics.

Socio-demographic and clinical characteristics N = 147	
Socio-demographic characteristics	**EoE, n (%) or median (IQR) (n = 147)**
Age, *years*	43 (29 - 52)
Gender, *male*	90 (61)
Level of education	
Low	49 (33)
High	98 (67)
In domestic partnership	
No	51 (35)
Yes	96 (65)
Clinical characteristics	
Atopic diatheses	119 (81)
Current clinical disease activity	
Dysphagia	97 (66)
Food impaction	41 (28)
Multiple endoscopic interventions with food bolus extraction	62 (42)
Diagnostic delay *, *years*	5 (2 - 14)
Disease duration, measured from year of diagnosis, years	3 (1 - 6)
Age at symptom onset, *years*	27 (19 - 38)
Previous dilation	21 (14)
Current treatment	
Topical steroids	35 (24)
Dietary restrictions	35 (24)
Topical steroids with additional dietary restrictions	15 (10)
PPIs	34 (23)
No treatment	28 (19)

IQR = Inter Quartile Range.
PPIs = Proton Pump Inhibitors.
* Diagnostic delay is the time interval between the first symptoms and the diagnosis.

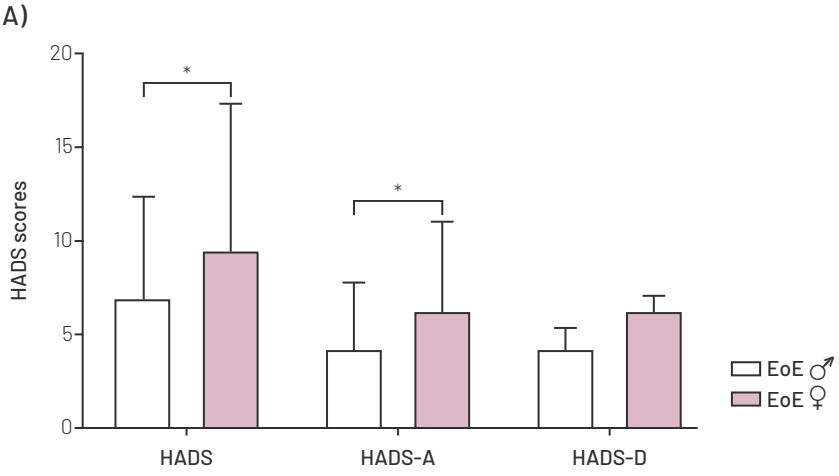

FIGURE 1A. | Anxiety and depressive symptoms (HADS) of eosinophilic esophagitis (EoE) patients vs. the general population.
HADS = Hospital Anxiety and Depression Scale, HADS-D = HADS Depression, HADS-A = HADS Anxiety .
* P-value of < 0.05, indicating a significant outcome.

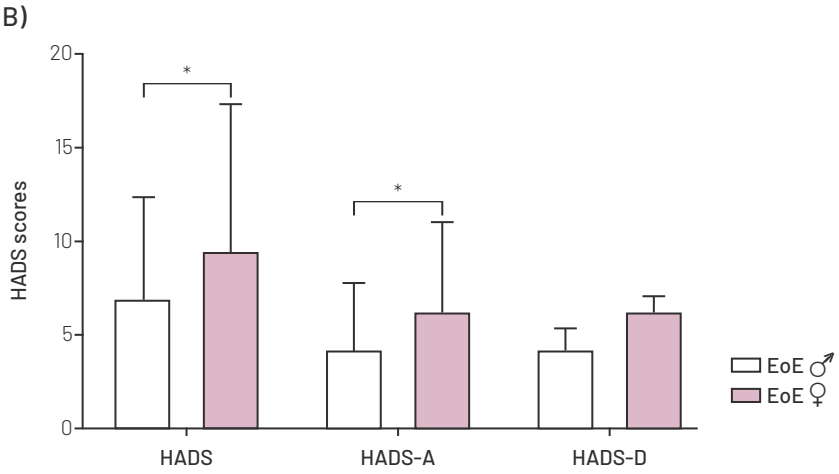

FIGURE 1B. | Anxiety and depressive symptoms (HADS) of male vs. female eosinophilic esophagitis (EoE) patients.
HADS = Hospital Anxiety and Depression Scale, HADS-D = HADS Depression, HADS-A = HADS Anxiety
* P-value of < 0.05, indicating a significant outcome.

ASSOCIATED FACTORS WITH HIGH LEVELS OF ANXIETY

Presence of high levels of anxiety (HADS-A ≥ 8; indicative of an anxiety disorder) was significantly more prevalent in young patients aged between 18 - 35 years (41%). Univariate analysis signified a possible trend between high levels of anxiety and younger age (18 - 35 years), female gender, not being in domestic partnership, current symptoms of daily dysphagia and food impaction (severe clinical disease activity) and a short disease duration (≤2 years). However, after multivariate logistic regression analysis, age between 18 - 35 years was the only independent factor associated with high levels of anxiety (Odds Ratio (OR) 3.0 | 95% Confidence Interval (CI) 1.3 - 6.9; p = 0.01 (**Table 2**).

TABLE 2. | Determinant factors associated with high levels of anxiety.

EoE patients N = 147		**Univariate analysis**			**Multivariate analysis**		
High levels of anxiety N = 35	**HADS-A ≥ 8, *n* (%)**	**OR**	**CI (95 %)**	**P-value**	**OR**	**CI (95 %)**	**P-value**
Demographic variables							
Female gender	18 (32)	1.982	0.919 - 4.274	0.081*	NS		NS
Age, *years*							
18 - 35	21 (41)	3.123	1.375 - 7.092	0.007**	2.999	1.307 - 6.881	0.01**
36 - 55	13 (18)	Ref.					
> 55	1/25 (4)						
In domestic partnership	19 (20)	0.540	0.248 - 1.173	0.119*			NS
Severe clinical disease activity	6 (46)	3.143	0.763 - 12.945	0.113*			NS
Short disease duration (≤ 2 years)	19 (31)	1.906	0.886 - 4.100	0.099**			NS

OR = Odds Ratio, CI (95 %) = 95 % Confidence interval, NS = Not significant, EoE = Eosinophilic esophagitis, P = percentile
Severe clinical disease activity = currently experiencing symptoms of daily dysphagia with foodimpaction.
* P-value < 0.2, indicating a possible trend.
** P-value of < 0.05, indicating a significant outcome.
Disease duration, measured from year of diagnosis.

GENERAL MENTAL DISTRESS

The general psychopathological profile of EoE patients was evaluated by means of self-reported symptoms of general mental distress (SCL-90-R), showing significantly higher levels of the GSI compared to the general population (135 ± 47.3 vs. 118.3 ± 32.3; $p < 0.001$). In addition, levels of the symptom subscales; depression, somatization, insufficiency of thinking and acting, hostility and sleep disturbance as well as anxiety and sensitivity in EoE patients were all significantly higher compared to the general population ($p < 0.001$ and $p < 0.05$), respectively (**Figure 2A**). In addition, GSI levels of both male and female EoE patients were significantly higher compared to the general population (males: 131.7 ± 44.9 vs. 118.3 ± 32.3; $p = 0.005$ and females: 140.5 ± 51 vs. 118.3 ± 32.3; $p = 0.002$), respectively. The subscales; anxiety, depression, somatization and insufficiency of thinking and acting were significantly higher in both male and female EoE patients compared to the general population (all; $p < 0.05$).

In our EoE cohort, female patients showed significantly higher levels of the GSI compared to male patients (127.3 ± 42 vs. 147.8 ± 52; $p = 0.017$) as well as the subscales; anxiety, depression, somatization and insufficiency of thinking and acting (male vs. female; $p < 0.05$) (**Figure 2B**).

Severe symptoms of general mental distress, indicated as GSI scores of 'above normal' and 'high' (corresponding to the 80th percentile of the norm group II), were observed in 51 (36%) EoE patients, of which the proportion of females was significantly higher than males (46% vs. 29%; $p = 0.048$). Evaluation of the symptom subscales for general mental distress (SCL-90-R) showed a significantly higher proportion of females with severe symptoms of depression (SCL-90-depression ≥ 80th percentile) and somatization (SCL-90-somatization ≥ 80th percentile) (male vs. female; $p = 0.029$ and $p = 0.001$), respectively. The percentages of patients in our EoE population exceeding the norm scores indicated as 'above normal' and 'high' (≥ 80th percentile) in all dimensions of the SCL-90-R are presented in **Figure 3**. In total, 22 (43%) patients with severe symptoms of general mental distress (GSI ≥ 80th percentile) reported to have current mental problems, of which only 8 (36%) patients received mental care and psychotropic medication (e.g., antidepressants or anxiolytics) was used in 7 (14%) patients. Fifteen (29%) patients with severe symptoms of general mental distress felt their mental problems were related to EoE. Of note, 29 (57%) patients with GSI scores exceeding the norm scores (≥ 80th percentile) denied having any mental problems.

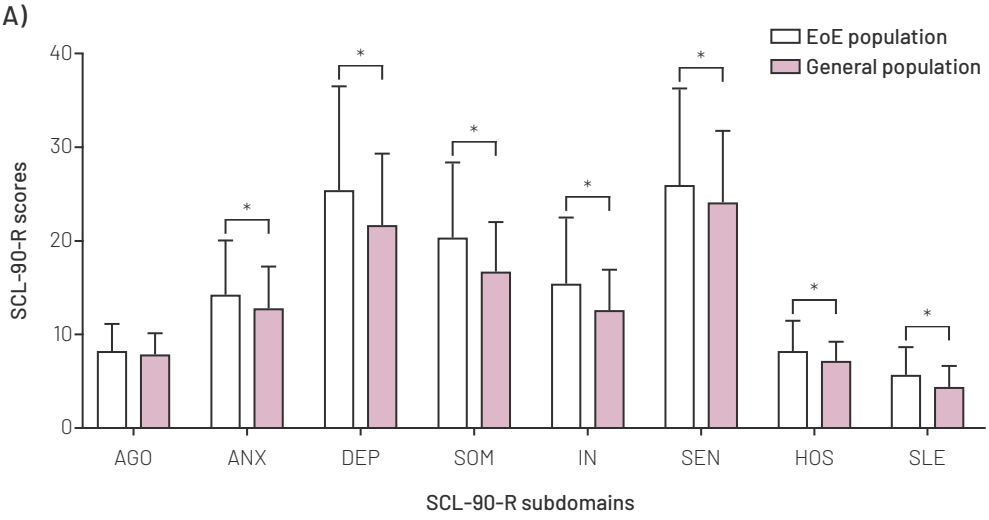

FIGURE 2A. | Mean scores on the subscales of the Symptom Checklist 90-Revised (SCL-90-R) of patients with eosinophilic esophagitis (EoE) vs. the general population.
AGO = Agoraphobia; ANX = Anxiety; DEP = Depression; SOM = Somatic Symptoms; IN = Inadequacy of Thinking and Acting; SEN = Distrust and Interpersonal Sensitivity; HOS = Hostility; and SLE = Sleeping.
* P-value of < 0.05, indicating a significant outcome.

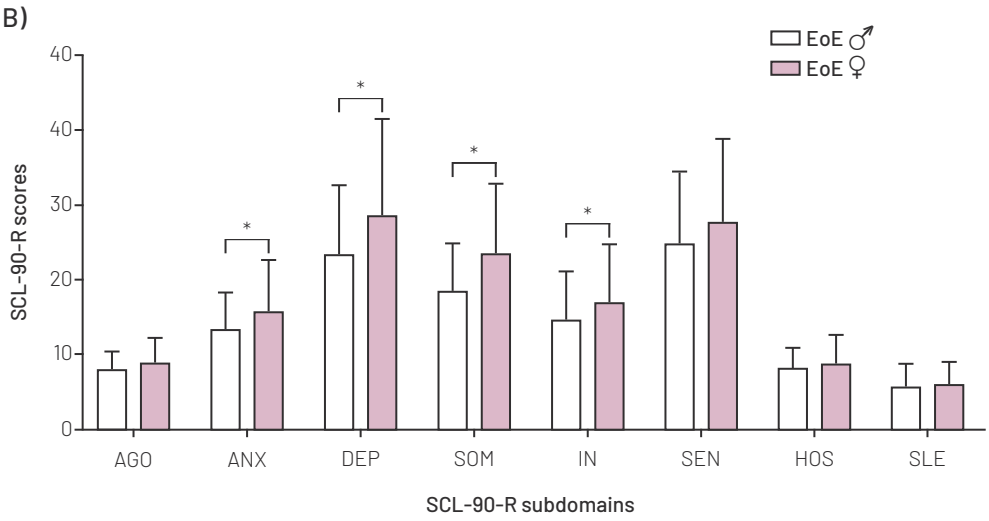

FIGURE 2B. | Mean scores on the subscales of the Symptom Checklist 90-Revised (SCL-90-R) of male vs. female patients with eosinophilic esophagitis (EoE).
AGO = Agoraphobia; ANX = Anxiety; DEP = Depression; SOM = Somatic Symptoms; IN = Inadequacy of Thinking and Acting; SEN = Distrust and Interpersonal Sensitivity; HOS = Hostility; and SLE = Sleeping.
* P-value of < 0.05, indicating a significant outcome.

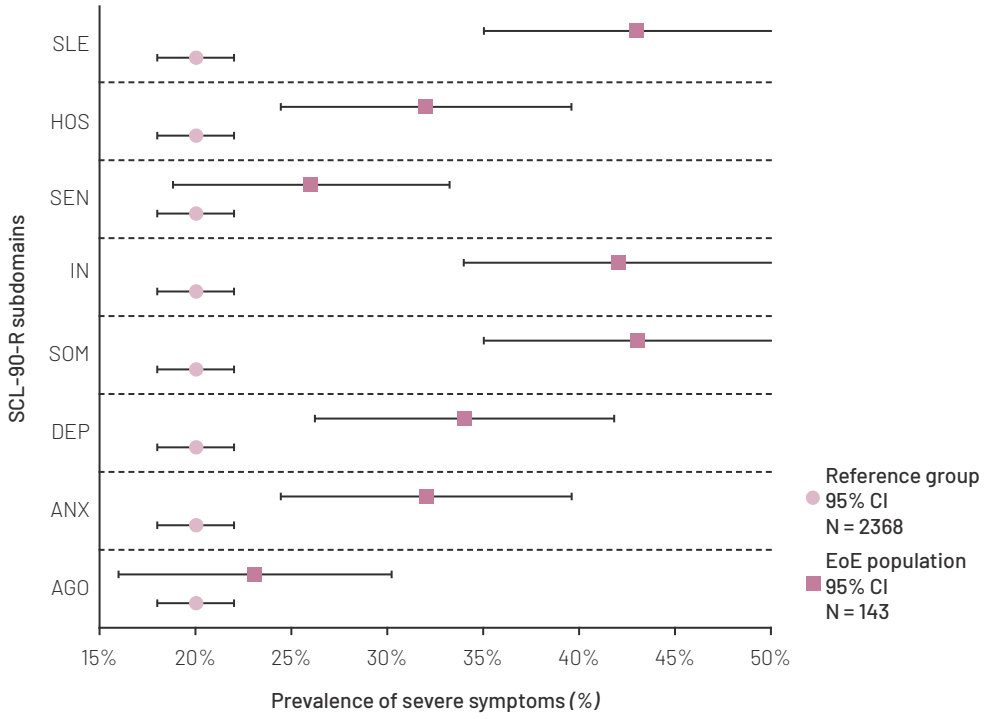

FIGURE 3. | Presence of severe symptoms on the subscales of the Symptom Checklist 90-Revised (SCL-90-R) in patients with eosinophilic esophagitis (EoE). Each dimension presents the percentage of EoE patients exceeding the norm scores indicated as 'above normal' and 'high' (≥80th percentile norm group II).[18]
AGO = Agoraphobia; ANX = Anxiety; DEP = Depression; SOM = Somatic Symptoms; IN = Inadequacy of Thinking and Acting; SEN = Distrust and Interpersonal Sensitivity; HOS = Hostility; and SLE = Sleeping.

ASSOCIATIONS BETWEEN CLINICAL DISEASE ACTIVITY AND SYMPTOMS OF MENTAL DISTRESS

Ninety-seven (66%) patients reported current symptoms of dysphagia and 41 (28%) food impaction, of which 33 (81%) stated to have multiple episodes a week. Comparison of self-reported clinical disease severity and HADS-scores, showed a significant positive correlation between the total SDI-scores and both the HADS-A ($r = 0.27$; $p = 0.001$) and HADS-D ($r = 0.19$; $p = 0.023$) scores (**Supplementary Table 1**). Additionally, SCL-90-R subscales; agoraphobia, anxiety, depression, somatization, sensitivity, insufficiency of thinking and acting, hostility and sleep disturbance all showed a significant positive correlation with the total SDI-score (all; $p < 0.05$). (**Supplementary Table 1**)

DISCUSSION

EoE is known to have impact on HRQOL of patients who suffer from the disease, although current literature is scarce on the understanding of mental health comorbidities in adult EoE patients. In this cross-sectional study, we observed a substantial presence of significant symptoms of mental distress among adult EoE patients. Although mean levels of anxiety and depression in our sample were not higher compared to the general population, relevant signs of anxiety (HADS-A ≥ 8; indicative of an anxiety disorder were seen in 24% patients. Moreover, high levels of depression (HADS-D≥ 8) were noted in 14 (10%) patients. These observed rates are comparable to a study of Lucendo *et al.*, in adult EoE patients, reporting significant signs of anxiety and depression in 31% and 10%, respectively.[10]

Furthermore, a remarkable finding in our study was the significant 3-fold risk for the presence of high levels of anxiety (HADS-A ≥ 8) in EoE patients between the ages of 18 and 35 years. The general onset of anxiety disorders usually occurs in childhood/adolescence, until they reach a peak in middle age, with tendency to decrease with older age.[22] With regards to EoE, a pediatric study suggested anxiety symptoms to increase with age, including rates of 9.3% in children (< 11 years) and 19% in adolescents (11 - 17 years).[23] In our EoE sample, 41% of young adults (18 - 35 years) and 18% of the middle aged (36 - 55 years) patients presented with significant signs of anxiety. Compared to prevalence rates of anxiety (HADS-A ≥ 8) in a general German population, which ranges from 14.4% -19.8% (< 40 years) and 19.8% - 25% (41 - 60 years),[24] it is certain that young adults diagnosed with EoE are more at risk for the development of significant signs of anxiety.

Overall, females showed significantly higher levels of mental distress compared to males in our EoE sample. This finding is consistent with previous literature reports on female predominance of common mental disorders in the general population.[25,26] For that reason, it seems notable that the proportion of males and females with significant signs of anxiety on both PRO measures (HADS-A ≥ 8 and SCL-90-anxiety ≥ 80th percentile) were equally distributed in our EoE sample. Since men are more prone of stricture development with consecutive risk of increased symptom severity,[27] one could argue that male EoE patients are more exposed to potential anxiety triggers such as impaction with need for upper endoscopy and food bolus dislodgement. This is supported by previous findings on the serious impact of dysphagia and food impaction on patients' fear, as well as identification of increased symptom severity as predictor of both disease and chocking anxiety.[8,11]

Although severe clinical disease activity was not independently associated with high levels of anxiety in our multivariate analysis, SDI scores significantly correlated with scores of the HADS-A and SCL-90-anxiety (**Supplementary Table 1**).

Compared to the general population, a greater severity of mental distress in EoE patients was observed, with a substantial proportion of patients (36%) with severe symptom levels (GSI ≥ 80th percentile) in our sample. Nevertheless, these results should be interpreted with caution, since the SCL-90-R is not corrected for somatic disorders.[20] In addition, the HADS-anxiety and depression scores were not higher compared to the general population, whereas the SCL-90-R-subscales anxiety and depression were significantly higher in EoE patients. Although a clear explanation is lacking, this inequality might be the result of the HADS being corrected for the presence of physical illness.[28] Also a more extensive screening as result of a higher number of items included in the SCL-90-R, in particular in the domain depression, might also be suggested as an explanation for this contrasting finding. Moreover, considering somatization (i.e., SCL-90-somatization) to be the most intense symptom in our sample, it could be argued that the presence of physical illness resulted in an overestimation of the GSI score (**Figure 3A** and **Figure 4**). However, only the questions 'pain in the chest or heart' (item 12) and 'having a lump in the throat' (item 53) fits with EoE related symptoms, suggesting these patients actually may experience somatic symptoms (e.g., difficulty to breath or dizziness) in response to their psychological distress. Moreover, the presence of EoE related symptoms (SDI-scores) significantly correlated with SCL-90-somatization levels ($r = 0.4$; $p < 0.001$), even if corrected for EoE-related symptoms by exclusion of SCL-90-items 12 and 53 (**Supplementary Table 1**). Generally, there is a moderate association between symptoms and biological disease activity (esophageal inflammation) in non-dilated EoE patients.[29,30]

We hypothesize that somatization of esophageal symptoms (e.g., dysphagia) in severe distressed EoE patients may help to explain additional variation in symptom severity, once variation in biological disease activity has already been taken into consideration. In IBD-patients, association between somatization and clinically active disease with absence of mucosal inflammation, was suggested to be secondary to somatoform-type behavior or a coexisting functional disease instead of being related to biological disease activity (i.e., mucosal inflammation or extra intestinal manifestations of IBD).[31] The concept of this so-called somatoform-type behavior might also play a role in EoE; the absence of histological data in our cohort did not allow us to further address this hypothesis.

Despite their clinical and public health importance, the presence of psychological disorders is often underdiagnosed and undertreated, in particular when coexisting with physical illness.[32] Significant signs of general mental distress (GSI ≥ 80th percentile) were observed in 51 (36%) EoE patients, of which 29 (57%) patients denied having any mental problems. Also, only 8 (16%) of these patients received mental care of which 7 (14%) patients reported current psychotropic medication use. Therefore, routine screening by gastroenterologists for symptoms of anxiety and depression in adult EoE patients through the mental health subscale of the Short Form (SF)-36 or Patient Health Questionnaire (PHQ)-4 could be suggested for clinical practice.[33-35] As such, several drivers of disease related anxiety, such as: symptom severity and need for long-term food restrictions have been indicated to be legitimate concerns for care givers in pediatric EoE. Significant impacts on eating and food-specific anxieties emerging into a newly classified eating disorder; Avoidant/ Restrictive Food Intake Disorder (ARFID) has already been observed in pediatric EoE and other digestive diseases.[36,37] ARFID is characterized by extreme restrictive eating behaviors (i.e., disturbed feeding patterns, highly selective eating habits) and awareness on the presence of this specific mental disorder in adult EoE patients should also be increased.

Based on our results it remains unclear whether distressed EoE patients' felt they received the mental care they need. The World Health Organization (WHO) studied the consultation process for mental health reasons, in which the preference for self-management (i.e., managing one's self) has been indicated as main barrier for not seeking mental treatment, even though need for mental care was perceived.[38-40] In addition, especially young and middle-aged patients are more likely to recognize need for treatment but experience more structural barriers to treatment seeking, such as: negative attitude towards help seeking, financial problems and time barriers.[41] Therefore, also a proactive approach towards (unmet) needs for mental care could be suggested for clinical practice.

Several limitations of our study merit attention. First, including patients from a tertiary center is known for limiting the generalizability of outcomes. However, as we included patients from our EoE cohort as well as new patients visiting the outpatient clinic, our study sample reflects a various population containing different stages of disease activity. Additionally, considering patients with mental disorders often face stigma, psychotropic medication use may have been underreported in our study. Nevertheless, these limitations are encountered by several strengths of our study design. To the best of our knowledge, this is the first cross-sectional study with specific interest of evaluating the presence

of mental distress among adult EoE patients and the extent to which clinical and socio-demographic factors are related. Considering the use of 2 validated PRO-measures (HADS/SCL-90-R), new insights are provided on the psychopathological profile of adult EoE patients. Another strength of our study lies in the large sample size of our cohort including EoE patients from various geographical areas in the Netherlands.

In conclusion, we observed a substantial presence of mental distress among adult EoE patients, with a compelling 3-fold risk of significant signs of anxiety during young adulthood (18 - 35 years). These findings are highlighting the need for future population-based studies on the prevalence of mental distress. Since EoE mostly affects young adults, screening for and treatment of mental health disorders should therefore become an integral part of the medical care of EoE patients.

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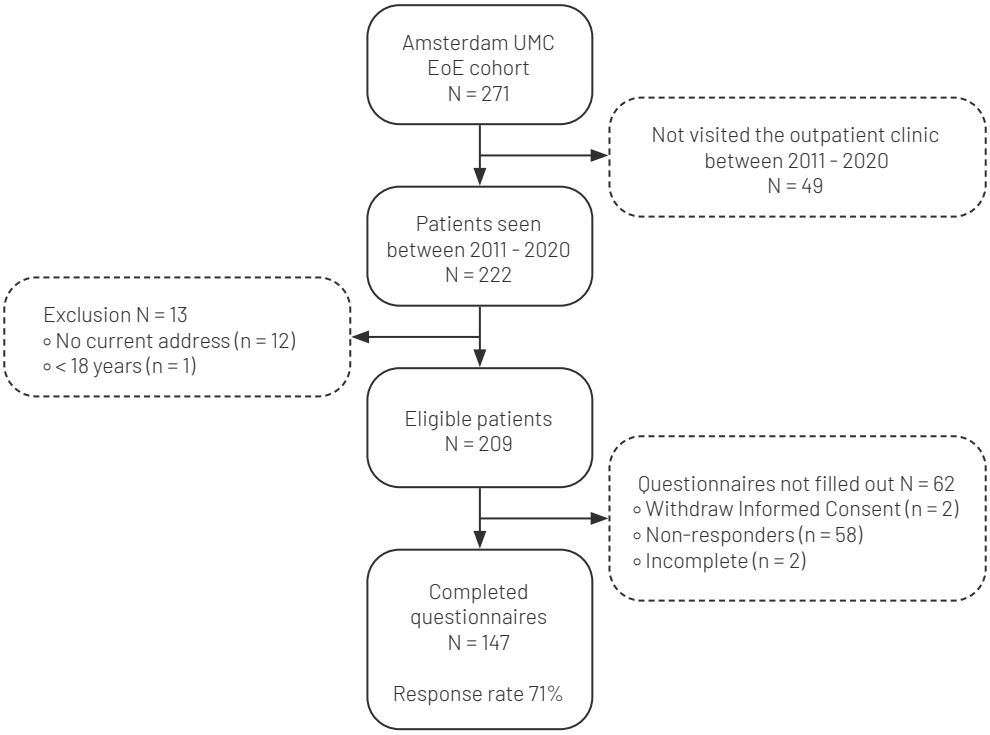
SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE 1. | Flowchart of patient's inclusion and response rate.

SUPPLEMENTARY TABLE 1. | Correlations between clinical disease activity and HADS and SCL-90-R in adult EoE patients.

	Clinical disease activity (SDI-score)	
Symptoms of mental distress	**r**	**P-value[a]**
HADS		
HADS-A	0.27	0.001 **
HADS-D	0.19	0.023 *
SCL-90-R		
Agoraphobia	0.17	0.042 *
Anxiety	0.3	< 0.001 ***
Depression	0.34	< 0.001 ***
Somatization	0.44	< 0.001 ***
Somatization (corrected)[i]	0.4	< 0.001 ***
Sensitivity	0.29	< 0.001 ***
Insufficiency of thinking and acting	0.34	< 0.001 ***
Hostility	0.27	0.001 **
Sleep disturbance	0.26	0.002 **

EoE = Eosinophilic esophagitis, HADS = Hospital Anxiety and Depression Scale, HADS-D = HADS Depression, HADS-A = HADS Anxiety, SCL-90-R = Symptom Checklist (90) Revised, SDI-score = Straumann Dysphagia Instrument score.

[i] SCL-90-R items 12 and 53 that may be related to EoE symptoms are excluded.

[a] P-value correlations between the total SDI-scores and HADS or SCL-90-R domains (Pearson's or Spearman's Rank correlation coefficients, as appropriate).

* P-value of < 0.05, indicating a significant outcome.

** P-value of < 0.01.

*** P-value < 0.001.

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CHAPTER 8

GENERAL WELL-BEING AND COPING STRATEGIES IN ADULT EOSINOPHILIC ESOPHAGITIS PATIENTS

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ABSTRACT

RATIONALE

Growing evidence suggests a negative effect of eosinophilic esophagitis (EoE) on patients' general Health-related quality-of-life (HRQOL). However, the relevance and use of coping strategies and its relation to (disease specific) HRQOL as well as its determinants have not been studied well.

METHODS

Adult EoE patients were invited to complete standardized measures on general HRQOL(SF-36) and coping strategies (UCL). Scores were compared to general population norms. The disease specific Adult Eosinophilic Esophagitis Quality-of-Life (EoE-QOL-A) measure was used to assess EoE-HRQOL. Socio-demographic-and clinical factors were also evaluated.

RESULTS

In total, 147 adult EoE patients (61% males), age 43 (IQR 29 - 52) years were analyzed. Mental health-scores (SF-36) were significantly lower in EoE patients, whereas Physical health scores (SF-36) were similar in EoE patients (vs. the general population; $p = 0.01$ and $p = 0.24$), respectively. The subdomain 'disease anxiety'(EoE-QOL-A) was mostly affected, determinants were; female gender, younger age, severe clinical disease activity, higher number of food bolus extraction and more recent EoE-diagnosis. Less effective coping styles (i.e., passive/palliative reaction) were associated with a significant impact on each individual EoE-HRQOL-subdomain as well as lower scores of the MCS in male EoE patients. Passive reaction in female EoE-patients correlated with impairment of the EoE-HRQOL-domains 'emotional impact' and 'disease anxiety'. Active problem solving was significantly related to better perception of mental HRQOL (SF-36) in both males and females.

CONCLUSION

EoE has a significant negative impact on mental HRQOL, with less effective copings strategies - specifically in males, being a relevant determinant. Thus, a pro-active approach towards coping mechanisms is needed in order to enhance HRQOL and manage patients' burden of EoE.

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic allergic disease, characterized by eosinophilic mucosal infiltration of the esophagus and symptoms of esophageal dysfunction.[1-3] Now widely recognized by gastroenterologists and allergists, EoE has rapidly evolved into an important cause of upper gastrointestinal morbidity in children and adults.[4-9] The first line management of EoE consists of medical therapy (i.e., proton-pump-inhibitors or topical corticosteroids) or dietary elimination of culprit foods and medications.[3] Maintenance treatment is indicated in EoE, since disease activity recurs quickly after cessation of therapy and ongoing eosinophilic inflammation is associated with narrowing of the esophagus and stricture formation.[10-13]

In general, Health-related quality-of-life (HRQOL) is a multi-dimensional concept that is determined by patients' physical, psychological and social status, as well as attitudes, concerns and behaviors in response to having a (chronic) disease.[14] Previous literature confirms that EoE significantly impacts on patients' daily life.[15,16] Yet, data remains scarce on to which demographic-, clinical- or cognitive/behavioral factors influences (illness specific) HRQOL in EoE. From the patients' perspective, being diagnosed with this 'relatively new' disease with need for life-long treatment and subsequent invasive procedures for disease monitoring may be of specific concern.[17] Moreover, most patients have developed adapted eating behaviors (e.g., taking smaller bites, avoid highly textured foods) or use dietary restrictions (avoidance of culprit foods) in order to manage symptoms and avoid food impactions in particular.[17] EoE patients generally display avoidance behaviors of eating (alone or with others) in daily social-life, due to swallowing anxiety or fear of giving others the impression of a state of illness.[18,19] Hence social situations may lead to stress, anxiety symptoms and embarrassment.

Coping refers to emotional, cognitive and behavioral efforts that affects the way each individual handles the physical, social and mental burden that is linked to stressful life events, such as having a chronic illness. Stress management seems to be crucial in general health. Despite a wide range of different coping mechanisms, two main categories are generally mentioned: problem- and emotion-focused coping.[20,21] Problem focused-coping refers to efforts to change a stressful situation (e.g., taking action, seeking information), whereas emotion focused coping involves strategies that regulate emotional distress that is being associated with the situation (e.g., expression of emotion and anger, distraction). Moreover, also gender differences in the selection of different coping styles have also

been well-described.[22-24] How individuals cope with a chronical illness determines patients' quality of life and has shown to be an important outcome in a number of chronic disease populations, e.g., rheumatoid arthritis and Inflammatory-Bowel-Disease (IBD).[25-30] At present, no studies are available that have evaluated coping strategies in adult EoE patients. More importantly, the degree to which different coping styles are related to (disease specific) HRQOL is unknown as well. Therefore, we aimed to assess i) general and disease specific HRQOL, ii) Coping strategies and their relationship with general and disease specific HRQOL, and iii) determinants (i.e., clinical and socio-demographic factors) of disease specific HRQOL.

METHODS

An observational cross-sectional study design was used to assess mental distress among adult EoE patients. Consecutive patients who attended the outpatient clinic of the Amsterdam UMC Motility Center, were invited to participate in the study. An informed consent letter including self-reported questionnaires was distributed at the outpatient clinic between July 2019 and February 2020. Patients with a documented diagnosis of EoE, aged 18 and over, with a sufficient command of written Dutch to complete a self-reported survey were considered eligible for inclusion.[32] Once consented, all patients completed a paper or digital version of the questionnaires. All data was safely collected and stored by using the Electronic Data Capture Castor.

PATIENTS AND PROCEDURES

A cross-sectional study design was used to assess for disease specific and general well-being (i.e., (EoE) HRQOL) and coping strategies in an adult EoE population. Inclusion criteria were a minimum age of 18, a sufficient command of written Dutch to complete self-reported questionnaires and documented diagnosis of EoE according to consensus guidelines.[3] All patients received an informed consent letter including questionnaires. Once written consent was obtained, all eligible patients completed a paper or digital version of the study questionnaires and data was subsequently stored by using the Electronic Data Capture Castor.

MEASURES

Demographics and clinical data

Socio-demographic characteristics (e.g., gender, marital status) as well as EoE related clinical information concerning year of symptom onset and diagnosis, history of

endoscopic interventions and dilation, EoE management (medical or dietary treatment), atopic comorbidities and adapted eating behavior (i.e., taking smaller bites, more chewing, eating slowly or drinking more water during meals) were evaluated by a standard fixed choice questionnaire. Clinical disease activity, defined as symptoms related to esophageal dysfunction (dysphagia and/or food impaction) were assessed by the Straumann Dysphagia Instrument (SDI)-measure.[33]

General health-related quality of life

General HRQOL was evaluated with the Short Form - 36 (SF-36) and has been widely validated for the use in different health care settings and patients. General HRQOL is measured in 36 items across eight domains, including physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, role limitations due to emotional problems, social functioning, and mental health. The items of the SF-36 are combined to form the physical health component scale (PCS) and the mental health component scale (MCS).[34] All SF-36 scores of our EoE patients, stratified for gender and age were compared to a national reference cohort, containing a random sample of the Dutch population (n = 1742).[35]

Disease specific health-related quality of life

The impact of EoE on psychosocial functioning was measured by means of the Adult Eosinophilic Esophagitis Quality of Life (EoE-QOL-A) measure.[36] This tool was developed to assess disease specific (EoE-)HRQOL in EoE populations and has not been validated in the Dutch population. The EoE-QOL-A consists of 24 items (Cronbach's $\alpha = 0.94$) across five subscales, including eating/diet impact, social impact, emotional impact, disease anxiety and swallowing anxiety (**Supplementary Table 1**). Each item score ranges from 4 (very good QOL) to 0 (very poor QOL). Overall scores range from 0 to 96, with higher scores indicating better quality of life. The total EoE-QOL-A index score includes the weighted average of all subscales.

Coping strategies

Patients coping styles were measured by using the validated Utrechtse Coping Lijst (UCL).[21,37] The UCL consists of 47 items, which represent seven different coping styles. The coping subscales are: active problem solving (i.e., not having to deal with a problem by looking for distraction, getting away from the situation) and palliative reaction, avoidance and passive expectancy, seeking social support, passive reaction (i.e., expression of

emotions and anger as well as fostering reassuring thoughts (**Supplementary Table 2**) Different coping strategies of our EoE sample were compared to a reference group, including normative data of the Dutch population, stratified by gender and age. The reference groups are described in the UCL manual.[38]

STATISTICAL ANALYSIS

Statistical analysis was performed with IBM SPSS Statistics (version 25.0)(SPSS, Chicago, USA). To characterize our sample, descriptive statistics were used to assess socio-demographic and clinical variables. Categorical data are described as percentages and continuous data are expressed as mean (± SD) or median (IQR). SF-36 scores and UCL scores were compared to previously published reference norms from the Dutch general population, stratified by gender and age.[35,38] Independent sample t-tests were used to compare SF-36 and UCL scores from our EoE cohort to the general population. Univariate linear regression analysis was used to assess clinical and socio-demographic factors (independent variables) that are possibly associated with EoE-QOL-A subscale scores (dependent variables). Subsequently, a multiple linear regression model was fitted for each subdomain of the EoE-HRQOL-A survey to identify determinants. Factors with a liberal p-value of < 0.2 were entered for multiple linear regression analysis with backward selection. A p-value of < 0.05 was considered to be statistically significant. Associations between coping styles and (EoE-)HRQOL were assessed by Pearson's or Spearman's rank correlations coefficients, as appropriate. Level of significance was set at < 0.05 for the PCS and MCS as well as $p < 0.01$ to correct for multiple testing for all 5 subdomains of the EoE-QOL-A.

ETHICAL CONSIDERATIONS

This study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO). Our study was reviewed by the Medical Ethics Committee of the Amsterdam University Medical Centre (UMC) and formal evaluation was waived according to Dutch law (W19_103#19.136). All participants provided informed consent before taking part and were given a unique study-ID.

RESULTS

PATIENT CHARACTERISTICS

In total, 147 patients (out of 209 patients) completed the self-reported questionnaires (response rate 71%). A male predominance (61%) was confirmed, with a median age of 43 (IQR 29 - 52) years. The median time interval between the first reported EoE symptoms and diagnosis (diagnostic delay) was 5 (IQR 2 - 14) years, with a median age at diagnosis of 39 (IQR 26 - 48) years. The majority of our cohort (66%) reported experiencing ongoing symptoms of dysphagia and/or food impaction. Ninety-three (64%) patients noted to have currently adapted their eating behavior (e.g., more chewing) as a result of EoE symptoms. Empiric elimination of causative foods was reported in 36 (25%) patients, of which 18 (12%) patients stated to be on an elimination diet under guidance of a specialized dietician at present. More details on patients' characteristics of our EoE sample are listed in **Table 1**.

GENERAL HEALTH-RELATED QUALITY OF LIFE

General HRQOL (SF-36) levels of the Physical health Component Scale (PCS) of EoE patients showed no difference compared to the general population (50.5 ± 8.6 vs. 51.4 ± 3.2; p = 0.244). Although PCS levels of male EoE patients vs. males of the general population were not significantly different (p = 0.453), female EoE patients scored significantly lower compared to females of the general population (p = 0.03). Moreover, female patients scored significantly lower on the PCS compared to males in our EoE cohort (52.5 ± 6.5 vs. 47.3 ± 10.4; p < 0.001) (**Figure 1A**).

Total levels of the Mental health Component Scale (MCS) were significantly lower in EoE patients compared to the general population (47.9 ± 10.4 vs. 50.1 ± 1.5; p = 0.01). Although MCS scores of females were similar (EoE vs. general population; p = 0.112), males scored significantly lower on the MCS (EoE vs. general population; p = 0.04). However, no differences between male vs. female patients on MCS scores were found in our EoE cohort (49.1 ± 9.4 vs. 46 ± 11.6; p = 0.076) (**Figure 1B**).

DISEASE SPECIFIC HEALTH-RELATED QUALITY OF LIFE AND ASSOCIATED FACTORS

Evaluation of the disease specific impact on psychosocial functioning (EoE-QOL-A) showed an average weighted score of 2.77 ± 0.81 (range 0.75 - 4) in our EoE cohort, with significant lower levels in females (vs. males; p = 0.002) (**Supplementary Table 3**). Lowest subdomain scores in our sample were observed on disease anxiety (2.46 ± 1.03 (range 0.2 - 4)) and eating/diet impact (2.47 ± 1.12 (range 0 - 4)), with lower levels in females on both

TABLE 1 | Socio-demographic and clinical characteristics.

Socio-demographic and clinical characteristics N = 147	**EoE, *n (%) or median (IQR)* (n = 147)**
Socio-demographic characteristics	43 (29 - 52)
Age, years	90 (61)
Gender, male	115 (78)
Alcohol use, yes	9 (6)
Smoking, yes	
Level of education	49 (33)
Primary or secondary school	98 (67)
College or University	96 (65)
In domestic partnership, living together or married	
Working status	9 (6)
Student	121 (82)
Employed	9 (6)
Unemployed	1 (1)
Stayed at home parent	7 (5)
Retired	
Clinical characteristics	
Atopic diatheses	119 (81)
Allergic rhinitis	106 (72)
Oral Allergy Syndrome (OAS)	52 (35)
Food allergy	50 (34)
Asthma	49 (33)
Atopic dermatitis	37 (25)
Clinical disease activity *	97 (66)
Adapted eating behavior	93 (64)
Number of endoscopic interventions	3 (2 - 6)
Diagnostic delay **, *years*	5 (2 - 14)
Disease duration ***, *years*	3 (1 - 6)
Age at diagnosis, *years*	39 (26 - 48)
Previous dilation	21 (14)
Current treatment	
PPIs	34 (23)
Topical steroids	35 (24)
Topical steroids with additional dietary restrictions	14 (10)
Empiric food elimination	36 (25)
No treatment	28 (19)

IQR = Inter Quartile Range, PPIs = Proton Pump Inhibitors.
* Clinical disease activity, defined as SDI-PRO score ≥ 1.
** Diagnostic delay is the time interval between the first symptoms and the diagnosis.
*** Disease duration, measured from year of diagnosis.

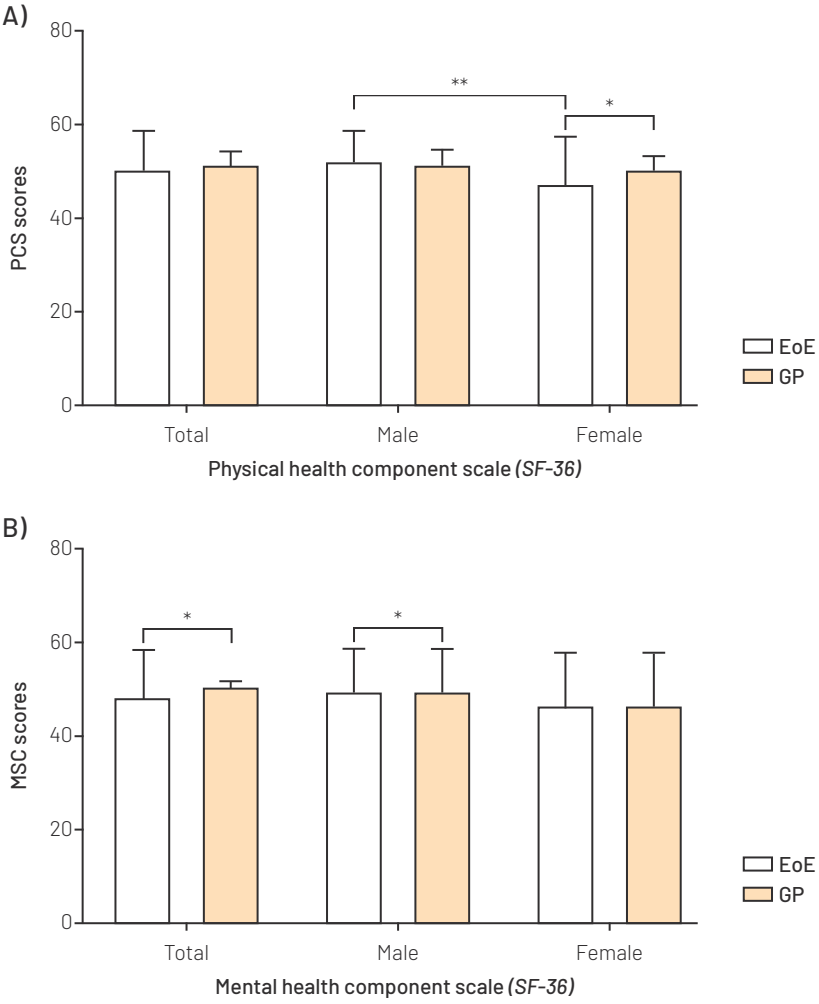

FIGURE 1. | A) Physical health component scale (SF-36) and B) Mental health component scale (SF-36) of EoE patients compared to the Dutch general population, stratified for gender.
SF-36 = Short Form (36) Health Survey, MCS = Mental health Component Scale, EoE = Eosinophilic esophagitis patients, GP = General population.
* P-value of < 0.05, indicating a significant outcome, ** P-value of < 0.01, *** P-value of < 0.001.

TABLE 2. | Factors associated with Disease specific Quality of Life in EoE patients.

Disease specific Quality of Life (EoE-QOL-A) N = 147	**Univariate analysis**	
	B	**ß**
Eating/diet impact		
Female gender	-0.589	-0.257
Age	0.025	0.313
Severe clinical disease activity	-1.301	-0.330
Dietary restrictions	-0.696	-0.294
Disease duration	0.075	0.269
Adapted eating behavior	-0.497	-0.213
Social impact		
Age	0.01	0.142
Severe clinical disease activity	-1.067	-0.323
Disease duration	0.031	0.131
Number of endoscopic interventions with food bolus extraction	-0.072	-0.154
Adapted eating behavior	-0.218	-0.111
Emotional impact		
Female gender	-0.504	-0.275
Age	0.018	0.283
Severe clinical disease activity	-0.828	-0.263
Disease duration	0.055	0.248
Adapted eating behavior	-0.232	-0.125
Disease anxiety		
Female gender	-0.478	-0.228
Age	0.019	0.256
Severe clinical disease activity	-0.422	-0.117
Disease duration	.061	0.239
Number of endoscopic interventions with food bolus extraction	-0.060	-0.117
Swallowing anxiety		
Age	0.018	0.244
Severe clinical disease activity	-1.095	-0.304
Disease duration	0.038	0.148
Adapted eating behavior	-0.397	-0.186

B = Unstandardized regression coefficient, where 1 point increase in the predictor variable (e.g., female gender) is associated with B point(s) increase in the dependent variable (EoE-QOL-A subscale score).
ß = Standardized regression coefficient, where 1 standard deviation increase in the predictor variable (e.g., female gender) is associated with ß point (s) increase in the dependent variable (EoE-QOL-A subscale score).
CI (95 %) = 95 % Confidence interval, NS = Not significant.
EoE-QOL-A = Adult Eosinophilic Esophagitis Quality of Life questionnaire, Disease duration = measured from year of diagnosis, Diagnostic delay = time interval between the first symptoms and the diagnosis.
* P-value < 0.2, indicating a possible trend, ** P-value of < 0.05, indicating a significant outcome.

Univariate analysis		Multivariable analysis			
95% CI (B)	P-value	B	ß	95% CI (B)	P-value
-0.953 to -0.225	0.002 **	-0.374	-0.163	-0.715 to -0.034	0.031 **
0.013 to 0.038	< 0.001 **	0.018	0.229	0.007 to 0.030	0.002 **
-1.911 to -0.691	< 0.001 **	-1.053	-0.267	-1.625 to -0.481	< 0.001 **
-1.069 to -0.324	< 0.001 **	-0.496	-0.209	-0.850 to -0.142	0.006 **
0.031 to 0.119	0.001 **	NS			
- 0.871 to -0.124	0.009 **	NS			
-0.001 to 0.02	0.086 *				NS
-1.580 to -0.553	< 0.001 **	-1.062	-0.321	-1.571 to -0.553	< 0.001 **
-0.007 to 0.069	0.114 *				NS
-0.148 to 0.004	0.062 **				NS
-0.537 to 0.101	0.179 *				NS
-0.793 to -0.214	0.001 **	-0.446	-0.212	-0.769 to -0.122	0.007 **
0.008 to 0.028	0.001 **	0.016	0.236	0.005 to 0.025	0.003 **
-1.326 to -0.330	0.001 **	-0.604	-0.199	-1.103 to -0.146	0.011 **
0.02 to 0.090	0.002 **				NS
-0.535 to 0.071	0.132 *				NS
-					
0.814 to -0.143	0.006 **	-0.446	-0.212	-0.769 to -0.122	0.007 **
0.007 to 0.030	0.002 **	0.015	0.204	0.003 to 0.027	0.001 **
-1.009 to 0.166	0.158 *	0.044	0.172	0.001 to 0.086	NS
0.02 to 0.101	0.004 **	-0.100	-0.196	-0.179 to -0.021	0.043 **
-0.143 to 0.023	0.157 *				0.014 **
0.006 to 0.03	0.003 **	0.015	0.186	0.002 to 0.025	0.02 **
-1.660 to -0.531	< 0.001 **	-0.913	-0.253	-1.477 to -0.350	0.002 **
-0.004 to 0.079	0.074 *				NS
-0.741 to -0.052	0.024 **				NS

domains (vs. males, all; $p < 0.01$). While emotional impact scores were also significantly lower in females (vs. males; $p = 0.001$), subdomain scores of social impact and swallowing anxiety were similar (male vs. female, all; $p > 0.05$) (**Supplementary Table 3**).

Multiple linear regression analysis indicated factors including female gender, younger age, severe clinical disease activity (i.e., current symptoms of daily dysphagia and food impaction) and dietary restrictions to be independently associated with impairment of EoE-HRQOL on the 'eating/diet' subdomain (**Table 2**). Moreover, severe clinical disease activity was only found to be significantly related to low levels of the EoE-HRQOL 'social impact' scores. Factors including female gender, younger age, and severe clinical disease activity were all independently associated with lower scores on the EoE-HROQOL 'emotional impact' subdomain. In addition, low scores of the EoE-HRQOL 'disease anxiety' subdomain were significantly associated with independent predictors, such as: female gender, younger age, more recent EoE-diagnosis and a higher number of endoscopic food bolus extractions. Finally, younger age and severe clinical disease activity were both indicated as significant determinants for low scores on the EoE-HRQOL 'swallowing anxiety' subdomain. More details on univariate and multivariable linear regression analysis of determinant factors for EoE-HRQOL are presented in **Table 2**.

COPING STRATEGIES

Coping styles (UCL) of EoE patients showed significantly more active problem solving, palliative reaction, avoidance and passive expectancy as well as seeking social support compared to the general population (all; $p < 0.05$). Moreover, passive reaction and expression of emotion and anger were significantly less reported in EoE patients (vs. general population; all; $p < 0.01$) (**Figure 2A**).

Male EoE patients showed significantly more active problem solving, palliative reaction, avoidance as well as seeking social support compared to males of the general population (all; $p < 0.05$). Moreover, passive reaction and emotion expressing were significantly less reported in male EoE patients (vs. general population; all; $p < 0.01$) (**Figure 2B**).

Female EoE patients showed more palliative reaction compared to females of the general population ($p = 0.035$), whereas no difference was found for other coping strategies (vs. general population; all; $p > 0.05$) (**Figure 2C**). Females in our EoE sample showed significantly more palliative reaction and seeking for social support compared to males (all; $p < 0.01$) (**Figure 2D**).

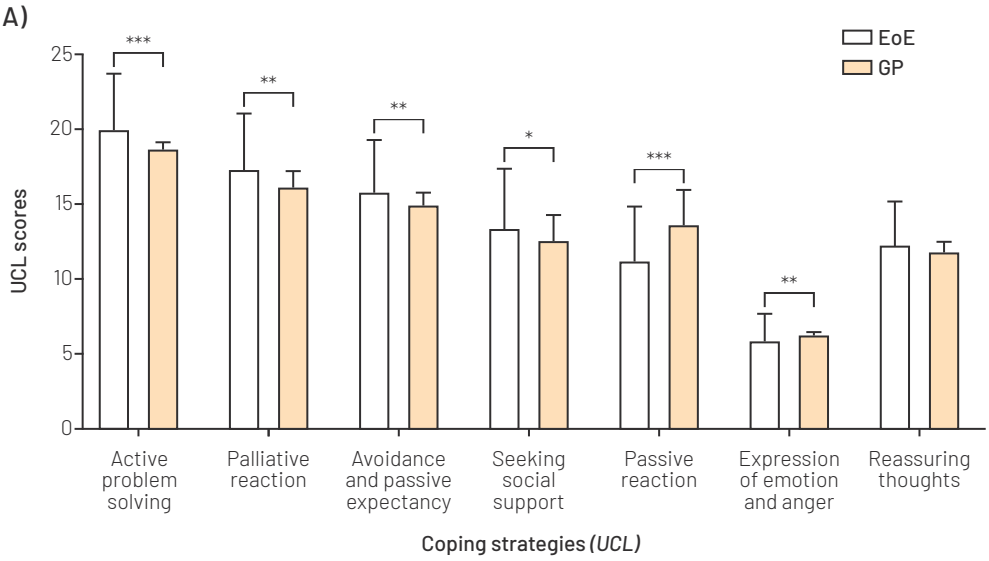

FIGURE 2A. | Coping styles of EoE patients compared to the Dutch general population.
UCL = Utrechtse Coping Lijst, EoE = Eosinophilic esophagitis patients, GP = general population.
* P-value of < 0.05, indicating a significant outcome, ** P-value of < 0.01, *** P-value of < 0.001.

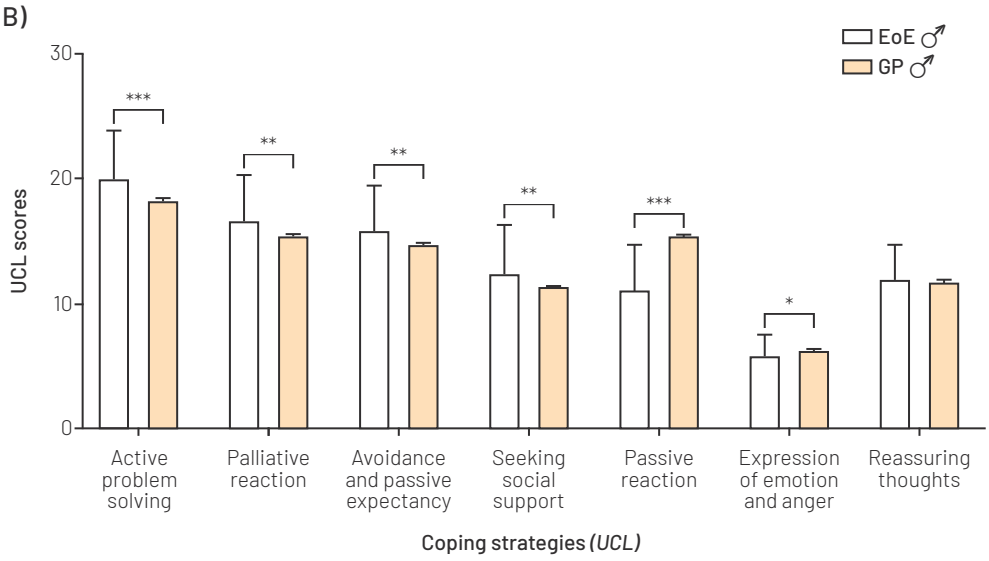

FIGURE 2B. | Coping styles of male EoE patients compared to the Dutch general population.
UCL = Utrechtse Coping Lijst, EoE = Eosinophilic esophagitis patients.
* P-value of < 0.05, indicating a significant outcome, ** P-value of < 0.01, *** P-value of < 0.001.

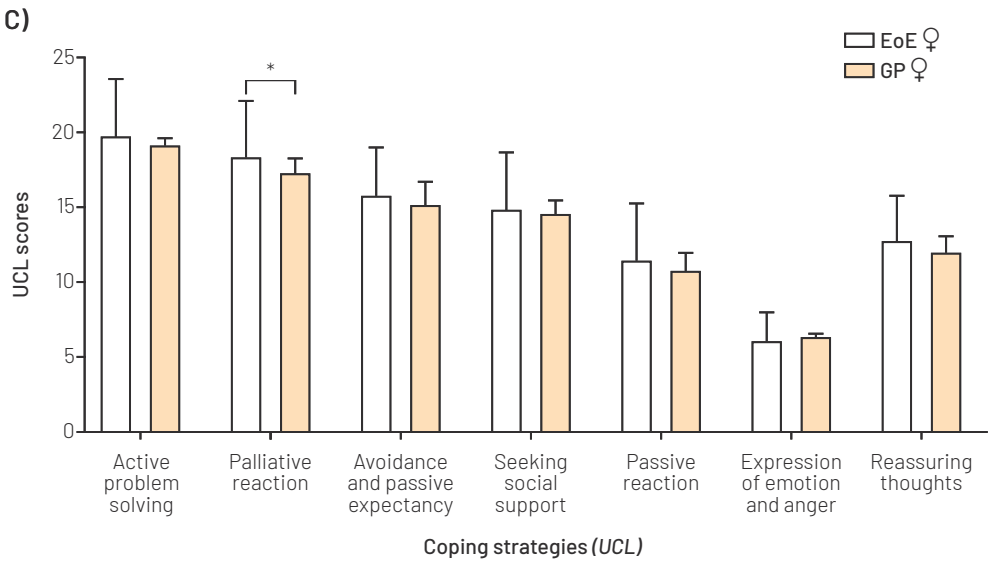

FIGURE 2C. | CCoping styles of female EoE patients compared to the Dutch general population .
UCL = Utrechtse Coping Lijst, EoE = Eosinophilic esophagitis patients, GP = general population.
* P-value of < 0.05, indicating a significant outcome, ** P-value of < 0.01, *** P-value of < 0.001.

TABLE 3A. | Correlations between coping styles and Disease specific Health related Quality of Life in male EoE patients.

Coping strategies (UCL) N = 89	**Disease specific Quality of Life (EoE-HR-QoL-A)**			
	Eating/diet impact		**Social impact**	
	r	**P-value [a]**	**r**	**P-value [a]**
Active problem solving [b]	0.086	0.421	0.084	0.434
Palliative reaction [c]	-0.376	< 0.001 **	-0.262	< 0.01 *
Avoidance and passive expectancy [c]	-0.117	0.275	-0.221	0.037
Seeking social support [b]	-0.026	0.806	0.023	0.832
Passive reaction [c]	-0.468	< 0.001 **	-0.264	< 0.01*
Expression of emotion and anger [c]	-0.182	0.087	-0.126	0.239
Reassuring thoughts [b]	-0.089	0.407	-0.097	0.365

UCL = Utrechtse Coping Lijst, EoE-HR-QoL-A = Adult Eosinophilic Esophagitis Quality of Life questionnaire.
[a] P-value correlation between UCL domains and EoE-QOL-A domains (Pearson's or Spearman's Rank correlation coefficients, as appropriate), [b] Problem-focused coping, [c] Emotional-focused coping.
* P-value of < 0.01, indicating a significant outcome, ** P-value < 0.001.

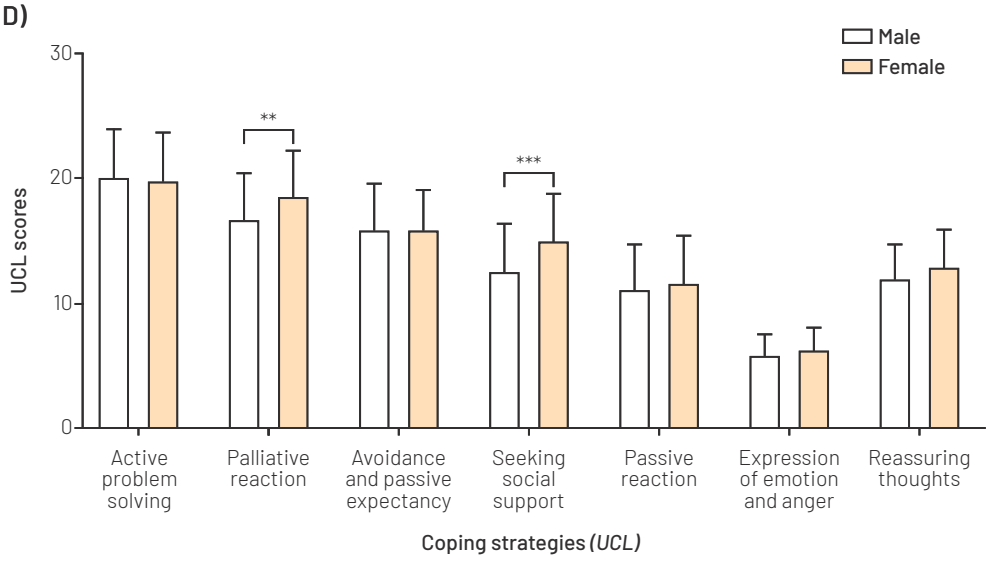

FIGURE 2D. | Coping styles of EoE patients, stratified for gender.
UCL = Utrechtse Coping Lijst, EoE = Eosinophilic esophagitis patients.
* P-value of < 0.05, indicating a significant outcome, ** P-value of < 0.01, *** P-value of < 0.001.

Disease specific Quality of Life (EoE-HR-QoL-A)					
Emotional impact		Disease anxiety		Swallowing anxiety	
r	P-value [a]	r	P-value [a]	r	P-value [a]
0.056	0.605	-0.043	0.689	0.200	0.060
-0.455	< 0.001 **	-0.451	< 0.001 **	-0.332	< 0.001 **
-0.148	0.167	-0.133	0.214	-0.152	0.155
-0.071	0.509	-0.102	0.341	0.000	1.000
-0.355	< 0.001 **	-0.372	< 0.001 **	-0.464	< 0.001 **
-0.184	0.085	-0.188	0.078	-0.213	0.045
-0.216	0.042	-0.254	0.016	-0.130	0.223

TABLE 3B. | Correlations between coping styles and Disease specific Health related Quality of Life in female EoE patients.

Coping strategies (UCL) N = 89	Disease specific Quality of Life (EoE-HR-QoL-A)			
	Eating/diet impact		Social impact	
	r	P-value [a]	r	P-value [a]
Active problem solving [b]	0.079	0.561	0.02	0.884
Palliative reaction [c]	-0.080	0.559	-0.187	0.167
Avoidance and passive expectancy [c]	-0.012	0.931	-0.240	0.075
Seeking social support [b]	0.022	0.872	-0.016	0.906
Passive reaction [c]	-0.286	0.033	-0.312	0.019
Expression of emotion and anger [c]	-0.086	0.529	-0.051	0.710
Reassuring thoughts [b]	-0.137	0.314	-0.158	0.244

UCL = Utrechtse Coping Lijst, EoE-HR-QoL-A = Adult Eosinophilic Esophagitis Quality of Life questionnaire.
[a] P-value correlation between UCL domains and EoE-QOL-A domains (Pearson's or Spearman's Rank correlation coefficients, as appropriate).
[b] Problem-focused coping.
[c] Emotional-focused coping.
* P-value of < 0.01, indicating a significant outcome, ** P-value < 0.001.

COPING STYLES IN MALES AND FEMALES CORRELATED TO PERCEPTION OF HRQOL

Considering the general differences in coping behaviors between males and females, the relationship between coping strategies and HRQOL were determined for both genders.[23,24] Less effective coping styles, such as: palliative reaction, avoidance and passive reaction significantly correlated with higher impairment of the MCS of the HRQOL (SF-36), whereas active problem solving was related with better perception of mental HRQOL (SF-36) in male EoE patients (all; $p < 0.05$) (**Supplementary Table 4A**). A passive coping style was significantly correlated with lower levels of the MCS in female EoE patients, whereas active problem solving also correlated with improvement of mental HRQOL (SF-36) (all; $p < 0.05$) (**Supplementary Table 4B**). With regards to disease specific HRQOL (EoE-QOL-A), both palliative- and passive coping styles were associated with a significant impact on each individual EoE-HRQOL subdomain in male EoE patients (all; $p < 0.01$) (**Table 3A**). Additionally, passive reaction significantly correlated with more impairment of the subdomains 'emotional impact' and 'disease anxiety' in female EoE patients (all; $p < 0.05$) (**Table 3B**).

Disease specific Quality of Life (EoE-HR-QoL-A)					
Emotional impact		Disease anxiety		Swallowing anxiety	
r	P-value [a]	r	P-value [a]	r	P-value [a]
0.205	0.130	0.146	0.283	-0.017	0.899
-0.228	0.092	-0.127	0.352	-0.188	0.165
-0.092	0.500	-0.108	0.428	-0.225	0.095
-0.110	0.418	-0.210	0.120	-0.092	0.501
-0.498	<0.001 **	-0.491	<0.001 **	-0.288	0.031
-0.225	0.096	-0.281	0.036	-0.124	0.361
-0.255	0.058	-0.207	0.126	-0.243	0.071

DISCUSSION

This is the first study, evaluating coping strategies and the degree to which different coping styles are related to (disease specific) HRQOL in adult EoE patients. Mental (SF-36) HRQOL was significantly affected in our EoE cohort in comparison to the general population norms. These observations are in line with a study by van Hewett *et al.*, also reporting lower MCS scores in EoE patients compared to sex and age matched controls.[39] Moreover, disease specific HRQOL (EoE-QOL-A) subdomains; 'disease anxiety' and 'eating/diet impact' were mostly affected. Our data show that less effective coping styles, such as passive- and palliative reactions were associated with a significant impact on each individual EoE-HRQOL subdomain as well as lower scores of the MCS in male EoE patients. Passive reaction in female EoE patients correlated with impairment of the EoE-HRQOL domains 'emotional impact' and 'disease anxiety'. Active problem solving was significantly related to better perception of mental HRQOL in both genders. Overall, determinants such as: severe clinical disease activity (i.e., daily dysphagia and food impaction), younger age and female gender were associated with impairment of EoE-HRQOL on most of the subdomains.

The subdomain 'eating/diet impact' of the EoE-HRQOL being mostly affected within our cohort supports also the former illustrated impact of dietary restrictions on patients' disease specific HRQOL.[18] We observed that total EoE-HRQOL and most subdomain scores were significantly lower in females compared to males (**Supplementary Table 3**). HRQOL related gender disparities are well-described in literature, with female gender being associated with poor HRQOL outcomes in multiple chronic health populations (e.g., rheumatoid arthritis and Asthma).[40-42] Within our sample, the subdomains 'social impact' and 'swallowing anxiety' did not differ between both genders. Factors such as: severe clinical disease activity and younger age were independently associated with impaired EoE-HRQOL in these 2 domains. This may be further supported by previous findings in adult EoE patients of males being more prone to stricture development, strong correlates between clinical disease activity and anxiety symptoms as well as a 3-fold risk of significant anxiety symptoms in young adulthood (18 - 35 years).[13,43] In fact, EoE patients generally display avoidance behaviors in social situations due to symptoms of food impaction with subsequent swallowing anxiety and fear of giving others the impression of a state of illness.[44] Hence social situations leads to stress, anxiety symptoms and embarrassment. Of note, also MCS scores were significantly lower only in EoE male patients compared to males of the general population (with no difference for females) (**Figure 1B**). Taken this together, particularly (young) males that are diagnosed with EoE may be more at risk of impaired HRQOL, specifically related to food impaction. In addition to this, a recent study by Taft *et al.*, observed that many EoE patients have elevated hypervigilance (i.e., heightened focus on physical symptoms) and symptom specific anxiety such as swallowing anxiety, both being associated with worst reported EoE symptoms and poor HRQOL.[45] These observations in mental health research emphasizes the importance of understanding the psychosocial issues being faced by EoE patients in clinical practice.

The subdomain 'disease anxiety' was the most affected in our cohort, with stress and anxiety related to 'having a chronic condition' being independently associated with factors such as: female gender, younger age, severe clinical disease activity, higher number of endoscopic interventions with food bolus extraction and a more recent EoE-diagnosis (**Table 3**). In particular, a more recent diagnosis of EoE being predictive of poor EoE-HRQOL seems to be in line with findings in the broader fields of IBD-research, with recently diagnosed patients having lower perception of disease specific HRQOL and greatest need of education and support.[46,47] Moreover, disease-related knowledge levels in IBD-patients are known to affect self-management (i.e., managing oneself) and

the ability to adapt mechanisms to manage the burden of having a chronic illness (i.e., coping strategies).[48] Therefore, specifically patients' education needs to be indicated as highly relevant for EoE-practice, since this relatively new disease still yields a scarcity of patients' information-resources.

According to the well-described Common-Sense Model (CSM) of self-regulation and health, HRQOL is suggested to be affected by 2 major determinants: illness perception and coping strategies.[49] The CSM postulates that coping strategies affects adjustment to an illness as indicated e.g., by physical, mental and social well-being.[50] Females are known to differently cope compared to males.[21] In general, females tend to use coping strategies that are aimed to change their emotional response to a stressful situation, whereas males uses more problem-focused methods for stress management.[23,24] Apart from the well-described association between female gender and seeking more social support as coping style, we observed also females using more palliative coping compared to males in our EoE cohort (**Figure 2C**) Interestingly, the use of less effective coping styles, such as: passive- and palliative reaction in male EoE patients was significantly associated with impaired perception of EoE-HRQOL on all 5 subdomains as well as lower perception of mental HRQOL. Additionally, passive reaction in females also significantly correlated with impairment of the EoE-HRQOL domains 'emotional impact' and 'disease anxiety' and lower scores of the MCS (**Table 3A, 3B, Supplementary Table 4A, 4B**). As illustrated by the CSM, it may be suggested that adapting these negative behavioral strategies into more effective coping mechanisms will effectively influence patients' perception of EoE-HRQOL. This is also further supported by our observation on active problem solving being significantly related to better perception of mental HRQOL in both genders (**Supplementary Table 4A, 4B**). Hence awareness (i.e., recognition and understanding) amongst gastroenterologists and allergists on the use of less effective coping strategies in adult EoE patients should be increased in daily practice. More importantly, when inadequate styles are identified, referral to a medical psychologist/psychotherapist for Cognitive behavioral therapy in order to support self-management may be helpful to adapt these coping strategies and improve EoE patients' perception of HRQOL.[51]

The study design has a few limitations that should be addressed. First, our observations are extracted from a large EoE sample of patients visiting the outpatient clinic of a tertiary health-care center, by that limiting its generalizability. However, it should be noted that our cohort, although not population-based actually reflects a diversified

population, including different stages of disease severity and treatment. Secondly, we did not assess patients' illness perception (i.e., individuals' beliefs and feelings about their disease). According to the CSM, cognitive and emotional illness perception also impacts directly or indirectly through influence on coping mechanisms on patients' HRQOL.[50] In a previous validation study of the EoE-QOL-A measure, HRQOL was linked to illness perception in adult EoE patients.[52] Therefore, the evaluation of illness beliefs amongst adult EoE patients, specifically related to HRQOL and coping styles may be implicated for future research. Nevertheless, we believe that this is the first study with specific interest of determining coping strategies in adult EoE patients and the degree to which different coping styles are related to (disease specific) HRQOL. Aside from the large sample size, also the use of multiple (validated) health outcome measures may be considered as another strength of our design.

In summary, our study confirms that EoE has a significant negative impact on mental HRQOL in adult EoE patients. Less effective coping strategies are related to poor perception of general and disease specific HRQOL, particularly in males. This study emphasizes the importance of HRQOL being a key health outcome in daily EoE practice and research evaluation effect of interventions. Therefore, a pro-active approach towards coping mechanisms and provision of sufficient mental care is needed to support adjustment to living with a chronic illness, and ultimately enhance EoE patients HRQOL.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE 1. | EoE-QOL-A domains.

Domains (EoE-QOL-A)	Explanation of Domains	Example of questions from the EoE-QOL-A questionnaire
Eating/diet impact	Modification of eating behavior or dietary restrictions	"I have to be cautious about eating because I have EoE"
Social impact	Impact on social interactions	"I try to hide my difficulty swallowing so that other people do not realize what is happening"
Emotional impact	Emotional impact	" I feel frustrated that I have EoE"
Disease anxiety	Issues related to having a chronic disease	"I worry about having to be on an EoE treatment for the rest of my life"
Swallowing anxiety	Issues related to food impactions	"I feel panicked or out of control when I have difficulty swallowing"

EoE-QOL-A = Adult Eosinophilic Esophagitis Quality of Life questionnaire.

SUPPLEMENTARY TABLE 2. | UCL domains.

UCL Domains	Explanation of Domains
Active problem solving *	Overlooking the situation, being focused on the problem and confidentially solving the situation
Palliative reaction **	Not having to deal with a problem by looking for distraction, getting away from the situation
Avoidance and passive expectancy **	Avoiding difficult situations and preferably attending as little as possible to an issue
Seeking social support *	Discussing the problem with family and/or friends or getting someone to help
Passive reaction **	Having a negative attitude towards the problem. Withdraw oneself, being overwhelmed by the situation, incapable of activity
Expression of emotion and anger **	Showing emotions as anger or fear
Reassuring thoughts *	Holding on to a positive attitude towards the problem or putting the situation in perspective

UCL = Utrechtse Coping Lijst.
* Problem-focused coping.
** Emotional-focused coping.

SUPPLEMENTARY TABLE 3. | Disease specific health-related Quality of Life in EoE patients.

Disease specific Quality of Life (EoE-QOL-A)	EoE (n = 147)	Male (n = 90)	Female (n = 57)	P-value [a]
Eating/diet impact (4 items, *weighted average*)	2.47±1.12 (0 - 4)	2.70±1.05	2.11±1.14	0.002 *
Social impact (4 items, *weighted average*)	2.80±0.94 (0.75 - 4)	2.88±0.88	2.68±1.03	0.22
Emotional impact (8 items, *weighted average*)	3.01±0.90 (0.38 - 4)	3.21±0.79	2.70±0.98	0.001 *
Disease anxiety (5 items, *weighted average*)	2.46±1.03 (0.2 - 4)	2.65±0.95	2.17±1.09	0.006 *
Swallowing anxiety (3 items, *weighted average*)	2.97±1.03 (0 - 4)	3.04±1.03	2.87±1.03	0.343
Overall EoE-HR-QoL score (24 items, *weighted average*)	2.77±0.81 (0.75 - 4)	2.93±0.75	2.51±0.86	0.002 *

EoE-QOL-A = Adult Eosinophilic Esophagitis Quality of Life questionnaire, EoE = Eosinophilic esophagitis patients.
[a] P-value male vs. female (Independent sample t-test).
* P-value of < 0.05, indicating a significant outcome.

SUPPLEMENTARY TABLE 4A. | Correlations between coping styles and General health related quality of life in male EoE patients

Coping strategies (UCL) N = 89	General health related quality of life (SF-36)			
	PCS		MCS	
	r	P-value [a]	r	P-value [a]
Active problem solving	-0.087	0.416	0.242	0.022*
Palliative reaction	-0.113	0.293	-0.224	0.035*
Avoidance and passive expectancy	-0.070	0.517	-0.307	<0.01**
Seeking social support	-0.054	0.618	0.026	0.809
Passive reaction	-0.215	0.043	-0.446	<0.001***
Expression of emotion and anger	-0.191	0.073	-0.196	0.066
Reassuring thoughts	-0.203	0.056	-0.016	0.885

UCL = Utrechtse Coping Lijst, PCS = Physical health Component Scale, MCS = Mental health Component Scale.
a P-value correlation between UCL domains and PCS or MCS (Pearson's or Spearman's Rank correlation coefficients, as appropriate).
* P-value of < 0.05, indicating a significant outcome.
** P-value of < 0.01, indicating a significant outcome.
*** P-value < 0.001.

SUPPLEMENTARY TABLE 4B. | Correlations between coping styles and General health related quality of life in female EoE patients.

Coping strategies (UCL) N = 56	General health related quality of life (SF-36)			
	PCS		MCS	
	r	P-value [a]	r	P-value [a]
Active problem solving	0.029	0.831	0.364	<0.01 *
Palliative reaction	0.063	0.645	-0.247	0.067
Avoidance and passive expectancy	-0.034	0.805	-0.77	0.573
Seeking social support	0.028	0.839	-0.042	0.758
Passive reaction	-0.202	0.135	-0.674	<0.001 **
Expression of emotion and anger	-0.180	0.184	-0.206	0.128
Reassuring thoughts	-0.185	0.173	-0.167	0.220

UCL = Utrechtse Coping Lijst, PCS = Physical health Component Scale, MCS = Mental health Component Scale.
[a] P-value correlation between UCL domains and PCS or MCS (Pearson's or Spearman's Rank correlation coefficients, as appropriate).
* P-value of < 0.05, indicating a significant outcome.
** P-value of < 0.01, indicating a significant outcome.

8

CHAPTER 9

SUMMARY DISCUSSION AND FUTURE PROSPECTS

SUMMARY

Eosinophilic esophagitis (EoE) is an allergen/immune-mediated esophageal disorder, characterized by symptoms of esophageal dysfunction (i.e., dysphagia and food impaction) and infiltration of the esophageal epithelium with eosinophils.[1-3] The frequency of EoE, as it is recognized today, has increased tremendously after its first description as a case series in the early 90s.[4,5] Since then, EoE has gone from an extremely rare condition to a more widely recognized major cause of upper gastrointestinal morbidity in children and adults. Overall, the evolution of EoE is thought to be an interplay between genetics, environment and host immune system factors that are involved in multiple pathways.[6,7] The proposed pathogenic mechanism is illustrated by an immune response that is primarily regulated by T-helper type 2 cells (Th2) against food- (and aero) allergens. At present, EoE-management strategies involve targeting the esophageal inflammation with medical therapy (such as proton pump inhibitors (PPIs) or swallowed topical steroids), dietary elimination of foods, and esophageal dilatation.[8] Over the past 25-years, the 'relatively new' field of EoE-research has rapidly evolved, with advanced understanding of its natural disease course, pathogenesis and more insights into diagnostics and effectiveness of various treatments. Yet, much remains to be uncovered on this emerging chronic disease.

EPIDEMIOLOGY, PATHOPHYSIOLOGY

In the first part of this thesis, we address the epidemiology and pathophysiology of EoE. **Chapter 2** presents the outcomes of a cross-sectional study that was conducted by using results from the nationwide network and registry from cyto- and histopathology in the Netherlands (PALGA). Between a time period of 25 years, 4061 patients were classified as EoE (71% male, mean age 37.9 years) of which 639 (16%) patients were children (< 18 years). The EoE incidence increased from 0.01 in 1995 to 3.16 per 100.000 inhabitants in 2019. A controversial topic still remains whether the overall dramatic rise of EoE frequency, in particular in developed countries, reflects a true disease expansion by paralleling other increasing Western diseases (e.g, allergic morbidities, Inflammatory Bowel Disease), or might be connected to improved medical awareness and diagnostic tests. Over the past 25-years we demonstrated a 2.6-fold raise of endoscopies with esophageal biopsy sampling, relatively modest given that the incidence of EoE has expanded a 316-fold within the same time window. From these results, it is clear that EoE incidence has not stabilized yet and continues to rise. Although genetic susceptibility is associated, this phenomenon indicates a prevalent role for environmental variations in disease manifestation and underscores the need to further investigate the mechanisms underlying its pathogenesis.

DIAGNOSTICS

According to consensus recommendations, if EoE is suspected, not only esophageal but also gastric and duodenal biopsy specimens should be sampled in order to exclude other generalized or eosinophilic gastrointestinal disorders, such as eosinophilic gastroenteritis or celiac disease. However, the diagnostic yield for this remains unclear. In **Chapter 3**, a retrospective chart-review was conducted in adult EoE patients that underwent upper endoscopy with biopsies sampled from the esophagus, stomach and duodenum. Standardized (electronic) case-report forms were used to extract clinical, endoscopic and histologic data. In total, 93 adults (71% males, age 36.4 years) with untreated EoE (≥ 15 eosinophils (eos)/high-power-field (hpf)) were included. The added diagnostic value of routine random sampling of gastric and duodenal biopsies yielded an additional histological diagnosis in 28 (30%) patients. However, most of these diagnoses were non-specific or *Helicobacter Pylori* gastritis, with no relevance to the management and/or impact on the previous diagnosis of EoE. Only 1 (1%) patient was diagnosed with eosinophilic gastroenteritis on duodenal biopsies. Therefore, we considered the observed diagnostic yield of 3.6% for a relevant other generalized or eosinophilic disorder in our cohort to be generally very low. From these results, future evidence-based statements on the limited utility of routinely sampled biopsy specimens from the stomach and duodenum in daily practice may be established.

MANAGEMENT

The following chapters of this thesis focuses on the medical and dietary treatment modalities for EoE. **Chapter 4** provides an overview of established EoE pharmacotherapies that have been evaluated as treatments (e.g., proton pump inhibitors, swallowed topical steroids), as well as other promising therapeutics that are in the drug development pipeline for EoE, such as monoclonal antibodies targeting IL-4/IL-13 (dupilumab), IL-13 (cendakimab = RPC4046) and the IL-5-receptor (benralizumab).

The management of EoE needs an integrated approach, with a fundamental role for identification and elimination of culprit foods. For patients, the major benefit of diet-based treatment is to potentially identify the root cause of their disease, so they are able to avoid these EoE culprits instead of being dependent on life-long medication. Elemental diets (i.e., amino-acid based formula (AAF) as sole source of nutrition) have proven to be highly effective (85% - 95% disease remission rates) in EoE patients of all ages.[9-14] However, adherence is challenged by its poor palatability and impact on social

life. Empiric removal of culprits (i.e., elimination of four-foods (FFED) or six-foods (SFED)) has been the most widely used diet for EoE patients in clinical practice. In **Chapter 5** we sought to determine if addition of AAF to an elimination diet (FFED; milk, wheat/gluten, egg and soy) might facilitate adherence and, therefore, enhance efficacy of dietary treatment. Patients were randomized (1:1) to groups given either a FFED or FFED with addition of AAF providing 30% of their daily energy needs (FFED + AAF). Patients (60% male, median age 34.5 years)) were randomized to FFED (n = 20) or FFED + AAF (n = 21); 40 patients completed the diet. Complete histological remission (< 15 *eos/hpf*) was achieved in 48% of FFED + AAF treated patients vs. 25% of FFED treated patients, respectively after 6 weeks. Eosinophils decreased significantly in both groups between baseline and week 6, but there was no difference in the change of eosinophils between groups. A significant but similar endoscopic and symptomatic reduction was observed in both groups. Disease-specific health-related Quality of Life scores significantly improved between baseline and week 6 in patients treated with the FFED + AAF and not in the FFED group. These findings could suggest that a combined approach of FFED and AAF may have benefits above FFED alone.

Dietary elimination of culprit foods is thought to target the adaptive immune system (i.e., suppress antigen-driven T-cell response), with no modification of signaling pathways or inflammatory cell-apoptosis as mostly follows after steroids or biological targets. Yet, there is a relative scarcity of studies evaluating the effect of dietary treatment on gene expression patterns in EoE patients, in particular, in the context of clinical features. In **Chapter 6**, we conducted an analysis of biopsy samples and data collected during a randomized controlled dietary intervention trial (2 types of diet) in adults with active EoE (≥ 15 *eos/hpf*)(details have been described in **Chapter 5**). Transcripts of 10 indicated genes were measured (qPCR) and compared to clinical correlates (eosinophils, symptoms, and endoscopic signs) at baseline and after treatment. Forty patients (pooled FFED + FFED + AAF)(60% male, age 34.5 years) completed the 6-weeks of dietary intervention. Eosinophils, symptoms and endoscopic signs were significantly decreased after the diet. We observed that multiple pathways that are leading to this common disease state are affected after dietary treatment, with significant changes of gene expression markers related to inflammation (IL-5, IL-13, TSLP, POSTN CPA-3, CCL-26, and IL-10), epithelial/barrier function (DSG-1 and CAPN-14) and fibrosis (TGF-β). Moreover, upregulation of CAPN-14 and lower levels of DSG-1 were associated with "fibrotic" phenotypes (i.e., presence of 'rings' and/or strictures' at upper endoscopy), whereas upregulation of IL-10 was linked to

"food impaction" phenotypes (i.e., patients presenting with symptoms of food impaction. These findings strongly suggest that elimination diets, besides inducing a clinical and histological response, are associated with a broad transcriptional response at the level of the esophageal epithelium.

IMPACT ON PATIENTS' DAILY LIFE

In the last part of this thesis, we focused on the mental issues being faced by EoE patients in daily life. The (long) road to an EoE diagnosis and onwards after can be a difficult journey, which also impacts on patients' mental and social health. However, the current literature still yields a significant gap on this important topic. Therefore, in **Chapter 7**, an observational cross-sectional study design was used to give more insights into the presence of mental distress among patients with EoE and its determinants (e.g., clinical and socio-demographic factors). Adult EoE patients were invited to complete standardized measures on anxiety/depressive symptoms (HADS) and general psychopathology (SCL-90-R). All scores were compared to general Dutch population norms. In total, 147 adult EoE patients were analyzed (response rate 71%). We observed that a considerable proportion (36%) of adult EoE patients suffers from mental distress (SCL-90-R Global Severity Index ≥ 80th percentile), with a compelling 3-fold risk of significant anxiety in those patients younger than 35 years. Since EoE mostly affects young adults, a proactive approach in the screening for and treatment of mental health disorders seems essential.

How individuals cope with the physical, social and mental burden that is linked to stressful life events (e.g., 'having a chronic illness') determines patients' health-related Quality of Life (HRQOL).[15] Since no studies were available on this relevant topic, we determined coping strategies and the degree to which different coping styles are related to (disease specific) HRQOL within a large cohort of 147 adults EoE patients (**Chapter 8**). EoE-patients were invited to complete standardized measures on general health-related quality of Life (Short Form-36 Health Survey (SF-36)) and coping strategies (Utrechtse Coping Lijst). Scores were compared to general population norms. The Adult Eosinophilic Esophagitis Quality of Life (EoE-QOL-A) measure was used to assess disease specific Quality of Life. We observed that Mental health-scores (SF-36) were significantly lower in EoE patients, whereas physical health-scores (SF-36) were similar to the general population. The subdomain "disease anxiety" (EoE-QOL-A; 'Issues related to having a chronic disease') was mostly affected and determinants were; female gender, younger age, severe clinical disease activity, higher number of food bolus extraction, and more recent EoE-diagnosis.

Moreover, we observed that less effective coping strategies are related to poor perception of general and disease specific HRQOL, particularly in males. This study emphasizes the importance of HRQOL being a key health outcome in daily EoE practice and research evaluation effect of interventions.

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DISCUSSION AND FUTURE PROSPECTS

EPIDEMIOLOGY AND PATHOPHYSIOLOGY

Eosinophilic esophagitis (EoE): a modern disease of the Western world?

Over a quarter century, the incidence of EoE in the Netherlands has expanded a 316-fold and continues to rise (**Chapter 2**). There still remains controversy on whether the overall dramatic rise of EoE frequency, in particular in developed countries, reflects true disease expansion by paralleling other increasing Western diseases (e.g., atopic morbidities, inflammatory Bowel disease (IBD)), or might be attributed to improved medical awareness and diagnostic methods. To answer this question, it first should be acknowledged that we did not assessed the actual disease awareness amongst clinicians in this thesis. However, in **Chapter 2** we observed that the rise in newly diagnosed EoE patients far outpaces any expansion in upper endoscopy with biopsy sampling, which is in line with multiple other studies in literature.[3,4,12] Although genetic predispositions are associated, this phenomenon indicates a prevalent role for environmental variations in disease manifestation. While progress has been made in understanding the role of genetics and the immune response, there is an emerging interest on the impact of 'Modern life' on the development and progression of EoE.

Early childhood is known to be important for immune maturation, thus early-life exposures originally shape the developmental susceptibility of the immune system.[5] It was hypothesized that 'modern hygienic condition' lead to less exposure to microbes during infancy, with subsequent increased sensitivity to allergic diseases.[6,7] Moreover, early-life events, e.g., formula feeding or Cesarean section, are considered to induce adverse effects on the microbiome.[8] Microbial dysbiosis may also arise from changes in environmental factors (e.g., food additives, genetic modifications) and a Western 'life-style' (i.e., limited physical activity, low in fibers and high saturated fats diets).[9-13] Moreover, the increasing prevalence of Gastroesophageal reflux disease (GERD) and reduced number of *Helicobacter Pylori* infections (i.e., polarizes towards T-helper (Th) type 1 immunity by that protecting against Th-2 induced EoE) in developed countries over the past two decades are both considered being relevant to the rapid surge of EoE.[14-16] Yet, it seems thus remarkable that the expanded use of acid-suppressant medication also parallels the emerging EoE incidence trends, by that connecting proton-pump-inhibitors (PPIs) as augmented factor (i.e., prevention of peptic digestion of food allergens, increased permeability of the gut and microbial dysbiosis) to EoE development.[17,18] A well-balanced microbiome plays a fundamental role in the development of the immune system, so

therefore the influence of the microbiota is essential to consider within the context of gastrointestinal (allergic) disorders, in particular EoE. Although progress in the microbiota research has been accelerating in the past years, literature still yields a significant gap in the understanding how alterations of the human microbiota influence esophageal tissue and whether dysbiosis contributes to inflammation in this organ. Hence, metagenomic sequencing, together with metatranscriptomics and metabolomics (i.e., multi-omics) can help to identify the functional relevance of bacterial gene expression, while also giving more insights into the mechanistic role of the microbiome in EoE. Taken together, putative environmental risk factors that might contribute to the development of this 'new Western disease' needs to be further elucidated, focusing on the possible preventive role of 'life-style' interventions, in particular in early-life.

DIAGNOSTICS

The (long) road to EoE diagnosis

Clinical symptoms are the main reason to perform upper endoscopy with biopsy sampling.[19] However, the process of EoE identification is complicated by various factors in practice, with a considerable delay between symptom onset and diagnosis.[20] Unfortunately, the association between EoE symptoms and biological disease activity is limited, so diagnosis and monitoring of disease requires the golden standard of upper endoscopy with biopsy sampling.[21] An invasive procedure, that either patients or clinicians may be reluctant to perform.[22] According to consensus guidelines, if EoE is suspected, not only esophageal but also biopsy specimens from the stomach and duodenum should be sampled in order to exclude other generalized eosinophilic gastrointestinal (GI) disorders, such as eosinophilic gastroenteritis or celiac disease. As is pointed out in this thesis, standard additional biopsies are not indicated in adult EoE patients without endoscopic abnormalities in the stomach and/or duodenum or suggestive symptoms (i.e., dyspepsia, abdominal pain, vomiting/nausea or diarrhea) and should be avoided in these cases (**Chapter 3**). Dispose of these unnecessary biopsy proceedings lowers health-care costs (i.e., reduced time and risk of complications) and promotes patients' acceptance of such procedures, which likely also reduces the risk of diagnostic delay.

EoE symptoms are often non-specific and may thus be under-recognized by patients and/or clinicians. Most patients have adapted their eating behaviors (e.g., taking smaller bites, chewing more carefully, eating slowly or drinking more water during meals) (**Chapter 8**) which hampers the recognition of dysphagia and other symptoms that may signal EoE.

Although dysphagia is the most common sign in EoE, it was observed in a population based study in the United States (US) that ~ 50% of dysphagia cases was not discussed with their clinicians.[23] Considering EoE symptoms gradually get worse over time, patients may not even be aware of those adapted eating strategies unless asked directly by the clinicians. Recently, also a novel 'non-dysphagia' related syndrome, expressed by an unpleasant or painful sensation occurring immediately after esophageal contact with specific foods or beverages was observed in EoE patients.[24,25] Although this 'food-induced immediate response of the esophagus' (FIRE) was theorized to derive from a different mechanism, a more detailed understanding of the pathogenic mechanism underlying this local reaction in the esophagus may improve the overall genesis of symptoms in EoE. Multiple factors contributing to its heterogeneous disease presentation are illustrating the importance of clinicians' awareness in order to avoid unnecessary diagnostic delay. Hence, it might be suggested that the calculated annual rates of newly diagnosed EoE patients presented in **Chapter 2** are even underestimated. Moreover, EoE is still rare in the world of general practitioners. Since general practitioners are the gatekeepers to hospital- and specialist care, it is essential for these specialists too to have basic understanding of EoE and its symptom presentation to increase disease identification, support their patients and refer them to the right services if required.

Non-invasive tests for diagnosis and disease monitoring

From the patients' perspective, there is a pressing need for non-invasive tests for diagnosis and disease monitoring, yet the question remains: are they ready for us? Multiple alternatives to (sedated) upper endoscopy were prompted for esophageal biopsy sampling, e.g., The esophageal string test, transnasal endoscopy, Cytosponge and biomarkers.[26,27] Although significant progress has been made in its development, more insights into whether these minimally-invasive tools could be used and implemented in practice are clearly needed. Given that patients being reluctant to undergo (multiple) upper endoscopies with biopsies, less invasive tests for EoE monitoring are urgently warranted. In addition to information on inflammation, identification of fibrosis in the lamina propria is important but requires deep-esophageal biopsies, which are not routinely sampled. Hence, identification of potential (serological) biomarkers to gain an objective measure of disease activity and severity would be very helpful. Therefore, the preliminary results of research on serum extracellular matrix (ECM) proteins as potential (prognostic) biomarkers for esophageal remodeling in EoE conducted by our research group are promising.[62]

MANAGEMENT

Precision medicine

Research into EoE mechanism and treatment has identified significant diversity at the cellular and clinical level, with newly defined EoE-endotypes that may serve as the foundation for a more personalized approach.[28] Stratification of EoE patients based on serological markers for different cellular processes (e.g., reflecting the process of fibrolysis) may potentially assist with a more efficacious therapy selection in future practice. However, to achieve a new degree of disease control by targeting traditional and biological therapies to particular EoE-endotypes, an in-depth characterization of patients' individual EoE signatures for future mechanistic studies are necessary.

Multiple consensus documents and guidelines have provided clinicians evidence-based frameworks for the management of EoE over the past decade.[29-32] Yet, many patients' still have unmet needs and significant disparities of adherence to guidelines for treatment choice and disease monitoring have been reported.[33-35] The ideal EoE-management strategy remains unclear, hence first-line treatment selection (i.e., PPIs, swallowed topical corticosteroids or dietary treatment) is a preference-sensitive choice and an optimal setting for shared decision making between patients and clinicians.[33,36] Data is scarce on this topic in EoE. Notwithstanding, little evidence suggests that shared decision making is practiced by most gastroenterologists, yet nearly ~ 50% of patients do not experience shared decision making in their perspective, which highlights a significant area of need in EoE.[39]

Tailored treatment: diet or medication?

At present, no topical corticosteroids have been approved by the the Food and Drug Administration (FDA), however, in 2017 the European Medicine Agency (EMA) authorized swallowed effervescent budesonide for the use in adult EoE.[40,41] Although swallowed steroids are generally safe and well-tolerated, patients' treatment barriers are; potential side effects (i.e., esophageal candidiasis ~ 3%) and preference of medication-free approach.[39] New medication and formulations (e.g., topical steroids) are being developed in response to unmet needs of a large proportion of patients, with novel therapeutic approaches directed at blocking the molecular inflammatory pathways that leads to EoE (**Chapter 4**). For patients, the major advantage of diet-based treatment is the possibility to identify the root cause of their disease, by doing so, they are able to avoid these EoE culprits instead of relying on daily medication. Efficacy of elimination diets were previously observed to parallel the number of restricted foods, yet, extensive diet restrictions with

risk of inadequate food intake (e.g., low calories, nutritional deficiencies) and subsequent need for repetitive endoscopies with biopsy sampling hampers patients' acceptability in daily life. In **Chapter 5**, we observed that the overall (complete) histological remission rate (< 15 *eos/hpf*) of ~ 38% after 6 weeks dietary restriction of milk, wheat, eggs and soy (Four food elimination diet (FFED) or combined dietary approach of FFED + amino acid-based formula (AAF)) was lower than expected based on previous studies (54% - 64%).[42,43] A recent multicenter trial in both pediatric and adult EoE also observed similar response rates, suggesting a potential bias in previous cohort studies.[44,45] In this multicenter study by Kliewer *et al.*, comparison of 1FED (milk) to elimination of six foods in adult EoE showed that histological response (< 15 *eos/hpf*) after 6 weeks was similar between groups (34% vs. 40%).[44] Taken this together, step-up- instead of top-down empiric elimination is currently accepted as the initial dietary approach for EoE, with by far cow's milk followed by wheat/gluten and egg being observed in most studies as common EoE triggers in patients of all ages.[46] Evidence suggests that the list of identified culprits in each setting might be related to regular consumption of local- foods e.g., legumes in Mediterranean countries.[47,48] Supported by current EoE-research and our findings from clinical practice, elimination of cow's milk from the daily food pattern (1FED) might be suggested as first-step approach in pediatric and adult EoE patients in the Netherlands. The specific antigens in cow's milk remains unclear, yet modification of its protein content (e.g., baked- or hydrolyzed milk) was recently found to be tolerated (i.e., < 15 *eos/hpf*) after reintroduction in patients having milk-induced EoE.[49,50] Therefore, prospective studies on the efficacy on initial milk elimination diets in EoE and its follow-up (e.g., are low amounts of food allergen exposure tolerated?) are definitely warranted.

IMPACT ON PATIENTS' DAILY LIFE

Understanding the mental and psychosocial burden of EoE

Illustrated by practice, the (long) journey to EoE diagnosis and from then on can be a difficult road. In **Chapter 7** and **Chapter 8**, the likely unseen or unspoken mental and psychosocial issues being faced by EoE patients were uncovered. In this thesis, it was confirmed that EoE has a substantial negative impact on patients' mental HRQOL, particularly in males (vs. general Dutch population norms). Moreover, significant signs of mental distress were observed in 36% patients of our EoE sample, of which 57% patients denied having any mental problems. From these results it is clear that a considerable proportion of adults suffers from mental distress, with a striking 3-fold risk of significant anxiety being observed in those patients younger than 35 years.

Female gender is being associated with poor HRQOL outcomes in multiple chronic health populations (e.g., asthma, rheumatoid arthritis).[51,52] However, based on the observations in this thesis, it could be suggested that particularly (young) males with EoE are more at risk of impaired HRQOL, specifically related to anxiety for food impaction. Considering that males are more prone to develop strictures, they are also more exposed to potential anxiety triggers such as impaction with need for upper endoscopy with food bolus extraction.[53]

From the patients' perspective, improvement of EoE symptoms and 'quality of life' are being prioritized as paramount treatment goals. Based on our findings of 'somatization' being the most intense symptom in our EoE-sample (**Chapter 7**), we hypothesized that somatization of esophageal symptoms in severe distressed EoE patients may also help to explain additional heterogeneity in symptom severity, once variation in biological disease activity has already been taken into account e.g., refractory dysphagia despite endoscopic or histological remission. Our concept of this so-called 'somatoform-type behavior' in EoE was further addressed in a recent study by Taft *et al.*, in which EoE patients were observed to have increased hypervigilance (i.e., heightened focus on physical symptoms) and symptom-specific anxiety such as swallowing anxiety, both being related to worst reported EoE symptoms and poor HRQOL.[54] Hence, what starts as a protective and instinctual response to a perceived threat - esophageal pain - can become a conditioned attentional response in the gut - brain axis. Substantial impacts on eating and food-specific anxieties emerging into a newly classified eating disorder; Avoidant/ Restrictive Food Intake Disorder (ARFID)(i.e., disturbed feeding patterns, highly selective eating habits), being already reported in pediatric EoE and other digestive diseases.[55,56]

Mental health care and psychosocial support in EoE

As pointed out by this thesis, a proactive approach towards the screening for- and treatment of psychological symptoms in EoE practice seems essential. In **Chapter 8** we observed that less effective coping strategies are related to poor perception of general and disease specific HRQOL, particularly in males. Cognitive behavioral therapy (CBT) is well-described in other (chronic) disorders and can be implemented to aid patients in gaining insights into how stressful events, thought patterns, emotions and psychological responses interact together. Hypervigilance and anxiety CBT in the form of relaxation training, cognitive restructuring and behavioral modification has been adapted for the use of other functional esophageal conditions, such as functional dysphagia and non-cardiac chest pain.[57] Hence, referral to a (medical) psychologist/psychotherapist for CBT

could be helpful to support 'self-management' and thus improve EoE patients' perception of their mental HRQOL. Self-management gives patients an active role in health-care, by that giving them more control of their illness, which seems to be part of a broader shift in health towards shared responsibility and decision making (i.e., shared decision making). Patients' disease-related knowledge levels are known to affect self-management and structured educational programs are associated with increased treatment adherence in other chronic conditions.[58,59] This relatively 'new Western disease' still yields a scarcity of patients' information-resources, so therefore provision of detailed patients' education in practice seems to be of importance.

Managing clinical disease outcomes

In the context of (industry-sponsored) clinical trials, further refinement of tools to assess disease outcomes are important steps towards improvement of medical or dietary treatment development, ultimately promoting patients' HRQOL. A collaboration of specialists has developed a Core Outcome Set, including several state-of-the-art outcomes e.g., eosinophilic esophagitis histology scoring system (EoEHSS) and the Endoscopic Reference Score (EREFS) that could be used in clinical trials to increase the quality of data extraction.[61] Although multiple symptom based indices have been developed in EoE, none of them were authorized by the FDA. Development of an accurate Patient Reported Outcome (PRO) measure according to- and approved by the FDA (Guidelines) to assess EoE symptom severity should thus be prompted for future EoE-research. Notwithstanding, this progress will likely be challenged by multiple factors that affect patients' symptom reporting, such as 'adapted eating behaviors', FIRE and the so-called 'somatoform-type behaviors'.

Multidisciplinary personalized approach

The clinical heterogeneity and complexity of this chronic disease implies the need for a multidisciplinary approach in practice. Apart from a (pediatric) gastroenterologist; an allergist should be involved to identify other coexisting atopic comorbidities (e.g., atopic dermatitis, Immunoglobuline (Ig) E-mediated food allergy) in order to provide adequate treatment. Moreover, nutritional monitoring by a (specialized) nutritionist of patients on dietary treatment to guarantee sufficient intake (e.g., nutritional deficiencies) and lower risk of diet errors (e.g., mistakes of food label reading) is important to minimize the impact of such restrictive food pattern on daily (social) life. Provision of mental care and psychosocial support by a psychologist should also be an integral part of EoE-

management, as this appears to be still an underexposed topic in EoE. Finally, involvement of a well-informed general practitioner is essential and has also an important role in the follow-up of EoE care in the maintenance treatment setting.

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ADDENDUM

NEDERLANDSE SAMENVATTING

Eosinofiele oesofagitis (EoE) is een steeds vaker voorkomende chronische, immuungemedieerde ontstekingsziekte van de slokdarm. Klinisch karakteriseert het ziektebeeld zich door symptomen van 'oesofageale dysfunctie', zoals dysfagie en voedselimpacties. Het histologisch beeld kenmerkt zich door infiltratie van meer dan 15 eosinofiele granulocyten per microscopisch gezichtsveld onder sterke vergroting ('high power field') in de slokdarmmucosa.[1,2,3] Begin jaren '90 werd EoE voor het eerst als unieke ziekte-entiteit beschreven.[4,5] Sindsdien wordt er wereldwijd - met name in het laatste decennium - een sterke toename van het aantal nieuwe EoE-patiënten per jaar gezien. Het ziektebeeld is inmiddels uitgegroeid tot een belangrijke hoofdoorzaak van klachten in het bovenste deel van het maag-darmkanaal bij kinderen en volwassenen.

Genetische aanleg en (niet-)allergische omgevingsfactoren spelen gezamenlijk een rol bij het ontstaan en de ontwikkeling van deze atopische ziekte.[6,7] Door wetenschappelijk onderzoek is er inmiddels aangetoond dat er sprake is van een T-helper type 2 cellen (Th2) geassocieerde immuunreactie, die voornamelijk wordt uitgelokt door voedselallergenen en mogelijk ook inhalatieallergenen. De huidige behandeling bestaat uit: protonpompremmers ('Proton-Pump-Inhibitors'), orale topicale glucocorticosteroïden, eliminatie van voedselallergenen (dieet) en slokdarmdilatatie.[8] EoE komt driemaal vaker voor bij mannen dan bij vrouwen; waarbij in h*et al*gemeen de diagnose het meest wordt gesteld in de leeftijdscategorie 20 - 40 jaar.[9] De afgelopen 25-jaar heeft het 'relatief nieuwe' EoE-onderzoeksveld belangrijke ontwikkelingen doorgemaakt, waarbij er onder meer inzicht is verkregen in het natuurlijke ziektebeloop, het werkingsmechanisme, de diagnostiek en effectiviteit van verschillende behandelingen. Ondanks deze vooruitgang in kennis over de ziekte zijn er rondom dit sterk toenemende ziektebeeld nog talloze belangrijke vraagstukken die in de toekomst zullen moeten worden onderzocht.

EPIDEMIOLOGIE EN PATHOFYSIOLOGIE

In **Hoofdstuk 2** van dit proefschrift staan de uitkomsten beschreven van een epidemiologische studie die is uitgevoerd met behulp van gegevens uit het Pathologisch Anatomisch Landelijk Geautomatiseerd Archief (PALGA). Binnen een tijdsbestek van 25-jaar hebben wij in totaal 4061 EoE-patiënten (71% man, gemiddelde leeftijd 37,9 jaar) kunnen identificeren, waarvan 639 (16%) kinderen (< 18 jaar). De incidentie van EoE steeg van 0,01 in 1995 tot 3,16 in 2019 per 100.000 inwoners. Een controversiële vraag blijft of de dramatische stijging van de jaarlijkse incidentie van EoE een reële toename van dit ziektebeeld betreft,

of dat dit kan worden verklaard door betere (h)erkenning en diagnostische middelen. Deze studie toont in **Hoofdstuk 2** aan dat in de afgelopen 25-jaar de incidentie van EoE met een factor 300 is toegenomen. In datzelfde tijdsbestek is het aantal uitgevoerde endoscopieën met afname van biopten uit de slokdarm slechts verdrievoudigd. Op basis van dit onderzoek kunnen wij dus vaststellen dat de incidentie van EoE in Nederland sterker is toegenomen dan het aantal verrichte endoscopieën de afgelopen 25 jaar. Bovendien lijkt de jaarlijkse incidentie van EoE fors door te blijven stijgen. Deze aanzienlijke groei loopt gelijk op met de zogenoemde wereldwijde 'allergie-epidemie'.[10,11] Dit fenomeen suggereert, naast genetische aanleg, een belangrijke rol voor (niet)allergische omgevingsfactoren bij de ontwikkeling van EoE. Meer onderzoek is dan ook noodzakelijk naar de variatie in omgevingsfactoren die mogelijk ten grondslag liggen aan de pathogenese van EoE.

DIAGNOSTIEK

Bij een klinische verdenking op EoE is een endoscopie met afname van biopten uit de slokdarm geïndiceerd. Volgens de huidige richtlijnen wordt, ter uitsluiting van andere eosinofiele ontstekingsziekten van het maag-darmkanaal zoals 'eosinofiele gastro enteritis' of 'coeliakie', geadviseerd om naast biopten uit de slokdarm, ook maag en de dunne darmbiopten af te nemen. Het wetenschappelijke bewijs voor het nut van deze aanvullende maag- en dunne darmbiopten is echter afwezig. In **Hoofdstuk 3** staat een retrospectief dossieronderzoek beschreven, waarbij wij door middel van standaard (elektronische) data-extractie klinische, endoscopische en histologische gegevens hebben verzameld. Dit studie-cohort bestond uit 93 volwassen patiënten (71% man, leeftijd 36,4 jaar) met onbehandelde EoE (≥ 15 eosinofiele granulocyten per 'high power field', *eos/hpf*), waarbij tijdens een endoscopie naast biopten uit de slokdarm, ook maag- en dunne darmbiopten werden afgenomen. Bij 28 (30%) patiënten leverde de afname van extra maag- en dunne darmbiopten een aanvullende histologische diagnose op. Het merendeel van deze diagnoses bestond echter uit niet-specifieke gastritis of *Helicobacter Pylori* gastritis, zonder relevantie voor de behandeling en/of impact op de (eerdere) diagnose EoE. Eén (1%) patiënt, waarbij eerder EoE werd vastgesteld, voldeed ook aan de klinische en histologische criteria voor de diagnose eosinofiele gastro-enteritis. De diagnostische opbrengst voor een relevante andere eosinofiele ontstekingsziekte van het maag- darmkanaal in dit cohort betrof 3,6% en bleek dus zeer klein te zijn. De resultaten uit **Hoofdstuk 3** kunnen mogelijk in de toekomst bijdragen aan vernieuwde aanbevelingen ten aanzien van het (routinematig) afnemen van maag- en dunne darmbiopten tijdens een endoscopie, indien er een klinische verdenking bestaat op EoE.

BEHANDELING

In het volgende deel van dit proefschrift gaan wij dieper in op de verschillende behandelingsopties van dit allergische ziektebeeld, waaronder (chronische) medicatie en eliminatie van voedselallergenen. **Hoofdstuk 4** geeft een uitgebreid overzicht van de medicamenteuze therapieën die reeds zijn onderzocht, zoals protonpompremmers en orale topicale glucocorticosteroïden. Daarnaast worden er andere veelbelovende therapeutische opties besproken die specifiek voor EoE in ontwikkeling zijn, zoals verschillende monoklonale antilichamen gericht tegen IL-4/IL-13 (dupilumab), IL-13 (cendakimab = RPC4046) en de IL-5-receptor (benralizumab).

Inmiddels is overtuigend gebleken dat voedselallergenen in een zeer belangrijke mate bijdragen aan de allergische ontstekingsreactie. De identificatie en vervolgens structurele eliminatie van uitlokkende voedselgroepen, zoals zuivel en gluten heeft dan ook een rol bij de behandeling van EoE. Het voordeel voor patiënten van deze aanpak is dat zij eventueel de oorzaak van hun allergie kunnen achterhalen. Dit geeft patiënten de mogelijkheid om, in plaats van levenslang noodzakelijke medicatie te gebruiken, deze specifieke voedselgroepen te weren uit hun dagelijkse voedingspatroon. Een elementair dieet bestaat uit hypoallergene drinkvoeding op basis van aminozuren (AAF), waarbij deze drinkvoeding voor een periode van 4 - 6 weken als enige voedingsbron fungeert. Uit klinisch onderzoek is gebleken dat een elementair dieet zeer doeltreffend is bij EoE-patiënten van alle leeftijden (85% - 95% bereikt histologische ziekte remissie; < 15 **eos/hpf**).[12–17] Helaas blijkt dit dieet in de praktijk echter geen geschikte behandelingsoptie voor de lange termijn. De monotone smaak en het gebrek aan vast voedsel maakt dat dit dieet lastig vol te houden is. In de klinische praktijk is empirische eliminatie van (de meest voorkomende) EoE-uitlokkende voedselgroepen een beter te aanvaarden dieet strategie. Eliminatie van 4 ('Four-food elimination diet', FFED) of 6 ('Six-food elimination diet', SFED) voedselgroepen wordt het meest frequent toegepast in de klinische praktijk. In Hoofdstuk 5 onderzochten wij of toevoeging van AAF aan een standaard eliminatiedieet (FFED; zuivel, tarwe/gluten, soja en ei) de therapietrouw en gemak rondom dieet zou kunnen vergroten, en daarmee ook de effectiviteit van de dieetbehandeling.

Patiënten werden gerandomiseerd (1:1) in 2 groepen, waarbij één groep werd behandeld met het standaard FFED en de andere groep met een combinatie dieet bestaande uit een FFED met toevoeging van AAF voor 30% van de dagelijkse energiebehoefte (FFED + AAF). Patiënten (60% man, gemiddelde leeftijd 34,5 jaar) werden gerandomiseerd

naar een FFED-groep (n = 20) en een FFED + AAF-groep (n = 21); 40 patiënten voltooiden het dieet na 6 weken. Complete histologische remissie (< 15 *eos/hpf*) werd bereikt in respectievelijk 48% van de patiënten die behandeld werden met het FFED + AAF vs. 25% van de patiënten behandeld met enkel het FFED na 6 weken. Dit verschil tussen beiden groepen was echter niet significant. Daarnaast werd in beiden groepen een significante afname gezien van het hoogst aantal eosinofielen vanaf de start van het dieet tot na 6 weken. Deze afname binnen het tijdsbestek van 6 weken was echter niet significant verschillend tussen beiden groepen. Symptomen en endoscopische kenmerken namen significant af in beiden groepen na 6 weken behandeling, hierbij was geen verschil in afname tussen beiden groepen te zien. Specifiek aan EoE gerelateerde kwaliteit van leven scores lieten een significante verbetering zien in de patiëntengroep die werd behandeld met het FFED + AAF, maar dit werd niet in de FFED-groep waargenomen na 6 weken. Deze bevindingen zouden kunnen suggereren dat een gecombineerde dieet strategie van FFED met toevoeging van AAF voordelen kan hebben ten opzichte van een FFED alleen.

Er wordt verondersteld dat eliminatie van voedselallergenen ingrijpt op het 'adaptieve immuunsysteem', doordat het de door antigenen aangedreven T-cel immuunrespons onderbreekt. Hierbij is er dus geen sprake van wijzigingen van signaalroutes of apoptose (geprogrammeerde celdood), zoals meestal volgt na behandeling met glucocorticosteroïden of monoklonale antilichamen. Toch zijn er nog weinig onderzoeksgegevens bekend over de effecten van dieetbehandeling op de genexpressie patronen bij (volwassen) patiënten met EoE, met name in relatie tot klinische kenmerken. In **Hoofdstuk 6** staan de resultaten beschreven van een analyse van gegevens die zijn verzameld tijdens een gerandomiseerd onderzoek, waarbij volwassen patiënten met actieve EoE (≥ 15 *eos/hpf*) werden behandeld met 2 verschillende vormen van een eliminatiedieet (FFED en FFED + AAF)(details staan beschreven in **Hoofdstuk 5**). De mate van expressie van 10 geselecteerde genen werd gemeten (qPCR) voor- en na dieetbehandeling, en vervolgens vergeleken met klinische gegevens (eosinofielen, symptomen en endoscopische kenmerken). In totaal werden 40 patiënten, waarvan 60% man met een gemiddelde leeftijd van 34,5 jaar na 6 weken dieetbehandeling in het onderzoek geïncludeerd en geanalyseerd (FFED en FFED + AAF samengevoegd). Er werd een significante afname gezien van het aantal eosinofielen, symptomen en endoscopische kenmerken vanaf de start van het dieet tot na 6 weken behandeling (allen; $p < 0,05$). Deze studie toont aan dat dieetbehandeling meerdere signaalroutes beïnvloedt, waarbij significante veranderingen van genexpressiemarkers voor inflammatie (IL-5, IL-13, TSLP, POSTN, CPA-3, CCL-26 en IL-10), epitheliale

barrièrefunctie (DSG-1 en CAPN-14) en fibrose (TGF- β) na 6 weken dieet werden gezien (allen; $p < 0,05$). Daarnaast werd in deze studie geobserveerd dat up-regulatie van CAPN-14 en lagere levels van DSG-1 beiden geassocieerd zijn met een 'fibrotisch fenotype' (d.w.z. endoscopische aanwezigheid van 'concentrische ringen' en 'stricturen' in de slokdarm). Up-regulatie van IL-10 werd in dit onderzoek geassocieerd met een 'voedselimpactie fenotype' (d.w.z. aanwezigheid van symptomen van voedselimpactie). Deze bevindingen suggereren sterk dat dieetbehandeling is geassocieerd met omvangrijke transcriptionele veranderingen op epitheel niveau van de slokdarm in volwassen patiënten met EoE.

IMPACT VAN DE DIAGNOSE EOE OP HET DAGELIJKSE LEVEN VAN PATIËNTEN

Het pad van- en naar de diagnose EoE kan lastig en langdurig zijn, en beïnvloedt naast de fysieke ook de sociale en mentale gezondheid van deze overwegend jonge patiëntenpopulatie. Het huidige EoE onderzoeksveld omvat echter tot op heden weinig studies met betrekking tot dit relevante onderwerp. Het laatste deel van dit proefschrift beschrijft dan ook de uitkomsten van een groot EoE cohort, waarbij aandacht wordt besteed aan de sociale en mentale impact van 'de diagnose EoE' op het dagelijkse leven van volwassen patiënten. In **Hoofdstuk 7** hebben wij de aanwezigheid van (ernstige) psychische klachten onderzocht met behulp van gestandaardiseerde vragenlijsten. Volwassen EoE patiënten werden uitgenodigd om vragen te beantwoorden over angst/depressieve klachten (Hospital Anxiety and Depression Scale (HADS)) en algemene lichamelijke en psychische klachten, in het kader van een screening op psychopathologie (SCL-90-R). De scores werden vergeleken m*et al*gemene normscores van de Nederlandse bevolking. Daarnaast hebben wij in **Hoofdstuk 7** onderzocht welke factoren voorspellend zijn voor psychische klachten, zoals klinische en sociaal-demografische kenmerken. In totaal werden de gegevens van 147 volwassen patiënten (61% man, gemiddelde leeftijd 43 jaar) met EoE geanalyseerd (responspercentage 71%). Met dit onderzoek hebben wij aangetoond dat een aanzienlijk aantal EoE patiënten (36%) ernstige psychische klachten ervaart (SCL-90-R; Globale psychische belasting score ≥ 80e percentiel). Daarnaast toont onze studie aan dat er sprake is van een driemaal verhoogd risico op significante angstklachten bij EoE-patiënten onder de 35 jaar. Aangezien de diagnose EoE het meest wordt gesteld in de leeftijdscategorie tussen de 20 en 40 jaar, is een proactieve benadering in relatie tot de screening op- en behandeling van psychische klachten in deze patiëntenpopulatie van essentieel belang.

Hoe mensen omgaan met lichamelijke, sociale en mentale gevolgen van een langdurige blootstelling aan stressvolle levensgebeurtenissen, zoals 'het hebben van een chronische ziekte', is bepalend voor aan gezondheid gerelateerde kwaliteit van leven.[18] Gezien het ontbreken van onderzoek in relatie tot dit onderwerp binnen de huidige literatuur, hebben wij in **Hoofdstuk 8** aandacht besteed aan de verschillende coping strategieën in volwassen EoE patiënten. Daarnaast hebben we ook gekeken naar de mate waarin verschillende coping stijlen zijn geassocieerd met (ziektespecifieke) kwaliteit van leven. Patiënten werden gevraagd om gestandaardiseerde vragenlijsten in te vullen met betrekking tot aan hun gezondheid gerelateerde kwaliteit van leven (Short Form-36 Health Survey) en coping strategieën (Utrechtse Coping Lijst). De gemiddelde scores werden vergeleken met de normgroep van de algemene bevolking. Ziektespecifieke kwaliteit van leven werd met behulp van de 'Adult Eosinophilic esophagitis Quality Of Life' (EoE-QOL-A) vragenlijst gemeten. In deze studie zagen wij dat de mentale gezondheid scores (SF-36) significant lager zijn bij EoE patiënten, terwijl scores voor de fysieke gezondheid (SF-36) gelijk zijn in EoE patiënten in vergelijking met de algemene bevolking. Met betrekking tot ziektespecifieke kwaliteit van leven was het subdomein 'Disease Anxiety' (EoE-QOL-A; 'zorgen in relatie tot het hebben van een chronische ziekte') het meest aangedaan in het EoE-cohort. Voorspellende factoren zijn; vrouwelijk geslacht, jongere leeftijd, ernstige klinische ziekteactiviteit, groter aantal endoscopische voedselbrok extracties en een meer recente EoE-diagnose. Bovendien zagen wij dat minder effectieve coping strategieën, met name bij mannen, gelinkt zijn aan verminderde mentale (ziektespecifieke) kwaliteit van leven. Deze onderzoeksresultaten benadrukken dan ook de relevantie van (ziektespecifieke) kwaliteit van leven als belangrijke uitkomstmaat voor de klinische praktijk en het wetenschappelijk onderzoek.

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PhD PORTFOLIO

Name PhD student: Willemijn E. de Rooij

PhD-period: July 2017 - August 2020

Supervisor: Prof dr. Albert J. Bredenoord

Co-supervisor: Dr. Marijn J. Warners

PhD training	Year	Workload (ECTS)
General Courses		
Basic course legislation & organization of clinical research (BROK)	2018	1.0
Practical Biostatistics	2018	1.1
Scientific writing course	2018	1.5
Advanced Immunology PhD Course	2019	2.9
Seminars, workshops and master classes		
Bi-weekly seminars in gastroenterology	2017 - 2021	1.5
Bi-weekly clinical motility meeting	2017 - 2021	1.5
Gut club	2017 - 2021	1.0
AG&M PhD Retreat	2018	1
Oral presentations		
United European Gastroenterology Week (2x)	2020	1.0
Digestive Disease Days (3x)	2017, 2020	1.5
AG&M PhD-retreat (1x)	2018	0.5
NVK-congres (1x)	2019	0.5
Poster presentations		
Digestive Disease Week (1x)	2020	0.5
United European Gastroenterology Week (1x)	2020	0.5
Attended (Inter)national conferences		
Digestive Disease Week (3x)	2018, 2019, 2020	1.5
United European Gastroenterology Week (2x)	2019, 2020	1.0
Federation of Neurogastroenterology and Motility Meeting	2018	1.0
Digestive Disease Days (2x)	2017, 2020	1.0
Amsterdam Live Endoscopy	2018, 2019, 2020	1.5
Teaching		
Tutoring		
Marielle Barendsen, Master Thesis	2020	1.0

ABOUT THE AUTHOR

Willemijn E. de Rooij was born in Bunnik, the Netherlands on the 24th of January in 1990. She grew up together with her younger brother Wouter and her parents Jos and Marie-José. In 2008, after finishing her secondary education at the St. Bonifatius College in Utrecht she started studying Psychobiology at the University of Amsterdam. Despite the interesting courses there, a medical career was still how she envisioned her future. She was successfully admitted to study Medicine at the University of Groningen in 2009. During her studies, she was member of the Committee of the Federation of Medical students (University of Medical Center Groningen and Deventer Hospital) and studied abroad in Uganda during her medical internship. After her clinical rotations in Deventer, she moved to Amsterdam and met the driven researchers dr. Marijn Warners and Prof. dr. Arjan Bredenoord, who supervised her during her master thesis on Eosinophilic Esophagitis (EoE), at the department of Gastroenterology and Hepatology, location AMC of the Amsterdam University Medical Centers (UMC). After her final rotation at the department of Gastroenterology and Hepatology at the St. Antonius Hospital Nieuwegein, she was registered as a medical doctor. In July 2017, Willemijn started a PhD-program under the supervision of Arjan Bredenoord and from then on, she worked as a clinical researcher focusing on multiple (clinical) EoE studies. During this period, she was also active as member of the working council of the AMC Medical Research BV and worked as a freelance medical examiner.

After a period of three years of fulltime EoE-research, she took up a position as resident Geriatric Medicine, Zorgbalans (Me-Doc) in January 2021. Meanwhile, she continued her research work and combined her clinical residency with (practical) guidance and personal development coaching of medical doctors at Me-Doc. In March 2022, Willemijn enthusiastically started her training as a General Practitioner (GP) at Amsterdam UMC, location VUmc. She worked as GP in training under supervision of drs. Jorrit van Lennep and drs. Anita Meiland (Huisartsenpraktijk Overtoom) while finishing her PhD thesis in the winter of 2022. In her free time, Willemijn enjoys sports (e.g., spinning, running), being creative, loves cooking, hosting dinners and spending time with family and friends.

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