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Classification of patients with esophageal eosinophilia by patterns of sensitization revealed by a diagnostic assay for multiple allergen-specific IgEs

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Abstract

Background

Eosinophilic esophagitis (EoE) is considered to be an immunoglobulin E (IgE)-mediated allergic disorder. Our goal was to examine IgE-mediated allergic sensitization patterns in patients with esophageal eosinophilia (EE).

Methods

We enrolled subjects with EE who underwent evaluation with a diagnostic panel to document multiple allergen-specific IgEs. Statistically significant groups were identified by cluster analysis. We also defined allergens based on their characteristics including outdoor, indoor, plant, and animal allergens.

Results

We classified patients with EE into 3 distinct groups, including cluster 1 (n = 62) who were minimally sensitized to most allergens except pollen and house dust, cluster 2 (n = 30) who were hypersensitized to outdoor and plant allergens, and cluster 3 (n = 15) who were hypersensitized to most allergens, most notably to indoor and animal allergens. Dysphagia reported among those in clusters 1, 2, and 3 at 35.5%, 46.7%, and 73.3%, respectively, ($p = 0.028$) and EoE endoscopic reference scores (EREFS) at 3.0, 6.0, and 8.0, respectively ($p < 0.001$) differed significantly between the 3 clusters. Those in cluster

3 had significantly higher prevalence of dysphagia (35.5% *vs.* 73.3%, $p = 0.030$), and higher EREFS with respect to rings (0.3 *vs.* 0.9, $p = 0.003$) and strictures (0.0 *vs.* 0.13, $p = 0.011$) compared to those in cluster 1.

Conclusions

IgE-mediated allergic sensitization patterns are associated with clinical features of patients with EE. Use of a diagnostic panel that detects multiple allergen-specific IgEs can help to explain the heterogeneous phenotype of this patient cohort.

Keywords: Eosinophilic esophagitis, Immunoglobulin E, Allergens, Sensitization patterns, Diagnostic panel

Abbreviations: BMI, Body mass index; EE, Esophageal eosinophilia; EoE, Eosinophilic esophagitis; EPZ, Esomeprazole; EREFS, Eosinophilic esophagitis endoscopic reference score**;** HPF, High-power field; IgE, Immunoglobulin E; LPZ, Lansoprazole; P-CAB, Potassium-competitive acid blocker; PPI, proton pump inhibitor; RPZ, Rabeprazole; Th2, T helper type 2; TSLP, Thymic stromal lymphopoietin; VPZ, vonoprazan; WBC, Peripheral white blood cell count

Introduction

Eosinophilic esophagitis (EoE) is an immune and allergen-mediated disorder that is characterized by eosinophil infiltration into the esophageal epithelium; it is also considered to be immunoglobulin E (IgE)-mediated, analogous to findings associated with bronchial asthma and atopic dermatitis [1, 2]. Patients with esophageal eosinophil infiltration are diagnosed with esophageal eosinophilia (EE) if an esophageal biopsy includes ≥ 15 eosinophils per high-power field (HPF); EE includes both symptomatic EoE which can be associated with dysphagia and chest pain as well as asymptomatic EE [3].

Molecular biological techniques have revealed that EoE is an eosinophil-predominant disorder characterized by a T helper type 2 (Th2) cytokine profile similar to that observed in association with other allergic disorders including bronchial asthma and atopic dermatitis. Acid and allergen-induced damage to the esophageal epithelium can trigger a Th2 immune response that results in the production of thymic stromal lymphopoietin (TSLP) and eotaxin-3. This response promotes influx of inflammatory eosinophils, mast cells, basophils, and lymphocytes into the esophageal mucosa [4-8], Th2-mediated inflammation also promotes epithelial and subepithelial remodeling with loss of barrier function, fibrosis, angiogenesis, and smooth muscle hypertrophy; these

features in turn promote esophageal stiffness, esophageal dysfunction and clinical symptoms including dysphagia and food impaction [9, 10]. In addition, pathways involving Th2 cytokines, including those mediated by interleukin (IL)-4 and IL-13, both key regulators of EoE, are similar to those involved in other IgE-mediated disorders [11, 12]. IL-4 mainly activates B-cell class switching which ultimately results in the synthesis and release of IgE in patients diagnosed with EoE [13]. Whether IgE plays an active role in the pathogenesis of EoE or serves only as a marker of disease pathogenesis remains unknown [14, 15]. Of note, pediatric patients with IgE-mediated food allergies are 100-times more likely to develop EoE than are members of the general public [16].

Clinically-significant allergen sensitization patterns have been identified in patients diagnosed with IgE-mediated allergic disorders such as bronchial asthma and seasonal allergic rhinitis. These patterns were identified using a multi-plex diagnostic to detect allergen-specific serum IgEs [17]. While the pathogenesis of EoE involves IgE-mediated allergic reactions, the precise patterns of allergic sensitization are not yet clear. In this study, our goal was to identify allergic sensitization patterns of patients diagnosed with EE using a diagnostic panel of multiple target antigen/allergens and to identify relationships between allergen patterns and clinicopathological features associated with EE.

Methods

Study design and participants

We conducted a single-center retrospective observational study. We enrolled patients diagnosed with EE who underwent diagnostic testing at the Osaka City University Hospital and between April 2016 and April 2019. In addition, patients with no history of obvious allergic disease who underwent a diagnostic test at Osaka City University Hospital between April 2016 and October 2018 were stratified as the non-allergic control group. Patient's serum samples were tested using View Allergy 39® to detect the presence of serum IgEs that target one or more specific antigens in a panel of multiple allergens (Thermo-Fisher Scientific Co. Ltd., Tokyo, Japan) [18-20]. EE was diagnosed using standard diagnostic criteria for EoE, including typical endoscopic findings such as mucosal edema (edema), esophageal rings (rings), white exudates or plaques (exudates), linear furrows (furrows), strictures, and ≥ 15 eosinophils per HPF in an esophageal biopsy specimen according to the 2017 United European Gastroenterology Guidelines [3]. In patients with EE, mucosal eosinophilia was restricted to the esophagus. Patients with obvious findings in the stomach and duodenal endoscopic examinations and severe eosinophil infiltration (≥20 eosinophils per HPF) on mucosal biopsy were excluded from

this study. Not all patients underwent mucosal biopsy of the entire gastrointestinal tract, including the stomach, duodenum, jejunum, and ileum. Demographic information was obtained from medical records, including the diagnostic assay inspection season, observation period, patient age, sex, body mass index (BMI), current smoking status (presence/absence), current drinking status (presence/absence), any allergic comorbidities including food allergy, bronchial asthma, seasonal allergic rhinitis, perennial allergic rhinitis, and/or atopic dermatitis, and medications for EoE. The observation period was defined as the date of the diagnostic assay or the date of consultation immediately before the assay to the date of the last consultation. We also obtained information on blood examinations such as peripheral white blood cell count (WBC), peripheral eosinophil count and percentage, peripheral basophil count and percentage, total serum IgE titer, and endoscopic findings including reflux esophagitis, atrophic gastritis and diagnosis of EoE. We evaluated endoscopic findings characteristic of EoE according to the eosinophilic esophagitis endoscopic reference score (EREFS). This scoring system features five endoscopic criteria including edema (0–2), rings (0–3), exudates (0–2), furrows (0–2), and strictures (0–1) [21]. We calculated a total EREFS score by adding the scores for each feature in both the distal and proximal or mid-esophagus [22]. The study protocol was approved by the Ethics Committee of the Osaka

City University Graduate School of Medicine (September 2018, Protocol number 4141 and September 2020, protocol number: 2020–151) and was performed in accordance with the principles of the Declaration of Helsinki.

Statistical grouping of allergens

Using the class values determined by the View Allergy 39® system, a two-stage cluster analysis (Ward's minimum variance method followed by the K-means method) was performed to classify the patients with EE into three clusters [23]. We defined allergens included in View Allergy 39® assay based on their characteristics. Cocksfoot, ragweed, mugwort, timothy, Japanese cedar, Japanese cypress, gray alder, and common silver birch were defined as outdoor allergens (i.e., pollens). House dust mite, house dust 1, cat dander, dog dander, cockroach, moth, latex, *Candida albicans*, *Alternaria alternata*, *Aspergillus fumigatus*, and *Malassezia spp*. were defined as indoor allergens (i.e., house dust, mold, and latex). Cultivated wheat, soybean, rice, sesame seed, buckwheat, peanut, apple, kiwi and banana were defined as plant allergens (i.e., grains, beans, nuts, and fruit). Tuna, salmon, chub mackerel, shrimp, crab, milk, pork, beef, chicken, egg white, and ovomucoid were defined as animal allergens (i.e., fish, crustacean, meat, and dairy/eggs). Similarly, the non-allergic control group was divided into three clusters.

Treatment efficacy

Proton pump inhibitors (PPIs) or potassium-competitive acid blocker (P-CAB), fluticasone propionate (800 μg/day) swallowing therapy, alone or in combination with other regimens, were administered to patients with EE. Lansoprazole (30 mg/day), rabeprazole (10 mg/day), and esomeprazole (20 mg/day) were administered as PPI treatments, while vonoprazan (20 mg/day) was administered as P-CAB treatment. To assess for treatment responses, we determined the number of patients with dysphagia, heartburn, and chest pain before and after treatment, and divided them into three groups: complete relief, partial relief, and no change group [24]. Symptom evaluation after medication administration was performed by interviewing the patients immediately after receiving each agent; the evaluation lasted for more than for 4 weeks. Complete relief was defined as absence of clinical symptoms. Partial relief was defined as partial disappearance of patients' symptoms. Endoscopically, treatment response was assessed as a percentage of change in EREFS total score before and after the administration of medications. Histologically, the treatment response was evaluated based on the number of eosinophils in the biopsy sample before and after the administration of medications, and the rate of change was confirmed.

Statistical analyses

Data are expressed as median and interquartile range (IQR) for continuous variables and as numbers for categorical variables. For categorical variables, comparisons were performed using Fisher's exact test, while continuous variables were compared using the Mann–Whitney U test or a Kruskal-Wallis test; p values < 0.05 were considered statistically significant. For comparison of individual factors between the clusters, we used Kruskal-Wallis test followed by Steel-Dwass multiple comparison for post hoc evaluation. Cluster analysis was performed by IBM SPSS Statistics22. Other statistical analyses were performed using EZR (version 1.34, Saitama Medical Center, Jichi Medical University, Saitama, Japan) which is a graphical user interface for R (version 3.3.2, The R Foundation for Statistical Computing, Vienna, Austria) [25].

Results

Allergic sensitization patterns within each patient cluster

Pattern of IgE-mediated sensitization facilitated classification of the patients with EE enrolled in our study into 3 clusters (Fig. 1). Cluster 1 (n = 62) were patients that were sensitized to some pollens and house dust, with low-level responses to nearly all the

allergens tested (Figs. 1 and 2a). Cluster 2 (n = 30) included patients that responded primarily to outdoor and to some plant allergens (Figs. 1 and 2b). Cluster 3 (n = 15) included patients that were sensitized to most allergens with particularly high-level responses to indoor and animal allergens (Figs. 1 and 2c). Table 1 includes the allergic sensitization status of each patient cluster. The number of positive allergens scored among patients in cluster 3 was significantly higher than that in cluster 1 (25.0 [20.0, 25.5] *vs*. 3.0 [1.0, 5.0], $p < 0.001$). Similarly, the sum of class values (Table 1) was significantly higher in cluster 3 than in cluster 1 (62.0 [56.0, 66.5] *vs.* 9.0 [4.0, 14.0], $p < 0.001$). Furthermore, the number of positive tests for outdoor allergens in cluster 2 was significantly higher than that in cluster 1 (6.0 [5.0, 7.8] *vs*. 1.0 [0.0, 2.0], $p < 0.001$); likewise, the number of positive tests for plant allergens in cluster 2 was also significantly higher than that in cluster 1 (1.0 [0.0, 5.0] vs 0.0 [0.0, 0.0], $p < 0.001$). The number of positive tests for indoor allergens among patients in cluster 3 was significantly higher than that in cluster 1 (8.0 [7.0, 8.0] *vs*. 2.0 [0.0, 2.8], $p < 0.001$) and the number of positive tests for animal allergens in cluster 3 was also significantly higher than that in cluster 1 (3.0 [2.0, 5.0] *vs*. 0.0 [0.0, 0.0], $p < 0.001$). Furthermore, similar tendencies were observed with respect to the sums of the class values for outdoor allergens, plant allergens, indoor allergens, and animal allergens with similar numbers of positive tests for all the

aforementioned allergens. Furthermore, cluster 2 was found to have significantly higher sensitization to outdoor and plant allergens than cluster 1 (the number of positive tests for outdoor and plant allergens: 6.0 [4.5, 7.5] vs. 1.0 [0.0, 2.0], $p < 0.001$ and 7.0 [5.0, 8.0] vs. 0.0 [0.0, 0.0], $p < 0.001$). The differences between cluster 2 and cluster 3 were as follows: cluster 2 was desensitized to indoor allergens (the number of positive tests for indoor allergens: 2.0 [1.0, 4.0] vs. 1.0 [0.0, 5.0], $p < 0.001$), while cluster 3 was desensitized to food allergens, especially those from animal sources (the number of positive tests for animal allergens: 0.0 [0.0, 1.0] vs. 3.0 [2.0, 5.0], $p < 0.001$). To rule out the effects of allergic comorbidities, we examined the baseline characteristics of patients with EE with and without allergic comorbidities, and no significant differences were found (Supplementary Table 1). The allergen-sensitized heatmap of patients with EE without allergic comorbidities was similar to that of all patients with EE, although a significant cluster could not be determined (Supplementary Fig. 1). The non-allergic control group had an extremely low IgE-mediated allergen sensitization compared with the patients with EE (Supplementary Table 2). When the non-allergic control group was classified into three clusters, no significant IgE-mediated allergen sensitization pattern was observed (Supplementary Fig. 2).

Clinical characteristics and laboratory values in patients from each cluster

As shown in Table 2, clinical characteristics of patients were examined within each cluster. There were no significant differences among the three clusters with respect to age, sex, BMI, current smoking, and current alcohol use. No significant differences were observed in the diagnostic assay testing seasons among the three clusters (Supplementary Table 3 and Supplementary Fig. 3). The observation period of cluster 3 was significantly longer than that of cluster 1 (679.0 [258.5, 1000.5] *vs.* 147.5 [63.0, 729.0]) (Supplementary Table 3). Patients assigned to cluster 3 included a large number of patients with bronchial asthma and atopic dermatitis (Table 2). Eleven patients in cluster 3 reported dysphagia; the prevalence of dysphagia in cluster 3 was significantly higher than in cluster 1 (73.3% *vs.* 35.5%; Table 2 and Fig. 3a). Patients in cluster 1 significantly had higher rates of heartburn among 3 clusters (Table 2). There were no significant differences among patients in any of the three clusters with respect to other clinical criteria. With respect to laboratory values, patients in cluster 3 had significantly higher titers of total serum IgE compared to those in cluster 1 (1200 [845, 2350] IU/mL *vs.* 103 [47, 223] IU/mL) (Table 3 and Fig. 3b). These were no significant differences among patients in any of the three clusters with respect to peripheral eosinophil and basophil counts.

Endoscopic findings

Table 4 includes the endoscopic findings associated with the patients in each cluster. The grade of edema, ring, and stricture were significantly different among these patient cohorts. The score for edema among patients in cluster 3 was significantly higher than that determined for patients in cluster 1 (Fig. 3c). The score for rings among patients in cluster 3 was also significantly higher than that in cluster 1 (Fig. 3d). The score for strictures in cluster 3 was also significantly higher than that in cluster 1 (Fig. 3e). Furthermore, EREFS total score in cluster 3 was also significantly higher than that in cluster 1 (Fig. 3f). There were no significant differences among patients in each of the three clusters with respect to other components of EREFS including exudates and furrows. Likewise, there were no significant differences with respect to esophageal eosinophil counts in biopsy specimens. Moreover, there were no significant differences with respect to the prevalence of reflux esophagitis and/or atrophic gastritis.

Response to medications

No significant difference was observed between the three clusters in terms of the choice of medications (Supplementary Table 4). The degree of decrease in EREFS total score and esophageal eosinophil count before and after treatment of cluster 3 were smaller than

those of cluster 1 (−78.9 [−99.1, 10.0]% *vs.* −92.9 [−98.7, −48.3]% and −75.0 [−100.0, −29.2]% *vs.* −100.0 [−100.0, −62.5]%). However, no significant differences were found among the three clusters in terms of symptoms, endoscopic findings, and esophageal eosinophil counts.

Discussion

Results of this study indicate that IgE-mediated allergic sensitization patterns are related to clinical features of patients with EE. In this study, we classified patients with EE into 3 clusters, each representing a distinct sensitization pattern that was determined using a multi-plex diagnostic panel to facilitate identification of unique allergen-specific IgEs. Patients in cluster 3 were sensitized to most allergens, most notably indoor and animal allergens, and were diagnosed with number of other allergic disorders including asthma and atopic dermatitis; these patients also experienced more prominent dysphagia and endoscopic findings indicative of more severe disease than found in patients in either of the other clusters. By contrast, patients in cluster 1 were sensitized to comparatively few allergens (some aeroallergens only), experienced only limited dysphagia with endoscopic findings that were significantly less severe than those in than patients assigned to either of the other clusters. Attempts have been made to generate consistent classifications of

EE/EoE phenotypes based on clinical, endoscopic and histological features but have been largely thwarted by the overall heterogeneity of this disorder [26]. For example, Shoda *et al.* [22] presented a framework to explain various common phenotypes by stratifying patients with EoE into three clinically-significant endotypes based on transcriptome analysis. Similarly, our results provide a consistent explanation of the distinct phenotypes associated with EE using a multi-plex diagnostic panel that facilitates simultaneous detection of multiple allergen-specific IgEs.

Our findings indicate that patients in cluster 3 who were sensitized to a variety of allergens, especially indoor and animal allergens, reported significantly more dysphagia in association with more serious endoscopic findings, including ring and stricture scores, than patients in cluster 1. Several groups have reported that esophageal fibrotic remodeling is associated with both dysphagia and with endoscopic findings including rings and strictures in patients diagnosed with EoE [27, 28]. Our results suggest that fibrotic remodeling may contribute to dysphagia, rings, and strictures and may be directly associated with specific IgE-mediated allergen sensitivity patterns. Previous studies revealed that adult patients with EE who had IgE-mediated allergies to soybean had severe obstructive symptoms and esophageal stricture [29]. IgE-mediated food sensitization induced the development of esophageal fibrosis in pediatric patients with

EE who had a TGFβ1 promoter single nucleotide polymorphism [30], and these findings support our results. Based on these findings, we suggest that IgE-mediated sensitization to a variety of allergens, notably those categorized as indoor and animal allergens, may be related to disease exacerbations of patients diagnosed with EE.

EoE has been associated with IgE-mediated allergic reactions to various allergens. Food allergens are among the known triggers of EoE; milk, egg, wheat, peanut, tree nuts, soy/legumes, fish, and shellfish are the eight commonly examined foods that can possibly trigger allergies [31]. Milk, egg, wheat and soy/legumes are among the common allergen triggers among patients with EoE in Western countries; elimination diets have been used successfully for the treatment of patients with EoE [32-39]. Elimination diets that focus on six of the eight aforementioned foods have been reported to result in 60% improvement in findings from esophageal biopsies [37, 38]. However, IgE-mediated allergy tests, including allergen-specific IgE serum testing and skin prick tests, have little utility in determining the need for elimination diets. Wechsler *et al.* [40] reported that the empiric elimination diet was effective in 72.1% of patients with EoE, whereas the allergy test-directed elimination diet was only effective in 45.5% of patients. Aeroallergens can also trigger in EoE; intranasal instillation of aeroallergens including dust mite, *Aspergillus fumigatus*, and cockroach antigen resulted features of EoE in

experimental mouse models [41, 42]. Furthermore, clinical reports reveal that aeroallergens prevalent in the environment during pollen season as well as mites and mold serve as triggers for relapses in patients with symptom-controlled EoE [43, 44]; our findings are consistent with these observations. Furthermore, Williamson *et al.* [45] found that that IgE-mediated allergies may play a role with respect to the severity and clinical course of EoE. Taken together, these reports reinforce our findings that suggest that IgE-mediated sensitization by a variety of allergens, including mainly indoor and animal allergens, may be related to exacerbation the clinical status of patients diagnosed with EE.

Aeroallergens are known to cause IgE-mediated sensitization to food allergens via the respiratory pathway due to antigenic cross-reactivity, a condition known as pollen-food allergy syndrome (PFAS) [46, 47]. Food allergens associated with PFAS include those derived from fruits and vegetables [48, 49]. Another group reported that sensitization with pollens associated with cross-reactivity to food allergens can trigger EoE [50]. In our study, the patients assigned to cluster 2 included those with EE who were highly sensitized to outdoor allergens; these mainly included pollen and plant allergens; as such, the results from this cluster may be related to PFAS. Aeroallergens other than pollens, including mites, insects such as cockroaches and pet dander from dogs and cats, which are included among the indoor allergens, may also be associated with cross-

reactivity to food antigens, most notably the animal antigens. For example, mites are associated with the mite-shrimp syndrome [51]. Furthermore, cockroaches contain cross-reactive tropomyosin, which may promote a greater risk of an allergic reaction to shellfish and snails [52, 53]. Likewise, cat dander has been associated with the cat-pork syndrome [54]. Cross-reactivities between indoor and animal allergens may also promote pathology associated with cluster 3, which includes a group of patients with EE who are sensitized to various allergens, mainly indoor and animal allergens in this study. A diagnostic panel that facilitates identification of multiple allergen-specific IgEs may be useful for evaluating cross-reactivity between aeroallergens and food allergens in patients diagnosed with EE.

A non-IgE-mediated mechanism is important for the development of EoE. For example, previous studies reported that non-IgE-mediated food hypersensitivity and IgG4-mediated food allergies may have a direct effect on the development of EoE [14, 55]. Our results suggested that IgE-mediated allergic sensitization patterns are associated with the clinical features of EE patients. Contrastingly, data regarding the pathophysiology of EE were limited as our study only analyzed the serum IgE levels. Majority of patients with EE in this study, who do not show remarkable IgE-mediated allergen sensitization, had non-obstructive endoscopic findings and lower prevalence of

dysphagia although they had higher prevalence of heartburn. The prevalence of dysphagia in cluster 3 was significantly higher than that in cluster 1. However, the proportion of asymptomatic patients was not different among the groups. Indeed, 3 (20.0%) patients in cluster 3 were asymptomatic or seemed to have milder symptoms. In addition, only 2 (13%) patients in cluster 3 had stenosis, which tended to be significantly lower than the prevalence (25%) reported in the US study [56]. These may be related to the fact that many Japanese patients with EE have seemed to have milder symptoms or are asymptomatic and show relatively few obstructive endoscopic findings [57-60]. In summary, the characteristics of many Japanese patients with EE may have relatively weak relationship with IgE-mediated allergen sensitization. It is difficult to explain the mechanism of symptom generation by conducting a serum IgE evaluation alone.

Among the limitations of this study, we recognize that we did not evaluate the EE patient clusters prospectively, as the study design was purely retrospective in nature. Because this study was retrospective, our results might be affected by confounding factors such as diagnostic assay inspection season, observation period, other allergic comorbidities, and the treatment method. We considered these effects as much as possible, however, it was difficult to eliminate these effects. We hope that prospective studies will be conducted in the future. Likewise, the diagnostic panel of used to detect allergen-

specific IgEs measures reactivity against the crude antigen, and does not permit assessment of reactivities to individual components. In addition, those with eosinophilic gastroenteritis with normal endoscopic findings may not be excluded because gastric and duodenal biopsies were not performed in all study patients. Finally, identification of IgE-mediated allergies, including those that are antigen-specific, are not helpful in determining with respect to determining the specific EoE-triggering allergens [14, 61]. The diagnostic panel facilitated identification of IgE-mediated sensitization patterns in patients diagnosed with EE. However, currently available allergy tests including non-IgE-specific methods including skin-patch tests, basophil activation tests, and tests to identify antigen-specific IgG also cannot predict triggers for EoE in adult patients; systematic re-introduction after a successful elimination diet trial appears to be the only effective means to identify trigger allergens at this time [37, 61].

In conclusion, our results reveal that IgE-mediated allergic sensitization patterns can be directly related to clinical features in patients diagnosed with EE. A diagnostic panel that facilitates identification of multiple allergen-specific IgEs can be used to generate a consistent explanation of the heterogeneous phenotypes of patients diagnosed with EE.

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Table 1 Sensitization status

Variable	Cluster 1 (n=62)	Cluster 2 (n=30)	Cluster 3 (n=15)	*p* value
Number of positive allergens	3.0 [1.0, 5.0]	11.0 [9.0, 13.8]	25.0 [20.0, 25.5]	< 0.001
Aero allergens	3.0 [1.0, 4.0]	9.0 [7.0, 10.8]	14.0 [12.5, 15.0]	< 0.001
Outdoor allergens *(Pollens)*	1.0 [0.0, 2.0]	6.0 [5.0, 7.8]	6.0 [4.5, 7.5]	< 0.001
Indoor allergen *(House dust, mold, and latex)*	2.0 [0.0, 2.8]	2.0 [1.0, 4.0]	8.0 [7.0, 8.0]	< 0.001
Food allergens	0.0 [0.0, 0.0]	2.0 [1.0, 6.8]	9.0 [7.0, 11.5]	< 0.001
Plant allergens *(Grains, beans, nuts, and fruit)*	0.0 [0.0, 0.0]	1.00 [0.0, 5.0]	7.0 [5.0, 8.0]	< 0.001
Animal allergens *(Fish, crustacean, meat, and dairy/eggs)*	0.0 [0.0, 0.0]	0.0 [0.0, 1.0]	3.0 [2.0, 5.0]	< 0.001
Sum of Class values	9.0 [4.0, 14.0]	34.0 [27.3, 36.8]	62.0 [56.0, 66.5]	< 0.001
Aero allergens	7.5 [4.0, 11.8]	26.0 [20.3, 29.0]	40.0 [37.0, 41.5]	< 0.001
Outdoor allergens *(Pollens)*	2.5 [0.0, 6.0]	17.5 [14.0, 21.0]	17.0 [14.0, 20.5]	< 0.001
Indoor allergens *(House dust, mold, and latex)*	4.0 [0.0, 7.0]	6.0 [3.0, 11.0]	21.0 [20.5, 25.0]	< 0.001
Food allergens	0.0 [0.0, 1.8]	5.0 [2.3, 15.5]	23.0 [17.5, 28.0]	< 0.001
Plant allergens *(Grains, beans, nuts, and fruit)*	0.0 [0.0, 0.0]	4.0 [2.0, 13.0]	15.0 [11.0, 18.5]	< 0.001
Animal allergens *(Fish, crustacean, meat, and dairy/eggs)*	0.0 [0.0, 1.0]	0.0 [0.0, 3.5]	6.0 [5.0, 11.5]	< 0.001

Data are expressed as median [IQR]

Table 2 Clinical characteristics

Variable	Cluster 1 (n=62)	Cluster 2 (n=30)	Cluster 3 (n=15)	*p* value
Age (years), mean±SD	47.5 ±10.3	45.3 ± 10.9	44.33 ± 10.0	0.444
Male (%)	37 (59.7)	21 (70.0)	9 (60.0)	0.612
BMI (kg/m^2), mean±SD	24.1 ± 3.7	23.2 ± 4.3	23.2 ± 3.5	0.489
Current smoking (%)	10 (16.1)	2 (6.7)	2 (13.3)	0.505
Current alcohol drinking (%)	32 (51.6)	12 (40.0)	9 (60.0)	0.445
Comorbidity of allergic diseases (%)				
Self-assessment food allergy	10 (16.1)	10 (33.3)	5 (33.3)	0.109
Bronchial asthma	11 (17.7)	2 (6.7)	6 (40.0)	0.023
Seasonal allergic rhinitis	18 (29.0)	12 (40.0)	2 (13.3)	0.204
Perennial allergic rhinitis	10 (16.1)	10 (33.3)	2 (13.3)	0.146
Atopic dermatitis	5 (8.1)	3 (10.0)	5 (33.3)	0.038
Clinical symptoms (%)				
Dysphagia	22 (35.5)	14 (46.7)	11 (73.3)	0.028
Heartburn	30 (48.4)	7 (23.3)	3 (20.0)	0.024
Chest pain	12 (19.4)	4 (13.3)	2 (13.3)	0.762
None	7 (11.3)	8 (26.7)	3 (20.0)	0.155

Table 3 Blood examinations

Variable	Cluster 1 (n=62)	Cluster 2 (n=30)	Cluster 3 (n=15)	*p* value
WBC (/ml)	5600 [5100, 6900]	5000 [4500, 5800]	5300 [4600, 6100]	0.041
Peripheral eosinophil count (/ml)	268 [163, 371]	278 [153, 384]	300 [174, 496]	0.621
Peripheral eosinophil ratio (%)	5.0 [2.9, 6.4]	6.0 [3.0, 7.8]	6.6 [3.2, 9.4]	0.191
Peripheral basophil count (/ml)	42 [29, 67]	39 [30, 56]	33 [20, 45]	0.248
Peripheral basophil ratio (%)	0.7 [0.5, 1.0]	0.8 [0.6, 1.1]	0.6 [0.4, 0.9]	0.435
Total titer of serum IgE antibody (IU/ml)	103 [47, 223]	250 [173, 555]	1200 [845, 2350]	<0.001

Data are expressed as median [IQR]

Table 4 Endoscopic findings

Variable		Cluster 1 (n=62)	Cluster 2 (n=30)	Cluster 3 (n=15)	*p* value
Reflux esophagitis (%)		7 (11.3)	6 (20.0)	0 (0.0)	0.175
Atrophic gastritis (%)		22 (35.5)	8 (26.7)	1 (6.7)	0.080
Edema (%)					<0.001
	Grade 0	17 (27.4)	6 (20.0)	0 (0.0)	
	Grade 1	45 (72.6)	22 (73.3)	8 (53.3)	
	Grade 2	0 (0.0)	2 (6.7)	7 (46.7)	
Rings (%)					0.006
	Grade 0	43 (69.4)	15 (50.0)	4 (26.7)	
	Grade 1	18 (29.0)	13 (43.3)	8 (53.3)	
	Grade 2	1 (1.6)	2 (6.7)	3 (20.0)	
	Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	
Exudates (%)					0.344
	Grade 0	18 (29.0)	8 (26.7)	2 (13.3)	
	Grade 1	41 (66.1)	20 (66.7)	10 (66.7)	
	Grade 2	3 (4.8)	2 (6.7)	3 (20.0)	
Furrows (%)					0.140
	Grade 0	5 (8.1)	4 (13.3)	0 (0.0)	
	Grade 1	57 (91.9)	26 (86.7)	14 (93.3)	
	Grade 2	0 (0.0)	0 (0.0)	1 (6.7)	
Stricture (%)					0.002
	Grade 0	62 (100.0)	30 (100.0)	13 (86.7)	
	Grade 1	0 (0.0)	0 (0.0)	2 (13.3)	
EREFS total score		3.0 [2.0, 6.0]	6.0 [3.0, 8.0]	8.0 [6.0, 10.0]	<0.001
Esophageal eosinophil counts (/HPF)		32.0 [20.0, 73.0]	50.0 [23.0, 94.0]	50.0 [34.5, 72.0]	0.272

Data are expressed as median [IQR]

Figure Legends

Fig. 1 Patterns of IgE-mediated sensitization in patients with esophageal eosinophilia

Figure 1


Cluster 1 (n=62)
Cluster 2 (n=30)
Cluster 3 (n=15)
Aero allergens
Food allergens
Outdoor
Indoor
Plant
Animal
Cocksfoot
Common ragweed
Mugwort
Timothy grass
Japanese cedar
Japanese cypress
Grey alder
Comon silver birch
House dust mite
House dust 1
Cat dander
Dog dander
Cockroach
Moth
Latex
Candida albicans
Alternaria alternata
Aspergillus fumigatus
Malassezia spp.
Wheat
Soybean
Rice
Sesame
Buckwheat
Peanut
Apple
Kiwi
Banana
Tuna
Salmon
Chub mackerel
Shrimp
Crab
Milk
Pork
Beef
Chiken
Egg white
Ovomucoid
Class of View allergy 39
0
1
2
3
4
5
6

Fig. 2 Class value of the elements included in each cluster, including **a** cluster 1, **b** cluster 2, **c** cluster 3. The median and interquartile range of class values based of the titer of specific IgE antibody detected for each allergen are shown.

Figure 2

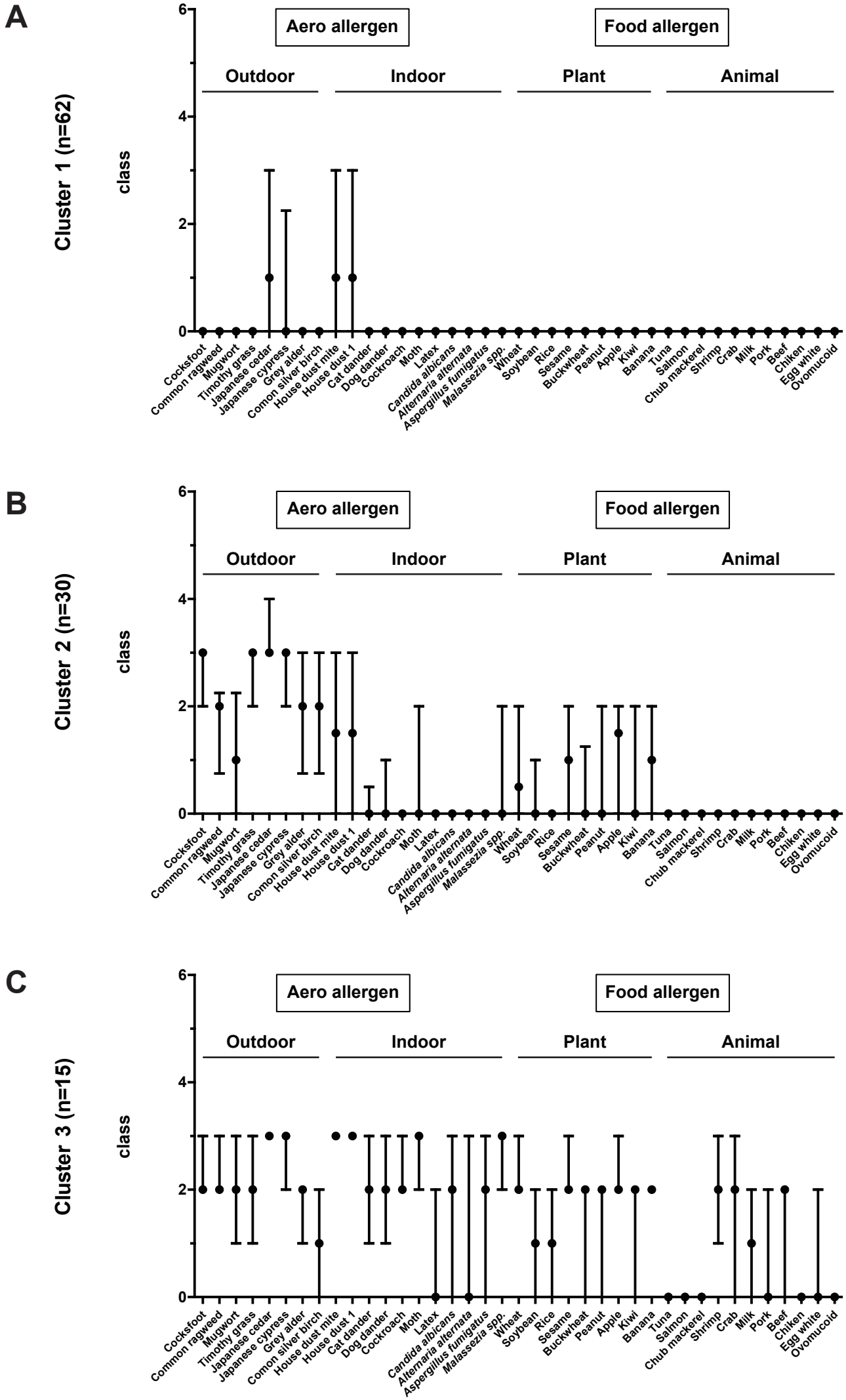
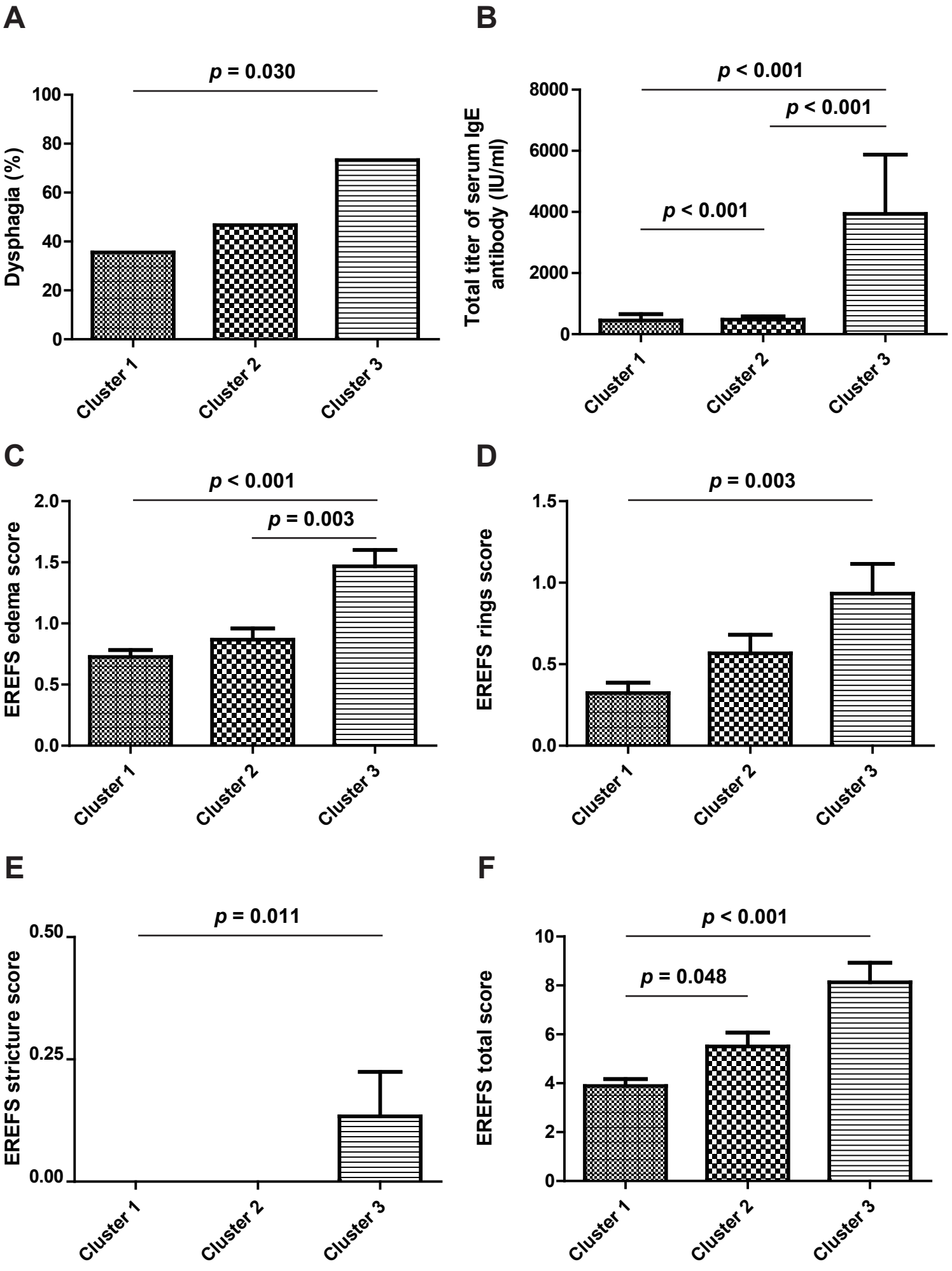

A
Cluster 1 (n=62)
class
Aero allergen
Food allergen
Outdoor
Indoor
Plant
Animal
0
2
4
6
Cocksfoot
Common ragweed
Mugwort
Timothy grass
Japanese cedar
Japanese cypress
Grey alder
Comon silver birch
House dust mite
House dust 1
Cat dander
Dog dander
Cockroach
Moth
Latex
Candida albicans
Alternaria alternata
Aspergillus fumigatus
Malassezia spp.
Wheat
Soybean
Rice
Sesame
Buckwheat
Peanut
Apple
Kiwi
Banana
Tuna
Salmon
Chub mackerel
Shrimp
Crab
Milk
Pork
Beef
Chiken
Egg white
Ovomucoid
B
Cluster 2 (n=30)
class
Aero allergen
Food allergen
Outdoor
Indoor
Plant
Animal
0
2
4
6
Cocksfoot
Common ragweed
Mugwort
Timothy grass
Japanese cedar
Japanese cypress
Grey alder
Comon silver birch
House dust mite
House dust 1
Cat dander
Dog dander
Cockroach
Moth
Latex
Candida albicans
Alternaria alternata
Aspergillus fumigatus
Malassezia spp.
Wheat
Soybean
Rice
Sesame
Buckwheat
Peanut
Apple
Kiwi
Banana
Tuna
Salmon
Chub mackerel
Shrimp
Crab
Milk
Pork
Beef
Chiken
Egg white
Ovomucoid
C
Cluster 3 (n=15)
class
Aero allergen
Food allergen
Outdoor
Indoor
Plant
Animal
0
2
4
6
Cocksfoot
Common ragweed
Mugwort
Timothy grass
Japanese cedar
Japanese cypress
Grey alder
Comon silver birch
House dust mite
House dust 1
Cat dander
Dog dander
Cockroach
Moth
Latex
Candida albicans
Alternaria alternata
Aspergillus fumigatus
Malassezia spp.
Wheat
Soybean
Rice
Sesame
Buckwheat
Peanut
Apple
Kiwi
Banana
Tuna
Salmon
Chub mackerel
Shrimp
Crab
Milk
Pork
Beef
Chiken
Egg white
Ovomucoid

Fig. 3 Clinical features associated with each cluster. **a** Proportion of patients reporting dysphagia among 3 clusters. **b** Total serum IgE titer, **c** edema score, **d** ring score, **e** stricture score and **f** total EoE endoscopic reference score (EREFS) within each cluster. Data presented mean with SEM.

Figure 3


A
p = 0.030
Dysphagia (%)
100
80
60
40
20
0
Cluster 1
Cluster 2
Cluster 3
B
p < 0.001
p < 0.001
p < 0.001
Total titer of serum IgE antibody (IU/ml)
8000
6000
4000
2000
0
Cluster 1
Cluster 2
Cluster 3
C
p < 0.001
p = 0.003
EREFS edema score
2.0
1.5
1.0
0.5
0.0
Cluster 1
Cluster 2
Cluster 3
D
p = 0.003
EREFS rings score
1.5
1.0
0.5
0.0
Cluster 1
Cluster 2
Cluster 3
E
p = 0.011
EREFS stricture score
0.50
0.25
0.00
Cluster 1
Cluster 2
Cluster 3
F
p < 0.001
p = 0.048
EREFS total score
10
8
6
4
2
0
Cluster 1
Cluster 2
Cluster 3

Supplementary

Supplementary Table 1 Clinical characteristics of patients with EE with and without allergic comorbidities

Variable	With allergic comorbidities (n=79)	Without allergic comorbidities (n=28)	*p* value
Clinical characteristics			
Age (years), mean ± SD	46.0 ± 11.1	47.6 ± 8.1	0.475
Male (%)	47 (59.5)	20 (71.4)	0.364
BMI (kg/m^2), mean ± SD	23.6 ± 4.0	24.2 ± 3.5	0.463
Current smoking (%)	9 (11.4)	5 (17.9)	0.514
Current alcohol drinking (%)	43 (54.4)	10 (35.7)	0.123
Comorbidity of allergic diseases (%)			
Self-assessment food allergy	25 (31.6)	0 (0.0)	<0.001
Bronchial asthma	19 (24.1)	0 (0.0)	0.003
Seasonal allergic rhinitis	32 (40.5)	0 (0.0)	<0.001
Perennial allergic rhinitis	22 (27.8)	0 (0.0)	0.001
Atopic dermatitis	13 (16.5)	0 (0.0)	0.019
Clinical symptoms (%)			
Dysphagia	37 (46.8)	10 (35.7)	0.378
Heartburn	28 (35.4)	12 (42.9)	0.503
Chest pain	11 (13.9)	7 (25.0)	0.239
None	12 (15.2)	6 (21.4)	0.557

Supplementary Table 2 Sensitization status of patients with EE and non-allergic control group

Variable	EE (n=107)	Control (n=37)	*p* value
Number of positive allergen	6.0 [2.5, 12.0]	2.0 [0.0, 4.0]	< 0.001
Aero allergen	5.0 [2.0, 9.0]	2.0 [0.0, 4.0]	< 0.001
Outdoor allergen *(Pollen)*	2.0 [0.0, 6.0]	1.0 [0.0, 2.0]	0.001
Indoor allergen *(House dust, Mold and Latex)*	2.0 [0.0, 4.0]	1.0 [0.0, 2.0]	0.001
Food allergen	0.0 [0.0, 4.0]	0.0 [0.0, 0.0]	< 0.001
Plant allergen *(Grain, Bean, Nut and Fruit)*	0.0 [0.0, 2.0]	0.0 [0.0, 0.0]	< 0.001
Animal allergen *(Fish, Crustacean and Meat)*	0.0 [0.0, 1.0]	0.0 [0.0, 0.0]	0.002
Sum of Class values	16.0 [7.0, 34.5]	7.0 [3.0, 11.0]	< 0.001
Aero allergen	14.0 [6.5, 27.0]	7.0 [3.0, 10.0]	< 0.001
Outdoor allergen *(Pollen)*	7.0 [1.0, 16.0]	3.0 [0.0, 7.0]	0.001
Indoor allergen *(House dust, Mold and Latex)*	6.0 [2.0, 10.0]	3.0 [0.0, 6.0]	0.003
Food allergen	2.0 [0.0, 9.0]	0.0 [0.0, 0.0]	< 0.001
Plant allergen *(Grain, Bean, Nut and Fruit)*	1.0 [0.0, 4.5]	0.0 [0.0, 0.0]	< 0.001
Animal allergen *(Fish, Crustacean and Meat)*	0.0 [0.0, 3.0]	0.0 [0.0, 0.0]	< 0.001

Data are expressed as median [IQR]

Supplementary Table 3 Diagnostic assay inspection season and observation period

Variable	Cluster 1 (n=62)	Cluster 2 (n=30)	Cluster 3 (n=15)	*p* value
the diagnostic assay inspection season (%)				0.210
Spring	18 (29.0)	9 (30.0)	3 (20.0)	
Summer	11 (17.7)	9 (30.0)	8 (53.3)	
Fall	17 (27.4)	7 (23.3)	3 (20.0)	
Winter	16 (25.8)	5 (16.7)	1 (6.7)	
Observation period	147.5 [63.0, 729.0]	90.0 [63.0, 325.5]	679.0 [258.5, 1000.5]	0.023

Spring: March, April, and May; Summer: June, July, and August

Fall: September, October and November; Winter: December, January, and February

Data are expressed as median [IQR]

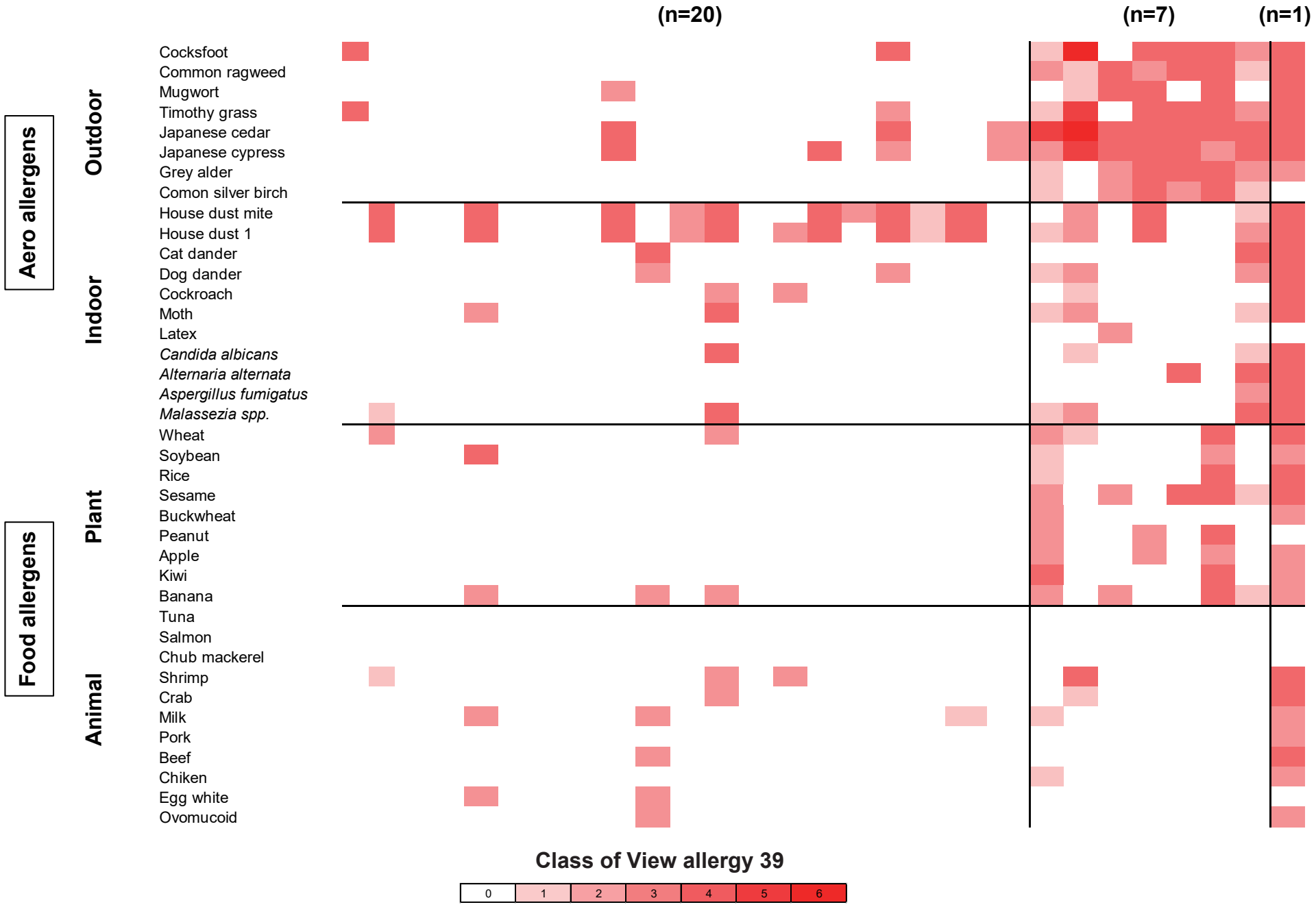
Supplementary Table 4 Medications and response

Variable		Cluster 1 (n=62)	Cluster 2 (n=30)	Cluster 3 (n=15)	*p* value
Medications (%)					0.759
PPI		30 (48.4)	15 (50.0)	9 (60.0)	
P-CAB		26 (41.9)	14 (46.7)	5 (33.3)	
Fluticasone		2 (3.2)	0 (0.0)	0 (0.0)	
PPI + Fluticasone		0 (0.0)	0 (0.0)	1 (6.7)	
P-CAB + Fluticasone		1 (1.6)	0 (0.0)	0 (0.0)	
None		3 (4.8)	1 (3.3)	0 (0.0)	
Response for medications					
Symptoms (%)	NC	10 (18.2)	2 (9.1)	2 (16.7)	0.433
	PR	13 (23.6)	10 (45.5)	4 (33.3)	
	CR	32 (58.2)	10 (45.5)	6 (50.0)	
EREFS total score (%)		-92.9 [-98.7, -48.3]	-88.5 [-99.5, -55.4]	-78.9 [-99.1, 10.0]	0.761
Esophageal eosinophil counts (%)		-100.0 [-100.0, -62.5]	-100.0 [-100.0, -78.1]	-75.0 [-100.0, -29.2]	0.438

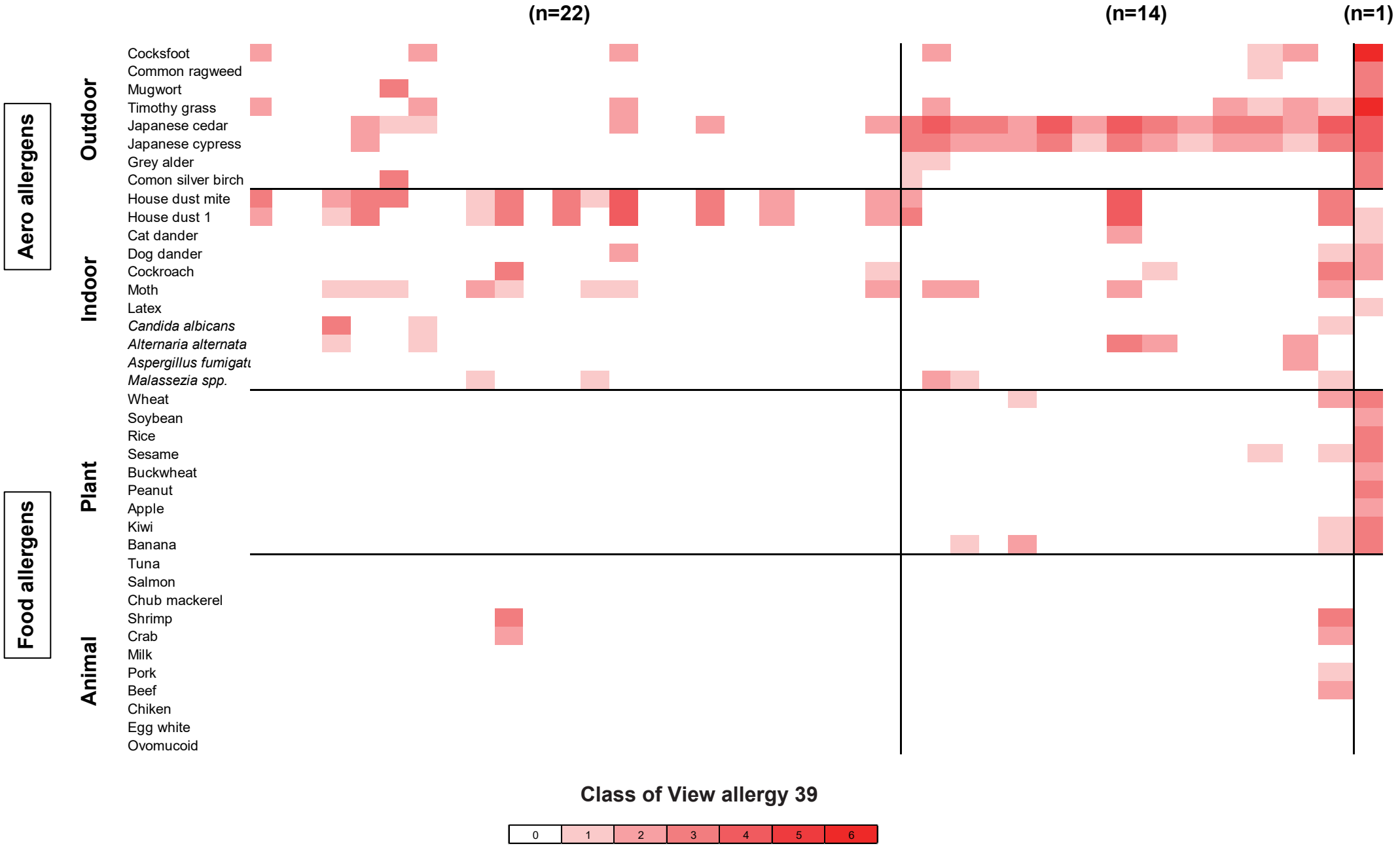
PPI: LPZ, RPZ or EPZ; P-CAB: VPZ; Fluticasone: swallowed fluticasone propionate

NC: no change; PR: partial relief; CR: complete relief

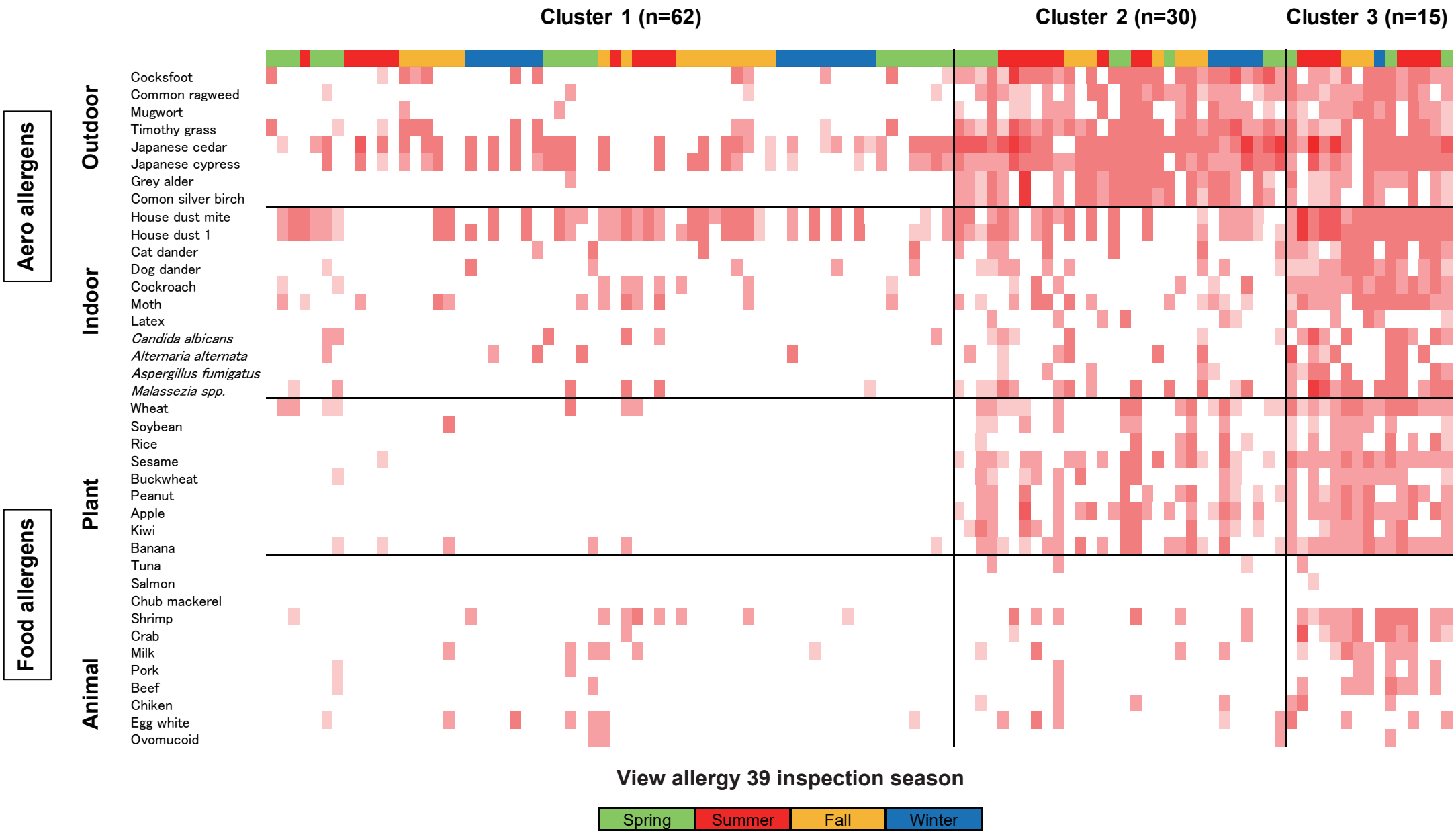
Data are expressed as median [IQR]



Supplementary Fig. 1 Patterns of IgE-mediated sensitization in patients with esophageal eosinophilia without allergic comorbidities



Supplementary Fig. 2 Patterns of IgE-mediated sensitization in the non-allergic control group



Supplementary Fig. 3 Patterns of IgE-mediated sensitization in patients with esophageal eosinophilia and the diagnostic assay inspection season