Pre-treatment allergen-specific IgE analysis and outcomes of allergen immunotherapy

Martin Berge[1], Lena Bertilsson[2], Olof Hultgren[2, 4], Svante Hugosson[1, 3], Amanj Saber[1, 3]

[1] Department of Otolaryngology, Örebro University Hospital, Örebro, Sweden

[2] Department of Laboratory Medicine, Örebro University Hospital, Örebro, Sweden

[3] School of Medical Sciences, Örebro University, Örebro, Sweden

[4] Department Clinical Immunology and Transfusion Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden

Address for correspondence: Amanj Saber

Email: amanj.saber@regionorebrolan.se

Telephone: +46 196023607

Fax: +46 19103301

ABSTRACT

Background: Patients show varied results to allergen immunotherapy (AIT. The reason for this variability is unclear.

Objective: To describe the relationship between AIT efficacy and demographic characteristics, as well as pre-treatment plasma levels of specific IgE-antibodies to grass and birch pollen.

Methods: A retrospective study was performed based on medical records of 128 patients who received AIT. The patients completed a questionnaire and pre-AIT plasma levels of allergen-specific IgE to grass and birch pollen were measured using EUROLINE DPA-Dx pollen 1 method.

Results: Seventy percent of patients classified their allergic symptoms as less severe after AIT. Twenty-seven percent had received AIT targeting only grass pollen, 19% targeting only birch pollen, and 55% targeting both grass and birch. A total of 35 different IgE profiles were found across our study population. On comparison of the demographic characteristics and concentration of allergen-specific IgE-antibodies no statistically significant differences could be found.

Conclusion: The majority of patients rated their allergic symptoms as less severe after AIT. No clear relationship could be demonstrated between pre-treatment allergen-specific IgE concentration, or demographic characteristics, and effect of AIT. There may be other factors underlying the different responses to AIT.

Keywords: Pollen allergy, IgE, allergen immunotherapy

INTRODUCTION

Allergic rhinitis (AR) affects up to 30% of the world's population and therefore poses a great socioeconomic burden [1]. Allergen immunotherapy (AIT) is the only disease-modifying treatment for immunoglobulin E (IgE)-mediated allergic disease [2]. In addition to being capable of modifying disease, it shows long-term effects after treatment is achieved; in other words, it is capable of curing allergies [3]. The treatment is based on the administration of allergen extracts which are complex mixtures, with not all extracts having the same allergenic properties [4,5]. Some extracts may lack some allergenic proteins or may be impaired during the production process and storage [5]. Moreover, the effectiveness of AIT can vary between patients [6,7]. Most patients are significantly improved after AIT, with a response rate of around 80% [8,9]. However, not all allergic individuals respond to AIT, and furthermore AIT is not as effective in treating hypersensitivity to all different allergens [2], and the reason for this is unclear. Furthermore, it has been shown that the effect decreases when treating for several allergens simultaneously [10]. It is difficult to predict how patients will respond to the treatment, but many studies have tried to find suitable biomarkers to predict the clinical effect of AIT [11–14].

More research is needed to gain a better understanding of exactly why AIT does not work for all patients. This knowledge will in turn provide opportunities for establishing the optimal dose and method of administration [2]. Diagnostic biomarkers help to select the patients who will be the best responders to a specific treatment [2]. Analysis of allergen-specific IgE (sIgE) has been proposed as a biomarker for AIT [1]. The use of allergen components is of great diagnostic importance to identify the main sensitizing component.

One of the aims of this study is to describe the relationship between AIT efficacy and demographic characteristics. Another aim is to study plasma levels of sIgE to grass and birch pollen prior to AIT, measured using the component-resolved, multiplex immunoblot test system, EUROIMMUNE (EUROIMMUN AG, Lübeck, Germany).

MATERIALS AND METHODS

Study design and population

This study is an observational, retrospective study on a cohort of patients with grass and/or birch pollen allergy who received AIT between 1999 and 2015 at the Otorhinolaryngology and Pulmonology Departments, Örebro University Hospital, Örebro,

Sweden. Adult patients with a history of AR, positive skin prick test, and/or allergen-specific IgE test were included in the study. The study was approved by the Swedish Ethical Review Authority. Written consent to participate in the study was collected from all included patients.

After completion, the effectiveness of AIT was assessed by the patients who completed a questionnaire for evaluation of allergic symptoms both before and after receiving AIT, using a 10 cm numeric rating scale (NRS) ranging from 0 (no symptoms) to 10 (severe symptoms). For subjects who reported suffering from asthma, both AR and asthmatic symptoms were assessed together on one single NRS. The study subjects were stratified into non-responders and responders based on whether their AR and/or asthmatic symptoms had improved, i.e., changed from severe symptoms before to moderate or mild symptoms after AIT. The questionnaire contained questions about demographic characteristics, asthma, duration of the patient's allergic symptoms, what medication the patient used, degree of satisfaction with the treatment, change in quality of life, and whether the patient had suffered from any side effects from the treatment.

Skin prick test and immunotherapy

Products from ALK- Abelló (Hørsholm, Denmark) were used for the skin prick test and AIT. Soluprick SQ® was used for the skin prick test. For AIT against grass pollen allergy, the majority of patients (n=101) received Alutard SQ® 5 Grasses, and only three patients received Alutard SQ® Timothy grass (*Phleum pratense*). The majority of patients with birch pollen allergy (n=86) were treated with Alutard SQ® Birch (*Betula verucosa*) and only eight patients were treated with Alutard SQ® 3 Trees (*Betula verucosa*, *Alnus glutinosa*, and *Corylus avellana*). Subjects who were treated simultaneously for both grass and birch allergy received a combination of either Alutard SQ® 5 Grasses and Alutard SQ® Birch (n=59), or Alutard SQ® 5 Grasses and Alutard SQ® 3 Trees (n=8), or Alutard SQ® Timothy grass and Alutard SQ® Birch (n=3).

Immunoglobulin E analysis

Serum IgE antibodies were measured using EUROBlotOne, EUROLINE DPA-Dx pollen 1 (EUROIMMUN AG, Lübeck, Germany), according to the manufacturer's instructions. The results were expressed in kU/L, with a cutoff value of 0.35 kU/L as a positive result. The test kit contained strips lined with parallel bands, for eleven different allergens, and a control band (indicator band). Serum samples were analyzed for specific IgE against eleven different allergens, *Betula verrucosa*, birch (t3), and the birch components

rBet v 1, rBet v 2, rBet v 4, rBet v 6, and *Phleum pratense*, Timothy grass (g6), and the Timothy components rPhl p 1, rPhl p 5, rPhl p 7, rPhl p 12, and cross-reactive carbohydrate determinants (CCDs). A known control sample, positive for birch (t3) and Timothy (g6), was run with each analysis to ensure that the method worked as intended.

Statistics

Microsoft® Excel (Microsoft, Seattle, W.A., USA) was used to store the data and to create the tables presented in this study. Mann-Whitney U-tests were used to compare interval or ordinal level variables between groups. Pearson's chi-squared tests (or Fisher's exact test where expected cell counts were <5 in the cross-tabulation) were used for categorical variables as well as for comparison of sIgE levels between the different groups. Alpha levels were set to 0.05 for all tests. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA).

RESULTS

Out of 353 patients who were contacted for participation in the study, 128 patients were included, as illustrated in Figure 1. Thirty-four (27%) patients underwent AIT targeting only grass pollen, 24 (19%) targeting only birch pollen, and 70 (55%) targeting both grass and birch. Twelve (9%) patients also received treatment targeting an additional allergen other than grass and/or birch at the same time (seven were treated for pet allergy, four for mugwort, and one for house dust mite (HDM)). In total, 74 (58%) subjects were treated with at least two allergens simultaneously (62 with grass and birch; three with grass, birch, and mugwort; three with grass, birch, and cat hair; two with grass and cat hair; one with grass, birch, and HDM; one with grass, birch, and dog hair; one with grass and mugwort; and one with grass, dog hair, and cat hair).

Ninety (70%) patients classified their AR and/or asthmatic symptoms as less severe after, compared to before, AIT, as shown in Figure 2. There were no statistically significant differences between non-responders and responders regarding age, sex, prevalence of asthma, or prior pharmacological treatment in any of the three treatment groups. Complete characteristics of the patients, stratified by target allergen, are given in Table I.

Responders were more satisfied with their AIT compared to non-responders across the subgroups treated for either grass, or grass and birch (8.75 vs. 5, p=0.014; 9 vs. 6, p=0.021). The responders were more satisfied also in the subgroup of patients treated for only birch allergy; however, without a statistically significant difference (9 vs. 7.75, p=0.214).

Across all three treatment groups, there were statistically significant differences in how non-responders and responders rated how much their quality of life was affected by their allergy after completed AIT (grass: 5.5 vs. 2, p<0.001; birch: 7 vs. 2.25, p=0.004; grass and birch: 6 vs. 2, p=0.001) (Table I).

In the group of patients treated for both grass and birch allergy, a total of 32 different molecular patterns were observed, 17 of which were found in the patients treated only for grass allergy, and nine in those treated for birch allergy only (Figure 3).

There were no large differences in molecular spread (defined as number of sIgE molecules to which a subject is sensitized) between non-responders and responders. However, within the subgroup of subjects treated for both grass and birch, the molecular spread was marginally lower in non-responders compared to responders (interquartile range (IQR) 4-5 vs. IQR 5-6, p=0.017) (Table II).

There was a significant difference in dispersion of concentration of sIgE to g6 molecules within subjects treated for both grass and birch. However, no obvious linear relationship could be seen. On comparison of the concentration of allergen sIgE antibodies within the other treatment groups, no statistically significant differences were found (Table II).

The levels of sIgE were also compared between non-responders and responders when stratified based on what type of symptoms the subjects experienced, AR only or AR and asthmatic, (Table III). There was only a statistically significant difference in dispersion of level of sIgE to g6 between non-responders and responders in the subgroup of patients who reported both AR and asthmatic symptoms.

DISCUSSION

We found that 70% of the patients ranked their symptoms as less severe after completion of, compared to before, AIT (65%, 67%, and 74% for the subgroups treated for grass, birch, and both grass and birch, respectively). Despite difficulties in comparing studies due to differences in outcome measures, our findings correspond relatively well to earlier studies, which found response rates to AIT of around 80% [8,9]. This indicates that, although most patients did benefit from AIT, 30% of the study subjects underwent a time-consuming and resource-intensive treatment without any obvious positive clinical effect. Although these proportions might change if using more refined outcome measures, this highlights the

importance of finding suitable predictive factors to better select the patients who will benefit from AIT.

In this study, the effect of AIT was assessed by a questionnaire using an NRS for the evaluation of allergic symptoms both before and after AIT. Although the NRS has not been validated for measuring the severity of AR, a similar instrument, the visual analog scale, has been shown to correspond well to AR severity [15] and evaluation of improvement in symptoms after AIT [16]. However, a less subjective measure of symptoms, as well the inclusion of a control group, would be required to accurately measure the effect size of AIT treatment in individual patients.

Our study included several patients aged 50 years or older, but the median age of the subjects was 32 years, which could indicate that the target population for AIT is of a relatively young age. However, AIT can be considered for the treatment of AR despite old age if no other contraindications exist [17].

In this study, 60% of the subjects were female. This distribution may have had some effect on our results as it has been shown that, despite the same immunological mechanisms of allergy, there is a clear clinical difference between female and male allergic patients. From adolescence onwards, female subjects suffer more often from allergies. This difference points to a role of sex hormones, intake of contraceptives, pregnancy, and hormone replacement therapy [18]. However, sex did not seem to have a significant influence on the effect of AIT in our findings.

Most of the subjects in this study were using antihistamines and local nasal steroids prior to receiving AIT, but a much smaller proportion of the subjects had been using systemic steroids. The proportion of patients who had used systemic steroids prior to AIT was slightly higher in the group who improved after AIT. Despite the lack of statistical significance, it is possible that use of systemic steroids could indicate a more severe form of allergy and support the current clinical practice of using AIT as a last line of treatment when conventional pharmacological treatment has failed [19].

Our findings showed that, across all subgroups, responders were more satisfied with their AIT compared to non-responders. Both patient-related factors such as age, and AIT-related factors such as duration and side effects, influence patients' satisfaction [20]. We also found statistically significant differences in how patients in all subgroups rated their quality

of life after completing AIT. This finding is well in accordance with reports by other researchers [21].

Serum IgE antibodies for birch and Timothy grass pollen were analyzed using the EUROLINE DPA-Dx pollen 1 method (EUROBlotOne, EUROIMMUN AG, Lübeck, Germany). The relevance of this method has been demonstrated [22].

Our results revealed a total of 35 different sIgE profiles across our 128 study subjects, which indicates the immense immunological heterogeneity in subjects who are allergic to the same pollen. Cipriani et al and Tripodi et al have shown that the number of these profiles varies greatly in different studies [16,23]. In our results, IgE profiles with a higher number of molecules (higher molecular spread) seemed to respond better to AIT targeting grass and birch simultaneously. However, it is important to note that no correction was made for multiple testing and, consequently, care should be taken when interpreting these p-values.

Our results revealed that Bet v 1 is the predominant birch pollen component, while Bet v 2, Bet v 4 and Bet v 6 are not common in the studied population. This finding confirms previously published data on sensitisation to these molecules in European populations [24].

Serum IgE to CCD was found in only 13% of the patients. This finding shows that most of the patients were not sensitive to this molecule, and there were no significant differences among the groups. Similarly, we could not find evidence that sIgE to panallergen molecules was more prevalent in subjects who responded to AIT than in those who did not. We could not show evidence of statistically significant differences in the levels of sIgE to panallergens (Phl p 7, Phl p 12, Bet v 2 and Bet v 4) among subjects who responded to AIT than in those who did not.

Moreover, from the outcome of this study no correlation could be seen between molecular sensitization profile for Phl p 7 and asthma. This is in contrast to what have been proposed that molecular sensitization to Phl p 7 is a reliable biomarker of asthma [25].

From our results, no clear association could be found between pre-AIT concentration of sIgE and AIT outcome. Using IgE as a biomarker in AIT has been reported to have conflicting outcomes [16]. One study found that a cutoff sIgE level >10 kU/L can be associated with perception of effective AIT [26]. In our study, there were no similar cutoffs.

We did find a statistically significant difference in levels of sIgE to g6 in the subgroup of subjects treated for grass and birch, where there was a higher percentage of

subjects in the responder group with sIgE to g6 >50 kU/L. This could indicate that the concentration of sIgE to g6 shows promise as a predictor for AIT outcome. However, this does not hold true in the subgroups treated for grass or birch separately. When the subjects were stratified based on type of symptoms, this difference in g6 level between non-responders and responders were only statistically significant within the subjects who reported both AR and asthmatic symptoms.

Overall, we expected to see more distinct differences in the levels of the allergen-specific IgE for birch and grass components between subjects who responded to AIT and subjects who did not.

The lack of statistically significant differences between responders and non-responders could indicate that there are other factors, not considered in this study, that dictate how well a patient will respond to AIT. An interesting yet unanswered question is whether sensitization profiles affect individual outcomes of AIT. It is not clear whether patients with different sensitization profiles respond differently to the same AIT [25]. Although there is significant interest in patient-tailored AIT, no such product has yet reached the commercial market.

A factor that may have influenced our results is related to which specific allergen components are found in the aluminium-adsorbed extract (Alutard SQ® products, ALK-Abelló). Alutard SQ products contain several different pollen proteins, both the main allergen and most minor allergens from grass or birch, but the exact ratios of these are unknown. As the European regulation of allergen products allows for great variation, different batches of the Alutard SQ product can have different allergenic content [27]. The standardization of allergen extract for diagnosis and therapy is still an open issue in allergology [25]. It is possible that the exact constituents of Alutard SQ vary with the variability in pollen intensity in different years, which could influence the efficacy of AIT. It is therefore possible that the subjects who did not respond or who reported only a moderate effect after AIT were sensitized to an allergen component that was not found in the allergen extract used in the AIT. One weakness of our study is that not all subjects were treated with the same allergen mixture, as some were treated with single allergen mixtures and some with compound mixtures containing allergens from several different grass/tree species. However, the proportion of patients who received a different allergen mixture was so small that we do not believe it has affected our results.

An environmental factor which may have affected the levels of IgE in our study subjects is when the samples were collected, as the blood samples in this study were collected a few months before the start of the actual pollen season.

The main limitation of this study is the low response rate to the patient questionnaires. The risk of non-response bias must be considered, as patients who had a positive experience of their AIT may have been more inclined to answer the questionnaire, and vice versa. We cannot exclude that the outcomes may have been different if more patients had participated in the study. However, our results on the overall effect of AIT are relatively equal to the results of previous, similar studies, which may indicate that the sample is representative [9,28].

Another limitation is the time elapsed from end of treatment to follow-up, since all patients answered the questionnaire in late 2018 or early 2019 while the time of the end of treatment varied from 2002 to 2018. Consequently, there is the possibility of recall bias and this may eventually have affected the low response rate. In future research, it might be possible to include a correction for time to follow-up in the analysis to avoid such limitation. Another limitation could be the long time the blood samples were stored, as this can affect the composition and quality of the biomolecules [29].

CONCLUSION

We found that the majority of the patients rated their allergic symptoms as less severe after, compared to before, AIT. No clear relationship was demonstrated between pre-treatment sIgE concentration or demographic factors, and effect of AIT. As the patients who did not respond to treatment had the same phenotype and IgE profiles as those who responded, this clearly indicates that there may be other factors underlying the different treatment responses. This urges us to conduct further studies to look for other substances (biomarkers) that predict or have an effect on the outcome of AIT.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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Table I. Characteristics of the study subjects both total and stratified by treatment groups. Categorical variables are presented as frequency (percentage), interval and ordinal level variables as median (IQR). P-values presented are from X^2- or Fisher's exact-tests for categorical variables, and Mann Whitney U-tests for interval and ordinal variables.
AIT – Allergen Immunotherapy; ICS – Inhaled Corticosteroids; LTRA – Leukotriene Receptor Antagonists; QoL – Quality of Life; * = p-value<0.05; ** = only reported for subjects with asthma according to medical records and/or questionnaire.

		Total			Grass			Birch			Grass and birch		
		Non-responders	Responders	p-value	Non- responders	Responders	p-value	Non- responders	Responders	p-value	Non- responders	Responders	p-value
N		38	90	–	12	22	–	8	16	–	18	52	–
Age, yrs	median (min–max)	32 (20–40.5)	32.5 (24.75–40)	0.518	37.5 (19.5–42.75)	37.5 (28.25–43.5)	0.631	36.5 (28.5–44.5)	35 (26–40)	0.610	25.5 (19.75–35.5)	29.5 (24–36.75)	0.160
	<30	18 (47%)	38 (42%)	0.592	4 (33%)	6 (27%)	0.714	2 (25%)	6 (38%)	0.667	12 (67%)	26 (50%)	0.221
Sex	Female	26 (68%)	51 (57%)	0.215	7 (58%)	12 (55%)	0.832	6 (75%)	13 (81%)	1.000	13 (72%)	26 (50%)	0.102
Additional target allergen		3 (8%)	9 (10%)	1.000	2 (17%)	2 (9%)	0.602	0 (0%)	0 (0%)	–	1 (6%)	7 (13%)	0.670
Pre-AIT treatment	Antihistamines	34 (89%)	80 (89%)	1.000	11 (92%)	21 (95%)	1.000	7 (88%)	15 (94%)	1.000	16 (89%)	44 (85%)	1.000
	Local nasal steroids	31 (82%)	61 (68%)	0.113	9 (75%)	16 (73%)	1.000	7 (88%)	10 (63%)	0.352	15 (83%)	35 (67%)	0.195
	Systemic steroids	11 (29%)	31 (34%)	0.545	3 (25%)	5 (23%)	1.000	3 (38%)	5 (31%)	1.000	5 (28%)	21 (40%)	0.340
Asthma	Diagnosed	10 (26%)	24 (27%)	0.967	2 (17%)	5 (23%)	1.000	3 (38%)	7 (44%)	1.000	5 (28%)	12 (23%)	0.753
	Self-reported	19 (50%)	42 (47%)	0.730	5 (42%)	9 (41%)	1.000	5 (63%)	10 (63%)	1.000	9 (50%)	23 (44%)	0.672
Pre-AIT asthma treatment**	Bronchodilator	11 (65%)	34 (72%)	0.555	4 (80%)	9 (90%)	1.000	3 (75%)	7 (64%)	1.000	4 (50%)	18 (69%)	0.410
	ICS	9 (53%)	30 (64%)	0.430	3 (60%)	8 (80%)	0.560	2 (50%)	8 (73%)	0.560	4 (50%)	14 (54%)	1.000
	LTRA	2 (12%)	2 (4%)	0.285	0 (0%)	1 (10%)	1.000	0 (0%)	0 (0%)	-	2 (25%)	1 (4%)	0.131
	None	5 (29%)	9 (19%)	0.495	1 (20%)	0 (0%)	0.333	1 (25%)	3 (27%)	1.000	3 (38%)	6 (23%)	0.649
Satisfaction with treatment		6.5 (0.5–10)	9 (0–10)	<0.001*	5 (1–10)	8.75 (1–10)	0.014*	7.75 (0.5–10)	9 (3–10)	0.214	6 (2.5–10)	9 (0–10)	0.021
QoL after treatment		6 (1–10)	2 (0–10)	<0.001*	5.5 (1–8)	2 (0–4)	<0.001*	7 (1.5–10)	2.25 (0–5)	0.004*	6 (1–9)	2 (0–10)	<0.001*

Table II. Comparison of molecular spread and specific immunoglobulin E (IgE) levels between improved and non-improved subjects, stratified by treatment group. Molecular spread is presented as median (interquartile range (IQR)) with p-values from Mann-Whitney U-test. Allergen-specific IgE levels are presented as frequency (%), with p-values from Fisher's exact test.
CCD = cross-reactive carbohydrate determinant; * = p-value<0.05.

		Total			Grass			Birch			Grass and birch		
		Non-responders	Responders	p-value	Non-responders	Responders	p-value	Non-responders	Responders	p-value	Non-responders	Responders	p-value
N		38	90	–	12	22	–	8	16	–	18	52	–
Molecular spread		4 (3.75–5)	5 (3–6)	0.097	4 (3–4.75)	4 (3–5.25)	0.929	2 (2–5)	3 (2–4)	0.573	5 (4–5)	5 (5–6)	0.017*
p12	<0.35	33 (87%)	76 (84%)	0.710	9 (75%)	18 (82%)	0.156	7 (88%)	15 (94%)	0.565	17 (94%)	43 (83%)	0.141
	0.35–3.4	2 (5%)	6 (7%)		2 (17%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	6 (12%)	
	3.5–49.9	1 (3%)	6 (7%)		0 (0%)	3 (14%)		1 (13%)	0 (0%)		0 (0%)	3 (6%)	
	>50	2 (5%)	2 (2%)		1 (8%)	1 (5%)		0 (0%)	1 (6%)		1 (6%)	0 (0%)	
p7	<0.35	37 (97%)	88 (98%)	0.359	12 (100%)	21 (95%)	1.000	7 (88%)	16 (100%)	0.333	18 (100%)	51 (98%)	1.000
	0.35–3.4	1 (3%)	0 (0%)		0 (0%)	0 (0%)		1 (13%)	0 (0%)		0 (0%)	0 (0%)	
	3.5–49.9	0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	
	>50	0 (0%)	2 (2%)		0 (0%)	1 (5%)		0 (0%)	0 (0%)		0 (0%)	1 (2%)	
p5	<0.35	14 (37%)	26 (29%)	0.124	1 (8%)	4 (18%)	0.866	6 (75%)	14 (88%)	0.196	7 (39%)	8 (15%)	0.077
	0.35–3.4	3 (8%)	1 (1%)		0 (0%)	0 (0%)		2 (25%)	0 (0%)		1 (6%)	1 (2%)	
	3.5–49.9	4 (11%)	9 (10%)		2 (17%)	3 (14%)		0 (0%)	1 (6%)		2 (11%)	5 (10%)	
	>50	17 (45%)	54 (60%)		9 (75%)	15 (68%)		0 (0%)	1 (6%)		8 (44%)	38 (73%)	
p1	<0.35	5 (13%)	16 (18%)	0.126	0 (0%)	1 (5%)	1.000	5 (63%)	10 (63%)	0.178	0 (0%)	5 (10%)	0.283
	0.35–3.4	0 (0%)	4 (4%)		0 (0%)	0 (0%)		0 (0%)	3 (19%)		0 (0%)	1 (2%)	
	3.5–49.9	5 (13%)	3 (3%)		0 (0%)	0 (0%)		2 (25%)	0 (0%)		3 (17%)	3 (6%)	
	>50	28 (74%)	67 (74%)		12 (100%)	21 (95%)		1 (13%)	3 (19%)		15 (83%)	43 (83%)	
g6	<0.35	5 (13%)	12 (13%)	0.029*	0 (0%)	0 (0%)	0.537	5 (63%)	10 (63%)	0.115	0 (0%)	2 (4%)	0.004*
	0.35–3.4	0 (0%)	6 (7%)		0 (0%)	0 (0%)		0 (0%)	4 (25%)		0 (0%)	2 (4%)	
	3.5–49.9	9 (24%)	6 (7%)		0 (0%)	3 (14%)		2 (25%)	0 (0%)		7 (39%)	3 (6%)	
	>50	24 (63%)	66 (73%)		12 (100%)	19 (86%)		1 (13%)	2 (13%)		11 (61%)	45 (87%)	
v6	<0.35	36 (95%)	82 (91%)	0.129	11 (92%)	22 (100%)	0.353	8 (100%)	14 (88%)	1.000	17 (94%)	46 (88%)	0.249
	0.35–3.4	0 (0%)	6 (7%)		0 (0%)	0 (0%)		0 (0%)	1 (6%)		0 (0%)	5 (10%)	
	3.5–49.9	1 (3%)	2 (2%)		0 (0%)	0 (0%)		0 (0%)	1 (6%)		1 (6%)	1 (2%)	
	>50	1 (3%)	0 (0%)		1 (8%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	
v4	<0.35	38 (100%)	88 (98%)	1.000	12 (100%)	21 (95%)	1.000	8 (100%)	16 (100%)	–	18 (100%)	51 (98%)	1.000
	0.35–3.4	0 (0%)	1 (1%)		0 (0%)	1 (5%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	
	3.5–49.9	0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	
	>50	0 (0%)	1 (1%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	1 (2%)	
v2	<0.35	33 (87%)	76 (84%)	1.000	8 (67%)	17 (77%)	0.726	8 (100%)	16 (100%)	–	17 (94%)	43 (83%)	0.299
	0.35–3.4	2 (5%)	7 (8%)		2 (17%)	2 (9%)		0 (0%)	0 (0%)		0 (0%)	5 (10%)	
	3.5–49.9	2 (5%)	5 (6%)		2 (17%)	2 (9%)		0 (0%)	0 (0%)		0 (0%)	3 (6%)	
	>50	1 (3%)	2 (2%)		0 (0%)	1 (5%)		0 (0%)	0 (0%)		1 (6%)	1 (2%)	
v1	<0.35	10 (26%)	10 (11%)	0.203	9 (75%)	10 (45%)	0.428	0 (0%)	0 (0%)	1.000	1 (6%)	0 (0%)	0.514
	0.35–3.4	1 (3%)	5 (6%)		1 (8%)	4 (18%)		0 (0%)	0 (0%)		0 (0%)	1 (2%)	
	3.5–49.9	4 (11%)	13 (14%)		1 (8%)	4 (18%)		1 (13%)	1 (6%)		2 (11%)	8 (15%)	
	>50	23 (61%)	62 (69%)		1 (8%)	4 (18%)		7 (88%)	15 (94%)		15 (83%)	43 (83%)	
t3	<0.35	10 (26%)	13 (14%)	0.433	10 (83%)	13 (59%)	0.562	0 (0%)	0 (0%)	1.000	0 (0%)	0 (0%)	1.000
	0.35–3.4	2 (5%)	4 (4%)		1 (8%)	2 (9%)		0 (0%)	0 (0%)		1 (6%)	2 (4%)	
	3.5–49.9	4 (11%)	13 (14%)		0 (0%)	3 (14%)		1 (13%)	1 (6%)		3 (17%)	9 (17%)	
	>50	22 (58%)	60 (67%)		1 (8%)	4 (18%)		7 (88%)	15 (94%)		14 (78%)	41 (79%)	
CCD	<0.35	35 (92%)	76 (84%)	0.526	11 (92%)	19 (86%)	1.000	7 (88%)	16 (100%)	0.333	17 (94%)	41 (79%)	0.338
	0.35–3.4	1 (3%)	8 (9%)		1 (8%)	2 (9%)		0 (0%)	0 (0%)		0 (0%)	6 (12%)	
	3.5–49.9	2 (5%)	6 (7%)		0 (0%)	1 (5%)		1 (13%)	0 (0%)		1 (6%)	5 (10%)	
	>50	0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	

Table III. Comparison of specific immunoglobulin E (IgE) levels between improved and non-improved subjects, stratified by symptom groups both as gathered from the subjects medical records as well as according to the questionnaires. Allergen-specific IgE levels are presented as frequency (%), with p-values from Fisher's exact test.
CCD = cross-reactive carbohydrate determinant; AR = allergic rhinitis; * = p-value<0.05.

		Symptoms according to medical records						Symptoms according to questionnaire					
		AR only			AR and asthma			AR only			AR and asthma		
		Non-responders	Responders	p-value	Non-responders	Responders	p-value	Non-responders	Responders	p-value	Non-responders	Responders	p-value
N		28	66		10	24		19	48		19	42	
p12	<0.35	26 (93%)	56 (85%)	0.521	7 (70%)	20 (83%)	0.690	18 (95%)	39 (81%)	0.247	15 (79%)	37 (88%)	0.648
	0.35-3.4	1 (4%)	4 (6%)		1 (10%)	2 (8%)		0 (0%)	4 (8%)		2 (11%)	2 (5%)	
	3.5-49.9	0 (0%)	5 (8%)		1 (10%)	1 (4%)		0 (0%)	4 (8%)		1 (5%)	2 (5%)	
	>50	1 (4%)	1 (2%)		1 (10%)	1 (4%)		1 (5%)	1 (2%)		1 (5%)	1 (2%)	
p7	<0.35	28 (100%)	65 (98%)	1.000	9 (90%)	23 (96%)	0.508	19 (100%)	47 (98%)	1.000	18 (95%)	41 (98%)	0.530
	0.35-3.4	0 (0%)	0 (0%)		1 (10%)	0 (0%)		0 (0%)	0 (0%)		1 (5%)	0 (0%)	
	3.5-49.9	0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	
	>50	0 (0%)	1 (2%)		0 (0%)	1 (4%)		0 (0%)	1 (2%)		0 (0%)	1 (2%)	
p5	<0.35	9 (32%)	17 (26%)	0.561	5 (50%)	9 (38%)	0.11	7 (37%)	10 (21%)	0.379	7 (37%)	16 (38%)	0.085
	0.35-3.4	1 (4%)	1 (2%)		2 (20%)	0 (0%)		0 (0%)	1 (2%)		3 (16%)	0 (0%)	
	3.5-49.9	4 (14%)	7 (11%)		0 (0%)	2 (8%)		3 (16%)	5 (10%)		1 (5%)	4 (10%)	
	>50	14 (50%)	41 (62%)		3 (30%)	13 (54%)		9 (47%)	32 (67%)		8 (42%)	22 (52%)	
p1	<0.35	4 (14%)	10 (15%)	0.615	1 (10%)	6 (25%)	0.180	3 (16%)	7 (15%)	0.533	2 (11%)	9 (21%)	0.284
	0.35-3.4	0 (0%)	2 (3%)		0 (0%)	2 (8%)		0 (0%)	1 (2%)		0 (0%)	3 (7%)	
	3.5-49.9	3 (11%)	3 (5%)		2 (20%)	0 (0%)		2 (11%)	1 (2%)		3 (16%)	2 (5%)	
	>50	21 (75%)	51 (77%)		7 (70%)	16 (67%)		14 (74%)	39 (81%)		14 (74%)	28 (67%)	
g6	<0.35	4 (14%)	9 (14%)	0.424	1 (10%)	8 (33%)	0.041*	3 (16%)	5 (10%)	0.647	2 (11%)	7 (17%)	0.022*
	0.35-3.4	0 (0%)	2 (3%)		0 (0%)	4 (17%)		0 (0%)	1 (2%)		0 (0%)	5 (12%)	
	3.5-49.9	5 (18%)	5 (8%)		4 (40%)	1 (4%)		3 (16%)	4 (8%)		6 (32%)	2 (5%)	
	>50	19 (68%)	50 (76%)		5 (50%)	16 (67%)		13 (68%)	38 (79%)		11 (58%)	28 (67%)	
v6	<0.35	26 (93%)	62 (94%)	0.085	10 (100%)	20 (83%)	1.000	17 (89%)	43 (90%)	0.198	19 (100%)	39 (93%)	1.000
	0.35-3.4	0 (0%)	4 (6%)		0 (0%)	2 (8%)		0 (0%)	4 (8%)		0 (0%)	2 (5%)	
	3.5-49.9	1 (4%)	0 (0%)		0 (0%)	2 (8%)		1 (5%)	1 (2%)		0 (0%)	1 (2%)	
	>50	1 (4%)	0 (0%)		0 (0%)	0 (0%)		1 (5%)	0 (0%)		0 (0%)	0 (0%)	
v4	<0.35	28 (100%)	65 (98%)	1.000	10 (100%)	23 (96%)	1.000	19 (100%)	47 (98%)	1.000	19 (100%)	41 (98%)	1.000
	0.35-3.4	0 (0%)	1 (2%)		0 (0%)	0 (0%)		0 (0%)	1 (2%)		0 (0%)	0 (0%)	
	3.5-49.9	0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	
	>50	0 (0%)	0 (0%)		0 (0%)	1 (4%)		0 (0%)	0 (0%)		0 (0%)	1 (2%)	
v2	<0.35	25 (89%)	56 (85%)	1.000	8 (80%)	20 (83%)	0.584	17 (89%)	41 (85%)	0.512	16 (84%)	35 (83%)	0.298
	0.35-3.4	2 (7%)	5 (8%)		0 (0%)	2 (8%)		2 (11%)	3 (6%)		0 (0%)	4 (10%)	
	3.5-49.9	1 (4%)	4 (6%)		1 (10%)	1 (4%)		0 (0%)	4 (8%)		2 (11%)	1 (2%)	
	>50	0 (0%)	1 (2%)		1 (10%)	1 (4%)		0 (0%)	0 (0%)		1 (5%)	2 (5%)	
v1	<0.35	8 (29%)	9 (14%)	0.1[illegible]	2 (20%)	1 (4%)	0.267	5 (26%)	8 (17%)	0.761	5 (26%)	2 (5%)	0.092
	0.35-3.4	0 (0%)	3 (5%)		1 (10%)	2 (8%)		0 (0%)	2 (4%)		1 (5%)	3 (7%)	
	3.5-49.9	3 (11%)	12 (18%)		1 (10%)	1 (4%)		3 (16%)	10 (21%)		1 (5%)	3 (7%)	
	>50	17 (61%)	42 (64%)		6 (60%)	20 (83%)		11 (58%)	28 (58%)		12 (63%)	34 (81%)	
t3	<0.35	8 (29%)	11 (17%)	0.639	2 (20%)	2 (8%)	0.699	5 (26%)	9 (19%)	0.940	5 (26%)	4 (10%)	0.282
	0.35-3.4	1 (4%)	3 (5%)		1 (10%)	1 (4%)		1 (5%)	3 (6%)		1 (5%)	1 (2%)	
	3.5-49.9	3 (11%)	0 (15%)		1 (10%)	3 (13%)		3 (16%)	8 (17%)		1 (5%)	5 (12%)	
	>50	16 (57%)	42 (64%)		6 (60%)	18 (75%)		10 (53%)	28 (58%)		12 (63%)	32 (76%)	
CCD	<0.35	27 (96%)	57 (86%)	0.443	8 (80%)	19 (79%)	0.821	18 (95%)	39 (81%)	0.462	17 (89%)	37 (88%)	0.453
	0.35-3.4	1 (4%)	6 (9%)		0 (0%)	2 (8%)		1 (5%)	5 (10%)		0 (0%)	3 (7%)	
	3.5-49.9	0 (0%)	3 (5%)		2 (20%)	3 (13%)		0 (0%)	4 (8%)		2 (11%)	2 (5%)	
	>50	0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)		0 (0%)	0 (0%)	

Figure 1. Flowchart of the inclusion process

Figure 2. Pre- vs. post-AIT symptom severity of the study subjects
AIT = Allergen ImmunoTherapy
* = Two patients rated their symptoms as moderate both before and after treatment, and thus are not considered as improved.

Figure 3. Specific Immunoglobulin-E profiles for the subjects, stratified by target allergen.