UNIVERSITY OF TURKU

Specific immunoglobulin E analysis in adults presenting with birch pollen allergy symptoms

Institute of Biomedicine
MDP in Biomedical Sciences, Drug Discovery and Development
Master's thesis

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The increase of IgE-mediated allergies and asthma is a socioeconomic challenge in developed countries. Although there are millions of people suffering from different types of allergies, they are often underdiagnosed, and almost half of the allergic people have not received a proper diagnosis for their symptoms.

Pollens are a major cause of allergic symptoms. Birch pollen is the most common cause of rhinitis and rhinoconjunctivitis in Finland. The major birch pollen allergen Bet v 1 is a protein that is associated with a majority of immunoglobulin E (IgE)-mediated allergies in the spring. It is very cross-reactive, so people who are sensitized to Bet v 1 may often react to many fruits and vegetables, too. Bet v 1 is the most commonly used allergen in immunotherapies of birch pollen allergy, but since also many other allergens are causing allergic symptoms, a specific diagnosis is important in order to treat the patient correctly.

This Master's thesis aimed to determine the current prevalence and specific allergens of birch pollen allergy in adults living in South-West Finland and suffering from significant symptoms of seasonal rhinitis or rhinoconjunctivitis, suspected on clinical grounds to be caused by allergy to birch pollen. The study included 148 volunteers (44 males and 104 females). A component analysis was performed to explore the presence of specific IgE antibodies against Bet v 1, other major birch pollen allergens (Bet v2 and Bet v 4) and several other known main airborne and food allergens in the Finnish adult population. Most (84 %) of the subjects were positive (positivity threshold, serum IgE concentration ≥ 0.35 kUA/L) for at least one specific IgE species against plant- or animal-derived allergens, and 80 % of the study participants were found to be sensitized to birch pollen, according to the serum IgE analysis. Specific IgE antibodies against Bet v 1 were observed in almost all participants who were IgE-positive for birch pollen antigens as a group (116 of 118 subjects). In 24 subjects (16 %), no IgE-based allergy diagnosis could be established. 30 subjects (20 %) were negative for IgE against birch pollen.

The average IgE concentration tended to be lower in older subjects. The mean IgE concentration of airborne allergens in the youngest age group (18-29 years) was significantly higher ($p<0.0001$) than in the oldest age group (50-65 years). No difference was observed between male and female subjects.

Many subjects who were found to be sensitized to food allergens had IgE antibodies against allergens that are known to cause cross-reactivity with birch pollen, for example, soy component Gly m 4 and peanut component Ara h 8. It has been previously shown that IgE antibodies to Gly m 4 and Ara h 8 allergens are generally due to sensitization to birch pollen since these allergens are Bet v 1 homologs, all belonging to the PR-10 protein family.

Based on the current results, it remains relevant to include specific serum IgE analysis in the diagnostic work-up of persons presenting with interfering symptoms of rhinitis or rhinoconjunctivitis during the birch pollen season and being considered for initiation of allergen immunotherapy with Bet v 1 –targeted products.

Key words: birch pollen allergy, immunoglobulin E, immunotherapy, Bet v 1

Table of contents

1 Introduction 4

1.1 Allergic disorders 4

1.2 Pollen allergies 5

1.3 Birch pollen allergens 6

1.4 Allergy treatments 10

1.4.1 Allergy treatments for mild symptoms 10

1.4.2 Allergen immunotherapy 11

1.5 Aim of the study 12

2 Results 13

2.1 Demographic and other baseline characteristics 13

2.2 Physical examination 14

2.3 Adverse events 16

2.4 Specific IgE analysis 17

3 Discussion 25

4 Materials and methods 28

4.1 Specific IgE analysis 28

4.2 Ethical issues 30

4.3 Statistical analysis 30

5 Acknowledgements 32

6 Abbreviations 33

References 34

Appendix 37

1 Introduction

1.1 Allergic disorders

There are many factors that affect the likelihood of an individual of developing an allergic illness. Genetics, exposure to allergens and other features in the environment all contribute to why some people are allergic and others are not. Children of allergic parents are very likely to develop allergies (Bønnelykke et al., 2015). The prevalence of allergies is increasing, and allergies are more common in developed countries, which has led to the "hygiene hypothesis", suggesting that in an environment lacking abundant pathogenic and non-pathogenic micro-organisms, the immune responses may not develop normally and allergies become more common (Galli et al., 2008).

Allergens are environmental substances that can either induce immunoglobulin E (IgE) production or be independent of IgE (Galli et al., 2008). In sensitized subjects, i.e. subjects with induced IgE production, re-exposure to the allergen typically causes an allergic reaction. This happens when the allergen cross-links with specific IgE molecules on the surface of mast cells, which then induce the release of mediators such as histamine and leukotrienes causing the onset of allergic symptoms (Royal College of Physicians of London. Working Party on the Provision of Allergy Services in the UK., 2003). Nickel is an example of another type of allergen that can induce immune responses with local inflammation causing the symptoms of allergic contact dermatitis, without the involvement of IgE (Galli et al., 2008). The main mechanisms of different types of allergic disorders are illustrated in Figure 1. This thesis deals solely with allergies where mast cells and IgE are involved.

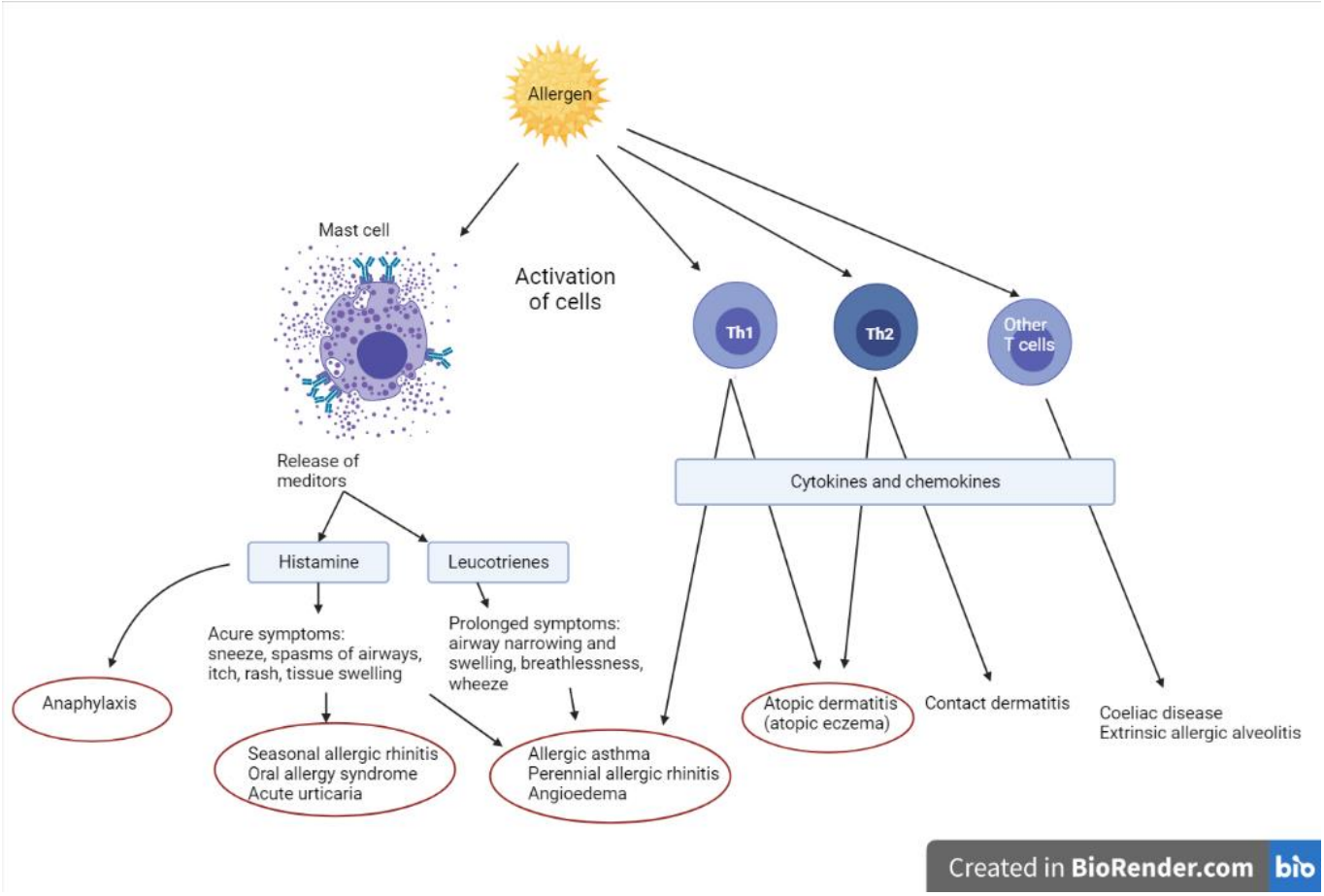

Figure 1. Illustration of the main mechanisms of allergic conditions

Th1 = type 1 T helper cells, Th2 = type 2 T helper cells. IgE-mediated allergic disorders are marked with red circles. (Modified from House of Lords Science and Technology, sixth report, 2007).

1.2 Pollen allergies

Respiratory allergies are the most common form of allergies in Europe. Currently, more than 150 million Europeans suffer from allergic rhinitis or asthma, which are common IgE hypersensitivity (type I) mediated immune disorders. Allergic diseases can disturb sleep, affect work or school performance, and have a generally negative impact on the quality of life. Each year, asthma and allergic rhinitis result in more than 100 million lost work and school days in Europe. Despite that, respiratory allergies are underdiagnosed. It has been estimated that approximately 45 % of all patients have never received a proper diagnosis. (European Federation of Allergy and Airways Diseases Patients Associations, 2011)

Pollens are one of the most frequent triggers of allergic symptoms. In Finland, 20 % of the population suffers from pollen allergy (Allergia-, iho- ja astmaliitto ry, 2021). The most important sources of allergenic pollen are birch, alder, various grasses, and mugwort. Of

these, birch pollen is the most common cause of allergic rhinitis. It is also very cross-reactive: patients allergic to birch pollen often react to alder, hazel, oak, ash, and several fruits and vegetables as well (Kleine-Tebbe & Jakob, 2017).

Seasonal pollen allergy can often be identified by the timing and nature of the allergic symptoms (rhinitis, conjunctivitis, sneezing, itching and watery eyes) if the patient is monosensitized to one type of pollen. For example, the peak birch pollen season in Finland depends on the latitude, beginning in April in Southern Finland and ending by Midsummer in the north (Allergia-, iho- ja astmaliitto ry, 2021). Early-pollinating cross-reactive species – such as alder that starts blooming in March-April – as well as wind-carried pollen from Central Europe, may, however, cause symptoms already in February, but the peak in symptoms usually follows the peak of the local birch pollen season (D'Amato et al., 1998).

Allergies are usually treated according to their symptoms, and the exact reason for the allergic symptoms does not needed to be tested and identified if the symptoms are mild (Blomgren, 2021). However, if the symptoms are severe or if there is a need to start specific immunotherapy, the sensitization to specific allergens should be confirmed.

IgE-mediated allergies can be confirmed with Skin Prick Tests (SPTs) and sometimes also with intradermal tests. SPT or "the scratch test" involves placing a small drop of a test liquid containg the suspected allergen on the skin, and then scratching or pricking the skin so that the allergen is introduced into the skin. The application site is then observed for signs of a reaction (usually, swelling and redness). Several suspected allergens can be tested at the same time within about 20 minutes (Henochowicz, 2020a). In intradermal allergy testing, a small amount of the suspected allergen is injected with a thin needle under the surface of the skin. This test is more sensitive than the SPT, and can usually provide more consistent results (Henochowicz, 2020b). Another approach to examine allergen-specific allergy is to determine allergen-specific IgE from serum. This is generally considered a sufficient method to confirm the cause of an allergy, but elevated IgE serum levels in serum alone are not enough to diagnose an allergy. For correct diagnosis, both clinical symptoms and relevant tests are evaluated. (European Academy of Allergy and Clinical Immunology, 2014)

1.3 Birch pollen allergens

Allergens that are specific to birch pollen, such as Bet v 1, Bet v 2 and Bet v 4, which are examined in this study, are named after the Latin name of white birch *Betula verrucosa*

(currently the synonym *Betula pendula* is favoured). These allergens belong to different protein families, including pathogenesis-related class 10 (PR-10) proteins and profilins. In addition to these three major previously birch pollen allergens, also other birch specific allergens (e.g. Bet v 6, Bet v 7 and Bet v 8) have been identified, but they are not examined in this master's thesis. It is now understood that Bet v 1 is the major pollen allergen of the white birch. It was first identified in 1989 in Austria, and after the first recombinant model was created, it has been one of the most commonly used allergens in allergy research (Kleine-Tebbe & Jakob, 2017). Bet v 1 is the main cause of type I (IgE-mediated) allergies observed in the spring. It is a 17 kD sized pollen protein belonging to the PR-10 protein family, and has high similarity, for example, with pollen allergens from to alder and hazel (Roth-Walter et al., 2014). Moreover, because of cross-reactivity, approximately 70 % of subjects allergic to fruits and vegetables are also sensitised to Bet v 1 (Vieths et al., 2002). Many fruits and vegetables contain allergens that are structural homologs of Bet v 1, which causes cross-reactivity. In Figure 2 there is a comparison between the structures of Bet v 1, a major celery allergen Api g 1 and a cherry allergen Pru av 1, showing the similarity of these molecules. In addition, symptoms caused by the cross-reactivity are similar to other allergic symptoms, and are presented in Table 1.

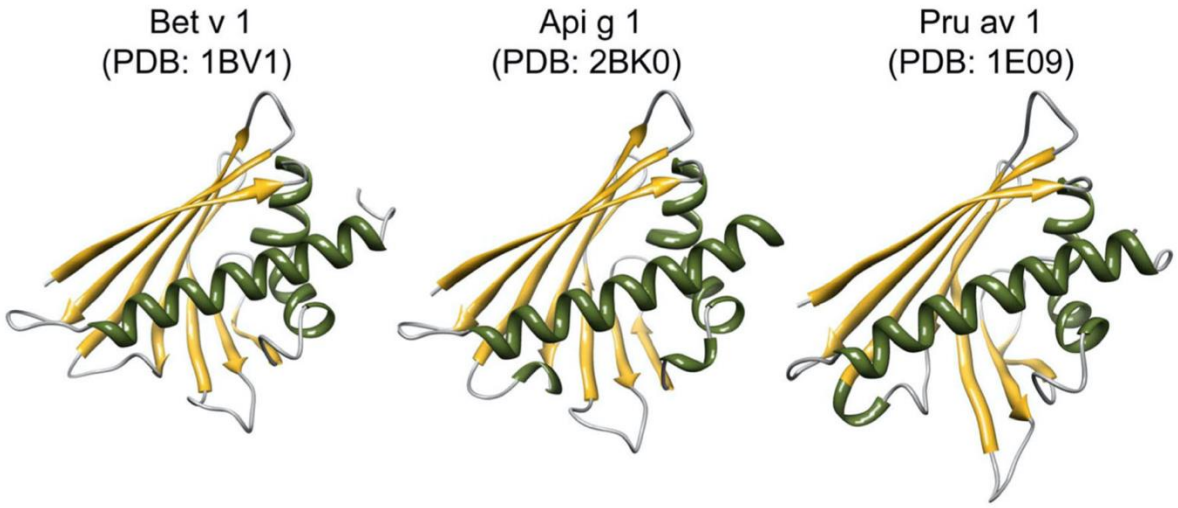

Figure 2. Structures of Bet v 1, Api g 1 and Pru av 1
(Adapted from Hauser et al., 2010)

The molecular structure of Bet v 1 is also similar to that of the protein lipocalin 2 (LCN2), which is mainly expressed in humans in the lungs, where it has many immunomodulatory functions. These functions depend on whether it binds iron (holo-form) or not (apo-form) (Roth-Walter et al., 2014). Also Bet v 1 has special pockets for binding iron. When there is no

iron ion bound in the pocket, the Bet v 1 molecule becomes an allergen and may cause allergic reactions. The protein manipulates type 2 T helper cells (Th2 cells) toward allergy. In allergic people, Th2 cells predominate over Th1 cells and there is an imbalance between Th1 and Th2 immune responses.

Research that has been done with Bet v 1 and other birch pollen allergens may help to understand the principles of allergy mechanisms, and the findings may also apply to other allergens with similar molecular structures. Consequently, it is also finally understood why certain allergies, such as pollen and food allergies, exist. (Roth-Walter et al., 2014)

Table 1. Possible symptoms caused by Bet v 1 cross-reactivity
(Modified from Klein-Tebbe et al., 2017)

Symptom complex	**Symptoms**	**Localization**
Solely oropharyngeal symptoms (frequent)	Itching	Labial mucosa, buccal mucosa, palate
	Burning, stinging	Palate, pharynx
	Mild mucosal swelling	Labial mucosa, buccal mucosa, palate, pharynx
Additional symptoms in the head area (in isolation or combination with symptoms above)	Itching, redness, watering of the eyes	Conjunctiva
(rare)	Itching, sneezing, runny nose, nasal congestion	Nose
	Itching	Ears, inner (Eustachian tubes)
	External swelling (angioedema)	Eyelids, lips, cheeks, ears, face
	Internal, pronounced swelling, globus hystericus, difficulty swallowing, hoarseness, respiratory distress, stridor	Palate, pharynx, larynx
Systemic manifestations (extremely rare)	Itching, redness, wheal formation, swelling	Localized, multifocal or generalized to the skin
	Nausea, vomiting, abdominal pain, diarrhoea	Gastrointestinal tract

	Difficulty breathing and chest pressure or tightness, respiratory distress, wheezing, coughing, possibly productive	Bronchi
	Vertigo (non-otologic), general weakness, syncope, circulatory collapse	Cardiovascular system

Bet v 2 is a 12-15 kDa protein, a member of the actin-binding profilin proteins that are ubiquitously expressed in cells of both animal and plant origin. It is considered a minor birch pollen allergen. (Sekerkova & Polackova, 2011)

Bet v 4 is also aanother minor birch pollen protein allergen, with a molecular weight of around 9 kDa. It belongs to polcalcins and has two binding sites for Ca2+, but also Bet v 4 specific IgE antibodies bind to these sites (Sekerkova & Polackova, 2011). Usually, fewer than under 10 % of allergic people are sensitised to polcalcins, but these proteinsy have a high ability to cross-sensitize allergic people (Asero et al., 2016).

There are both geographical and age-dependent differences in the reactivity of IgE antibodies in allergic individuals around the world. For example, in a study by Sekerková & Poláčková (2011), in which the presence of Bet v 1, Bet v 2 and Bet v 4 specific IgE antibodies in birch pollen allergic people living in the Czech Republic (107 children, 71 adults) was examined, Bet v 1 specific IgE antibodies were identified in most subjects, without any significant difference between children and adults. Bet v 2 positivity was found more frequently in children than in adults. Bet v 1 monospecificity was more common in adults compared to children. Specific IgE antibodies against Bet v 1 were not detected in 10 % of the subjects. Half of these lacked specific IgE antibodies against any of these three birch allergens (Sekerkova & Polackova, 2011).

Some previous information exists on specific serum IgE reactivity profiles to individual allergens also in Finland. Serum samples were collected from 51 sensitized individuals in two cross-sectional surveys performed in 1973 and 1994 in Vammala, in southwest Finland. The sera were analyzed for IgE reactivity to the main allergens present in timothy grass and/or birch pollen extracts. The median concentrations of IgE antibodies to timothy grass and birch pollen were higher in 1994 than in 1973, and the prevalence of IgE reactivity to some of the tested allergens was also higher in 1994 than in 1973, particularly concerning Bet v 1 (100 %

vs. 29 %). The authors suggested that the increase in specific IgE levels together with a possible increase in the prevalence of IgE reactivity to the major allergens Phl p 5 and Bet v 1 between 1973 and 1994 may have contributed to the observed increase in atopic disorders in Finland. (Movérare et al., 2006)

As Bet v 1 is only one of the allergenic molecules implicated in birch pollen allergy, there is a need to try to delineate its causal role in the clinical manifestations of birch pollen allergy-associated symptoms. Such knowledge may help to guide the development and use of specific immunotherapies.

1.4 Allergy treatments

In food or animal allergies, avoiding the allergen is the first line of treatment, but avoidance is usually not feasible in the case of airborne allergens (aeroallergens). H_1 -antihistamines, steroid-containing nasal sprays (i.a. beclometasone dipropionate, budesonide and fluticasone furoate) and eye drops that are available without prescription are recommended for controlling mild or intermittent symptoms, but they are often insufficient for controlling moderate/severe or persistent allergies (Pesonen, 2022). For patients having more severe symptoms, allergen immunotherapy is the only way to reduce or eliminate the symptoms (James & Bernstein, 2017).

1.4.1 Allergy treatments for mild symptoms

H1 -antihistaminics are the most commonly used allergy medication to treat allergic rhinitis and allergic conjunctivitis. H1 -antihistaminics for treating allergy are pharmacologically inverse agonists of H1 –receptors. They downregulate the activity of histamine at H1-receptors by binding to the inactive form of the receptor and locking it (Pesonen, 2022). This results in a reduction of allergy-related vasodilation, swelling and the accumulation of inflammatory cells at the site of inflammation and reduces itching, sneezing and watery eyes (Simons, 2003).

So-called first-generation H1 -antihistaminics cross the blood-brain barrier and therefore may cause fatigue and impaired performance. In addition, they may also have anticholinergic effects. Second-generation H1 -antihistaminics are more water-soluble so they have less potential to cross the blood-brain barrier. That is why they are devoid of sedative effects; in all, current second-generation H1 -antihistaminics have very few side effects and are well

tolerated (Kawauchi et al., 2019). Second-generation H_1 -antihistaminics used in Finland include i.a. cetirizine and levocetirizine, loratadine and desloratadine, and ebastine (Lehtimäki & Moilanen, 2018a).

If the antihistaminic alone is not efficacious enough, it can be combined with, for example, a leukotriene receptor blocker or glucocorticoid-containing nasal sprays or eye drops. Nasal sympathomimetics are also used to reduce nasal congestion but they must only be used over a few days, up to 10 days continuously, since they may cause rhinitis medicamentosa, meaning rebound rhinitis. (Lehtimäki & Moilanen, 2018b)

1.4.2 Allergen immunotherapy

It is estimated that around 15 million Europeans have birch pollen allergies, of whom about 10 % are believed to have symptoms that are not well controlled by conventional, symptom-relieving medications (ALK-Abelló A/S, 2018). In such cases, more detailed investigations are usually performed to map the person's allergies (and possible concomitant asthma, if suspected), and prescription medication is initiated. Allergen immunotherapy (AIT) may be considered when bothersome symptoms persist despite these measures. Currently, specific AIT is the only therapeutic modality that can achieve effective symptom control, associated with long-lasting changes in the underlying immune mechanisms that lead to increased tolerance to allergen exposure, modulation of disease progression and potentially remission. (Alvaro- Lozano et al., 2020)

Natural low-dose exposure to allergens induces allergen-specific IgE production in sensitive individuals by stimulation of a T helper cell immune response. In AIT, high-dose exposure to an allergen leads to suppression of the Th2 response and stimulation of T helper 1 and T regulatory cell pathways leading to the generation of allergen-specific IgG (mainly IgG4) antibodies (Eifan et al., 2011). This would suppress mast cell and basophil activation and lead to modulation of the immune response (Calderon et al., 2012).

AIT involves repeated administration of the relevant allergen for long periods, often for 1 to 3 years. AIT usually starts with a build-up phase during which the allergen doses progressively escalate, followed by a maintenance phase where a high, well-tolerated, allergen dose is given regularly for a prolonged period. Conventional forms of AIT include the administration of natural allergen extracts. There are different administration routes for AIT but the most

common routes are subcutaneous and sublingual, and both of them are equally efficacious when compared in clinical trials. (Calderón et al., 2011)

When a patient seeks medical care for his/her allergy, a more detailed specific diagnosis is often sought by SPT or specific IgE testing from a blood sample. Both will give information about specific allergic sensitizations, but they measure different aspects of sensitization. An SPT measures the local allergic reaction elicited by intradermal exposure to small amounts of a panel of allergens, whereas a blood IgE test measures the concentrations of circulating IgE types specific to different allergens. When AIT is considered as a treatment, a specific diagnosis is essential, because AIT involves administering increasing doses of the sensitizing allergen(s) with an aim to gradually desensitize the patient. Using incorrect allergens for AIT risks creating new sensitizations. (Alvaro- Lozano et al., 2020)

1.5 Aim of the study

The aim of this cross-sectional diagnostic clinical study was to determine the current prevalence and specific allergens of birch pollen allergy in adults living in South-West Finland and suffering from significant symptoms of seasonal rhinitis or rhinoconjunctivitis, suspected on clinical grounds to be caused by allergy to birch pollen. A component analysis was to be performed in order to explore the presence of specific IgE antibodies against Bet v 1, other major birch pollen allergens and several other known main allergens in the Finnish adult population. Such information may help to plan new immunotherapy strategies for birch pollen allergy and to guide the implementation of such strategies. Additionally, the prevalence of birch pollen allergy and birch-specific IgE in the Finnish population had not been examined after the study by Movérare et al. (2002). The current study participants might also be considered as candidates for specific AIT, and for correct targeting of AIT, information on specific allergens is needed, both on the individual and on the population level. The prevalence of pollen allergies is increasing so there is a continuous need for new effective treatments.

2 Results

2.1 Demographic and other baseline characteristics

All study subjects were Finnish Caucasian males (n = 44) or females (n = 104) aged 19–65 years. On Visit 1, their mean (SD) height was 169 (9) cm, weight 80 (19) kg and BMI 28 (6) kg/m^2. Summaries of the demographic and other baseline characteristics of all included subjects are presented in Table 2 below.

Table 2. Summary of demographic and baseline characteristics

N = total number of subjects, SD = standard deviation, [1]Yes = currently using (irregular and regular use); no = never used; quit = former user

Variable		Male (N = 44)	Female (N = 104)	All (N = 148)
Age in years, mean (SD)		39 (12)	40 (12)	40 (12)
Height in cm, mean (SD)		179 (6)	165 (6)	169 (9)
Weight in kg, mean (SD)		92 (18,3)	75 (17)	80 (19)
BMI, kg/m^2, mean (SD)		29 (6)	28 (6)	28 (6)
Heart rate, mean (SD)		65 (11)	68 (11)	67 (11)
Systolic BP, mmHg, mean (SD)		131 (13)	123 (15)	126 (15)
Diastolic BP, mmHg, mean (SD)		77 (10)	78 (10)	78 (10)
ECG findings, n (%)				
	Normal	34 (77,3%)	84 (80,8%)	118 (79,7%)
	Abnormal	10 (22,7%)	20 (19,2%)	30 (20,3%)
Use of nicotine products, n (%)				
	Yes, current	6 (13,6%)	7 (6,7%)	13 (8,8%)
	No, never	32 (72,7%)	81 (77,9%)	113 (76,4%)
	Quit (former user)	6 (13,6%)	16 (15,4%)	22 (14,9%)

N = Total number of subjects, SD = Standard deviation

141 subjects (95 %) reported any medical history events (in addition to the history of allergic symptoms). 11 (7 %) subjects reported rhinitis only, 7 (5 %) subjects reported rhinoconjunctivitis only and 130 (88 %) subjects reported both of them when the allergy history was recorded at Visit 1. All study participants evaluated their allergy symptoms as interfering with daily activities or sleep. 146 (99 %) of the study participants used allergy medications to relieve their symptoms, some of them also outside of the birch pollen allergy season. None of the individuals had undergone allergen-specific immunotherapy (AIT) within the past 5 years, but 13 (8.8 %) subjects had undergone immunotherapy against birch pollen

more than 5 years ago. Summary information of the subjects' allergies is presented in Table 3 below.

Table 3. Summary of the study subjects' allergy history

		Male (N=44)	Female (N=104)	All (N=148)
Rhinitis only		3 (6.8%)	8 (7.7%)	11 (7.4%)
Rhinoconjunctivitis only		3 (6.8%)	4 (3.8%)	7 (4.7%)
Both (Rhinitis+Rhinoconjunctivitis)		38 (86.4%)	92 (88.5%)	130 (87.8%)
Symptoms interference				
	Yes	44 (100.0%)	104 (99.0%)	148 (100.0%)
	No	0 (0.0%)	0 (0.0%)	0 (0.0%)
Use of symptomatic medication				
	Yes	43 (97.7%)	103 (99.0%)	146 (98.6%)
	No	1 (2.3%)	1 (1.0%)	2 (1.4%)

2.2 Physical examination

At Visit 1, there were 36 (24 %) abnormal physical examination findings. Abnormal physical examination findings are not listed in this research report since none of them was considered clinically significant.

At Visit 1, the mean (SD) HR of the participants was 67 (11) beats/min, the mean systolic BP (SD) was 126 (15) mmHg and the mean diastolic BP (SD) was 78 (10) mmHg. There were 30 (20 %) abnormal 12-lead ECG findings, of which two were assessed as clinically significant. One subject had left bundle branch block and another had atrial fibrillation, which was also recorded in this subject's previous medical history (Table 2).

Spirometry results indicated ventilatory dysfunction in 25 (17 %) subjects. 21 (14 %) subjects had mild or moderate impairment ($-2.5 \leq$ FEV1 z-score < -1.65) and 4 (3 %) subjects had moderately severe or severe impairment (FEV1 z-score < -2.5). Those subjects who had moderately severe or severe ventilatory dysfunction were all found to be sensitized to birch pollen allergens, based on their IgE results. Three of these subjects were nonsmoking and one

had quit smoking. 4 (3 %) subjects with mild or moderate ventilatory dysfunction had negative serum IgE results. A summary of the spirometry results is presented in Table 4.

Table 4. Summary of the spirometry results
FEV1 = first second of forced expiration, FVC = forced vital capacity, PEF = peak expiratory flow rate

Variable		Male (N=44)	Female (N=104)	All (N=148)
FEV1, mean (range) (litres)				
	FEV1 abs	4 (2.65-5.06)	2.86 (1.43-4.28)	3.2 (1.43-5.06)
	FEV1 pred	91 (72-110)	91 (55-118)	91 (55-118)
	FEV1 z-score	-0.78 (-2.51-0.92)	-0.8 (-3.48-1.6)	-0.79 (-3.48-1.6)
FVC, mean (range) (litres)				
	FVC abs	5.15 (3.43-6.59)	3.59 (1.63-5.11)	4.06 (1.63-6.59)
	FVC pred	93 (70-120)	92 (50-119)	93 (50-120)
	FVC z-score	-0.65 (-2.8-1.85)	-0.6 (-3.74-1.67)	-0.62 (-3.74-1.85)
FEV1/FVC, mean (range)				
	FEV1/FVC abs	0.78 (0.63-0.91)	0.80 (0.64-0.96)	0.79 (0.63-0.96)
	FEV1/FVC, pred	98 (79-118)	98 (79-113)	98 (79-118)
	FEV1/FVC z-score	-0.29 (-2.94-2.5)	-0.32 (-3.48-2.26)	-0.31 (-3.8-2.5)
PEF, mean (range) (litres per minute)				
	PEF abs	8.92 (6.6-12.07)	6.16 (3.45-8.82)	6.98 (3.45-12.07)
	PEF pred	83 (61-109)	83 (47-111)	83 (47-111)
	PEF z-score	-1.36 (-3.15-0.8)	-1.2 (-3.71-0.75)	-1.25 (-3.71-0.8)
Obsctruction (FEV1 z-score < -1.65), n (%)				
Mild or moderate (-2.5 ≤ z-score < -1.65)		6 (13.6 %)	15 (14.4%)	21 (14.2%)
Moderately severe or severe (<-2.5)		1 (2.3 %)	3 (2.9 %)	4 (2.7 %)

Many subjects had some laboratory values outside of the reference ranges of the clinical laboratory, but only four of the abnormal laboratory values were defined as clinically significant. These assessments were repeated at Visit 2, and appropriate health-related instructions were given to the affected subjects. Summaries of all laboratory variables are presented in Table 5.

Table 5. Summary of subjects' clinical laboratory variables at baseline

Variable		Male (N = 44)	Female (N = 104)	Total (N = 148)
Heamatology, n (%)				
	Normal	17 (38.6%)	45 (43.3%)	62 (41.9%)
	Abnormal	27 (61.4%)	59 (56.7%)	86 (58.1%)
Clinical Chemistry, n (%)				
	Normal	21 (47.7%)	70 (67.3%)	91 (61.5%)
	Abnormal	23 (52.3%)	34 (32.7%)	57 (38.5%)
HIV and hepatitis serology, n (%)				
	Negative	44 (100.0%)	104 (100.0%)	148 (100.0%)
	Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)
Urine dipstick, n (%)				
	Normal	41 (93.2%)	70 (67.3%)	111 (75.0%)
	Abnormal	3 (6.8%)	34 (32.7%)	37 (25.0%)
Drugs of abuse, n (%)				
	Negative	43 (97.7%)	99 (95.2%)	142 (95.9%)
	Positive	1 (2.3%)	5 (4.8%)	6 (4.1%)
Pregnancy test, n (%)				
	Negative	0 (0.0%)	92 (88.5%)	92 (62.2 %)
	Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)
	NA	44 (100.0%)	12 (11.5%)	56 (37.8%)

N = total number of subjects
NA = not applicable

2.3 Adverse events

24 AEs were reported in 22 (15 %) subjects during the study. All of the AEs were assessed as mild in severity. No AEs of special interest (study procedure-related), SAEs or other significant AEs were reported.

The most common AE during the study was common cold, which was reported by 9 subjects (6 %). Sinusitis (3 subjects, 2 %) and headache (3 subjects, 2 %) were also reported. All of the AEs were assessed as not related to the study procedures. A summary of the reported adverse events is presented in Table 6.

Table 6. Summary of adverse events

AE term	Frequency
Urinary tract infection	2
Headache	3
Common cold	9
Hematuria	1
Fracture of the left 3rd toe	1
Tonsillitis	1
Back pain	1
Sinusitis	3
Jaw pain	1
Type II diabetes	1
Elevated GT	1

2.4 Specific IgE analysis

The severity of an allergy can be divided into allergy classes from 0 to 6, based on IgE concentration results in serum (Kleine-Tebbe & Jakob, 2015). When the IgE results for the airborne allergen group were examined, it was seen that most of the subjects belonged to allergy class 3 or 4 (Figure 3). None of the subjects had an allergy regarded as a class 6 allergy when taking into account the IgE concentrations of the airborne allergen group. When specific birch pollen IgE concentrations were examined, 11 subjects had IgE concentrations against Bet v 1 corresponding to values of allergy class 6, in which IgE concentrations are greater than 100 kUA/L (Kleine-Tebbe & Jakob, 2015).

When the correlation was examined between the allergy classes and the FEV1 z-scores from the spirometry tests of the subjects, no statistically significant association between them was detected ($r^2=0.046$, $p=0.603$). Some subjects having mild to severe obstruction had no detectable IgE concentrations while some subjects had concentrations at moderately high levels.

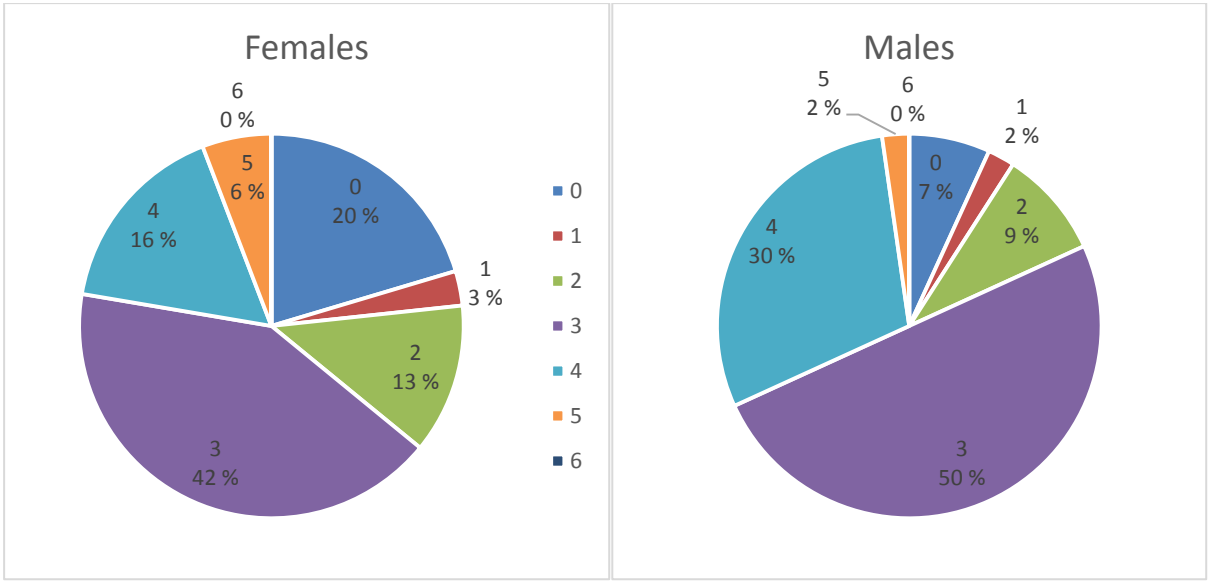

Figure 3. Subjects' allergy classes by serum IgE concentration, in females and males. Total IgE concentrations against airborne allergens.

Allergy is divided in seven allergy classes from 0 to 6 based on serum IgE concentration results. Allergy class 0 = IgE concentration 0,1 – 0,34 kUA/L (very low, allergy unlikely, does not rule out IgE-mediated symptoms) ; 1 = 0,35 – 0,69 kUA/L (low, symptoms are rare); 2 = 0,7 – 3,49 kUA/L (moderate, symptoms occur in many people); 3 = 3,5 – 17,49 kUA/L (high - symptoms occur in most people); 4 = 17,5 – 52,49 kUA/L (high or very high antibody levels, levels may be associated with the severity of the symptoms); 5 = 52,5 – 99,99 kUA/L (very high or extremely high antibody concentrations); 6 = > 100 kUA/L (extremely or exceptionally high antibody levels)(Kleine-Tebbe & Jakob, 2015).

124 subjects (84 %) had positive IgE results for at least one airborne allergen, and 17 (11 %) subjects had positive IgE results for at least one food allergen (Figures 4 and 5). Based on the IgE results for the birch allergen group, 118 of 148 (80 %) subjects were birch pollen-sensitized (Figure 4). Many subjects were also sensitized to cats (58 subjects, 39%), dogs (54 subjects, 36 %) and timothy grass pollen (82 subjects, 55 %). However, the mean IgE concentrations for these allergens were lower than for birch pollen (2.69 – 7.39 kU_A/L vs. 34.45 kU_A/L).

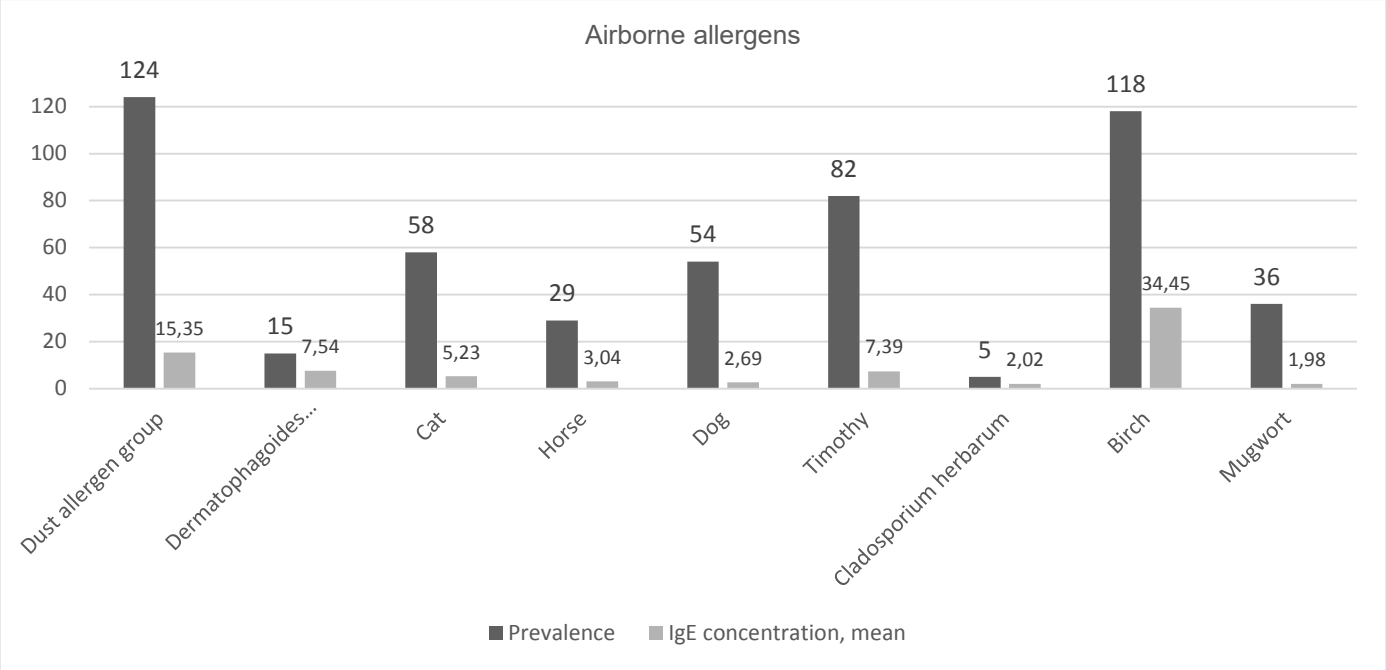

Figure 4. Prevalence and serum IgE concentrations of airborne allergens

Concentration means have been calculated for subjects exceeding the positivity threshold (≥ 0.35 kU_A/L). Concentration values >100 were treated as = 100 in the calculation of the mean.

The most prevalent food allergens were soy (7 subjects, 5 %), soy component Gly m 4 (7 subjects, 5 %), peanut (10 subjects, 7 %) and peanut component Ara h 8 (9 subjects, 6 %). A few subjects were also sensitized to egg white (4 subjects, 3 %), milk (3 subjects, 2 %) and wheat (6 subjects, 4 %). The highest IgE levels were against Gly m 4 (mean 27.14 kU_A/L) and Ara h 8 (mean 24.22 kU_A/L), which are both Bet v 1 homologs.

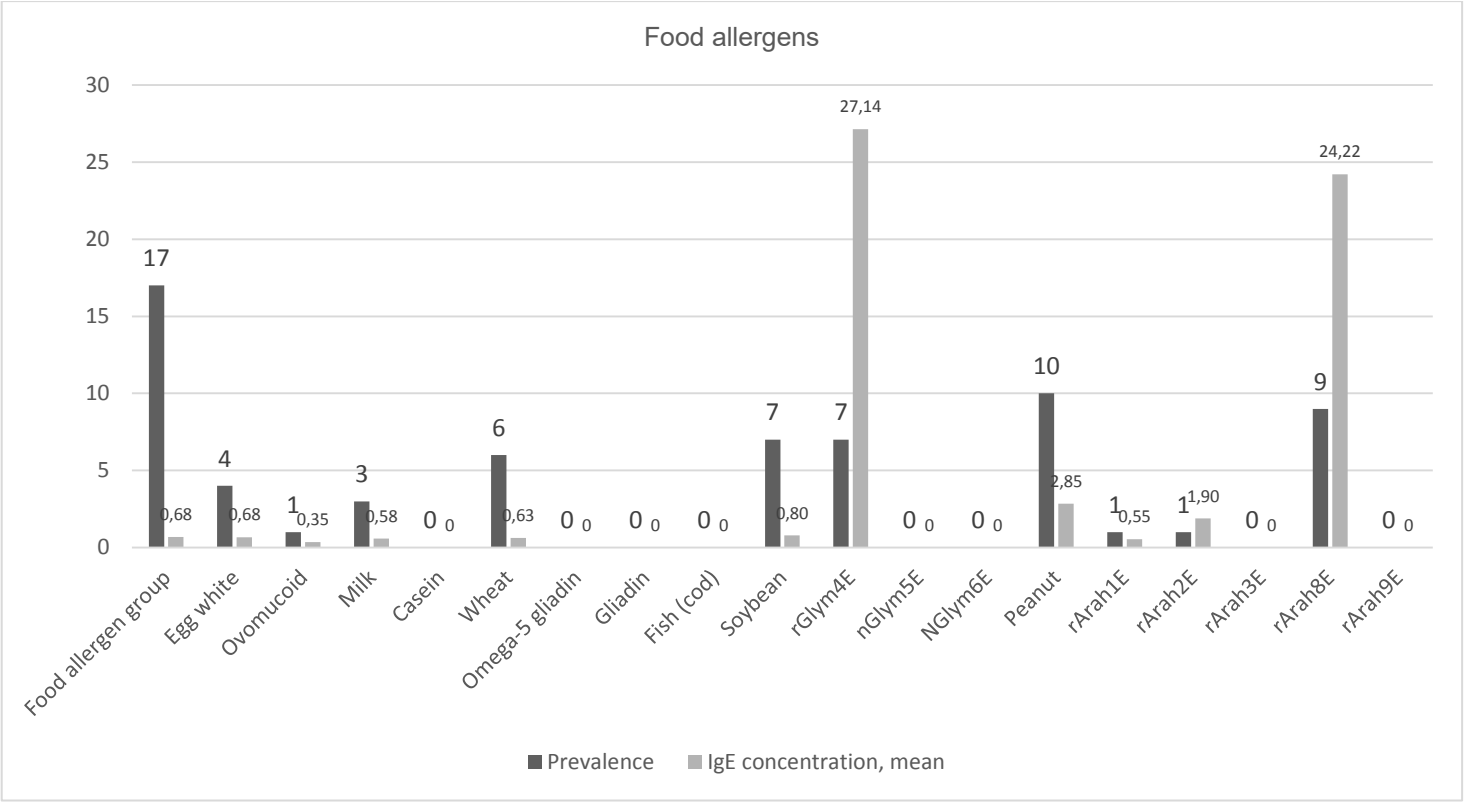

Figure 5. Prevalence and serum IgE concentrations of food allergens

Concentration means have been calculated for subjects exceeding the positivity threshold ≥ 0.35 kU_A/L). Concentration values >100 were treated as = 100 in the calculation of mean. Om5gliE = wheat component omega-5 gliadin; rGlym4E = soybean component rGly m 4 PR-10; nGlym5E = soybean component rGly m 5; nGlym6E = soybean component rGly m 6; rArah1E = peanut component rAra h 1; rArah2E = peanut component rAra h 2; rArah3E = peanut component rAra h 3; rArah8E = peanut component rAra h 8 PR-10; rArah9E = peanut component rAra h 9 LTP

In 116 of the 118 birch pollen IgE positive subjects (98 %), the IgE concentration for the major birch pollen allergen Bet v 1 was ≥ 0.35 kUA/L (Table 7). Only 6 (5 %) subjects had a positive IgE result for Bet v 2 and only 3 (3 %) subjects were positive for Bet v 4 IgE. Bet v 1 monospecificity was found in 108 of the 118 birch-allergic subjects (92 %).

Bet v 2 or Bet v 4 monospecificity was only seen in one subject, whose Bet v 2 IgE concentration was 8.2 kUA/L and that of Bet v 1 IgE was 0.31 kUA/L, remaining just below the positivity threshold. In 11 (9 %) birch-allergic subjects, the birch pollen IgE concentration exceeded the measurement range of the assay (>100 kUA/L); 4 of these subjects also tested positive for food allergy. All of these 4 subjects were positive for Bet v 1 IgE, but not Bet v 2 or Bet v 4. The 6 subjects who were not found to be sensitized to birch pollen, but had some IgE antibodies against airborne allergens, were identified to be sensitized to cat, timothy grass pollen or Cladosporium herbarum.

Concentrations and prevalence of IgE antibodies to birch pollen, and its major allergens Bet v 1, Bet v 2 and Bet v 4 are listed in Table 7. Specific IgE levels to birch pollen, Bet v 1, Bet v 2 and Bet v 4 in subjects are presented in Figure 6.

Table 7. Prevalence and concentrations in serum of IgE antibodies to birch pollen and specific birch pollen allergens

The criterion for inclusion in the analysis was a positive IgE test result (concentration ≥ 0.35 kU_A/L).

Test	Prevalence, n (%)	IgE concentration, mean[1] (range; median), kU_A/L
Birch pollen	118 (79.7 %*)	34.45 (0.76-100; 24.50)
Bet v 1	116 (98.3 %**)	29.20 (0.52 – 100.00; 18.00)
Bet v 2	6 (5.1 %**)	5.91 (0.44 – 17.00; 4.30)
Bet v 4	3 (2.5 %**)	4.03 (1.10 – 7.30; 3.70)

[1]concentration values >100 were treated as = 100 in the calculation of mean, median and range

*out of 148 subjects

**out of 118 subjects

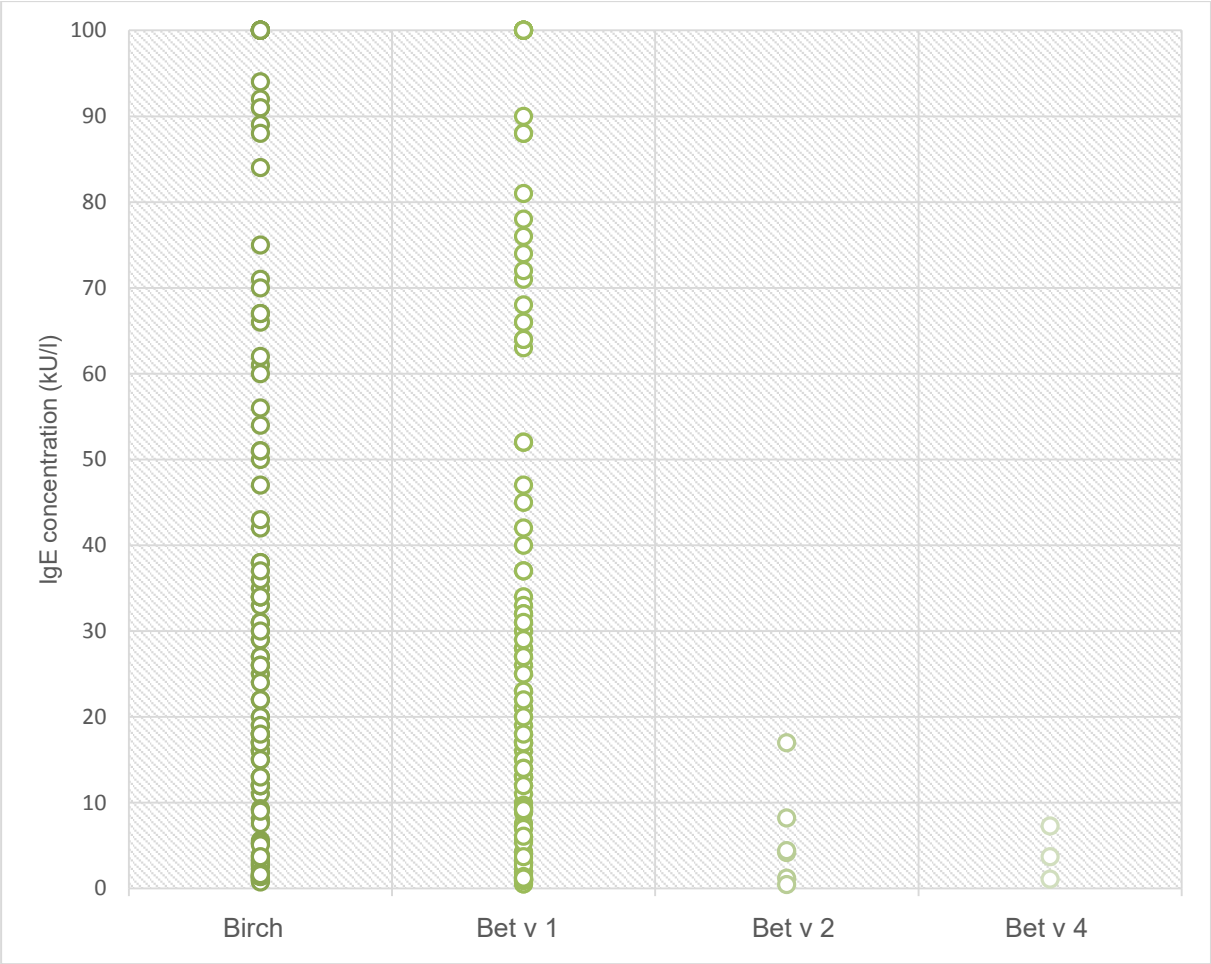

Figure 6. Specific IgE concentrations in serum against birch pollen and its major allergens Bet v 1, Bet v 2 and Bet v 4

Results from 148 subjects. Measurement range 0.35-100 kUA/L, concentration values >100 were treated as = 100.

There were no significant differences in any IgE results between females and males (Table 3 in Appendix 1). However, when analyzing the airborne allergen group IgE data, there were statistically significant differences between the age groups (p<0.0001, Figure 7). In the youngest age group (18–29 years), the mean concentration of IgE antibodies against airborne allergens was 23.01 kUA/L, whereas in the oldest group (50-65 years) the mean IgE concentration was 8.46 kUA/L. There were 35 subjects (24 %) in groups 1 (18-29 years) and 2 (30-39 years), 26 subjects (18 %) in group 3 (40-49 years) and 28 subjects (19 %) in group 4 (50-65 years).

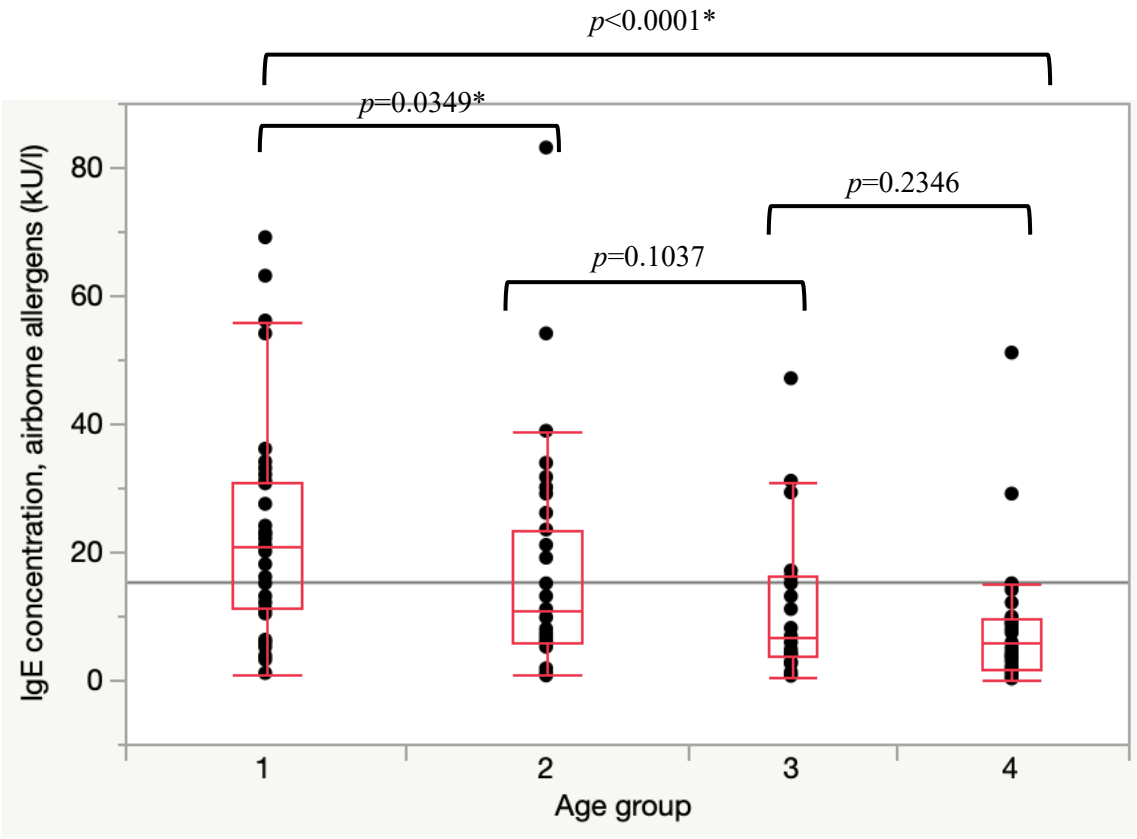

Figure 7. Serum IgE concentrations by age group, airborne allergens

Group 1 = 18-29 years, mean IgE concentration 23.01 kU_A/L; group 2 = 30-39 years, Mean IgE concentration 16.71 kU_A/L; group 3 = 40-49 years, mean IgE concentration 10.96 kU_A/L; group 4 = 50-65 years, mean IgE concentration 8.56 kU_A/L. Statistical test: Kruskal-Wallis rank sum test and nonparametric comparisons with Wilcoxon method. P<0.05 (marked with and asterisk) indicates statistically significant difference.

There was a statistically significant positive correlation between the birch pollen and Bet v 1 IgE concentrations ($r^2 = 0.9321$, $p<0.0001$, Figure 8). No significant associatios were detected between Ige concentrations for birch pollen and Bet v 2 or between birch pollen and Bet v 4.

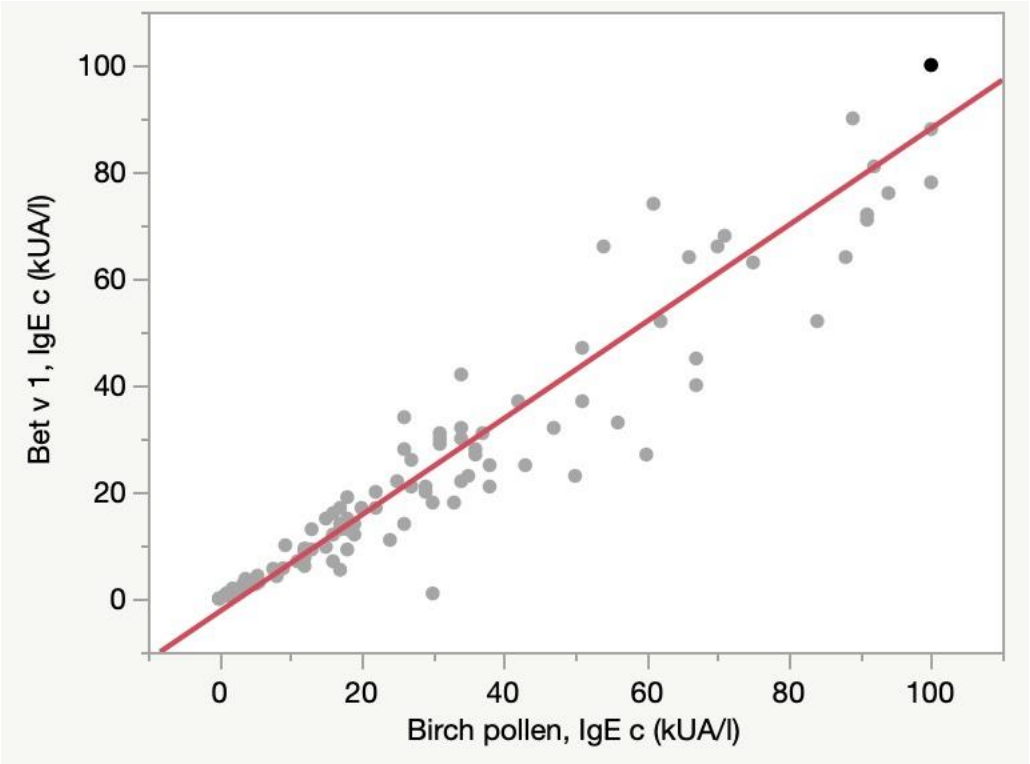

Figure 8. Correlation between the concentrations of IgE against birch pollen and its specific allergen Bet v 1. Pearson's product moment correlation, r2 = 0.9321, p<0.0001.

91 subjects (61 % of all study participants) reported having a runny nose during the birch pollen allergy season and 109 subjects (74 %) had itching in the nose, eyes or throat. Additionally, sneezing was reported by 42 (28 %) subjects and eye-watering by 43 (29 %) subjects. Those subjects whose IgE test results for airborne allergens turned out to be negative reported similar symptoms as the subjects with positive IgE results for the tested airborne allergens.

3 Discussion

The aim of this master's thesis research project was to determine the prevalence and specific allergens of birch pollen allergy, as determined by specific IgE analysis in serum, in subjects who reported having interfering symptoms of rhinitis or rhinoconjunctivitis during the birch pollen season. The main variable of interest was the IgE concentrations in serum against specific allergen components of birch pollen.

All 148 study participants (100 %) reported interfering symptoms of rhinitis or rhinoconjunctivitis, and almost all (99 %) used symptomatic medication to relieve their symptoms, some of them also outside of the birch pollen season. 84 % of the subjects were tested positive (positivity threshold, serum IgE concentration ≥ 0.35 kUA/L) for at least one specific IgE against plant- or animal-derived allergens, and 80 % were found to be sensitized to birch pollen, according to this serum IgE analysis.

Specific IgE antibodies against the major birch pollen antigen Bet v 1 were observed in almost all participants who were IgE-positive for birch pollen antigens as a group (116 of 118 such subjects). Only 6 subjects were positive for IgE against Bet v 2 and only 3 subjects were positive for IgE against Bet v 4. This is consistent with earlier studies carried out in Finland (Rossi et al., 2003; Movérare et al., 2002), reporting that subjects with symptoms of birch pollen allergy are much more likely to have IgE antibodies against Bet v 1 than the other known birch pollen allergens. Also in other European countries, it has been found to be rare that birch pollen sensitized people would be negative for IgE against Bet v 1 (Biedermann et al., 2019).

The results of this master's thesis project confirmed that Bet v 1 remains a major birch pollen allergen in the Finnish population. No evidence was gained that the IgE concentrations would have decreased since the latest previous studies on this topic were conducted (Rossi et al., 2003; Movérare et al., 2002). However, if climate change will impact the concentrations and prevalence of different types of pollens in Northern Europe, it may also change the IgE patterns of the allergic population. As previous studies have shown, in people living in Southern Europe, for example, IgE antibodies against Bet v 2 and Bet v 4 are more common than in Finnish population and, in addition, people with allergies are more sensitive to pollens from other species than birch, such as cypress or grasses. This could mean that if the trees and

other plants that currently only grow in Southern Europe become more common also in the North, it may affect allergies and serum IgE levels in the population.

The mean IgE concentrations varied by age and decreased in older subjects. The mean IgE concentration of airborne allergens in the youngest age group (18-29 years) was 23.01 kU_A/L and in the oldest age group (50-65 years) it was significantly lower ($p<0.0001$), 8.56 kU_A/L. Similar results have been reported previously (e.g. Ciprandi et al. 2017 & De Amici 2013), and it is known that ageing is related to declines in the different functions of the immune system. However, such decreases in older individuals usually concern only antibodies against specific allergens or allergen groups, but the total IgE levels in serum usually remain stable (de Amici & Ciprandi, 2013).

Many subjects who were found to be sensitized to food allergens had IgE antibodies against allergens that are known to cause cross-reactivity with birch pollen allergens, for example, soy component Gly m 4 (7 subjects of 17 food allergen sensitized subjects) and peanut component Ara h 8 (9 subjects of these 17). IgE antibodies to Gly m 4 and Ara h 8 allergens are likely due to sensitization to birch pollen. These allergens are Bet v 1 homologs belonging to the PR-10 protein family, and they are heat sensitive or destroyed during digestion, so cooked food rarely causes symptoms. However, consuming Gly m 4 containing products, especially beverages, during the peak birch pollen season may cause severe allergic reactions. (Mastrorilli et al., 2019)

Not all persons reporting interfering symptoms of rhinitis or rhinoconjunctivitis during the birch pollen season, and thus likely to be clinically diagnosed with birch pollen allergy, were found to be sensitized to the allergens covered by this thesis project when using a positivity threshold of ≥ 0.35 kUA/L for specific serum IgE concentrations. In 24 subjects (16 % of the total study population of 148), no IgE-based allergy diagnosis could be established. 30 subjects (20 %) were negative for IgE against birch pollen. One reason for this discrepancy could be that serum IgE levels against seasonal allergens do not remain stable throughout the year, peaking in the birch pollen season, and a large proportion of the serum samples included in the present study were collected later in the year, outside of the local birch pollen season.

Nevertheless, based on the current results, it remains relevant to include specific serum IgE analysis in the diagnostic work-up of persons presenting with interfering symptoms of rhinitis or rhinoconjunctivitis during the birch pollen season and being considered for initiation of AIT with Bet v 1 –targeted products.

As mentioned, IgE reactivity to birch pollen may also be caused by sensitization to Bet v 2 and/or Bet v 4 without reactivity to Bet v 1, although this appears to be rare in Finland. However, it is clinically important to establish a specific diagnosis prior to commencement of the allergen immunotherapies, since Bet v 1 is much more abundant in therapeutic birch pollen extracts than the minor birch pollen allergen components, so this type of therapy for patients allergic to Bet v 2 or Bet v 4 may not be efficacious. Additionally, the IgE reactivity profiles vary in different countries (Movérare et al., 2002; Sekerkova & Polackova, 2011), which should be kept in mind when developing new allergy therapies and therapeutic guidelines.

4 Materials and methods

The present clinical study was performed at Clinical Research Services Turku – CRST Oy in Turku, Finland, and the information collected from the study subjects was analysed for this thesis. The clinical study was funded by the Finnish biopharmaceutical company Desentum Oy who has granted permission to use the data in this thesis project. The study included 148 adult volunteers (males and non-pregnant females) with clinically documented symptoms of allergic rhinitis or rhinoconjunctivitis, whose participation consisted of two study visits.

On the first visit, the subjects underwent a general health examination, including i.a. medical history, physical examination, and standard spirometry measurement, and gave a blood sample for determination of serum levels of IgE class antibodies directed towards Bet v 1 and other major birch pollen antigens and other common antigens possibly causing symptoms of allergic rhinitis or rhinoconjunctivitis. Blood and urine samples were also collected for general health-related evaluations, and a 12-lead ECG was recorded. On visit 2, an individual health report with laboratory results was provided to the subjects, together with an interpretation of their clinical significance. No treatment intervention was involved in this study.

The analyses of haematology and clinical chemistry were performed at TYKSLAB, the accredited clinical laboratory of Turku University Hospital, and the specific IgE analysis was carried out by Yhtyneet Medix Laboratoriot Oy (currently Synlab Suomi Oy) in Helsinki.

4.1 Specific IgE analysis

For serum IgE analyses, a venous blood sample of 4 ml was collected into a serum blood tube (without gel) by the study nurses. Immediately after blood collection, the tube was inverted 10 times. The blood samples were kept at room temperature for 30 min. The serum was separated within 60 min from the sampling by centrifuging for 10 min using a centrifugal force equal to 2500 g at +24 ºC. The samples were stored (for up to 48 h) in a refrigerator until shipment to the laboratory. The serum samples were shipped to Medix Yhtyneet Laboratoriot for ImmunoCAP (Phadia AB, Uppsala, Sweden) analysis for specific IgE species.

Serum IgE levels for two main allergen classes, airborne allergens and food allergens, were measured. Specific IgE concentrations were analysed and expressed in terms of allergen-specific units (U_A). The measurement ranges for the IgE concentration assays were set as 0.10

– 100 kUA/L. Values below 0.35 kU_A/L were regarded and classified as negative results and concentrations exceeding the measurement range of the assay were set as >100 kU_A/L. Specific allergens were determined only from those samples where the allergen group result was positive (≥0.35 kU_A/L). The airborne allergens included Dermatophagoides pteronyssinus, cat, horse, dog, timothy grass, Cladosporium herbarum, birch and mugwort allergens, and the food allergens included egg white, milk, wheat, fish (cod), soybean and peanut allergens. Again, if the IgE result was positive (≥0.35 kU_A/L) for birch, egg white, milk, wheat, soybean or peanut, the analysis was continued by testing for specific components of this allergen. Associations with subjective symptoms and other subject characteristics were explored without any formal hypothesis testing. The conduct of the IgE analyses is illustrated below in Figures 9 and 10.

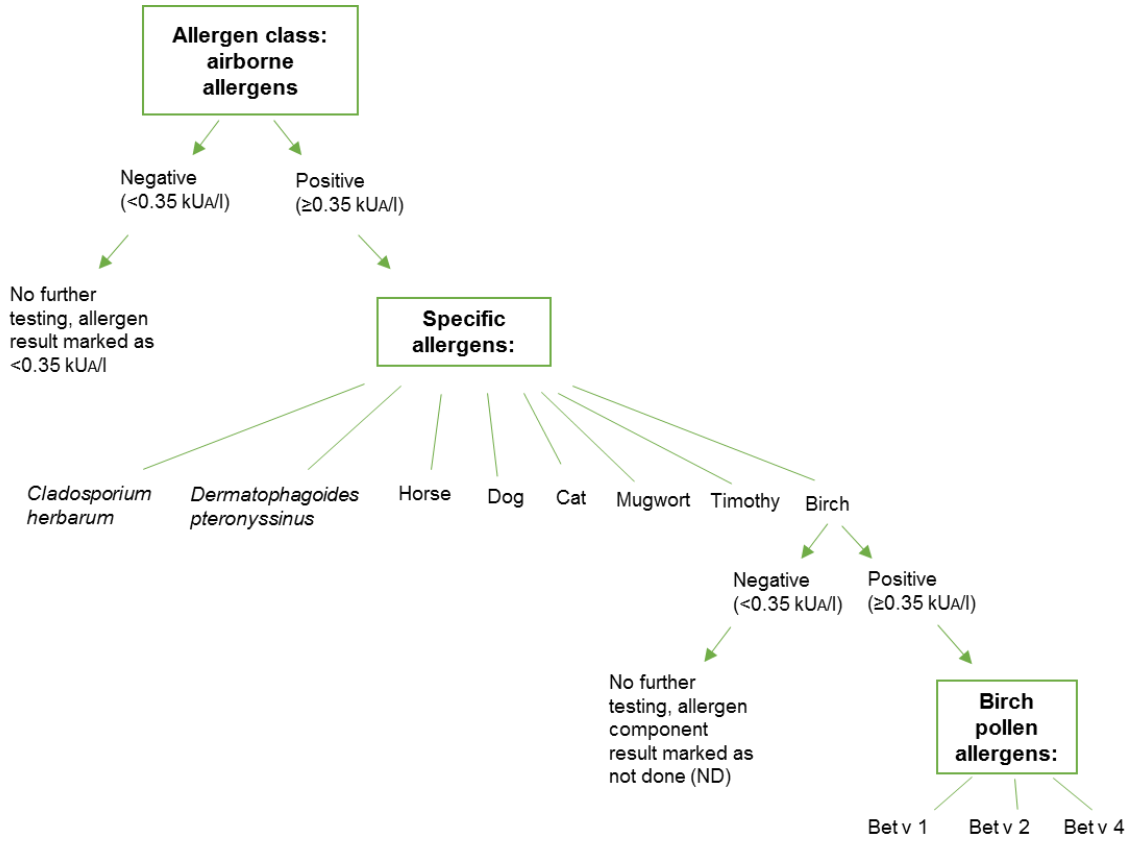

Figure 9. Flowchart of the airborne allergen analyses

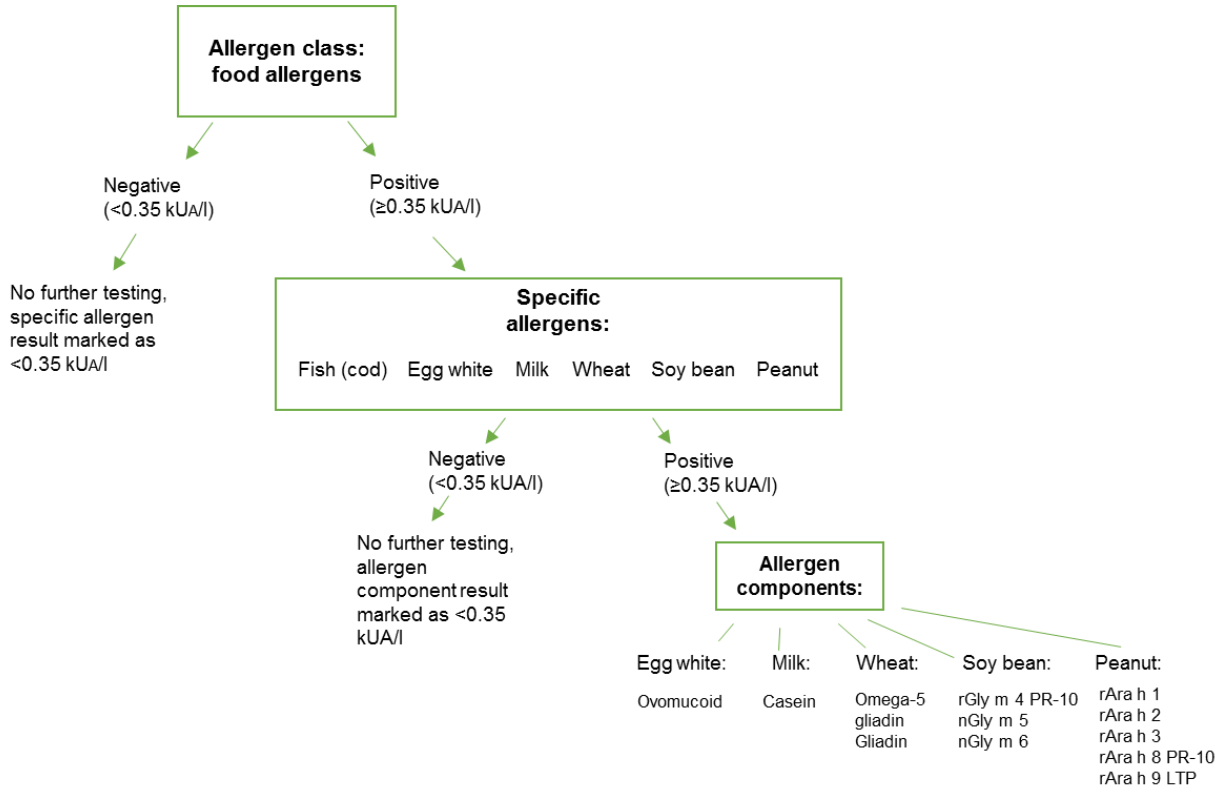

Figure 10. Flowchart of the food allergen analyses

4.2 Ethical issues

This study was conducted according to the principles of the Declaration of Helsinki and in accordance with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP). In addition, all relevant regulations and guidance for biomedical research involving human subjects were followed, with special emphasis placed on subject wellbeing. All participants provided written informed consent for the study before any other study-related procedures were commenced. The study protocol was approved by the Ethics Committee (EC) of the Hospital District of South-West Finland. As CRST Oy was the data registrar in this study, no information revealing the identity of the participating individuals is included in this thesis, in accordance with the General Data Protection Regulation.

4.3 Statistical analysis

The study was explorative and descriptive, so no formal statistical hypotheses were stated. The evaluation for the objectives of this study was based on descriptive statistics. Normality assumptions were checked visually and with the Shapiro Wilks test. Demographic data were expressed as means and standard deviations. Mann-Whitney U-test was performed when

comparing the IgE results between men and women and the nonparametric Kruskal-Wallis rank test to evaluate differences between age groups. Additionally, the correlation between the IgE results and spirometry data and the allergy class were evaluated by Spearman's rank order correlation and by Pearson's product moment correlation. *P* values less than 0.05 indicated statistical significance. Statistical analyses were performed with the software JMP Pro 16.0 for macOS.

The study subjects' medical history information, physical examination findings, blood pressure values, ECG findings, clinical laboratory values and spirometry results were tabulated, evaluated, and summarized using descriptive statistics.

5 Acknowledgements

The clinical conduct of the study was carried out at Clinical Research Services Turku – CRST Oy and this whole Master's thesis project was done under the supervision of Professor emeritus Mika Scheinin and Professor emeritus Markku Koulu. I want to sincerely thank my supervisors for their valuable support and guidance throughout the project. I feel privileged to have conducted my thesis work with such experienced supervisors. Also special thanks go to my colleagues at CRST Oy, who helped me to carry out the project and introduced me to the world of clinical trials. I would also like to thank Desentum Oy for providing me an opportunity to utilize the information gathered during the clinical trial. Finally, last but not least, I want to warmly thank my classmates and my family for motivating me from the beginning to the end of this project.

6 Abbreviations

AE	Adverse event
AIT	Allergen immunotherapy
FEV1	First second of forced expiration
FVC	Forced vital capacity
IgE	Immunoglobulin E
LCN2	Lipocalin 2 protein
PEF	Peak expiratory flow rate
PR-10 protein	Pathogenesis related class 10 protein
SPT	Skin prick test
Th1	Type 1 T helper cell
Th2	Type 2 T helper cell

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Appendix

Table 1. Summary information of medical history and physical examination findings

Variable		Male (N = 44)	Female (N = 104)	Total (N = 148)
Medical history(relevant medical/surgical history), n (%)				
	Yes	43 (97.7%)	98 (94.2%)	141 (95.3%)
	No	1 (2.3%)	6 (5.8%)	7 (4.7%)
Concomitant medications, n (%)				
	Yes	42 (95.5%)	104 (100%)	146 (98.6%)
	No	2 (4.5%)	0 (0%)	2 (1.4%)
Physical examination findings				
Normal findings, n (%)				
	Yes	34 (77.3%)	78 (75.0%)	112 (75.7%)
	No	10 (22.7%)	26 (25.0%)	36 (24.3%)
Allergies, n (%)				
	Yes	44 (100%)	104 (100%)	148 (100%)
	No	0 (0%)	0 (0%)	0 (0%)
Anaphylactic reactions, n (%)				
	Yes	0 (0%)	0 (0%)	0 (0%)
	No	44 (100%)	104 (100%)	148 (100%)

N = total number of subjects

Table 2. Serum IgE positivity by allergen (group); n (%). Measurement range 0.10-100 kU_A/l. Values below 0.35 kU_A/L were regarded and classified as negative results

Test	Male (N = 44)	Female (N = 104)	Total (N = 148)
Airborne allergen group	41 (93.2 %)	83 (79.8 %)	124 (83.8 %)
Dermatophagoides pteronyssinus	2 (4.5 %)	13 (12.5 %)	15 (10.1 %)
Cat	17 (38.6 %)	41 (39.4 %)	58 (39.2 %)
Horse	6 (13.6 %)	23 (22.1 %)	29 (19.6 %)
Dog	17 (38.6 %)	37 (35.6 %)	54 (36.5 %)
Timothy	28 (63.6 %)	54 (51.9 %)	82 (55.4 %)
Cladosporium herbarum	1 (2.3 %)	4 (3.8 %)	5 (3.4 %)
Birch	39 (88.6 %)	79 (76.0 %)	118 (79.7 %)
Mugwort	13 (29.5 %)	23 (22.1 %)	36 (24.3 %)
Food allergen group	7 (15.9 %)	10 (9.6 %)	17 (11.5 %)
Egg white	1 (2.3 %)	3 (2.9 %)	4 (2.7 %)
Ovomucoid	1 (2.3 %)	0 (0.0 %)	1 (0.7)
Milk	1 (2.3 %)	2 (1.9 %)	3 (2.0 %)
Casein	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
Wheat	4 (9.1 %)	2 (1.9 %)	6 (4.1. %)
Omega-5 gliadin	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
Gliadin	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
Fish (cod)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
Soybean	4 (9.1 %)	3 (2.9 %)	7 (4.7 %)
rGly m 4 PR-10	4 (9.1 %)	3 (2.9 %)	7 (4.7 %)
rGly m 5	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
r Gly m 6	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
Peanut	6 (13.6 %)	4 (3.8 %)	10 (6.8 %)
rAra h 1	1 (2.3 %)	0 (0.0 %)	1 (0.7 %)
rAra h 2	1 (2.3 %)	0 (0.0 %)	1 (0.7 %)
rAra h 3	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
rAra h 8 PR-10	5 (11.4 %)	4 (3.8 %)	9 (6.1 %)
rAra h 9 LTP	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)

Table 3. Serum IgE concentrations, by allergen (group); mean (range; median), kU_A/l, in the subjects who had a positive IgE concentration result for each allergen. Values below 0.35 kU_A/L were regarded and classified as negative results, concentration values >100 were treated as = 100 in the calculation of mean, median and range

Test	Male (N = 44)	Female (N = 104)	Total (N = 148)	p-value
Airborne allergen group	16.24 (0.63-83.00; 11.10)	15.05 (0.58-69.00; 9.80)	15.35 (0.58-83.00; 11.00)	0.38
Dermatophagoides pteronyssinus	1.08 (0.36-1.80; 1.08)	8.54 (0.83-70.00; 0.96)	7.54 (0.36-70.00; 0.96)	0.44
Cat	3.71 (0.65-12.00; 1.70)	5.87 (0.36-41.00; 2.70)	5.23 (0.36-41.00; 2.55)	0.56
Horse	2.31 (0.53-4.20; 2.12)	3.22 (0.35-22.00; 1.20)	3.04 (0.35-22.00; 1.20)	0.73
Dog	1.85 (0.38-11.00; 1.10)	3.08 (0.40-19.00; 1.30)	2.69 (0.38-19.00; 1.30)	0.31
Timothy	7.52 (0.45-41.00; 4.00)	7.19 (0.35-71.00; 4.00)	7.39 (0.35-71.00; 4.00)	0.85
Cladosporium herbarum	0.49 (0.49-0.49; 0.49)	2.84 (0.36-10.00; 0.50)	2.02 (0.36-10.00; 0.49)	NA
Birch	40.77 (1.10-100.00;31.00)	31.34 (0.76-100.00; 18.00)	34.45 (0.76-100; 24.50)	0.52
Mugwort	1.44 (0.39-3.50; 0.95)	2.28 (0.40-12.00; 1.30)	1.98 (0.38-12.00; 1.20)	0.87
Food allergen group	0.84 (0.36-2.92; 0.53)	0.57 (0.35-1.33; 0.46)	0.68 (0.35-2.92; 0.49)	0.81
Egg white	0.47 (0.47-0.47; 0.47)	0.74 (0.41-1.40; 0.42)	0.68 (0.41-1.40; 0.45)	NA
Ovomucoid	0.35 (0.35-0.35; 0.35)	NA	0.35 (0.35-0.35; 0.35)	NA
Milk	0.42 (0.42-0.42; 0.42)	0.66 (0.41-0.91; 0.66)	0.58 (0.41-0.91; 0.42)	NA
Casein	NA	NA	NA	NA
Wheat	0.61 (0.35-0.94; 0.58)	0.66 (0.61-0.70; 0.66)	0.63 (0.35-0.94; 0.66)	0.82
Omega-5 gliadin	NA	NA	NA	NA
Gliadin	NA	NA	NA	NA
Fish (cod)	NA	NA	NA	NA
Soybean	0.67 (0.58-0.76; 0.66)	0.97 (0.85-1.10; 0.96)	0.80 (0.58-1.10; 0.76)	0.052
rGly m 4 PR-10	25.25 (2.00-61.00; 19.00)	29.67 (15.00-57.00; 17.00)	27.14 (2.00-61.00; 17.00)	0.86
rGly m 5	NA	NA	NA	NA
r Gly m 6	NA	NA	NA	NA
Peanut	1.83 (0.98-3.00; 1.70)	4.38 (1.00-12.00; 2.25)	2.85 (0.98-12.00; 1.70)	0.67
rAra h 1	0.55 (0.55-0.55; 0.55)	NA	0.55 (0.55-0.55; 0.55)	NA
rAra h 2	1.90 (1.90-1.90; 1.90)	NA	1.90 (1.90-1.90; 1.90)	NA
rAra h 3	NA	NA	NA	NA
rAra h 8 PR-10	25.34 (2.50-64.00; 17.00)	22.83 (7.30-47.00; 18.50)	24.22 (2.50-64.00; 17.00)	0.81
rAra h 9 LTP	NA	NA	NA	NA