Subcutaneous allergen specific immunotherapy – clinical efficacy assessment and side-effects and their prevention

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This thesis is based on the following previously published papers, which will be referred to in the text by the numbers I-VI:


Denne afhandling er i forbindelse med ovenstående tidligere publicerede artikler af Det Lægevidenskabelige Fakultet ved Københavns Universitet antaget til offentligt at forsvares for den medicinske doktorgrad.

Københavns Universitet, den

Dekan
Preface

The studies forming the basis of this thesis have been carried out during my employment at the Allergy Clinic, National University Hospital, Copenhagen, Denmark.

During the years I have had the privilege to work with inspiring colleagues, all contributing to a unique research environment, where sharing of knowledge is a common policy and personal support is generously provided.

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Definitions

Allergens
Allergens are antigens which cause allergy. In the present context allergens are antigens that have given rise to an IgE antibody response. Most allergens are proteins, often with carbohydrate side chains.

Allergen extracts
Allergen extracts are an aqueous solution of allergens such as pollens extracted from natural sources.

Allergen specific immunotherapy
Allergen specific immunotherapy is the practice of administering gradually increasing quantities of an allergen product to an individual with IgE-mediated allergic disease in order to ameliorate the symptoms associated with subsequent exposure to the causative allergen. Allergen specific immunotherapy can be provided by both injective and non-injective methods. In the present context allergen specific immunotherapy refers to subcutaneous immunotherapy. In subcutaneous allergen specific immunotherapy the allergen product is an allergen extract.

Allergenic source
The allergenic source is the material from which allergen extracts are prepared.

Allergic anaphylaxis
Allergic anaphylaxis is a severe immediate systemic reaction occurring in IgE sensitized individuals after exposure to an allergen. It is caused by the rapid release of vasoactive mediators from mast cells and basophils.

Allergic rhinoconjunctivitis
Allergic rhinoconjunctivitis is symptoms of an immune-mediated hypersensitivity reaction in the nose and conjunctiva. Most cases are IgE-mediated. Based on duration of symptoms, it can be useful to differentiate between intermittent and persistent allergic rhinitis. The severity of allergic rhinitis can be classified as mild or moderate/severe.

Allergy
Allergy is a hypersensitivity reaction initiated by immunological mechanisms. Allergy can be antibody and/or cell-mediated. In the majority of cases the antibody typically responsible for an allergic reaction belongs to the IgE isotype and these individuals may be referred to as suffering from an IgE-mediated allergy. In this thesis allergy will refer to an IgE-mediated mechanism.

Cluster immunotherapy
Cluster immunotherapy is the term used for the administration of two or more injections with allergen extract per visit with the aim to achieve the maintenance dose more rapidly than by the conventional “one-injection-per-week” schedule.

Conjunctival provocation test
Conjunctival provocation testing means application of allergenic material to the mucosa of the eye. A conjunctival allergen provocation test is used to objectively confirm a case history suggesting ocular allergy and to evaluate changes in sensitivity.

Hypersensitivity
Hypersensitivity is a condition where objectively reproducible symptoms or signs are initiated by exposure to a defined stimulus that is tolerated by normal subjects.

Major allergen
A major allergen refers to an allergen from a complex allergen extract that binds IgE in > 50% of patients sensitive to the allergen.

Nasal provocation test
Nasal provocation testing means application of allergenic material to the mucosal lining of the nose. A nasal allergen provocation test is used to objectively confirm a case history suggesting allergic rhinitis and to evaluate changes in sensitivity.
Pollen count
The pollen count is a measure of pollen grains in the atmosphere expressed as number of pollen grains/m³ air.

Pollen season
The pollen season is the period over which a given percentage of the total annual pollen count is recorded in the atmosphere.

Rush immunotherapy
Rush immunotherapy is a form of allergen specific immunotherapy in which several injections are administered at 30- to 60-min intervals with the aim to maintenance dose within hours or days.

Skin prick test
Skin prick testing means introduction into the superficial layer of the skin of allergenic material. Skin tests are used to confirm IgE-sensitization supporting an allergy diagnosis.

Systemic reaction
Systemic reactions to subcutaneous immunotherapy are any symptoms from organs distant from the injection site.
Abbreviations

A  asthma
ANTI  antazoline-naphazoline, eye drops
CPT  conjunctival provocation test
DBPC  double-blind placebo-controlled
Dp  *Dermatophagoides pteronyssinus*
EAACI  European Academy of Allergology and Clinical Immunology
ECP  eosinophil cationic protein
EOS  eosinophil
EPX  eosinophil protein X
HDM  house dust mite
HR  histamine release
HR<sub>15</sub>  the lowest concentration of allergen or anti-IgE releasing ≥ 15 ng/ml of histamine
IgE  immunoglobulin class E
LLR  late local reaction
MAX  the maximum concentration of histamine (ng/ml) released at any concentration
NPT  nasal provocation test
PEF  peak expiratory flow
PLS  partial least square analysis
PRED  prednisolone tablet
SC  subcutaneous
SCIT  subcutaneous allergen specific immunotherapy
SEMP  acrivastine, antihistamine tablet
SPT  skin prick test
SR  systemic reaction
SYMP  symptom score
1. Introduction and background

The prevalence of allergic rhinitis is high with a huge variation worldwide (3.2-66.6%).\(^1\) International surveys have shown that despite pharmacological treatment allergic rhinitis is associated with a high symptom burden and impaired health related quality of life.\(^{2-4}\) Allergen avoidance is not possible with outdoor inhalant allergens and conventional pharmacotherapy, although in general efficient, does not provide long-lasting efficacy. Allergen specific immunotherapy is a treatment that can lead to, not only improvement in allergic rhinitis symptoms, but also to long-term benefit on symptoms following discontinuation of therapy, including long term disease remission as well as reduction in disease progression from allergic rhinitis to asthma. Data also suggest a benefit in prevention of new atopic sensitizations.\(^{5-13}\)

The most prevalent seasonal inhalant allergies in the Nordic European countries are birch- and grass-pollen induced allergic rhinoconjunctivitis. Published randomised and double-blind placebo-controlled (DBPC) subcutaneous allergen immunotherapy (SCIT) studies with birch- or grass-pollen extracts give evidence for a significant reduction in symptom score and medication use in birch- and grass-pollen allergic rhinitis patients, respectively.\(^{14-41}\) These SCIT studies concern single allergen pollen extracts, but in fact patients are rarely monosensitized. The majority of pollen allergic rhinoconjunctivitis patients in the Nordic European countries are sensitized to both birch- and grass-pollen with symptoms of allergic rhinoconjunctivitis in both birch- and grass-pollen seasons. One could speculate that a prior pollen season could influence symptoms in the following pollen season, thereby introducing a possible bias in the evaluation of efficacy of SCIT. The efficacy of SCIT with birch- and grass-pollen extracts in patients with allergy to both birch and grass has not, so far, been investigated in this patient population.

There are several important methodological considerations in the evaluation of the efficacy of the treatment, including study design and outcome measures. Short time efficacy of SCIT evaluated by one-year placebo-controlled pollen SCIT studies require a high number of patients and might be subject to randomization bias, with two groups differing in terms of disease severity. The inclusion of a baseline year enables the investigator to stratify according to disease severity, and to use the patient as his own control with fewer patients needed to obtain sufficient power to detect clinically relevant effects. The amount of pollen grains in the air, however, influence on the degree of symptoms of allergic rhinitis, and the annual variation of pollen exposure might have an effect on results of SCIT studies with a duration of more than one year.

Symptom score and use of medication are considered primary outcomes in the assessment of efficacy of SCIT. Other parameters could potentially be used as surrogate markers of efficacy, provided they are correlated to symptom/medication score. Several parameters including measurements of allergic sensitization and inflammation have been investigated, but results have not been consistent and correlations to clinical symptoms rarely examined.

Allergen specific immunotherapy involves the risk of side-effects. The majority of side-effects are harmless reactions responding well to treatment and the risk of inducing anaphylactic side-effects is small, but fatalities have been caused by immunotherapy.\(^{42-44}\) Possible risk factors have been identified by reviewing case studies of fatal and near fatal reactions to SCIT.\(^{45}\) The risk factors, include errors in dosage, presence of symptomatic asthma, high degree of hypersensitivity (by skin tests or specific IgE), use of beta-blockers, injections from new vials and injections during seasons of exacerbations. These results need support from prospective studies. Not only efforts to identify risk factors for side-effects with SCIT, but also investigations of how to prevent side-effects during treatment are needed to minimise the incidence of both severe and mild side-effects. It has been debated if antihistamine premedication could reduce side-effects of SCIT, but no consensus has been reached due to lack of double-blind placebo-controlled studies.\(^{46-49}\)

The aims of the present work were threefold. First we wanted to investigate the efficacy SCIT in an allergic population with both birch and grass-pollen hayfever and to analyse results according to design and influence of pollen exposure. For this we chose a design with a baseline year to be able to stratify according to disease severity as measured by symptom score during pollen season, and to be able to use the patients as their own controls, thereby limiting the number of patients required. The baseline year was also used to correlate different paraclinical measurements to the symptom score and use of medication, to investigate the relevance of these variables as a secondary outcome. The first treatment year the patients were treated double-blinded with either birch or grass SCIT, enabling us to look at the influence of birch SCIT on symptoms in the following grass-pollen season. The following year the patients were treated with both allergens. The influence of pollen load on symptoms and assessment of efficacy of treatment were investigated by a mathematical model. Secondly, we wanted to study possible risk factors of side-effects. This was prospectively investigated in two different patient populations. Thirdly, the onset of action of antihistamine on hayfever symptoms was investigated followed by a DBPC study of the efficacy of antihistamine premedication on side-effects of SCIT.
2. Material and methods (I-VI)

Study populations and designs

In articles I-III, the same study population of 52 adult patients with rhinoconjunctivitis and allergy to birch as well as grass-pollen (dual sensitivity) were investigated. All had a history of moderate to severe allergy to birch and grass-pollen with symptoms in April and May, and May through July, corresponding to the local birch- and grass-pollen seasons respectively. Seventeen of the patients also suffered from mild to moderate asthma with occasional symptoms; six of these patients had symptoms only during the pollen season. They all fulfilled the criteria for SCIT according to the position paper of the European Academy of Allergology and Clinical Immunology (EAACI).\(^{(49)}\) The patients were included in a 3-year study consisting of a baseline year without immunotherapy, obtaining pre-treatment values, followed by two years of SCIT. The patients were characterised at inclusion by symptoms of their birch- and grass-pollen allergy and different inflammatory markers. During the baseline year measurements of allergic inflammatory markers were obtained during and outside birch- and grass-pollen seasons, in the allergic study population as well as in a non-allergic control group. During the three consecutive pollen seasons, the rhinoconjunctivitis symptom score and use of antihistamines, eye-drops and oral prednisolone were recorded. After the baseline year, patients were allocated to double-blinded clustered SCIT with aluminium-adsorbed extract produced from either birch (Betula verrucosa) or grass (Phleum pratense) pollen. After one year of treatment, the patients continued SCIT with their original extract and in addition received the other extract. The treatment code was broken at the end of the study. The side-effects of SCIT with birch- and grass-pollen extracts were registered. In article IV the study population comprised 1038, primarily adult patients, with allergic rhinoconjunctivitis, allergic asthma or allergic reaction to hymenoptera. They all fulfilled the EAACI criteria for SCIT and registered side-effects during the induction phase with the allergens in question, primarily Betula verrucosa and Phleum pratense.

In article V the study population comprised 43 adult grass-pollen allergic rhinoconjunctivitis patients, with or without mild asthma. The patients participated in a placebo-controlled, randomized, and double-blind 1-day field study exploring the onset of action of antihistamine on allergic rhinoconjunctivitis symptoms during natural grass-pollen exposure.

In article VI another adult population of 45 birch- or grass-pollen allergic rhinoconjunctivis registered side-effects during SCIT with birch- or grass-pollen extract and pre-treatment with antihistamine in a DBPC study design.

Extracts

All extracts for skin prick test (SPT) (Soluprick SQ); for conjunctival provocation test, nasal provocation test and titrated SPTs (Aquagen SQ); and for SCIT (Alutard SQ) were supplied by ALK-Abelló, Hørsholm, Denmark. The extracts for immunotherapy were in an aqueous solution with aluminium hydroxide. The activity of the extracts for SCIT was given in standardized units (SQ-U). The amount of major allergens per top dose (100,000 SQ-U) were Betula verrucosa (SQ 108 silver birch, 12.3 µg Bet v 1), Phleum pratense (SQ 225 timothy, 20.2 µg Phl p 5), Dermatophagoides pteronyssinus (Dp) (SQ 503 house dust mite (HDM), 9.8 µg Der p 1), cat hair (SQ 555, 14.6 µg Fel d 1), and Vespula spp. (SQ 802 wasp venom, 100 µg dry weight).

Skin prick test (SPT)

Skin prick tests were performed according to the recommendations of the EAACI.\(^{(48)}\) The size of the wheal was determined either by wheal diameter or wheal area. The area was determined by a SPT scanner.\(^{(49)}\) A SPT response (wheal diameter ≥ than 3 mm; wheal area > 7 mm\(^2\) with Soluprick SQ was considered positive for inclusion. In article III, SPT was performed in quadruplicate on the volar site of the forearm with the allergen (Aquagen SQ) dilutions (100, 1000, 10,000, 100,000 SQ-U/ml), and the skin index defined as the SPT area of 100,000 SQ-U/ml divided by the SPT area of histamine (10 mg/ml). This index was used for calculations.

Conjunctival provocation test (CPT)

Conjunctival provocation test was performed by applying one drop of diluent in the lower conjunctival sack and then increasing allergen concentrations (100, 1000, 10,000, 100,000 SQ-U/ml) in alternate eyes every 10 min until a positive reaction (conjunctival redness and itching) occurred.\(^{(50)}\)

Nasal provocation test (NPT)

Nasal provocation test was performed by instillation of 0.1 ml of diluent in each nostril by a mechanical pump and then increasing allergen concentrations (100, 1000, 10,000, 100,000 SQ-U/ml) every 10 min until two of three criteria were fulfilled: more than five sneezes, more than 0.5 ml nasal secretion, or more than 50% reduction in nasal peak flow.\(^{(51)}\)

Basophil histamine release (HR)

HR from basophil leukocytes was performed by the glass microfiber method (Refib, Copenhagen, Denmark), described in article II and in detail elsewhere.\(^{(52)}\) The histamine release from basophil leukocytes (HR) was measured and expressed as basophil releasability (the maximum concentration of histamine (ng/ml) released at any concentration [MAX]) and basophil cell sensitivity (the lowest concentration of allergen or anti-IgE releasing ≥ 15 ng/ml of histamine [HR\(_{50}\) ]).\(^{(52-55)}\)
Specific IgE and total IgE
Total serum IgE was measured by the Microparticle enzyme immunoassay on a IMx reader, according to the manufacturer (Abbot Laboratories, Abbot Park, IL, USA). The sensitivity was 0.1 kUA/L. Specific IgE was measured by an enzyme fluorescence assay with a solid phase of covalently bound allergen to cellulose (former Pharmacia, now Phadia CAP system, Phadia, Upplands, Sweden), according to the instructions of the manufacturer. Results are reported in kUA/L, with a cut-off value of 0.35 kUA/L.

Eosinophil cationic protein (ECP) and Eosinophil protein X (EPX)
Plasma and serum for ECP and EPX were prepared from blood samples drawn in vacutainers with EDTA or without anticoagulant. Serum and plasma were recovered after 2 h at room temperature. Serum and plasma levels of ECP and EPX were determined by ELISA procedures.  

Eosinophils (EOS)
The counting of eos was performed automatically by the Technicon H1™ peroxidase method and measured by a Technicon counter (Technicon Instruments Corp., New York, NY, USA).

Subcutaneous allergen specific immunotherapy (SCIT)
SCIT was performed according to the EAACI guidelines, with the exception of the recommended reduction in dosage with late local reactions > 8 cm.  

Systemic reaction – grading
All systemic reactions (SR) in this thesis are described according to the proposal by EAACI (33) published in 1993: 0 = no symptoms; 1 = unspecific symptoms (reactions probably not IgE-mediated, i.e. discomfort, headache, arthralgia, etc.); 2 = mild systemic reactions (mild rhinitis or asthma responding adequately to antihistamines or beta2-agonist spray); 3 = non-life-threatening systemic reactions (urticaria, angioedema, or severe asthma, responding well to treatment) and 4 = anaphylactic shock (rapidly progressing reaction of itching, flushing, erythema, bronchial obstruction, etc. requiring intensive treatment).

Pollen counts and pollen seasons
The Burkard recording volumetric spore trap was used for collecting pollen grains (Burkard Manufacturing Ltd, Rickmansworth, Hertfordshire, UK). The pollen count was expressed as number of pollen grains/m3 air. The method is described elsewhere. The trap was placed on a roof, 15 m above ground level, in Copenhagen city. In article V the pollen concentration was also measured 1.5 m above ground level. The birch- and grass-pollen seasons were defined as the period including the mid 90% of total pollen of birch and grass, respectively.

Statistics
Statistics are described in outline of the various studies. In general, probability (P) values of< 0.05 were considered statistically significant.

3. Assessment of efficacy of allergen specific immunotherapy (I, II)
A relevant method to evaluate the efficacy of allergen specific immunotherapy with pollen is to monitor the clinical manifestations of the allergic disease (rhinitis, conjunctivitis, and asthma) by keeping symptom diaries during the pollen seasons.  

Commonly used designs are randomized (with or without stratification), one-year, placebo-controlled pollen IT studies comparing seasonal symptom score and medication from two patient groups (actively and placebo-treated). Randomization is expected to balance treatment groups among covariates (factors or variables presumed to influence the primary variable to be analysed), but, in practice, it is not unusual to observe differences post hoc. Stratification by potentially important covariates e.g. age, gender, and duration of allergy is often applied. The number of covariates in the stratification procedure is however, limited, especially in studies with few participants. Even if these procedures balance the groups according to potentially important parameters of severity of allergic rhinitis e.g. duration of allergy, and results of specific IgE, SPT, and CPT, it does not imply identical clinical severity of the disease. A false conclusion of efficacy could be the consequence. Another approach is to investigate the reduction in symptom scores/medication in relation to a pretreatment period, enabling the investigator to stratify according to the clinical parameters in question. It also gives the possibility to compare the individual patient before and during treatment. However, pollen studies carried out over several years are complicated by annual variations in seasonal pollen counts, a complication which could interfere with an observed reduction in symptom score/medication.

Allergen specific immunotherapy has been shown to reduce both symptoms and use of medication. The frequency of symptoms and use of rescue medication are interdependent, and a combined symptom/medication score might be a more relevant primary outcome of SCIT, compared to symptom or medication score alone. The relative importance of symptom scores and medication respectively, is debatable and varies between studies. A pragmatic solution assumes equivalent importance of symptoms and medication scores indicating that each of these account for half of the clinical burden of the disease. The power to discriminate between effective treatment and placebo with this outcome measure remains to be investigated. One study has addressed this question with
data from a previously published study of sublingual grass-pollen immunotherapy.\textsuperscript{62,63} The scoring of symptoms and use of medication depends on the individual patient, and a method of weighting symptom and medication scores dependent on the actual data of the population might be a solution.

Most pollen SCIT studies concern single allergen pollen extracts, but in fact patients are rarely monosensitized. A major part of pollen allergic rhinoconjunctivitis patients in the Nordic European countries are sensitized to both birch and grass-pollen with symptoms of allergic rhinoconjunctivitis in birch as well as grass-pollen seasons. These two seasons are related in time, starting with birch-pollen followed closely by grass-pollen. The outcome of the birch-pollen season, in terms of pollen load, symptoms and treatment, might influence the outcome of the grass-pollen season. The efficacy of SCIT with birch- and grass-pollen extracts in patients with dual sensitivity has never been described.

Symptom/medication scores are recommended as primary outcome measures when efficacy of SCIT is evaluated.\textsuperscript{65,66} Symptom scores are, however, timeconsuming. Provocation tests as well as paraclinical parameters associated with allergic rhinitis are potential surrogate markers for symptom scores and in theory could be used in the evaluation of efficacy of SCIT, provided that they are well correlated with the symptom/medication scores. Allergic inflammation is initiated by allergen cross-linking of specific IgE antibodies, which are attached to mast cells and basophil leukocytes. During the subsequent late phase, the eosinophil leukocyte is the most characteristic cell of the inflammatory reaction in the nasal mucous membrane.\textsuperscript{2,65-76} Analyses of markers of these cornerstones of the allergic inflammation could prove relevant as a practical and objective replacement of or supplement to symptom/medication scores.

3.1 Aim of study

The main aim was to investigate the efficacy of subcutaneous allergen specific immunotherapy in a study population with dual sensitivity i.e. birch as well as grass-pollen allergic rhinoconjunctivitis. For the assessment of efficacy further aims were included:

1) We wanted to determine whether a number of different measurements of allergic inflammatory markers would correlate with seasonally induced symptoms in patients, and, if so, to identify the parameter with the optimal correlation with symptoms, which would thus be the best suited to supplement/replace symptom scores.

2) We wanted to investigate the influence of pollen load on symptoms and efficacy assessment, when a design including a baseline year followed by two years of immunotherapy was chosen.

3.2 Outline of study

For three consecutive years (1992-94), from April to August, 52 adult patients with both birch- and grass-pollen allergic rhinoconjunctivitis recorded symptom score and medicine intake once a day. The severity of each of five symptoms (sneezing, rhinorrhea, nasal congestion, itchy nose and/or throat, and itchy eyes) was scored on an arbitrary scale of 0-3. The patients were instructed to use medication only when needed, and to observe changes in medication requirement. The basic treatment was oral antihistamine (acrivastine, 8 mg), with a maximum intake of three capsules daily, and antihistamine eye-drops (antazoline-naphazoline), with a maximum dosage of six drops in each eye daily. If symptoms were not sufficiently alleviated at the maximum dosage of antihistamine capsules and eye-drops, oral prednisolone in 10-mg tablets was prescribed. If more than 20 mg was needed in one day, the patient was asked to contact the investigator for a 1-week prednisolone treatment. The mean daily values of symptom score, number of antihistamine capsules, antihistamine eye-drops, and doses (mg) of prednisolone were calculated for each patient. After the birch- and grass-pollen season in 1992 (baseline), patients were assigned to active SCIT treatment with birch (group 1) or grass (group 2) by dynamic allocation with the method of minimization.\textsuperscript{77,78} This method stratifies according to numerous covariates, to achieve balance across several factors simultaneously. In this study seven covariates were chosen: total symptom score for a week in the birch-pollen and grass-pollen peak seasons, specific IgE and SPT for birch and grass, and age. The covariates were weighted, with 3 for symptoms and 1 for the remaining factors. Twenty-six were assigned to group 1 (birch SCIT) and 26 patients to group 2 (grass SCIT). Patient groups were comparable with respect to sex, age, duration of allergy symptoms, specific IgE, and SPT and CPT sensitivity. From November 1992 to autumn 1993, the patients received double blinded clustered SCIT with either Betula verrucosa (group 1) or Phleum pratense (group 2). From November 1993 to the end of 1994, the patients continued SCIT with their original extract and in addition received the other extract, and were subsequently treated with both Betula verrucosa and Phleum pratense pollen extract. The order of allergen was not known until the end of study.

The area under the curve (AUC) and seasonal mean were used to describe the severity of seasonal symptoms and medication. Longitudinal data analysis was used to investigate the relation between different pollen counts and the magnitude of clinical efficacy. The longitudinal model strengthens the reliability of the results by including the single patient as his own reference. A statistical fixed-effect model was formulated, basically following the structure of the usual multiple regression analysis, to investigate the influence of pollen level on symptom score/use of medication, the effect of SCIT treatment, and the SCIT treatment effect dependent on pollen levels.\textsuperscript{80}
During the baseline year blood samples were drawn in the birch-pollen season (last week of April, visit 1), the grass-pollen season (last week of June, visit 2) and out of season (October, visit 3). Similar blood samples were collected from eight non allergic persons. Total IgE and specific IgE and HR to birch and timothy grass were measured. Eosinophils were counted and eosinophil cationic protein (ECP) and eosinophil protein X (EPX) were measured in serum as well as plasma. Mean symptom scores and medication for one week prior to blood tests were calculated. The partial least square analysis (PLS) is an alternative to the more classical multiple linear regression and is considered to be more robust. It was used to establish an optimal statistical link between the symptom score and medication (Y) and the laboratory tests (X). The PLS analysis predicts the Y data as well as possible, and at the same time produces a linear relationship between X and Y.[81,82] The multivariable PLS analysis solves the problem of intercorrelation between variables.

### 3.3 Results and discussion

#### 3.3.1 Baseline year

**Symptom score and consumption of medication**

In the baseline year, groups 1 and 2 were, as expected, similar in symptom score (Fig.1), but minor differences were seen in medication; group 1 had a higher consumption of antihistamine tablets during the birch-pollen season (P = 0.017) and group 2 had greater usage of eye-drops during the grass-pollen season (P < 0.001). This illustrates that, even with a dynamic allocation by the method of minimization, imbalance between groups can be observed and all the important covariates have to be accounted for before allocation.

The symptom score increased during the birch-pollen season, with a further increase during the period between the defined birch- and grass-pollen season (where the birch-pollen grains decreased and grass-pollen grains emerged and started to increase). Maximum symptom scores were seen in the beginning of the grass-pollen season. The same picture was observed with the use of antihistamines, whereas the use of eye-drops seemed to follow the pollen seasons more precisely. The population also had a higher seasonal mean symptom score in the grass-pollen season than in the birch-pollen season (mean 5.3 vs 3.8; P < 0.001), as well as higher seasonal use of antihistamine tablets (mean 1.2 vs 0.5; P < 0.001) and antihistamine eye drops (mean 1.1 vs 0.7; P = 0.01). These results could be due to priming. Priming was first defined and described in 1968 when pollen allergic subjects were challenged on successive days and the amount of pollen needed to induce a clinical relevant reaction decreased.[83] Priming has since then mostly been investigated by repetitive allergen challenges but also by allergen challenge during the pollen season.[84] Priming occurring during a pollen season has been suggested, as a birch-pollen allergic population increased the intake of medicine during the pollen season despite a constant pollen load.[85] Other factors could have contributed to the higher score in the grass-pollen season. Our study population might have had a more severe grass-pollen allergic rhinitis compared to birch-pollen allergic rhinitis. At inclusion the population had a higher grass specific IgE compared to birch specific IgE (mean 30.5 vs 13.7 kU/l; P = 0.003), as well as a higher sensitivity towards grass in CPT (mean 23,050 vs 42,076 SQ-U/ml; P= 0.017), although the difference in SPT wheal area was not significant (mean 49.8 vs 42.3 mm²; P= 0.059). Difference in the allergenicity of pollens could be another factor, including a difference in the content and release of allergens from birch and grass-pollen grains. Also a differences in the course and duration of pollen seasons (birch: abrupt increase in pollens, 24-day season; grass: slow increase in pollens, 58-day season), could add to the differences observed.[86,87]

The allergic sensitization and inflammation during the two subsequent pollen seasons were studied (birch-pollen season = visit 1; grass-pollen season = visit 2). The associations between symptom scores and use of medication (antihistamine tablets, antihistamine eye drops, prednisolone tablets) and laboratory findings were also investigated. The mean birch-pollen count was low the week before the blood tests as it was in the beginning of the season in this particular year[1992], with 62 birch-pollen grains/m³ (corresponding to the 25% fractile of the daily count of birch-pollen in the peak seasons for the years 1977-89). The symptom scores and use of medication the week before blood tests were correspondingly low, with no use of prednisolone (mean average daily symptom score: 1.77, SD = 1.86, range 0-6.14; antihistamine tablets: 0.16, SD 0.42, range 0-1.71; antihistamine eye drops: 0.24, SD 0.52, range 0-2.29). In contrast the grass-pollen counts were moderate, with 47 grains/m³ (corresponding to the 50% fractile of the daily count of birch-pollen in the peak seasons for the years 1977-89). A significant increase from visit 1 to visit 2 was observed in symptom score (visit 2: mean 6.26; SD 3.12; range 1-13.86; P<0.001), use of antihistamine tablets (visit 2: mean 1.36; SD 0.93; range 0-3.14; P<0.0001), and antihistamine eye drops (visit 2: mean 1.47; SD 1.8; range 0-7.86; P<0.0001). Prednisolone was used by 21 % of the patients in the grass-pollen season.
**In vitro measurements**

Total IgE was higher in the allergic group compared to the control group, with no seasonal increase (Figure 2). A seasonal increase in specific IgE was observed from visit 1 to visit 2 for both birch and grass ($p < 0.05$) (Figure 2).

An increased release of histamine was again observed from visit 1 to 2, for both birch- and grass-pollen allergens ($p < 0.05$) (Figure 3). The MAX calculations were not expressed as "percent release of total histamine content", and it cannot be excluded that the observed increase in basophil releasability or reactivity reflected an increase in the number of peripheral basophils. However, there was no difference in basophil counts between visit 1 and 2. For the HR15 the increase from visit 1 to 2 was only evident for birch. The explanation could be that the HR15 to grass was high even at visit 1; i.e. the amount of membrane-bound grass-specific IgE was so high that the lowest concentration of the grass-extract was sufficient to induce significant HR (cut-off 15 ng histamine/ml blood). A weak but significant correlation was observed between the HR15 and MAX ($r^2$ between 0.29 and 0.36, $p<0.0001$). In the birch- and grass-pollen seasons, there was a relationship between the anti-IgE response and the specific birch or grass response in both MAX and HR15 (birch: $r^2=0.81$ and 0.47 for MAX and HR15, respectively; grass: $r^2=0.81$ and 0.34 for MAX and HR15, respectively; $p<0.0001$ in all correlations). This could be explained by a high proportion of the total IgE bound to the basophils being specific IgE to the relevant allergens. There was an increase not only in grass-specific IgE and grass HR but also for the corresponding birch measurements. The birch-pollen load increased just after visit 1. A peak response in birch-specific IgE and birch HR after visit 1, which persisted for some time, could explain the observed increases from visit 1 to visit 2.

![Figure 2. Seasonal changes in blood concentration of total IgE in allergic patients and control subjects, and seasonal changes in birch- and grass specific IgE (measured by CAP) of allergic patients. Box-and-whisker plot showing 10, 25, 50, 75, and 90% cumulative relative frequencies (centiles). Visit 1) early birch-pollen season; visit 2) mid-grass-pollen season; visit 3) after pollen seasons. *P<0.05](image)

![Figure 3. Seasonal changes in basophil histamine release (MAX and HR15) of allergic patients, performed with anti-IgE, and birch- and grass specific IgE. Box-and-whisker plot showing 10, 25, 50, 75, and 90% cumulative relative frequencies (centiles). HR15 at visit 2 for anti-IgE and grass-specific IgE was maximal at 25% cumulative relative frequency. Visit 1) early birch-pollen season; visit 2) mid-grass-pollen season; visit 3) after pollen seasons. *P<0.05](image)
A significant increase in EOS, serum ECP, serum EPX and plasma EPX from visit 1 to visit 2 was observed in the allergic patients, whereas no difference was observed in any parameter between visits in control subjects (Figure 4). In the birch- and grass-pollen seasons, the EOS correlated well with serum values of ECP and EPX ($r^2$ = 0.40 and 0.53, respectively; $p$ < 0.0001). The correlation between serum ECP and EPX was $r^2$ = 0.42 ($p$ < 0.0001). The relationship between EOS and plasma values was weak (plasma ECP: $r^2$ = 0.09, $p$ = 0.0036; plasma EPX: $r^2$ = 0.15, $p$ = 0.0001), as was the relationship between serum and plasma values (serum ECP vs plasma ECP: $r^2$ = 0.05, $p$ = 0.037; serum EPX vs plasma EPX: $r^2$ = 0.15, $p$ = 0.0001).

As the eosinophil counts were significantly correlated with serum ECP and serum EPX, and we did not find an individual increase in ratios serum ECP/EOS and serum EPX/EOS from visit 1 to visit 2, we cannot exclude the possibility that the increase in serum ECP and EPX observed was partly due to an increased number of eosinophils.

Overall the results indicated an activation/up-regulation of the IgE synthesis, and the basophil and eosinophil leukocyte, from the birch-pollen season to the grass-pollen season, which supports the idea of priming. No prior studies have investigated the changes in birch- and grass-pollen seasons in patients with dual sensitivity, but the literature gives several examples of an increase within a single pollen season: variations in IgE serum levels, basophil histamine release (HR), and eosinophil activity. Fewer studies have investigated the relationship between parameters and allergic symptoms, and no clear pattern has emerged when correlated with symptoms of allergic rhinitis.

Association between symptom score/consumption of medication and in vitro measurements
Study I showed a relationship between results of in vitro tests and symptoms of allergic rhinitis. There was, however, an intercorrelation between the different in vitro tests, that would influence the interpretation of the usual Spearman regression correlations between symptom/medication score and laboratory tests. A recent study revealed that when bivariate correlation
When multiple linear regression was used only nasal eosinophil count and the amount of specific IgE were independent predictors of the severity of perennial allergic rhinitis. In our study the PLS analysis was chosen to solve this collinearity problem. It analyzes the variation in parameters between patients; i.e. if for example, symptom scores change, do HR tests change as well, and can the change in symptom scores be explained by the change in HR? The PLS analysis may possibly explain to what extent the different in vitro parameters express the severity of allergic rhinitis. Only results from visit 2 are presented. The results from visit 1 revealed the same pattern; however, the background noise in the data was greater than at visit 2.

Results are presented in Table 1. When all symptom and medication scores (Y) and in vitro tests (X) were considered together, a significant component was found (F-test value 40.2, correlation 0.34 for the cumulative relation, cross validated). This component explained 46% and 19% of the variance in combined laboratory tests, and combined symptom and medication score, respectively; i.e., 19% of the total variation in symptom and medication scores was explained by 46% of the total variation in laboratory tests (Table 1). As the results indicate, factors other than these chosen blood test parameters influenced the severity of allergic rhinitis (and other factors than the severity of allergic rhinitis contributed to the changes in the blood-test results). The factor loadings show the relative significance of the variables in relation to each other, as defined by the PLS model (Table 1). For the independent X variables, all histamine parameters were highly intercorrelated, and contributed about 70% of their total variance; i.e., 70% of the variation in HR could be explained by the variation in symptom-therapy score. For the independent Y variables the relative weights (factor loadings) were 0.7328:0.5613:0.3844:0.0113, which can be normalized to 1:0.76:0.52:0.02. For this particular study population the estimation of a patient’s total weighted symptom medication score then became: 1 symptom score + 0.76 antihistamine tablets + 0.52 eye drops + 0.02 prednisolone.

The results indicate that the chosen in vitro parameters do not necessarily reflect the actual disease status of the target organ. If we had measured and included the number of eosinophils/concentration of ECP/EPX in the tissue, and the releasability of tissue basophil/mast cells this might have increased the explained percentage of the total variation in disease severity. Other immunologic cells such as CD4⁺ T-helper cells and Langerhans cells, have been shown to be related to allergic inflammation, indicating that allergic inflammation and its relation to the severity of the allergic disease is very complex, and that different components of the immune response are connected. A seasonal increase in the blood measurements of basophil HR, eosinophils, serum ECP and EPX, and specific IgE was observed in allergic rhinitis patients, with a high degree of intercorrelation within tests describing the activity of the individual cells.

The PLS analysis seems a good choice when multiple parameters are assessed, as this multivariable model solves the collinearity problem (intercorrelation between variables). The model showed a significant relationship between clinical symptoms and the blood measurement markers of immunoinflammation, with an indication of different importance among the individual tests. It showed that a few selected tests had as much impact as all the tests put together. The HR test seemed to be the most relevant parameter for clinical symptoms during the pollen season. However, although a significant relationship was found between in vitro tests and the symptom and medication scores, the PLS model explained only less than one quarter of the total variation in combined symptom and medication score. This strongly indicates that other factors than the inflammatory status influence the patients’ perception of the severity of symptoms.

Table 1. Partial least square analysis of in vitro tests (X), symptom scores and medication (Y)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor loading</th>
<th>Variance explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>X (total)</td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>grass HR15</td>
<td>0.3339</td>
<td>0.78</td>
</tr>
<tr>
<td>anti-IgE MAX</td>
<td>0.3200</td>
<td>0.72</td>
</tr>
<tr>
<td>birch MAX</td>
<td>0.3164</td>
<td>0.70</td>
</tr>
<tr>
<td>anti-IgE HR15</td>
<td>0.3159</td>
<td>0.70</td>
</tr>
<tr>
<td>birch HR15</td>
<td>0.3154</td>
<td>0.70</td>
</tr>
<tr>
<td>grass MAX</td>
<td>0.3013</td>
<td>0.64</td>
</tr>
<tr>
<td>grass specific IgE</td>
<td>0.2938</td>
<td>0.60</td>
</tr>
<tr>
<td>birch specific IgE</td>
<td>0.2404</td>
<td>0.40</td>
</tr>
<tr>
<td>eosinophils</td>
<td>0.2297</td>
<td>0.36</td>
</tr>
<tr>
<td>total-IgE</td>
<td>0.2253</td>
<td>0.35</td>
</tr>
<tr>
<td>serum EPX</td>
<td>0.2252</td>
<td>0.35</td>
</tr>
<tr>
<td>plasma EPX</td>
<td>0.2066</td>
<td>0.29</td>
</tr>
<tr>
<td>serum ECP</td>
<td>0.2034</td>
<td>0.28</td>
</tr>
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<td>plasma ECP</td>
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<td>0.08</td>
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<tr>
<td>Y (total)</td>
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<td>0.19</td>
</tr>
<tr>
<td>symptom score</td>
<td>0.7328</td>
<td>0.44</td>
</tr>
<tr>
<td>antihistamine tablets</td>
<td>0.5613</td>
<td>0.25</td>
</tr>
<tr>
<td>antihistamine eye drops</td>
<td>0.3844</td>
<td>0.11</td>
</tr>
<tr>
<td>prednisolone</td>
<td>0.0113</td>
<td>0.00</td>
</tr>
</tbody>
</table>
3.3.2 Changes during treatment
The mean daily symptom score and use of medication for the birch- and grass-pollen seasons 1992-4 for group 1 (birch SCIT in 1993) and group 2 (grass SCIT in 1993) are described in Figure 1. After 1 year of treatment (1993), the birch SCIT group 1 had statistically significant fewer symptoms and used less medication during the birch-pollen season than the untreated group 2. Like several other SCIT studies, this study also showed an important annual variation in pollen counts as well as duration of season: Birch 1992: mean 101 pollen grains, 24-day season; 1993: mean 932 pollen grains, 16-day season; 1994: mean 165 pollen grains, 17-day season. Grass 1992: mean 31 pollen grains, 58-day season; 1993: mean 23 pollen grains, 58-day season; 1994: mean 64 pollen grains, 44-day season. For birch SCIT, symptom score and medication rose after the first treatment year, as such questioning the efficacy of treatment. However, the birch-pollen season during the first treatment year was the most intensive season registered in Denmark so far, and a statistical difference between groups was found this year, indicating efficacy after all. For grass, symptom score and medication decreased after the first treatment year, but for both groups. No statistical difference was seen after the first treatment year, except for antihistamine intake. This could be caused by a low grass-pollen season in 1993, a tendency (although not significant) towards a higher pretreatment value of symptom score/medication in grass-pollen season before SCIT for group 2, and/or reduction of priming (group 1 was treated with birch SCIT and had less symptoms and medication use in the preceding birch-pollen season).

Like other randomized and controlled or DBPC birch immunotherapy efficacy studies in allergic rhinitis, a significant effect of the treatment was indicated. In contrast the efficacy of grass SCIT could be questioned in this study, but the results of this long term study however, revealed, that the evaluation of clinical efficacy in pollen SCIT lasting several years has to take differences in pollen counts into consideration.

Figure 5. Calculated seasonal mean of symptom score, and seasonal mean of medication of average birch- and grass-pollen-allergic patients at different pollen counts, based on model. Dotted line represents untreated group, and solid line treated group.
### Table 2. Results from longitudinal variance analysis (equation 1). Values shown are estimated values, standard error in parenthesis, and \( P \) values in italics.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>( \mu ) constant</th>
<th>( \sigma_i^2 ) between patient variance</th>
<th>( \sigma^2 ) residual variance</th>
<th>( \alpha ) group effect</th>
<th>( \delta_1 ) pollen effect</th>
<th>( \delta_2 ) treatment effect</th>
<th>( \delta_3 ) pollen-treatment interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birch:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>symptom score</td>
<td>284</td>
<td>0.274</td>
<td>0.224</td>
<td>-0.024</td>
<td>0.334</td>
<td>-2.703</td>
<td>0.348</td>
</tr>
<tr>
<td></td>
<td>(0.079)</td>
<td>(0.036)</td>
<td>(0.101)</td>
<td>(0.071)</td>
<td>(0.687)</td>
<td>(0.0001)</td>
<td>(0.122)</td>
</tr>
<tr>
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<td>-771</td>
<td>0.106</td>
<td>0.083</td>
<td>0.032</td>
<td>0.292</td>
<td>-0.912</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>(0.030)</td>
<td>(0.013)</td>
<td>(0.062)</td>
<td>(0.043)</td>
<td>(0.419)</td>
<td>(0.029)</td>
<td>(0.074)</td>
</tr>
<tr>
<td>eye drops</td>
<td>-210</td>
<td>0.122</td>
<td>0.130</td>
<td>-0.008</td>
<td>0.170</td>
<td>-1.441</td>
<td>0.175</td>
</tr>
<tr>
<td></td>
<td>(0.038)</td>
<td>(0.021)</td>
<td>(0.070)</td>
<td>(0.054)</td>
<td>(0.523)</td>
<td>(0.059)</td>
<td>(0.093)</td>
</tr>
<tr>
<td><strong>Grass:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>symptom score</td>
<td>-1893</td>
<td>0.234</td>
<td>0.173</td>
<td>0.006</td>
<td>1.111</td>
<td>2.386</td>
<td>-0.738</td>
</tr>
<tr>
<td></td>
<td>(0.066)</td>
<td>(0.027)</td>
<td>(0.091)</td>
<td>(0.242)</td>
<td>(1.050)</td>
<td>(0.285)</td>
<td></td>
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<tr>
<td>antihistamine tablets</td>
<td>-1411</td>
<td>0.116</td>
<td>0.057</td>
<td>-0.019</td>
<td>0.658</td>
<td>1.398</td>
<td>-0.411</td>
</tr>
<tr>
<td></td>
<td>(0.031)</td>
<td>(0.009)</td>
<td>(0.062)</td>
<td>(0.140)</td>
<td>(0.606)</td>
<td>(0.164)</td>
<td></td>
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<tr>
<td>eye drops</td>
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<td>0.878</td>
<td>2.726</td>
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<tr>
<td></td>
<td>(0.052)</td>
<td>(0.015)</td>
<td>(0.080)</td>
<td>(0.178)</td>
<td>(0.774)</td>
<td>(0.210)</td>
<td></td>
</tr>
</tbody>
</table>

(1) \( Y_{ik} = \mu + \alpha_i + \delta_1 p_j + \delta_2 t_{ij} + \delta_3 t_{ip} + y_{ik} + \xi_{ik} \)

Here \( i \) denotes the treatment group, i.e., group 1 or 2; \( j \) denotes the year, i.e., 1, 2 or 3; and \( k \) identifies patients. \( \mu \) is a constant, and \( \alpha_i \) is the group influence. \( \alpha_i \) sums to zero over all \( i \). \( p_j \) is the influence of pollen level; \( t_{ij} \) is the treatment effect, with the value 0 for no treatment, and 1 for treatment; \( t_{ip} \) is the treatment-pollen interaction; and \( \delta_1, \delta_2, \delta_3 \) are their coefficients. \( y_{ik} \) is random patient influence assumed to be independent and normally distributed with mean zero and variance \( \sigma_i^2 \left[ N(0, \sigma_i^2) \right] \). The errors \( \xi_{ik} \) are independent and normally distributed with mean zero and variance \( \sigma^2 \left[ N(0, \sigma^2) \right] \). \( y_{ik} \) and \( \xi_{ik} \) are independent. The expected symptom score/use of medication, with and without IT, can be calculated according to the estimated \( \delta \)'s in the above model. A significant \( \delta_1 \) indicated an influence of pollen level on symptom score/use of medication. A significant \( \delta_2 \) indicated a treatment effect of IT. A significant \( \delta_3 \) indicated an IT treatment effect dependent on pollen levels.
3.3.3 Variability in pollen exposure

A longitudinal model was made to reduce the bias of differences in pollen counts, and to further strengthen the reliability of the results by including the individual patient as his own reference. Table 2 shows the estimated parameters of the model. Figure 5 shows the effect of treatment, as a function of mean seasonal pollen counts calculated from equation 1, and the estimated parameters in table 2. No significant group effect (α) was observed; i.e. the two groups of patients could be regarded as taken from the same population. There was a significant pollen effect (δₜ), and symptom score and medication were positively dependent on pollen counts; i.e. the higher the pollen count, the higher the symptom/medication score. A significant effect of SCIT (δₜ) was found for both birch and grass; i.e. treated patients had lower symptom scores and used less medication. However, a seasonal grass-pollen load above a count of 20-30 pollen grains/m³ was necessary for an effect on symptom score/medication to emerge. As such the study indicated that the efficacy of grass SCIT does not show below a certain grass-pollen count. Similarly, another study was able to show a significant difference in symptom and medication scores between actively treated and placebo-treated grass-pollen allergic patients only during the period with high pollen exposure. In a multicentre study, a significant difference between actively treated and placebo-treated grass-pollen-allergic patients was found only in centres with an average weekly pollen count of more than 40 pollen grains/m³.

In most cases the SCIT effect was dependent on pollen counts (δₜ). For grass, the difference in symptom score and medication were positively dependent on pollen counts; i.e. the higher the pollen count, the higher the symptom/medication score. A significant effect of SCIT (δₜ) was found for both birch and grass; i.e. treated patients had lower symptom scores and used less medication. However, a seasonal grass-pollen load above a count of 20-30 pollen grains/m³ was necessary for an effect on symptom score/medication to emerge. As such the study indicated that the efficacy of grass SCIT does not show below a certain grass-pollen count. Similarly, another study was able to show a significant difference in symptom and medication scores between actively treated and placebo-treated grass-pollen allergic patients only during the period with high pollen exposure. In a multicentre study, a significant difference between actively treated and placebo-treated grass-pollen allergic patients was found only in centres with an average weekly pollen count of more than 40 pollen grains/m³.

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3.4 Conclusions

The results of this part of the thesis indicate that patients with pollen allergic rhinitis, who are sensitized to both birch and grass, might have a prolonged and more severe grass-pollen season due to priming, and one could speculate that SCIT with birch-pollen might decrease the severity of grass-pollen allergy.

When a clinical SCIT trial is designed several considerations are taken into account, not only the statistical principles but also regulatory and political/economic issues. Whether a one-year, randomized placebo-controlled double-blind study design, or a study including inclusion of a baseline period of observation is preferred could be discussed. However, a significant treatment effect was found in this study.
The evaluation of efficacy of long-term SCIT in seasonal allergic rhinitis has to take the differences in seasonal pollen counts into consideration. We applied a statistical longitudinal model for the evaluation of the efficacy of pollen SCIT, which takes into consideration the differences in pollen counts. By this model we demonstrated the clinical efficacy of grass-pollen SCIT to be dependent upon the magnitude of the pollen counts, and that efficacy of grass SCIT seems to increase with higher pollen exposure.

Not surprisingly a great variability in symptoms and use of medication was found between patients. The results indicated that the individual patient profile did not change, that is if the patient tended to have the highest score and use of medication this ranking did not change during subsequent years irrespective of SCIT. Thus it can be discussed if symptom scores and medication use can be used as a universal measure for the severity of allergic disease. Pure random allocation may not evenly distribute “high reporters” and “low reporters”, and study designs including a baseline year with stratification according to individual scores or calculations of changes in efficacy parameters could be an alternative to a placebo-controlled study design with no pretreatment assessment, when efficacy of SCIT is evaluated.

SCIT potentially reduces both symptoms and the use of medication. Symptoms and consumption of medication are interdependent, and a combination of symptom score and medication may provide clinically more relevant information than each of these parameters alone. The relationship is complex, however, with many potential methods for adjusting the symptom score for use of rescue medication. Further the relationship might differ dependent on the study population. Thus it would be preferable to use a method of weighting symptom and medication scores that is dependent on the actual data, and not on a somewhat arbitrary weight chosen by the investigator.

The partial least square analysis was used to explore the optimal weighting scheme between symptom score and medication and the relationship with blood-measurement markers of immunoinflammation, chosen to be potential surrogate markers of allergic rhinitis symptoms. A seasonal increase in basophil HR, eosinophils, serum ECP and EPX, and specific IgE was observed in the allergic rhinitis patients, with a high degree of intercorrelation within tests describing the activity of the individual cells. The presented PLS analysis therefore seems a good choice when multiple parameters are assessed, as this multivariable model solves the collinearity problem (intercorrelation between variables). The model showed a significant relationship between clinical symptoms and the blood-measurement markers of immunoinflammation, with an indication of different importance among the individual tests. It showed that a few selected tests tell as much as all the tests put together. The HR test seemed to be the most relevant predictor of clinical symptoms during the pollen season. However, although a statistically significant relationship was found between in vitro tests and the symptom and medication scores, the overall result indicated only a minor association with this primary efficacy parameter. This emphasizes the complexity of the allergic inflammation with many factors contributing to the severity of the disease, i.e., there is no simple coherence. It can be discussed if the chosen surrogate markers of allergic rhinitis can be used in the evaluation of efficacy of SCIT. They cannot replace the primary clinical outcome, but might be used as secondary outcomes e.g., to investigate mechanisms behind the actions of SCIT.

4. Side-effects of allergen specific immunotherapy (III, IV)

The literature on allergen specific immunotherapy shows a huge variation in frequency and severity of systemic reactions (SR), with SRs in 0-100% of patients. However, the risk of side-effects reported in different studies is difficult to compare due to differences in study designs, differences in parameters that might influence the outcome e.g., patient related factors (ex. disease), manufactory differences in allergen standardization, formulation and potency of the extracts, and differences in dose schedules. Missing or less detailed reporting of side-effects could also influence results. In a recent Cochrane review on efficacy and safety of subcutaneous allergen specific immunotherapy for seasonal allergic rhinitis (placebo-controlled studies) only 65% of the studies that satisfied the inclusion criteria provided data on systemic reactions, and only 59% reported local reactions. Differences in recording and grading of SRs could also contribute to the differences. For instance, some studies only include more severe side-effects such as urtication, asthma and anaphylactic shock, whereas others also include milder side-effects such as rhinitis and unspecific symptoms like fatigue and headache. A recent review article from the World Allergy Organization (WAO) taskforce recommended that all adverse events which occur in more than 5% of subjects should be described, and all adverse events specifically related to treatment must be described in detail. It is also recommended to use the EAACI grading system of severity for systemic reactions, which has recently been revised.

Most data on side-effects derive from smaller controlled or placebo-controlled efficacy studies, and only few SCIT studies have been designed to investigate differences in frequency of side-effects. As many factors might contribute to the risk of side-effects, both patient related and treatment related, it is very important, especially with small studies, that the populations are comparable. Due to the low frequency of side-effects large scale studies are needed to achieve more reliable data on the prevalence, character and severity of side-effects as well as the influence of patient and treatment
parameters on these data. Such studies should include statistical analysis able to determine the influence of the various parameters on the occurrence of SRs in the individual patient.

When side-effects are evaluated, not only the magnitude and severity but also the onset time of a systemic reaction are important factors. Reactions with early onset seem to be more severe in nature and often require intensive monitoring and treatment.[123] Mild reactions with later onset might not require observation and can be handled by the patients themselves. This has practical implications for the observation time after an allergen injection.

4.1 Aim of study
The aim of study III and IV was to investigate the occurrence, onset, type and severity of side-effects of SCIT. Study III investigated birch- and grass-pollen SCIT within the same small study population, whereas study IV was a multicentre study with a high number of patients, and included birch and grass-pollen extracts as well as other allergen extracts.

4.2 Outline of study
The EAACI guidelines were followed with regards to inclusion and exclusion of patients and the practical performance of the treatment. In study III a 7 week clustered induction schedule (14 injections to maintenance dose) was used, whereas in the multicentre study IV different modified cluster regimens or a conventional 1 injection per week schedule were used. The centres also differed in terms of use of antihistamine pretreatment, and number of allergens used on the same day. As the significance of individual allergens on the occurrence of side-effects is blurred when more than one allergen is injected on the same day, it was decided to analyze data from single allergen treatment separately.

All treatment related systemic reactions of grades 2-4 during the induction phase were registered.[43] Grading of SRs was performed by the principal investigator. The number of patients with SRs and the number of SRs per injection were calculated. The difference in severity of SRs, as well as symptoms and onset time were described. Systemic reactions with onset later than 30 min, were defined as late SRs. Late local reactions (LLR), were defined as local reaction registered later than 30 min) and subcutaneous nodules where registered in study III, only.

4.3 Results and discussion

Occurrence of side-effects
In study III, where patients were treated with both birch-pollen and grass-pollen extracts, the number of patients with SRs differed, with five (11%) and 21 (45%) patients in the birch and grass groups, respectively (P<0.001). A difference between birch and grass-pollen SCIT was also found in study IV (P=0.004), see figure 6. The corresponding values were 18% and 30% in birch and grass groups, respectively. In study III, there was a difference in the number of systemic reactions per injection, with SRs in 0.7% of birch-extract injections and 3.3% of grass-extract injections (P<0.0001). In study IV, the frequency also differed between allergens, with wasp venom (0.8%), birch (2.0%), grass (4.1%), Dp (5.9%) and cat (7.5%) (P<0.0001).

Another study using extracts from ALK-Abelló for SCIT with hymenoptera, birch-pollen, grass-pollen, HDM and cat dander, found fewer patients with grade 2-4 SRs than the reported study (mostly wasp) and birch compared to the other allergens, including grass.[125] This was in accordance with our study. Small differences were seen; in the reported study the highest number of patients with SRs were seen with cat extract followed by Dp and grass, whereas we found the grass extract to cause most SRs followed by cat and Dp. The number of side-effects per injection to the maintenance dose in our study was higher with cat and Dp compared with grass, and thus in accordance with this study. The minor differences could be due to the small number of side-effects. A recent study found a higher number of patients with SRs when treated with grass and ragweed SCIT compared to mite, birch and parietaria.[136]

Other large scale studies have mainly focused on HDM and grass-pollen extracts, with diverging results. In a
study with pollen (pollen species not specified) or HDM extracts there was also a higher frequency of SRs per injection in HDM compared to pollen SCIT, but multivariate logistic analysis did not demonstrate that HDM was a risk factor associated with SRs, and there was no difference in the number of patients with SRs between pollen and HDM.\(^{(127)}\) In a prospective study of children, both the number of children with side-effects and the number of systemic reactions per injection were significantly higher with HDM extract in comparison with grass-pollen extract,\(^{(128)}\) a second study found a lower frequency of side-effects in patients treated with HDM extract compared to pollen extract\(^{(129)}\) whereas two studies showed no significant difference in the incidence between pollen and HDM.\(^{(130;131)}\) In three of these studies the extracts were provided from different manufacturers,\(^{(127;129;131)}\) which could influence on results.\(^{(132;133)}\)

In a multicentre study comparing extracts from different manufacturers, there was no difference in HDM extracts, but in relation to pollen extracts (gramineae), the preparation from one of the manufacturers had a significantly lower frequency of systemic reactions.\(^{(129)}\) A different study found no difference in number of patients with SRs due to grass-pollen extracts from different manufacturers, but the frequency differed with the type of extracts, with fever SRs with phleum pratense extract compared to lolium perenne and mix grass.\(^{(134)}\) Standardization methods for extracts were different between the manufacturers and not all manufacturers provided information on the amount of major allergen in the extracts.

The results of our studies indicate that the incidence of side-effects of SCIT is related to the allergens and that the grass extract is more likely to induce side-effects compared with the birch extract. The most obvious explanation for this difference between allergens would be a difference in the strength or potency of the extracts. The strength of the extract depends on the magnitude of the response it elicited in the specific allergic population which was used historically for the assessment of allergen extract potency by skin prick testing. If the grass-pollen allergic patient population investigated had a low sensitivity towards grass-pollen allergens, and the birch-pollen allergic population had a high sensitivity towards birch-pollen allergens, consequently the strength of the grass-pollen extract would be higher, compared to the birch-pollen extract. The birch extract in our study contained 12.3 µg major allergen Bet v 1 per 100.000 SQU compared with 20.2 protein µg major allergen Phl p 5 in the grass extract. Differences in potency of extracts could also be part of the explanation for the difference in incidence of SRs observed between studies.\(^{(125-127;129-133)}\)

The nature of the allergen itself including different numbers and proportions of major and minor allergens in the extracts, as well as the heterogeneity between patients in terms of the IgE reactivity to the individual allergen components, may also be a part of the explanation.\(^{(122;127;137-139)}\)

### Table 3. Number and distribution of systemic reactions.

<table>
<thead>
<tr>
<th></th>
<th>Birch IT</th>
<th>Grass IT</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>U + Q</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>U + A</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>U + RC</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>U (only)</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>U (total)</td>
<td>0</td>
<td>8</td>
<td>0.005</td>
</tr>
<tr>
<td>I + Q</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>I + RC</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>I + OAS</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>I (only)</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>I (total)</td>
<td>0</td>
<td>8</td>
<td>0.005</td>
</tr>
<tr>
<td>A + RC</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A (only)</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>A (total)</td>
<td>0</td>
<td>6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RC + OAS</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>RC (only)</td>
<td>3</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>RC (total)</td>
<td>4</td>
<td>11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OAS (only)</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>OAS (total)</td>
<td>5</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>rash(^{3})</td>
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<tr>
<td>facial erythema</td>
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<td></td>
</tr>
<tr>
<td>Systemic reaction (total)</td>
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<td></td>
</tr>
<tr>
<td>Early systemic reaction</td>
<td>1</td>
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</tr>
<tr>
<td>Late systemic reaction</td>
<td>7</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Systemic reaction (grade 2/3/4)</td>
<td>31/10/0</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

**U:** urticaria; **A:** asthma; **RC:** rhinoconjunctivitis; **I:** itching without urticaria; **Q:** angioedema of lips and face; **OAS:** oral allergy syndrome i.e., oral-pharyngeal itching; \(^{3}\) patient with atopic dermatitis who repeatedly developed 1 to 5 mm large macules at predisposed sites, 3 to 6 hours after injections . * Chi square test.

### Type of side-effects

In study IV, SRs with all inhalant allergens included rhinoconjunctivitis of the same magnitude, around 61% of reactions, whereas in study III the birch extract gave rise to rhinoconjunctivitis in 50% of the reactions compared to 27% of the reactions with the grass extract, see Table 3 (P < 0.01). In study IV, all allergens gave rise to urticaria, only rarely with birch (2%), and more often with grass (26%) (p = 0.003) (Figure 7). In study III no urticaria was observed with the birch extract, but might have been observed with a larger study group. The reason for these differences is not clear. As with urticaria, asthma was observed with all allergens in study IV, but not with the birch extract in study III, again possibly due to the small number of patients. The frequency of asthma was significantly higher in SCIT with cat (66%) and HDM (42%) compared to birch (13%) and grass (28%)
(P < 0.001) (Figure 7). This could be explained by a higher number of asthmatic patients in the cat and HDM group, although the tendency to a higher frequency of pre-existing asthma in the cat and HDM group, seen in our study, was not significant. In a prospective study with pollen allergic rhinitis patients with or without seasonal asthma and SCIT with individual pollens, SRs were classified as asthma, urticaria or anaphylaxis. When patient groups were divided in an asthma + rhinitis group and a rhinitis only group there was a statistically higher number of SRs with asthma in the asthma group compared to the rhinitis only group.\(^{123}\) The higher frequency of side-effects in asthmatic patients could be related to the size of the affected organ (lungs vs. upper airways in rhinitics), and a higher degree of airway hyperreactivity. When looking at SRs with urticaria and anaphylaxis there was, however, no difference between asthmatics and rhinitics, and pre-existing asthma might thus only increase risk of asthma symptoms during SCIT.\(^{123}\) In another SCIT study with asthma and rhinoconjunctivitis patients treated with either HDM or grass-pollen, there was a higher proportion of SRs and number of asthmatics in the HDM group. Again it seemed that most of the SRs in the asthma group included asthma, which was not the case for the rhinitis group.\(^{146}\)

The allergen products used most often in asthma are perennial allergens including house dust mites and pets (cats and dogs), i.e. allergens to which the patient may have recently been exposed before injections. This could induce subclinical asthma which would increase the susceptibility of the patient. Persistent inflammation caused by low grade exposure to perennial allergen might also increase the risk of systemic side-effects. The severity of asthma was not registered in our study but could have contributed to the differences in frequency of SRs between pollen and perennial allergens observed in our study. In 1989, 125 asthma patients were investigated during a three day rush immunotherapy protocol with a standardized, aqueous extract of Dermatophagoides pteronyssinus.\(^{142}\) It was observed that a significantly higher proportion of patients with FEV1 < 80% from predicted values developed asthma, compared to patients with FEV1 > 80%. Asthma as a predictor of side-effects in SCIT is discussed in the next chapter.

Inclusion of adjuvants in immunotherapy extracts is important to enhance immune responses to allergens, and different adjuvant formulations have been developed in an attempt not only to make immunotherapy more effective but also to reduce side-effects. Aluminium has traditionally been added to aqueous extract, and with a slow release of the allergen in theory the risk of side-effects could be reduced. The results have, however, not been conclusive,\(^{123,142,144}\) and aluminium has been shown to induce subcutaneous nodules.\(^{145,146}\) It has previously been shown that the frequency of patients with subcutaneous nodules is proportionate to the quantity of aluminium in the extracts.\(^{124}\) This study found subcutaneous nodules in 60% of patients, in contrast to approximately 90% in our study III, with the same amount of aluminium. In study III, patients were actively asked about nodules in questionnaires, and their arms were palpated. This could explain the difference. Only 50% of patients with nodules felt inconvenience, mostly occasional, and the majority of nodules were transient; however, other adjuvants with no long-term accumulation might help to reduce local side-effects further. Calcium phosphate-adsorbed allergen extracts have been developed to avoid the subcutaneous nodules seen with aluminium adjuvants,\(^{34}\) but no comparative prospective studies between calcium phosphate and aluminium adsorbed extracts concerning efficacy and safety have been made.

![Figure 7. Percentage of side-effects to each allergen presenting with asthma: white column and urticaria: black column. For asthma, P < 0.001 and for Urticaria, P = 0.003](image)

**Severity of side-effects**

In study III, there were no grade 4 reactions. All SRs after birch SCIT were mild (grade 2) reactions consisting of rhinoconjunctivitis and oral allergy syndrome, whereas 24% of the SRs after grass SCIT were grade 3 reactions including asthma and urticaria (Table 3). The tendency towards milder SRs with birch extracts compared to grass extracts was confirmed in study IV, see figure 8. The SRs of birch SCIT were mainly mild grade 2 reactions, whereas grass was the only allergen with severe grade 4 reactions after injection. The difference was mainly due to urticaria, but also due to anaphylactic reactions. A higher potency of the extract could again be part of the explanation. Another study has investigated if different allergens were prone to induce more severe reactions, but found no difference in grade 1-4 SRs between pollens, mite and hymenoptera.\(^{131}\) Further studies are needed.
In study III and IV patients were observed for 30 min, and were then allowed to leave if no symptoms had emerged. A substantial part of SRs occurred later than 30 min, 86% in study III and 50% in study IV, respectively. All grade 4 reactions in study IV occurred when the patient was still at the centre, whereas 54% of the grade 3 reactions occurred after the patient had left the centre, see Table 4. In 72% of these, late grade 3 reactions, urticaria was the reason for grading the SR as 3. The severity of urticaria was not evaluated. SCIT studies where side-effects are reported do not always present the onset of systemic reactions. In 3 studies with rush protocols and aqueous extract most of the SRs occurred within 15 minutes and none were observed later than 45 minutes.\(^{122,123,141}\). In contrast two studies with conventional updosing and aluminium extracts reported that all SRs were late reactions (occurred later than 30 minutes).\(^{35,124}\) A significantly higher frequency of late SRs (59%) was observed in patients treated exclusively with depot allergen extracts, compared to 26% in patients treated with non-depot extracts from same manufacturer.\(^{125}\) In later studies with depot extracts the frequency of early (within 30 minutes) SRs varied between 14% and 71%.\(^{130,146,148,150}\)

Anaphylactic reactions are rare, but it is crucial that all severe reactions, requiring immediate treatment occur when the patient is still in the clinic. An observation period of minimum 30 min has been proposed in international guidelines.\(^{43,47}\) In two recent surveys from the United States of America (USA), 10 (77%) patients with fatal reactions and 65 (96%) patients with near fatal reactions, for whom information on the timing of the onset of symptoms was available, had symptoms within 30 min of the injection.\(^{151,152}\) The SCIT approach differs between USA and Europe. Mostly plural allergens in individual mixed-extracts are used in the USA and mostly single allergens are used in Europe. A large survey of onset of anaphylactic reactions to SCIT in Europe does not exist. Most studies, that report anaphylactic reactions with information of time of onset, report debut of symptoms within 30 minutes. In different studies including both depot and non-depot extracts anaphylaxis was reported occurring within 15 min,\(^{38,123,141}\) within 20 min\(^ {122}\) and within 30 min.\(^ {125,130,136,148}\) Onset of anaphylactic symptoms later than 30 minutes is only occasionally described.\(^ {149}\)

Though grade 4 reactions in our study as well as other studies seem to appear within the observation period of 30 minutes, a high proportion of grade 2-3 side-effects still occur after the patient has left the clinic. Increasing the observation period to 1 hour in study IV did not seem to change the proportion of side-effects occurring at the clinic significantly (Table 4). The patients managed the majority (93%) of late side-effects, and consequently a high degree of patient education is essential when performing SCIT.

When comparing the LLRs after equal doses, irrespective of the number of injections given on the same day, the LLRs were significantly smaller in the birch group at all SCIT stages \(p<0.0001-0.03\). There were 100 (8.1%) LLRs > 8 cm in the grass group, compared to 16 (1.4%) in the birch group \(p<0.0001\). In six of these 116 LLRs (5%), injections at the next visit resulted in SRs. Ten percent of the SRs were preceded by a LLR greater than 8 cm, and in 6% of the SRs a LLR greater than 8 cm developed after the injection causing the SR (all grass (NS). The results indicate that delayed skin reactions greater than 8 cm after SCIT do not seem to predict SRs at the next visit, and SRs were not followed by great LLRs. Local reactions as a predictive parameter for SRs will be discussed in chapter 5.
4.4 Conclusions

Even though subcutaneous birch and grass-pollen immunotherapy convincingly show efficacy of the treatment, the treatment is lengthy, costly and not without risk. Our studies show that the incidence of systemic reactions seems to differ with the allergen in question, and for seasonal pollen allergic rhinitis, immunotherapy with grass-pollen extract seems to be associated with a higher number and more severe SRs than birch-pollen extracts. These results were consistent in two different patient populations: 1) a small group, where each patient was treated with both birch- and grass-pollen SCIT, and 2) a larger population with different updosing schedules. The most obvious explanation for these results would be a difference in the potency of the extracts. Possible differences in potencies of the extracts are, however, difficult to investigate, as allergen manufacturers express potencies of their products in company specific units that do not allow product comparison.\(^{(132;135)}\) International references for allergen content in extracts are being produced and are needed for comparing efficacy and side-effects of individual allergens.\(^{(133;134)}\)

Our studies also indicated that there seems to be a different profile of side-effects related to each allergen. This could in part be due to an influence of patient disease, with more asthma patients receiving SIT with perennial allergens, and a higher frequency of asthma symptoms in patients with previous asthma in their history. But it does not explain the higher incidence of urticaria in grass-pollen patients, and the results need to be further investigated.

Aluminium has traditionally been added to aqueous extract to give a depot effect. With a slow release of allergen in theory the risk of side-effects could be reduced. The drawback of aluminium is the tendency to induce subcutaneous nodules, and results from our study showed a high proportion of subcutaneous nodules. However, only 50% of the patients felt inconvenience, mostly occasional, and the majority of nodules were transient; however, other adjuvants with no long-term accumulation might help to reduce local side-effects further. Our studies and other studies indicate that addition of adjuvant for slow release of allergen may increase the proportion of side-effects occurring late, even though the most severe side-effects seem to occur within 30 minutes regardless of aluminium content. In our study increasing the observation period to 1 h, would not have changed the proportion of side-effects occurring outside the clinic significantly, so the recent guideline for practical allergen-specific immunotherapy with an observation period of 30 min can be supported.\(^{(139)}\) Recent advances in the understanding of immunological mechanisms underlying immunotherapy has increased the interest in design of new adjuvants including virus-like particles, Th1-deviating agents such as bacterial cell wall lipopolysaccharide derivates (monophosphoryl lipid A) and bacterial DNA CpG-containing oligonucleotides.\(^{(27;155)}\) Comparative clinical trials are needed to conclude if these approaches improve efficacy and safety of allergen immunotherapy.

5. Predictive factors of side-effects (III, IV)

Investigation of possible risk factors and prevention of side-effects are needed to minimize the incidence of both severe and mild side-effects. It would be of great benefit if one could identify parameters predictive of systemic reactions before the patient started SCIT, to help identifying the patients where special precautions are needed. During treatment, preventive measures such as premedication with antihistamine could be an option.

Predictive factors for side-effects can be patient- or treatmentrelated. Patient-related factors most likely concern the severity of the allergic disease and the sensitivity/reactivity to the allergen in question. The severity of seasonal allergic rhinitis can be measured by symptom- and medication diaries during season. It seems relevant to include demographic data of the patient and diagnostic tests, which is not only IgE related, but also include markers of immunoinflammation. These factors must be easily obtainable within the daily routine to make it relevant for clinical decisions, such as simple diagnostic tests like SPT, specific IgE measurements, basophil histamine release test as well as CPT and NPT. Local allergic inflammatory reactions have been related to the severity of allergic symptoms upon allergen exposure\(^{(22)}\) but biopsies are costly, inconvenient to the patient and not possible to obtain in routine clinical practice. The local immunoinflammation has been reflected in the blood with regard to eosinophils which is easily measured in daily routine.

5.1 Aim of study

The aim of this part was to investigate possible risk factors for side-effects. In study III the aim was to identify parameters predictive of SRs, with focus on the diagnosis and severity of the allergic disease, described by seasonal symptoms and medication requirements; the outcome of SPT, CPT, and NPT; and results from blood tests such as total and specific IgE, HR, eosinophil numbers, serum ECP and EPX. The LLRs as predictor of side-effects was also discussed. In the large multicentre study IV the aim was to obtain a description of the side-effects with sufficient data to allow identification of possible risk factors, with focus on patient disease and allergen.
Table 5. Possible risk factors for having systemic side effects during dose increase phase (Binary logistic regression analysis) on patients reaching maintenance dose with or without side effects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance</th>
<th>OR</th>
<th>95% CI for OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female/male)</td>
<td>0.090</td>
<td>1.41</td>
<td>0.95 - 2.08</td>
</tr>
<tr>
<td>Indication (+/- asthma)</td>
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<td>0.80</td>
<td>0.52 - 1.22</td>
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<tr>
<td>Centre (reference is centre 4)</td>
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<td>0.81</td>
<td>0.46 - 1.40</td>
</tr>
<tr>
<td>centre 1</td>
<td>0.439</td>
<td>0.81</td>
<td>0.46 - 1.40</td>
</tr>
<tr>
<td>centre 2</td>
<td>0.387</td>
<td>1.45</td>
<td>0.62 - 3.37</td>
</tr>
<tr>
<td>centre 3</td>
<td>0.378</td>
<td>0.81</td>
<td>0.50 - 1.30</td>
</tr>
<tr>
<td>Allergen (reference is wasp)</td>
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<td>cat hair</td>
<td>0.029</td>
<td>2.78</td>
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</tr>
</tbody>
</table>

5.2 Outline of study

In study III, the clinical and paraclinical data were compared in patients with and without side-effects to SCIT, and the relationship between numbers of birch SCIT side-effects per patient and these data were investigated. The individual tests are described in chapter 2.

In study IV, the possible risk factors for having systemic side-effects during the up-dosing phase was investigated by binary logistic regression analysis in patients reaching the maintenance dose with or without side-effects. The parameters included were gender (female/male), indication (+/- asthma), allergen, and centre. The centres differed in the practical performance of SCIT in more than one possible important parameter (updosing regimen, antihistamine premedication, number of allergens, etc.).

5.3 Results and discussion

In study III, the results from pretreatment, birch- and grass-specific SPT, CPT; NPT; IgE and HR, as well as total IgE; EOS and serum ECP and EPX, were similar in the group with and without SRs to SCIT. Furthermore, these groups did not differ with respect to age, duration of allergic symptoms, and prevalence of asthma, or in seasonal means of birch/grass symptom score and antihistamine intake. No correlation was found between the number of SRs and the above mentioned parameters for either birch or grass, except that the number of eosinophils correlated to the number of side-effects to grass SCIT. In study IV, the binary logistic regression analysis in patients reaching the maintenance dose with or without side-effects showed that only the type of allergens and not parameters of gender, asthma or centre were predictive for side-effects during the updosing phase (Table 5).

Asthma

Earlier studies could indicate that asthma as such and the severity of asthma are a risk factor for SRs, which might be explained by a higher risk of asthma symptoms (See chapter 4). However later studies, including larger multicentre studies, have not been in total agreement with these results. In our study III, the frequency of concomitant asthma was not higher in the patients with side-effects compared to the patients without side-effects. Total number of patients, and number of patients with asthma was however low. A retrospective study of SCIT with different allergens showed that SRs were less pronounced among patients with rhinitis and hymenoptera venom allergy compared to exclusively asthmatic patients. The study included 3 different cluster up-dosing schedules with aqueous extract alone, aqueous followed by depot Alutard and Alutard alone, which might contribute to the difference, and no multivariate analysis was made. In a multicentre study considering SCIT with HDMs, pollen and hymenoptera there was no difference in number of patients with SRs in patients suffering from rhinitis or asthma. The frequency of side-effects in this study was, however, low and multivariate analysis was not performed. In a large scale study on SCIT with HDM, grass-pollen, olive and parietaria pollen - with no multivariate analysis and only few systemic reactions - there was a significantly higher frequency of SRs in asthmatics both in number of patients and number of injections. In another multicentre prospective study SCIT study there was a higher percentage of SRs among asthmatic compared with non-asthmatic patients. However, multivariate logistic analysis did not demonstrate this difference, and there was no difference when looking at the number of patients with SRs. Again the number of side-effects was low in this study. In a multicentre study where patients were treated with an allergen not related to their asthma (venom immunotherapy) pre-existing asthma did not influence the risk of side-effects.

The reason for the conflicting and mostly negative results could also be that the studies, according to guidelines, have excluded patients with severe asthma and only vaccinated the patients if the the asthma was well treated. So in general our results support that asthma is no risk factor, if the guideline for practical allergen-specific immunotherapy is followed.

Patient demographic data

Gender and age as well as duration of allergic symptoms have been considered as risk factors of side-effects. Our studies could not confirm this, and other studies have presented diverging but mostly negative results by
different methods. Gender: No correlation to gender, higher risk in females, higher risk in males (not significant after multivariate analysis). No difference in gender between groups with and without side-effects. Age: No correlation. No difference between groups with and without side-effects. Duration of allergic symptoms: No difference between groups with and without SRs.

Severity of allergic rhinitis
In our study III there was a higher frequency of side-effects when the same patients were treated with grass-pollen extract compared to birch-pollen extract. One could argue that the difference was caused by a difference in the severity of clinical birch- and grass-pollen allergic rhinitis. In fact, during the initial observation period, the patients had a higher seasonal mean symptom score in the grass-pollen season than in the birch-pollen season (medians 5.4 and 3.4; p < 0.001). However, differences in duration of pollen seasons and in pollen load might explain this finding. Furthermore, no difference in mean birch- and grass-seasonal symptom score, and use of medication in the pollen seasons was found between the groups with and without side-effects. This is in accordance with an earlier grass-pollen SCIT study. Also a mite study did not find a difference in symptom score between a group with a high SR rate per injection compared to a group with a low SR rate.

Skin prick test and provocation tests
In our study III the results from pretreatment birch- and grass-specific SPT (skin index), CPT and NPT were similar in the group with and the group without SRs to SCIT. Furthermore no correlation was found between the number of SRs and the above-mentioned parameters for either birch or grass. In an earlier grass-pollen SCIT study there was no correlation between skin thresholds and SRs, but most patients with major SRs were predicted by high nasal sensitivity. Another grass study did not find a difference in SPT between groups with and without systemic reactions. A mite study found that patients with a high systemic reaction rate per injection were characterized by a high skin sensitivity to Dp before SCIT. In contrast no difference was found in nasal or conjunctival sensitivity. Another mite study found that the skin test end point was higher in the group with severe SRs compared to the group with no SRs, whereas yet another mite study found no significant difference. Differences in patient selection, sample size or definitions of skin results may explain the conflicting results.

Specific IgE and basophil histamine release
We found no difference in pretreatment birch- or grass-specific IgE between groups with and without side-effects, and no correlation between the number of SRs and these parameters. The same negative results were observed with the basophil histamine release test. Similarly no correlation to specific IgE was found in a grass-pollen study, no difference in specific IgE was found between groups with and without side-effects in a mite study, and mite allergic patients with low and high SR rate had similar specific IgE and histamine release in another mite SCIT study.

Blood eosinophils, ECP and EPX
We found a correlation between the number of grass SCIT-induced SRs per patient and the concentration of eosinophils in the blood. This finding was not observed for birch IT and there was no difference in concentration of eosinophils in the groups with and without SRs, bringing into question the relevance. Furthermore, there was no difference or correlations concerning the eosinophil markers ECP and EPX.

All parameters were taken outside the pollen seasons, which might explain the negative results. No other studies have investigated the correlation between side-effects and eosinophils.

Local reactions
According to the EAACI Immunotherapy Position Paper guidelines from 1993 no increment in allergen dose is recommended if the immediate local reaction exceeds 5 cm or if the late local reaction is greater than 8 cm or inconvenient to the patient. In our study III we only took the late local reaction into consideration if it was inconvenient to the patient.

Our study III revealed that only 5% of the late local reactions > 8 cm resulted in a SR at the next visit, and only 10% of the SRs were preceded by a late local reaction > 8 cm, thereby questioning the limit of the late local reaction. Other studies have questioned the local reactions as predictor for systemic reactions. A study with aqueous extract showed that immediate (within 20 minutes) local reactions (present at any size) were few, with a low positive predictive and negative predictive value. This study did however adjust the next dose if the induration was > 1 cm. Another study found no correlation of immediate skin reactions (local reactions of 2 cm or greater) with SRs, and a late local reaction (greater than 10 cm) did not correlate to SRs at the next injection. The paper did not describe if a local reaction as such caused a modification of injection dosage. A recent study compared two large, age and gender matched groups with and without side-effects. There was a higher rate of early large local reactions (> 25 mm) in the group with side-effects, but still there were a lot of false positive local reactions in the group with side-effects. Another study investigated whether dose adjustment versus no adjustment for local reactions during SCIT influenced the occurrence of subsequent systemic reaction. Retrospectively the change to no local reaction adjustment policy did not increase the rate of local reactions immediately preceding a systemic reaction, and among those experiencing a systemic reaction, the rate of local reactions were unchanged. The positive predictive value of a local reaction for a
subsequent systemic reaction was again low.\textsuperscript{(159)} A later study again confirmed, that a change in protocol from adjustment to no adjustment did not change the rate of systemic reactions.\textsuperscript{(160)} As such the studies support the notion that individual early and late large local reactions cannot reliably predict systemic reactions to the subsequent dose of SCIT. On the other hand in the most recent study\textsuperscript{(158)} the frequency of early large local reactions was categorized by either low or high frequency, and the result showed significant and increasing OR with increasing percentage of large local reactions for the individual patient. This indicates that patients with higher rates of large local reactions are at increased risk of experiencing systemic reactions.

**Allergens and schedules**

Our study IV revealed that type of allergen was predictive of side-effects, and this knowledge could be used when f.ex. up-dosing schedule and top dose are considered. The influence of up-dosing schedules and top dose on side-effects, have not been investigated in this thesis. Cluster and rush schedules have been developed to achieve a maintenance dose more rapidly than with conventional schedules, thereby reaching a therapeutic maintenance dose more rapidly. The advantage of fast up-dosing schedules might, however, be at cost of increased risk of side-effects. The maintenance dose of SCIT is often predefined by the manufacturer which in turn has defined the top dose from performed efficacy studies that mostly do not involve dose-response curves. Earlier studies on SCIT with especially hymenoptera venom have indicated that rush schedules are associated with an increased risk of side-effects.\textsuperscript{(142;144;161)} Comparative studies with inhalant allergens comparing different schedules are few. Two mite studies showed no difference between a cluster and a conventional schedule, whereas a ragweed study showed a significantly higher frequency of side-effects per injections with a modified rush schedule compared to conventional schedule.\textsuperscript{(150;162;163)} Only a few studies have compared the relevance of top dose for side-effects, but indicate a higher risk with a high maintenance dose compared to a low dose.\textsuperscript{(28;119)}

### 5.4 Conclusion

It would be a great benefit to be able to identify patient-related factors that could predict side-effects in SCIT. Studies on risk factors of side-effects with SCIT are few and data have not been conclusive, which might reflect the broad spectrum of possible risk factors. We have been investigating the possible risk of side-effects including a large number of relevant demographic data, diagnostic tests of allergy as well as inflammatory markers, but with negative results. Only the type of allergen seemed predictive of side-effects and might result in modification of the SCIT procedure. Larger studies are needed for evaluation of other possible risk factors.

### 6. Antihistamine treatment (V,VI)

Since 1988 it has been discussed whether to use H1-antagonists/antihistamine as a preventive measure for side-effects to allergen specific immunotherapy.\textsuperscript{(164;165)} In a prospective rush protocol with HDM, premedication with methylprednisolone, ketotifen and a long lasting theophylline reduced the incidence of patients with systemic reactions, defined as asthma, urticaria and anaphylaxis.\textsuperscript{(122)} The same premedication protocol was used in a later prospective study with pollen extracts.\textsuperscript{(123)} The percentage of patients with anaphylaxis decreased, and the need for adrenaline was similarly reduced. These studies were, however, neither blinded nor controlled. In 1992 the first placebo-controlled, double-blind study of pretreatment with antihistamine was performed. Fifty-two patients allergic to honeybee venom were treated by a 4-day rush immunotherapy protocol.\textsuperscript{(166)} Although no overall difference was observed in systemic subjective or objective side-effects between groups, the terfenadine group developed itching, and urticaria/angioedema less frequently. The results were encouraging but a major concern was that the premedication might mask a minor systemic reaction that with a future non-reduced injection dose would give rise to a more serious reaction. By using premedication with antihistamine one could also speculate if a delay in the onset of systemic reaction would occur, with a possible serious reaction developing after the observation period in the clinic has ended. The above uncontrolled studies performed with aqueous extract showed that all serious reactions occurred within 30 minutes, with no delay when using premedication, whereas the above DBPC study did not present data of onset time.

Antihistamines have been proven to be effective in the treatment of allergic rhinitis as well as urticaria. Onset of action of antihistamines is a clinically relevant parameter in the treatment of allergic rhinitis and urticaria. Onset of action is also an important parameter if pretreatment with antihistamines is considered in SCIT. Onset of action of antihistamines has mostly been evaluated by studies of wheal and flare reactions to skin prick tests with histamine.\textsuperscript{(167-169)} There might be a discrepancy between the effect on histamine-induced wheal and flare reactions and clinical onset of action, but clinical studies on onset of action are few. A relevant method for evaluating the clinical onset of action in allergic rhinitis would be to estimate the time to a reduction in allergic rhinitis symptoms caused by challenge with pollen in exposure chambers\textsuperscript{(170)} or by exposure to pollen in nature.\textsuperscript{(171-173)} Results might, however, differ with pollen load\textsuperscript{(174)} and methods of evaluation of clinical efficacy.

#### 6.1 Aim of study

The aims of this part were:

1) To investigate the clinical onset of action of an antihistamine in a 1 day field study and discuss the influence of different methods of evaluation on results (V).
2) To elucidate whether antihistamine pretreatment in allergic rhinitis patients would influence the frequency and severity of side-effects to SCIT. Furthermore, we wanted to investigate a possible time lag from injections to SRs and on local reactions (VI).

6.2 Outline of study

Study V was a placebo-controlled, randomized, and double-blind 1-day field study, investigating the efficacy and onset of action of capsule acrivastine 8 mg in 42 patients suffering from allergic rhinoconjunctivitis elicited by natural grass-pollen exposure. Upon arrival at the hayfield, symptom scores were registered every 10 min (sneezing, rhinorrhea, nasal congestion, itchy nose or throat, and itchy eyes; scores of 0-5). Number of sneezes were registered every 10 min. When the patient reached a total symptom score of 10 points (40% of maximum total score), the first capsule was administered and the time recorded. This was done to ensure sufficient and even amount of symptoms. The patients were instructed to report when they felt relief of symptoms, and patients recording more than 50% reduction of symptoms were allowed to leave the field, as this reduction was considered clinically relevant. If less than 50% relief or no relief was observed after 1 hour, another capsule was administered. Symptoms were assessed for 2 hours after capsule administration. Unblinded acrivastine 8 mg was given if the patient felt no relief 2 h after administration of the first capsule. The onset time was evaluated by different criterias. We estimated the onset time by an exponential decay model with estimation of onset defined as the timelag after dosing of acrivastine until reduction from placebo commenced. Survival of time to 50% reduction in scores (half-lives) was analysed. The time point to a significant difference in symptom score between the antihistamine- and placebo-group was evaluated. The median time for a 50% reduction in total symptom score, and the percentage of patients with a 50% reduction in symptom score at each time point were evaluated as well. The numbers of sneezes in 30-min periods were compared. Study VI included 45 birch or grass-pollen allergic rhinitis patients. The patients were randomized to active treatment or placebo tablets by the method of minimization. In a double-blind treatment schedule, loratadine or identical placebo tablets were administered 2 hours before the first injection at each visit during the up-dosing phase. The patients received weekly cluster immunotherapy (7 weeks, 14 injections) with either Betula verrucosa or Phleum pratense pollen extracts. The practical performance of immunotherapy followed the EAACI Immunotherapy Position Paper guidelines. Before each injection the physician evaluated the patient’s condition including peak expiratory flow (PEF), and assessed the patient’s suitability for immunotherapy. The patients received injections at 30-minute intervals and stayed in the clinic for at least 30 minutes after the last injection. Dose modifications were made if SRs occurred, with either no increment, or reduction of the dose up to 4 steps. Patients who experienced severe SRs, repeated allergic rhinitis, or inconvenient large LRs during cluster immunotherapy were transferred to the department’s conventional (one injection a week) schedule. If SRs persistently occurred despite these preventive measures, the patients were excluded from the study. Thirty minutes after each allergen injection, the swelling at the injection site (early LR) was measured (mean of the longest diameter and the midpoint orthogonal diameter). Measurement of PEF was repeated, and any signs or symptoms of SR were recorded by the investigators. After each injection series, the patients filled in a diary for the next 2 days. Swelling at the site of injection (late LR) was measured at 2 to 4, 4 to 6, 6 to 8, 10 to 14, 24, and 48 hours after injection. PEF was measured at the same intervals. Patients registered the time of onset of symptoms such as rhinitis, asthma, urticaria, angioedema, or any other symptoms. Drug consumption to alleviate symptoms (loratadine and/or β2-agonist) was recorded. SRs were classified as early (i.e., those occurring within 30 minutes) and late (those occurring 31 minutes to 48 hours after the last injection). SRs were graded from 2 to 4, as previously described. **Figure 9.** Nonlinear regression of median relative TSS after first dosing. Average difference between time of first dosing and time of scoring immediately before dosing was 4 min. Symptoms were scored every 10 min, and average scoring times from dosing were therefore -4, 6, 16, ... 116 min. Circle = acrivastine, Triangle = placebo. *P < 0.05; ** P < 0.01; *** P < 0.001.
6.3 Results and discussion

6.3.1 Onset of action of antihistamine (V)

With this 1-day field study it was possible to expose the patients to the same amount of pollen, and it showed that exposure time before significant symptoms varied. The local pollen exposure measured 1.5 meter above ground level was 11 to 26 times higher than the official Danish pollen counts measured 15 meter above ground level.\(^{(176;177)}\) The pollen counts were constant for the first 1.5 hours and then increased, presumably due to activity in the field. The base-line median total symptom scores were not different between the two groups. Twenty six patients received their first capsule within 30 minutes, and only four patients did not receive the first capsule within 60 min, suggesting a sufficient exposure to grass-pollen in view of patient sensitivity.

The onset time was evaluated by different criterias. The course of median total symptom score relative to baseline was modelled with an exponential decay process with half-lives of 79 and 911 min after antihistamine and placebo, respectively. Time to onset of action was estimated to be 19 minutes (95% confidence interval 0-39 min), calculated as the time lag after dosing of acrivastine until reduction from placebo level commenced, Figure 9. When looking at total symptom scores at different time points a statistically significantly different reduction in median total symptom score relative to baseline was seen for the first time between antihistamine and placebo at 46 minutes after initial dosing. The reduction at that time point was 22% in the antihistamine group and 0% in the placebo group. If looking at a 50% reduction in symptom score which might be more clinically relevant, the onset time increased. The median time to 50% reduction was 80 min (95% CI 57-112) in the antihistamine group and > 120 minutes in the placebo group (\(p = 0.0032\)). At 80 minutes there was also a statistically significant difference in the number of patients experiencing a 50% reduction of total symptom score for the first time (\(p < 0.05\)). Symptom scores are subjective, whereas sneezes are an objective parameter describing a part of the symptomatology of allergic rhinitis. The mean number of sneezes in periods of 30 minutes was calculated. Reductions in sneezes could not be calculated as few patients had a pretreatment period exceeding 30 min. The difference between the antihistamine group and the placebo group became statistically significant from 61 min onwards (\(p < 0.05\)), whereas the individual symptom score of sneezes was significant from 31 minutes and onwards (\(p < 0.01\)). The scores and numbers were closely related.

This study highlights that different onset times are found, depending of the methods used in the evaluation. The definition of a clinically relevant reduction in symptoms is debatable. In our study the course of symptom score relative to baseline was modelled with the exponential decay process which gave an overall good impression of onset of action. The time to onset of action calculated as the time lag after dosing of antihistamine until reduction from placebo level commenced, however, although informative, might not be clinically relevant to the patient. In an earlier field study comparing three different antihistamines including loratadine, onset of relief was determined as the time to 33% reduction of symptom score of any rhinitis symptom. Mean time to relief of any symptom was 20 min for all three groups, whereas a 33% reduction in all common symptoms occurred after 4-6 hours.\(^{(178)}\) The study was not placebo-controlled. In our study, the median time to 25% reduction in any symptom was 9 minutes with antihistamine and 20 minutes with placebo, and the difference was not significant. Furthermore, 71% in the placebo group and 91% in the antihistamine group had a 25% reduction in total symptom score, bringing into question if a 33% reduction in symptoms is sufficient for evaluation of efficacy. In a review of double-blind, placebo-controlled studies on onset of action for the relief of allergic rhinitis symptoms with second generation antihistamines including loratadine, the results again depended on definition, with onset for loratadine ranging from 1 hour and 42 minutes to 3 hours.\(^{(178)}\) Based on the data above an interval of 2 hours from loratadine intake to injection of allergen extract was chosen for the premedication SCIT study to be presented below.

**Figure 10.** Median diameter of LRs after immunotherapy at different time intervals. *Open triangles:* Placebo. *Filled Triangles:* Loratadine. Parameters were assessed by the exact two-sample Wilcoxon rank-sum test (Mann-Whitney). \(*p < 0.05, ***p < 0.001.\)

6.3.2 Antihistamine premedication (VI)

The study showed a significant difference in the number of patients with SRs, seven (33.3%) and 19 (79.2%) patients in the loratadine and placebo groups, respectively (\(p = 0.002\)) (Table 6). No other double-blind placebo-controlled study using antihistamine alone as premedication has been performed with pollen...
immunotherapy. In a study of 22 patients\textsuperscript{149} receiving rush SCIT with non-standardized individual mixtures of allergen extracts (which could include both pollens, moulds and HDM), premedication with a combination of H\textsubscript{1}- and H\textsubscript{2}-antagonists and corticosteroid also reduced the number of patients with SRs significantly. However, the study was small, and as multiple allergens could be used, individual allergens could be present at different concentrations in different extracts. One later study with bee venom SCIT and antihistamine premedication and one study with hymenoptera venom SCIT and premedication with a combination of H\textsubscript{1}- and H\textsubscript{2}-antagonists could not confirm these results.\textsuperscript{179,180} A more recent DBPC study with premedication (H\textsubscript{1} and H\textsubscript{2} antagonists and corticosteroid) in rush immunotherapy with imported fire ant had too few SRs to draw any conclusion.\textsuperscript{181}

The number of SRs per injection in our study was 5.8% in the loratadine group and 9.5% in the placebo group, but the difference did not reach statistical significance ($p = 0.072$). No DBPC study with antihistamine premedication published so far has presented data of SRs per injection.

No life-threatening (grade 4) SRs were observed in our study neither in the loratadine group nor in the placebo group (Table 6). SRs were more severe in the placebo group than in the loratadine group, with 28.2% versus 5.9% of the SRs graded as 3 ($p = 0.037$). This was due to the higher incidence of urticaria in the placebo group: The grade 3 SRs involved 10 episodes of urticaria and 1 episode of asthma in the placebo group versus 1 episode of asthma in the loratadine group. Moreover, the difference in number of patients with urticaria (25.0% in placebo group vs. 4.8% in loratadine group) did not reach statistical significance ($p = 0.062$). Two other studies also reported fewer cutaneous reactions (itching, urticaria, angioedema) as well.\textsuperscript{116,180}

Neither our study nor trials published so far has shown any indication of an increased risk of severe systemic allergic reactions with antihistamine pretreatment as a result of “masking” of warning from previous milder allergic symptoms.

In our study pretreatment with antihistamine did not delay the onset of SRs, with late reactions in 72% vs. 67% in the loratadine and placebo groups, respectively ($p = 0.214$) (Table 6). Even though the number was high in both groups, the reactions were mostly mild with only 3 reactions necessitating contacts to medical doctors (all in the placebo group). The time interval from last injection to onset of urticaria symptoms was 30 minutes for the one patient in the loratadine group, whereas in the placebo group the median time was 2 hours after the last injection (range 5 min - 9 h). Median interval to onset of asthma symptoms was 6.5 hrs (range 20 min – 24 hrs) in the loratadine group and 1.6 hrs (range 5 min – 24 hrs) in the placebo group, with no significant difference. The onset time of SRs is only presented in one other DBPC study\textsuperscript{179} where all but one patient with objective systemic adverse reactions reacted within 30 minutes after injection; this patient experienced urticaria after 2 hours. Our study was performed with depot extract in contrast to the other studies using aqueous extracts, which could explain the higher proportion of SRs occurring late.

### Table 6. Number and distribution of injections and systemic reactions.

<table>
<thead>
<tr>
<th>Loratadine group</th>
<th>Placebo group</th>
<th>$P$ Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>U+A+RC (no.)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>U+A (no.)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>U+RC (no.)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>U (single symptom) (no.)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>U (total)</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>A+RC (no.)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>A (single symptom) (no.)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Asthma (total)</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>RC (single symptom) (no.)</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>RC (total)</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Itching without U (no.)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>SR (total)</td>
<td>18</td>
<td>39</td>
</tr>
<tr>
<td>Early SR (no.)</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Late SR (no.)</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Grading of SR (2/3/4) (no.)</td>
<td>(17/1/0)</td>
<td>(28/11/0)</td>
</tr>
<tr>
<td>Patients with SR (%)</td>
<td>33.3</td>
<td>79.2</td>
</tr>
<tr>
<td>Patients with RC (single symptom) (%)</td>
<td>23.8</td>
<td>33.3</td>
</tr>
<tr>
<td>Patients with asthma (%)</td>
<td>4.8</td>
<td>25.0</td>
</tr>
<tr>
<td>Patients with urticaria (%)</td>
<td>4.8</td>
<td>25.0</td>
</tr>
<tr>
<td>Total injections (no.)</td>
<td>309</td>
<td>411</td>
</tr>
<tr>
<td>SR per injection (%)</td>
<td>5.8</td>
<td>9.5</td>
</tr>
</tbody>
</table>

U, urticaria; A, asthma; RC, rhinoconjunctivitis.* Chi square test.

The LRs in our study were significantly smaller in the loratadine group between 30 minutes and 24 hours after the injection (Figure 10). Similar results were seen in the above mentioned study.\textsuperscript{166} The early local reactions seemed greater than in our study presumably due to an aqueous extract and the allergen used (hymenoptera). The reduction in size of LRs was small in our study, and the clinical relevance doubtful. No patients had an early LR greater than 5 cm. There was no difference in the number of late LRs greater than 8 cm between the loratadine and placebo groups, with late LRs greater than 8 cm in 6.5% and 9.5% of injections, respectively ($p = 0.144$). A later study\textsuperscript{180} with bee venom and aqueous extract recorded LRs 30 min to 3 hours after the last injection and observed more patients with extended local reactions greater than 6 cm in diameter in the placebo group than in the group treated with fexofenadine. We did not ask about subjective symptoms, but another study graded the immediate local symptoms (after 30 min) of erythema, swelling and
pruritus from 0-3. There were significantly higher symptom scores in the placebo group for all 3 parameters.\(^{179}\) Not only the intensity but also the duration of symptoms is relevant to the patient. In our study the local reactions seemed to have disappeared within 48 hours for both the actively treated group and the placebo group, whereas in another study there was a tendency towards a higher number of patients with a longer duration of local reactions in the placebo group.\(^{180}\)

6.4 Conclusions

The information on clinical onset of action of antihistamines is relevant when deciding which antihistamine to use for symptoms already present, such as treatment of side-effects to SCIT, and also has an impact if pretreatment with antihistamines in SCIT is considered. The results of study V showed as expected that the estimation of clinical onset of action of antihistamines depends on the method chosen. In our study the course of symptom score relative to baseline was modelled with an exponential decay process which gave an overall good impression of onset of action. The half-life or time to a 50% reduction in symptoms score may prove clinically relevant. The time to onset of action, calculated as the time lag after dosing of antihistamine until reduction from placebo level commenced, however, although informative, might not be clinically relevant to the patient.

Study VI shows that premedication with antihistamine may reduce the incidence and severity of side-effects, by reducing local reactions and systemic reactions of the urticaria/angioedema type. There is no evidence of masking the early warning signs and of delaying the onset of systemic reactions, which have been an argument against the pre-treatment. The study showed a reduction in the size of LR when treated with antihistamine, but the reductions were small, with limited clinical relevance.

7. Conclusions and perspectives

Pollen allergic rhinitis is a common disease. Most studies regarding pollen allergic rhinitis, including investigations on SCIT, focus on a single pollen allergen, despite the fact that many patients are polysensitized and thus suffer from allergy to more than one pollen. In the Northern European countries dual allergy to birch- and grass-pollen is frequent and the pollen seasons are very close to each other. We have presented the first prospective study of pollen allergic patients with dual sensitivity and symptoms in birch- AND grass-pollen seasons. The results of our study could indicate that allergy to birch-pollen could enhance the allergic rhinitis symptoms in the grass-pollen season, possibly due to a priming inflammatory effect. In theory immunotherapy with birch-pollen extract could, reduce priming with a subsequent less severe grass-pollen season in patients with dual sensitivity. Further studies are needed to study the possible priming during natural pollen exposure, as well as reduction of priming during immunotherapy. A four armed study (placebo, birch SCIT, grass SCIT, birch and grass SCIT) is proposed.

The methods for assessment of efficacy of allergen specific immunotherapy can be discussed according to design of the study, primary outcomes and statistical methods. As an alternative to a one year placebo-controlled study, we presented a study including a baseline year with the possibility to use the patient as his own control. The importance of the variability of pollen exposure for efficacy assessment in studies including more than one year was highlighted in our study. A statistical longitudinal model for the evaluation of the efficacy of pollen immunotherapy, which takes into consideration the differences of pollen counts, has been presented and proven useful. The model demonstrated a beneficial role of both birch- and grass-pollen immunotherapy. The ability to demonstrate clinical efficacy of grass-pollen immunotherapy appeared to be dependent upon the magnitude of pollen counts. However, the model should be used in further longitudinal studies, lasting several years, with other seasonal pollen courses, to confirm its effectiveness.

Symptom and medication scores are considered primary outcome measures for efficacy. Surrogate markers of allergen specific reactivity could potentially be used as parameters of clinical efficacy, but although our study found a correlation between different markers and symptom and medication scores of allergic rhinitis, the association was not impressive and in vitro markers can not replace the primary clinical outcomes.

Immunotherapy is time consuming and not without risk. Systematic attempts have been made to make the treatment as rational as possible, balancing time consumption and patient inconvenience and the risk of inducing systemic reactions. Major issues in this aspect are to identify risk factors and to improve safety. There is a broad spectrum of possible risk factors of side-effects including patient related parameters as well as treatment related factors. Potential risk factors have mostly been proposed from retrospective reviews on fatal or nearly fatal reactions to the treatment, and from efficacy studies reporting side-effects. Few studies have been designed to investigate side-effects and risk factors for side-effects. We have compared the side-effects of birch- and grass-pollen allergen extracts in a design where the same patients were treated with both allergens. The conclusion was that the grass-pollen extract was associated with a higher number and more severe SRs than birch-pollen extract. This study was small, but we confirmed the result in a large multi centre safety study. This study also concluded that allergens and not the different practical performance of SCIT seemed predictive of side-effects. When designing future studies on side-effects and risk factors for side-effects the difference in tendency to produce side-effects between allergens should be taken into consideration. Clinical and
paraclinical factors that, to some extent, describe the severity of allergic rhinitis could be associated to side-effects. Despite including several potential parameters in our studies we were not able to identify clinical or paraclinical factors as a risk factors for side-effects. Further large prospective studies are needed for evaluation of possible risk factors.

It can be debated when the frequency of side-effects with subcutaneous immunotherapy is too high, but novel approaches are needed in order to reduce side-effects. For some years antihistamine pretreatment has been suggested as an approach to reduce side-effects, but no consensus has been reached due to insufficient data. We performed the first placebo-controlled study investigating antihistamine pretreatment in pollen immunotherapy in a double-blinded fashion. The results were promising, as it seems to reduce both the number and severity of systemic reactions. Moreover there was no evidence of masking the early warning signs and of delaying the onset of systemic reactions, which have been arguments against antihistamine pre-treatment.

8. Summary
Pollen allergic rhinoconjunctivitis is a common disease and in the Northern European countries many pollen allergic patients are sensitized to more than one pollen type, typically birch and grass. However, previous studies on pollen allergy, and allergen specific immunotherapy efficacy studies, have focused on individual allergens. We have prospectively followed a patient population, who suffered from both birch- and grass-pollen allergic rhinoconjunctivitis. Symptom score and use of medication were registered over a time interval including the birch- and grass-pollen season. Results indicated that priming occurs during the birch-pollen season, resulting in a relatively higher symptom score in the grass-pollen season, when related to exposure. Thus 1-year placebo-controlled grass-pollen immunotherapy efficacy studies could be biased, if the study population includes an uneven distribution of patients with allergy to both birch- and grass-pollen.

Our study is the first study investigating the efficacy of subcutaneous immunotherapy (SCIT) in patients sensitized to both birch- and grass-pollen. We chose a design including a baseline year without immunotherapy followed by double-blind SCIT with either birch- or grass-pollen extract. This design made it possible to include patients according to the severity of rhinoconjunctivitis as described in symptom score diaries. The study showed that symptom score and use of medication are dependent on pollen exposure. A mathematical model for the evaluation of efficacy of pollen SCIT, which takes into consideration the differences in pollen counts, was introduced. The model demonstrated a beneficial role of both birch- and grass-pollen SCIT, and that the efficacy was dependent on pollen exposure.

The assessment of efficacy is most often based on changes in symptom- and medication scores. The collection of these data requires a lot of resources, and paraclinical data could be an alternative, if results are closely correlated to the clinical outcome. Serum specific IgE, basophil histamine release and eosinophil parameters are associated with allergic rhinitis, but studies investigating the relationship to symptom scores are few and present diverging results. Our study showed, that the severity of allergic rhinitis was only partly explained by specific IgE levels, basophil and eosinophil function, and that these parameters can not replace symptom score in the assessment of efficacy of SCIT.

Immunotherapy is an efficient treatment of allergic rhinitis, but it carries a significant risk of side effects. The ability to identify high-risk patients before SCIT is initiated, would be a step forward. Risk factors can depend on the practical performance of the treatment, and on patient related factors, such as severity of allergic rhinitis and the allergic sensibility and reactivity. We investigated possible risk factors for side effects of SCIT in the population of birch- and grasspollen allergic rhinitis patients, as well as in a large Danish multicentre study of SCIT. Neither severity of allergic rhinitis measured by symptom- and medication- scores, demographic data such as age, duration of allergic disease and asthma status, diagnostic tests for allergy such as specific IgE, histamine release, skin prick test, conjunctival- and nasal provocation test, nor markers of inflammation such as number and function of eosinophils (eosinophil cationic protein and eosinophil protein X) could predict side effects. The investigations showed a difference in number and profile of side effects depending on allergen extract and SCIT with grass pollen extract carried a higher risk of side effects, of greater severity, when compared to SCIT with birch extract.

It has been debated, whether antihistamine premedication could reduce side effects of immunotherapy while at the same time masking the early warning signs of severe reactions. It has also been considered, if pretreatment with antihistamine merely delayed the onset of systemic reactions, with a possible debut after the completion of the observation period. We performed the first double-blind placebo-controlled antihistamine pretreatment study in pollen immunotherapy. The results indicated that pretreatment with antihistamine reduced both the number and severity of side effects, mainly due to a reduction in urticaria. There was no indication that antihistamine pretreatment masked a mild reaction, which with a future unreduced dose, would result in a more serious reaction, nor that pretreatment with antihistamine delayed the onset of side effects.
9. Summary in Danish

Pollen allergisk rhinoconjunctivitis er en hyppig sygdom, og i de nordiske lande er mange pollenallergikere sensibiliseret overfor flere pollentyper, typisk birk og græs. På trods af dette har tidligere undersøgelser af pollenallergi og effektstudier af allergen specifik immunterapi kun fokuseret på enkelt allergener. Vi har prospektivt fulgt en patient population, som havde både birke- og græspollen udløst allergisk rhinoconjunctivitis. Symptom- og medicinscore udført i en periode, som inkluderede både birke- og græspollensæsonen, tydede på, at der sker en priming i birkepollen sæsonen, således at dobbeltsensibiliserede (birk og græs) får en forholdsmæssig højere score i græssæsonen i forhold til eksponeringen. Dette kan have betydning for forklaring af resultater for 1 års placebo kontrollerede immunterapi studier med græs, hvor populationen indeholder en ujævnhed af dobbeltbensibiliserede.


Vurdering af effekt af immunterapi er typisk baseret på ændringer i symptom- og medicinscore. Disse målinger er meget ressourcerækvende, hvorfor parakliniske data kunne være et alternativ, hvis disse er tæt korrelerede til de kliniske scores. Serum specifik IgE, basofil histamin release og eosinofil parametre er associeret til allergisk rhinitis, men undersøgelse af deres relation til symptomsværhedsgrad er få og viser divergerende resultater. Vores undersøgelse viste, at sværhedsgraden af allergisk rhinitis kun er delvis forklaret af specifik IgE niveauer, basophil og eosinophil funktion, og de kan ikke erstatte symptom score, når effekten af immunterapi skal vurderes.


Det er dog væsentligt at diskutere om antihistamin præmedicinerne kunne reducere bivirkningsfrekvensen ved immunterapi, men at det samtidig var årsag til sværere bivirkninger på grund af manglende varsel. Det har også været overvejet, om antihistaminbehandlingen blot forsinkede bivirkningerne, med mulighed for debut af symptomer efter observationsperioden. Vi udførte det første dobbeltblindede placebo-kontrollerede antihistamin studie med pollen immunterapi. Resultaterne viste, at antihistamin præmedicinerne reducerede antallet af bivirkninger, samt at det reducerede sværhedsgraden af bivirkninger, overvejende pga. reduktion i antallet af urticariatifælde. Der var ingen tegn på, at præmedicinerne med antihistamin maskerede de tidlige advarsler om sværere reaktion, eller at det forsinkede debut af bivirkninger.
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