SUBLINGUAL IMMUNOTHERAPY IN CHILDREN WITH ALLERGIC RHINITIS

Esther Röder

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SUBLINGUAL IMMUNOTHERAPY IN CHILDREN WITH ALLERGIC RHINITIS

Sublinguale immunotherapie bij kinderen met allergische rhinitis

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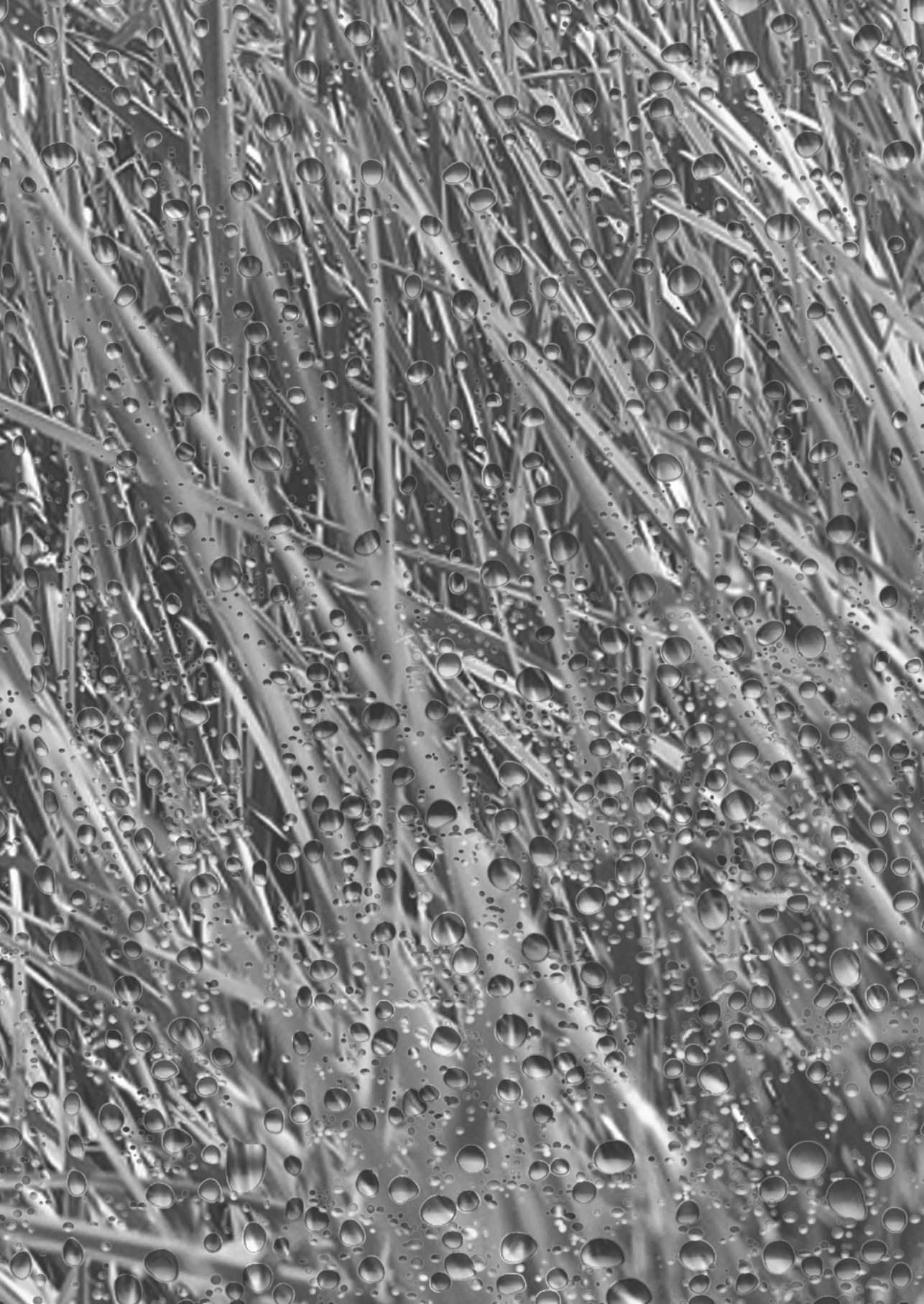

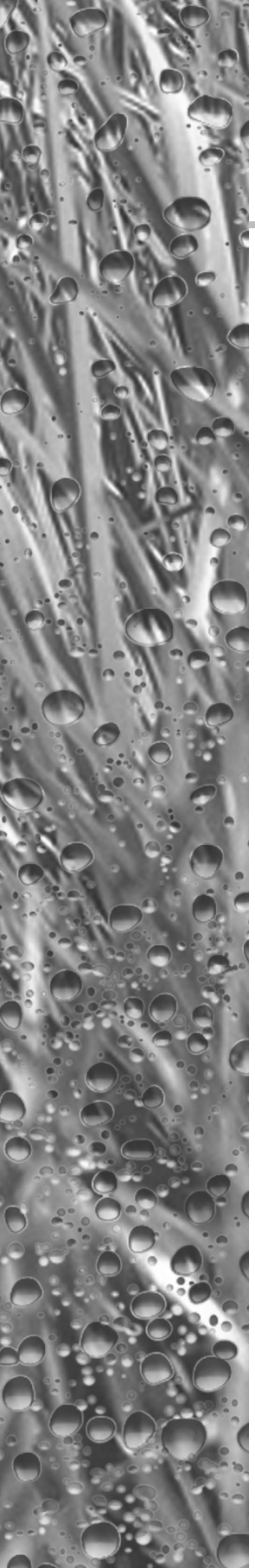

Chapter 1

General introduction

Introduction

ALLERGIC RHINITIS

Prevalence and impact

Allergic rhinitis is one of the most prevalent chronic diseases and like all IgE mediated diseases its prevalence has increased during the last decades, affecting around 20% of children[1] and adults[2] in Europe. In The Netherlands, an incidence rate of approximately 9 per 1000 patient-years in general practices has been reported for children as well as adults.[3] Most patients develop symptoms of allergic rhinitis before the age of 20.[4]

The typical clinical symptoms of allergic rhinitis are an itchy runny nose, sneezing and nasal congestion.[5] These symptoms are the result of an excessive inflammatory reaction of the immune system to normally harmless allergens, like grass pollen, house dust mite and animal dander. In spite of the high prevalence, allergic rhinitis is often considered a trivial disease. During the last two decades awareness of the impact of allergic rhinitis is increasing. Besides the fact that a substantial proportion of patients have conjunctivitis and asthma, it has also been acknowledged that patients suffer from general complaints like fatigue, sleeping problems and difficulty concentrating.[5] Allergic rhinitis can significantly influence the patient's quality of life due to its impact on daily activities, school- and work performance.[5] According to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines, allergic rhinitis is classified by the duration (persistent or intermittent) and severity (mild or moderate/severe) of the symptoms. In line with the focus on the impact of allergic rhinitis, the level of severity is determined by assessing the consequence of the disease on daily life.[5]

The annual costs of allergic rhinitis vary between European countries[6,7], but can be as high as € 4000 per patient per year. The economic burden in The Netherlands is unknown. Costs can be divided into direct and indirect costs. The most important direct costs include prescribed medications and physician consultations. Examples of indirect costs are absence from work or school and reduced productivity. Besides costs directly related to allergic rhinitis there are also costs related to the conditions associated with allergic rhinitis, for instance healthcare costs and productivity losses due to asthma.

Although not associated with severe morbidity and mortality, due to the high prevalence, the impact on daily life and the related costs, allergic rhinitis can be considered a significant public health concern.

Diagnosis

The patient's perception of their allergic status (presence/absence of allergy) and the allergic trigger is often inaccurate. A structured allergy history alone is also insufficient

to correctly diagnose allergy.[8] Diagnostic testing is therefore necessary, especially when treatment is directed at a specific allergen. For instance, in case of house dust mite allergy when indoor environmental control measures are indicated or when treatment with specific allergen immunotherapy is considered. Sensitisation to aeroallergens can be assessed by a skin test or measuring specific IgE antibodies in blood. The diagnostic performance of these two tests in inhalant allergy is equal, provided that they are performed by trained personnel and standardised extracts are used.[9] Assessment of a sensitisation should always be combined with a structured allergy history to determine the clinical relevance of the allergic trigger.

Treatment

The cornerstones of allergic rhinitis treatment are patient education, allergen avoidance, symptomatic drug therapy and specific immunotherapy. The management of allergic rhinitis in children resembles that in adults.

Education

Educating the patient is important, because the patient's knowledge and expectations influences the success of treatment in chronic conditions like allergic diseases.[10] Patients should be informed about the cause and course of allergic rhinitis, know how to avoid contact with the allergen and understand the methods, instructions, goals and risks of a treatment. This information should be repeated regularly, because patients immediately forget approximately half of the information and of what is remembered, about half is incorrect.[11] Education will optimise the effect of treatment, lower the risk of adverse effects and improve compliance.

Avoidance

Avoiding the allergen completely is effective, but not always feasible. For instance, patients with hay fever, who are allergic to the grass and/or tree pollen present in the air, will not be able to completely avoid contact in daily life. Patients with a house dust mite allergy can reduce the concentration of the allergen in their homes by removing carpets and using special bedding covers. However, it is still unclear how much allergen exposure needs to be reduced to have a beneficial effect. Although these measures are effective in asthmatic children sensitised to house dust mite, so far there is no evidence that effective allergen avoidance in rhinitis can be achieved.[12] Because allergic reactions often are attended with non-specific hyperreactivity, it is also important to reduce the exposure to irritative substances like tobacco smoke.

Symptomatic treatment

Symptomatic treatment focuses on blocking the release or the actions of the chemicals involved in the inflammatory reaction, such as histamine. The most frequently used symptomatic medications include antihistamine tablets, intranasal steroids and topical antihistamines and cromoglycates. Intranasal steroids are the most effective treatment of allergic rhinitis in adults. They reduce symptoms and the use of other drugs and improve overall daily functioning. Intranasal steroids are more effective than antihistamine tablets and the new generation is not associated with significant systemic side effects in children. Antihistamine tablets (second generation) are effective and safe in children. They reduce malaise and may improve learning performance. Topical antihistamines have the advantage of a rapid onset of action, but only reduce nasal symptoms. Topical cromoglycates have the best safety profile, but are less effective and the frequent administration can hamper adherence.[13]

The disadvantage of symptomatic treatment is that it doesn't always sufficiently control the symptoms and some patients are reluctant to use them for longer periods. Furthermore, the ideal treatment not only ameliorates symptoms, but also influences the natural course of the disease. Allergen avoidance and symptomatic treatment will only result in short-term relief. Allergen-specific immunotherapy on the other hand targets the cause of the disease and can induce long-term remission of the allergic symptoms.[14,15]

Immunotherapy

Allergen-specific immunotherapy, also called desensitisation or hyposensitisation, is the administration of gradually increasing amounts of the allergen to which the patient is allergic. The idea behind this treatment is that due to the induction of immunological tolerance the patient will become less sensitive to the allergen.

Subcutaneous immunotherapy

The first scientific publications on allergen-specific immunotherapy for inhalant allergens in humans appeared in the Lancet a century ago. In 1911 Noon, and later his successor Freeman, published the results of their experiment where they injected a grass pollen derived allergen extract under the skin of patients with hay fever. They found that their "prophylactic inoculation" (later called "subcutaneous immunotherapy") resulted in a decrease of the immediate conjunctival reaction to the extract and amelioration of symptoms.[16,17] In the following decades immunotherapy was extended to more allergens and the design and quality of the studies evolved. The first double-blind placebo-controlled trial with subcutaneous immunotherapy (SCIT) was published by Frankland and Augustin in 1954.[18]

Nowadays, SCIT with grass, birch, ragweed and *Parietaria* pollen, house dust mite and cat dander is considered effective in adults with allergic rhinitis.[19] SCIT is indicated for patients with severe and/or prolonged symptoms poorly controlled by adequate symptomatic treatment or when symptomatic treatment is refused by the patient or leads to significant side effects. Furthermore, SCIT is particularly indicated when persistent allergic rhinitis is associated with mild or moderate asthma. The indications and contraindications for immunotherapy are the same for adults and children (>5 years).[20]

The major advantage of SCIT is that it is an etiology-based treatment and the effects, reduction of both symptoms and medication requirements, persist at least 3 years after discontinuation of treatment.[14,15] To realise these long-term effects SCIT should be given for a minimum of 3 years.[14,15] For children, SCIT has the additional advantage that it may prevent the onset of new sensitisations in mono-sensitised patients[21] and might reduce the progression of the disease from allergic rhinitis to allergic asthma[22]. Unfortunately, SCIT also has several disadvantages. First, the injections can be inconvenient, in particular for children. Secondly, serious adverse events have been reported and the administration of the injections is therefore restricted to experienced physicians or specialised centres.[20]

Alternative administration forms

To overcome the disadvantages of SCIT, alternative routes of administration were explored in an attempt to make immunotherapy more acceptable and safer, particularly for children. Administration by the oral, bronchial and nasal route was investigated, but mainly due to side effects these alternatives were abandoned. In 1986 the first results of a randomised controlled trial with sublingual immunotherapy (SLIT) in adults were published.[23] In SLIT the allergen, in drops or tablets, is kept under the tongue for at least 1 minute and subsequently swallowed. Nowadays, SLIT is considered the only good alternative for SCIT. It is not only patient-friendlier but also has a favourable safety-profile, making administration at home possible. These advantages make SLIT not only an ideal treatment option for children but facilitate the use of immunotherapy in a primary care setting.

Mechanisms of immunotherapy

The goal of immunotherapy treatment is to modulate the response of the immune system to the allergen. The influence of immunotherapy on several features of the allergic reaction, like allergen specific antibodies, T-lymphocytes and inflammatory cells, has been investigated.

Induction of different types of regulatory T-lymphocytes (Treg cells) seems to be the key to induce tolerance. They suppress allergen-specific IgE production and increase the production of specific IgG (which acts as a "blocking" antibody). Furthermore, Treg cells

alter the characteristics of T-lymphocytes from an allergic (Th2) profile to a non-allergic (Th1) profile. In addition, they reduce allergen-specific inflammation by decreasing inflammatory cell recruitment, activation, and mediator release (such as histamine and eosinophil cationic protein). The exact working mechanism of immunotherapy, however, is still not elucidated.[24]

SUBLINGUAL IMMUNOTHERAPY IN CHILDREN

Its disease-modifying and possible preventive qualities make immunotherapy an attractive treatment option for children. Considering convenience and side effects, sublingual immunotherapy seems the ideal administration form in this population. Although the first paediatric study was published in 1990[25], ten years later the information on SLIT in this population was still limited. Only five randomised placebo-controlled trials in children with allergic rhinitis were available.[25-30] The main results and limitations of these trials are summarised in the following section.

Efficacy

All five trials were small (30 to 66 randomised participants) and only two trials were of high methodological quality.[26,27] Different allergen extracts - house dust mite[25,26], grass pollen[29], *Parietaria* pollen[28] and olive pollen[27] - were evaluated. The duration of treatment varied from 12 to 24 months. In one trial the treatment wasn't given throughout the year, but pre- and co-seasonal.[27] Various methods were used to record symptoms and medication use and analyse efficacy. A positive effect on symptoms, i.e. a statistically significant difference between treatment groups in favour of SLIT, was presented in three trials.[25,28,29] Only one study showed a positive effect on rescue-medication use.[29] The limited number of trials and participants, the diversity in allergen extracts and study design as well as the often insufficient description of the results made it difficult to draw a conclusion on the efficacy of SLIT in children at that time.

Quality of life

Although allergic rhinitis can have a significant impact on daily life, school performance and social activities, the quality of life of the participants was not investigated in any of the trials.

Adherence

An important issue in the evaluation of the efficacy of a drug is adherence. Adherence (or compliance) is generally defined as the extent to which patients use their medication as prescribed. Medication intake can be assessed using direct or indirect methods. Direct

methods measure the concentration of a drug or its metabolite in blood or urine. For SLIT only indirect methods like questionnaires and counting left-over tablets are available. Efficacy and adherence are inter-related. Non-adherence compromises efficacy and inefficacy may lead to non-adherence. The administration at home is considered a great advantage of SLIT, because it takes away an important reason for non-adherence with the subcutaneous form.[31-33] On the other hand, self-administration and consequently lack of medical supervision might lead to incorrect or irregular use. In only one of the five trials medication intake was registered and described. Hirsch et al.[26] showed that 53% and 67% of the participants in the SLIT and placebo group respectively reported complete regular intake. Factors that could influence adherence to SLIT were never investigated.

Adverse events

The interpretation of the data on adverse events was hampered by the often unclear or lacking definition of an adverse event. Four trials presented data on adverse events.[25-28] Local side effects, like swelling and itching in the oropharyngeal area, were more frequently reported in the intervention groups. Systemic side effects (e.g. lower airway symptoms, urticaria) were rare and mostly mild. In one trial an acute asthma exacerbation requiring hospitalisation was described in the placebo group.[28] Systemic anaphylactic reactions did not occur.

Immunology

Immunological outcomes mainly focused on the assessment of total IgE[25,26] and allergen specific IgE[25-28], IgG[25,26], IgG1[25] and IgG4[25-28] levels in serum. Although allergen specific IgE, IgG and IgG4 levels increased significantly in the intervention group compared to the placebo group in some trials, others could not demonstrate a difference between treatment groups. The inflammatory status of the disease was assessed in two trials by measuring ECP (eosinophil cationic protein) in serum[27] and cysteinyl leukotrienes LTB4 and LTE4 in urine[29]. The levels of ECP did not differ between treatment groups. Both LTs, however, showed a significant decrease in the intervention group.

Primary care

The majority of patients with allergic rhinitis are treated by their general practitioner.[34,35] The good safety profile of SLIT increases the availability of immunotherapy in primary care. Prescription by general practitioners could have the advantage of introducing immunotherapy at an early stage of the disease, potentially preventing the progression of the disease. So far, all trials were performed in referral centres.

Aims and outline of this thesis

Although allergic rhinitis is a serious health concern and immunotherapy a potentially ideal treatment option for children, 90 years after the first immunotherapy trial was published there were still several unmet needs.

First, the majority of the information on the efficacy of immunotherapy came from trials performed in an adult population. In general, the assumption was made that these results could be extrapolated to children. But are children in this respect little adults? Since no overview on the efficacy of immunotherapy in children and adolescents was available, there was not enough evidence to answer this question. Therefore, a systematic review of the literature on the efficacy of immunotherapy with inhalant allergens in all its different administration forms in youngsters with allergic rhinitis was performed. The results of this review are described in **chapter 2**.

Secondly, in 2001 there was a need for large well-designed trials with SLIT in youngsters, studying not only symptoms and medication use, but also quality of life, adherence and immunological effects. To meet this demand, the STARDROP-study was designed. In this large randomised trial 204 youngsters (6-18 years), known to their general practitioner with allergic rhinitis, received either grass pollen extract or placebo for two years. The primary outcome of the study was the nose-eye symptom score after two years of treatment. Additionally, the use of symptomatic medication, overall evaluation of treatment, quality of life and side effects were evaluated. **Chapter 3** focuses on the results of these primary and secondary outcomes. The adherence of the youngsters to the study protocol and medication intake, as well as factors that might influence adherence were also analysed and are presented in **chapter 4**. Besides a diary card, several other patient-reported outcomes were assessed, including generic and disease-specific quality of life and a global assessment of symptoms. The relevance of these outcomes is described in **chapter 5**. The immunological response to the treatment was explored and the results are reported in **chapter 6**. For the latter analysis an advanced statistical method, new to the field of immunology, was used. This method is described in **chapter 7.**

In the general discussion, **chapter 8,** the main findings of the review and the results of the STARDROP-study are reflected on.

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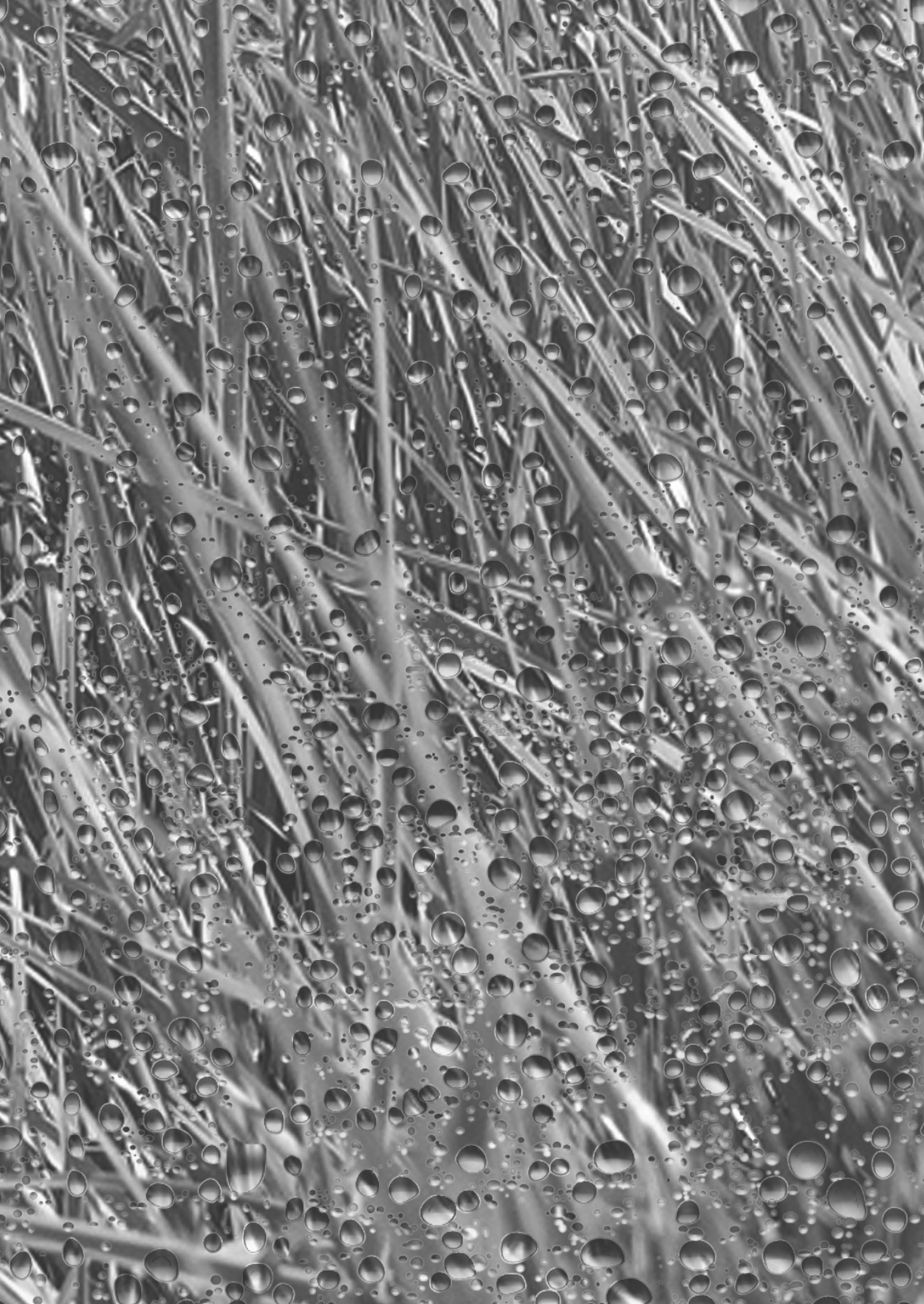

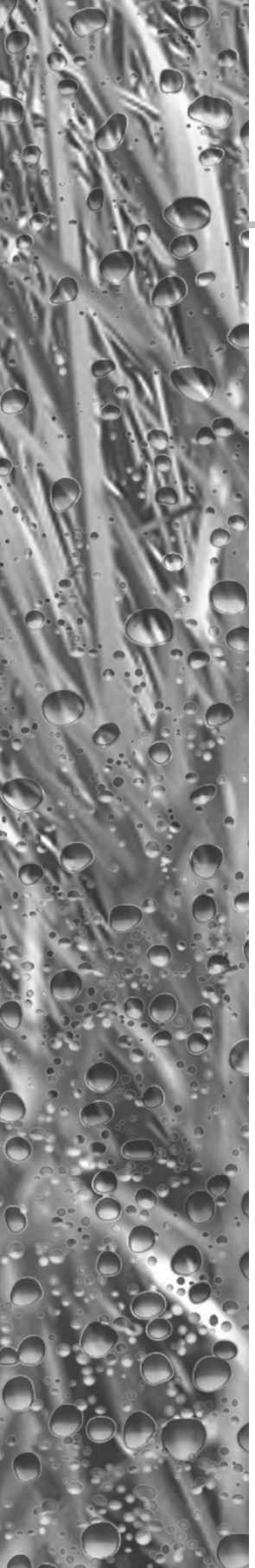

Chapter 2

Immunotherapy in children and adolescents with allergic rhinoconjunctivitis: a systematic review

Esther Röder

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Pediatr Allergy Immunol 2008;19:197-207

ABSTRACT

Allergen-specific immunotherapy is one of the cornerstones of allergic rhinoconjunctivitis treatment. Since the development of non-invasive administration forms with better safety profiles, there is an increasing tendency to prescribe immunotherapy in youngsters. However, no overview is available on the efficacy of immunotherapy in all its different administration forms in youngsters. Therefore, we systematically reviewed randomised controlled trials (RCTs) to evaluate the effect of immunotherapy with inhalant allergens on symptoms and medication use in children and adolescents with allergic rhinoconjunctivitis. Medline, EMBASE, the Cochrane Controlled Clinical Trials Register and reference lists of recent reviews and published trials were searched. RCTs including youngsters aged 0-18 yr with allergic rhinoconjunctivitis and comparing immunotherapy with placebo, symptomatic treatment or a different administration form of immunotherapy were included. Primary outcome measures were rhinoconjunctivitis symptom and/or medication scores. Methodological quality was assessed using the validated Delphi list. A method of best evidence synthesis, a rating system with levels of evidence based on the overall quality and the outcome of the trials, was used to assess efficacy. Six subcutaneous (SCIT), four nasal (LNIT), seven oral (OIT) and eleven sublingual (SLIT) immunotherapy trials, comprising 1619 youngsters, were included. Only 39% of the trials were of high methodological quality. For the SCIT and OIT subgroups the level of evidence for efficacy was conflicting. Moderate evidence of effect was found for LNIT. Analysis of the SLIT subgroup showed no evidence of effect. The evidence for the perennial and seasonal allergen trials within the subgroups varied from moderate evidence of effect to no evidence of effect. In conclusion, there is at present insufficient evidence that immunotherapy in any administration form has a positive effect on symptoms and/or medication use in children and adolescents with allergic rhinoconjunctivitis.

INTRODUCTION

The cornerstones of allergic rhinoconjunctivitis treatment are allergen avoidance, symptomatic drug therapy and specific immunotherapy. Allergen-specific immunotherapy, i.e. the administration of increasing amounts of allergen, in the traditional subcutaneous form (SCIT) prevents symptoms by altering the natural course of the disease.[1] The reduction of both symptoms and medication requirements even persists after discontinuation of treatment.[2] SCIT is an attractive treatment option for children, because it might also prevent the onset of asthma and the development of new sensitisations.[3,4] Unfortunately, SCIT has several disadvantages. The inconvenience of injection therapy hampers the administration to children, whereas the risk of serious adverse events restricts administration to experienced physicians or specialised centres.[1,5] Consequently, alternative routes of administration have been developed to make immunotherapy more acceptable and safer, particularly for children. So far, articles have been published on nasal (LNIT), oral (OIT) and sublingual immunotherapy (SLIT). However, no overview is available on the efficacy of immunotherapy in all its administration forms in children. Therefore, we performed a systematic review of the literature to evaluate the effect of immunotherapy with inhalant allergens on symptoms and medication use in children and adolescents with allergic rhinoconjunctivitis.

METHODS

Search strategy

One of the reviewers (ER) searched Medline, EMBASE and the Cochrane Controlled Clinical Trials Register. The search strategy combined disease and therapy specific terms, [(rhin* OR hay fever) AND (immunotherap* OR desensiti*)], with the optimal search strategy for controlled trials.[6] Additionally, reference lists of recent reviews and published trials were searched. Only full text articles were considered and language was not restricted. The search was completed in June 2006.

Study selection

Two reviewers (ER and MB) independently selected the trials, initially based on title and abstract. Of the selected abstracts the full text article was retrieved. Final selection was independently performed using a standardised form. Disagreement was resolved in a consensus meeting.

We selected randomised controlled clinical trials (RCTs) including youngsters aged 0-18 yr with allergic rhinoconjunctivitis, with or without asthma. Trials using inhalant allergens were considered irrespective of route, dose or duration of treatment. We

included only trials comparing immunotherapy with placebo or symptomatic treatment and trials comparing different administration forms of immunotherapy. Trials exclusively comparing different doses of the same product were excluded. Sensitivity to the administered allergen had to be confirmed by a positive skin test and/or serum allergen-specific IgE. Our primary outcome measures were rhinoconjunctivitis symptom and/or medication scores. Therefore, at least one of the following outcomes had to be presented: a rhinoconjunctivitis symptom score, a (anti-allergic) medication score and/or a composite symptom-medication score. If a separate rhinitis score was absent, a total symptom score including a rhinitis score was evaluated. In case an overall evaluation of the treatment was presented but no composite symptom-medication score, the former was evaluated instead. Secondary outcome measures in this review were quality of life, adverse events and compliance with medication intake.

Methodological quality assessment

Two reviewers (ER and either MB, HG or RG) independently assessed the methodological quality using the Delphi list.[7] Two items were added concerning the drop-out rate (Table 1).

Each item (D1-D11) was scored as yes (=1), no (=0) or unclear (=0). Disagreement between the two reviewers was resolved in a consensus meeting. The overall methodological quality of a trial was computed by counting the number of positive scores, with equal weights applied on all items. We arbitrarily regarded trials with a score ≥6 (range 0-11) of high quality.

The inter-assessor agreement on the methodological quality was calculated using Cohen's Kappa-scores (κ), ranging from –1 (perfect disagreement) to +1 (perfect agreement).[8] A κ more than 0.7, between 0.5-0.7 or less than 0.5 corresponded with a high, moderate or poor level of agreement between assessors respectively.

Table 1. Items of the Delphi-list for the assessment of the methodological quality of the articles

D1	Was a method of randomisation performed?
D2	Was the treatment allocation concealed?
D3	Were the groups similar at baseline regarding the most important prognostic indicators?
D4	Were both inclusion and exclusion criteria specified?
D5	Was the outcome assessor blinded?
D6	Was the care provider blinded?
D7	Was the patient blinded?
D8	Were point estimates and measures of variability presented for the primary outcome measures?
D9	Did the analysis include an intention-to-treat analysis?
D10	Was the withdrawal/drop-out rate <20% of the total study population?
D11	Was the withdrawal/drop-out rate unlikely to cause bias?

Data extraction and analysis

Two reviewers (ER and either MB, HG or RG) independently extracted data on a standardised form regarding trial design, treatment, participants and outcome measures. In principle, the last treatment period was evaluated, unless the data presented for this period were insufficient or a control group was no longer present.

Considering the outcome measures, our initial intention was to extract means and SDs or RRs and 95% CIs to pool the data. In case of a statistical significant difference between treatment groups, the clinical relevance of this difference would be assessed. A difference in symptom/medication scores between treatment groups of at least 30% was considered the minimal clinical important difference.[9] However, the description of the various scores was often insufficient as for instance information on the range and composition of the score was lacking. Different point estimates (e.g. mean or ranks) were presented and the measures of variability (e.g. SD or 95% CI) were not always stated. Moreover, the trials were heterogeneous concerning participants, allergen (type and dose), and duration of treatment. Therefore, the results could not be pooled.

Instead, for each trial we present the presence or absence of a positive effect of the intervention on the symptom/medication scores compared with the control group. An effect was considered positive, if a statistically significant difference was presented between intervention and control group. If a positive effect was found for at least one of the three scores (symptom score, medication score, composite symptom-medication score) efficacy was considered demonstrated.

To analyse the efficacy for different subgroups we used the method of best evidence synthesis, a rating system with levels of evidence based on the overall quality and the outcome of the trials.[10] First, the total number of high-quality trials within each group was evaluated. In case of at least two high-quality RCTs, only these trials would provide the evidence. All trials were considered if no or only one high-quality RCT was available. Secondly, the consistence of the findings was calculated. Findings were considered consistent if ≥75% of the trials reported comparable results (i.e. positive or negative effect) on the demonstrated efficacy. A score <75% was considered inconsistent. Lastly, the level of evidence (conflicting/strong/moderate/limited) was determined. Conflicting evidence existed, if multiple RCTs showed inconsistent findings. Strong evidence was defined by consistent findings among at least two high-quality RCTs. Moderate evidence existed if one high-quality RCT and/or at least two low-quality RCTs showed consistent findings. If only one low-quality RCT was found, the evidence was considered limited. This method of best evidence synthesis was applied to each administration form subgroup separately. Within these subgroups, the best evidence synthesis was also applied to the seasonal and perennial trials separately.

RESULTS

Identification and selection of the literature

The search strategy resulted in 1629 titles and abstracts. The majority (n=1274) did not meet the inclusion criteria, mainly because 1040 articles did not refer to RCTs. A total of 23 potentially eligible manuscripts were excluded because of language barriers (10 Polish, 6 Russian, 4 Spanish, 2 Romanian, 1 Croat). We retrieved 332 full text articles for detailed evaluation. Another 304 trials were excluded, mainly because they included only adults (n=125) or a mixed adult-child population from which the data for children could not be extracted (n=85). Eventually 28 RCTs were included in this review.[3,11-37]

Description of the included trials

All trials were performed in referral centres and all except two were carried out in Europe.[12,13] Eleven trials reported financial or intellectual support from a pharmaceutical company.[3,14,22,24,27,28,30-33] Study medication was supplied by the manufacturer in four trials.[11,16,17,35]

Treatment

Six SCIT, four LNIT, seven OIT and eleven SLIT trials were included. In the majority of trials (n=19) a seasonal allergen, mainly grass pollen (n=13), was used. Nine trials used a perennial allergen, i.e. house dust mite[15,19,25,26,34-37] and mould[14]. Only nine trials, all within the SLIT subgroup, stated the cumulative dose of allergen in micrograms.[27,28,30-36] The duration of treatment varied from 1 to 36 months. Most trials (n=23) were placebo-controlled. In four trials the control group received symptomatic treatment[3,15,18,30] and in one trial OIT was compared to SCIT[21]. All children were allowed to use rescue medication in addition to their study medication. Allergen exposure was measured in 17 trials[11-14,17,20-22,24,27,28,30-33,36,37], but only five trials incorporated allergen exposure in their analysis[12,17,20,24,28] (Table 2).

Participants

A total of 1619 children (ranged: 15-205) were included. Seventeen trials included <50 participants[11,13,14,16-20,23-26,29,31,32,35,36] and only four trials comprised ≥100 children[3,12,27,30]. The participants were aged between 3 and 18 year. Generally more boys were included. All trials, except for five[16,18,19,24,30], included a mixed rhinitis-asthma population. One trial did not state whether participants with asthma were included[21] (Table 3).

Methodological quality assessment

Eleven trials were of high methodological quality[11,19,20,25,27,30,32-36] of which the majority was found within the SLIT subgroup. The description of "treatment allocation" (D2),

"similarity of the treatment groups at baseline" (D3), "blinding of the outcome assessor/care provider" (D5/D6) and "inclusion of an intention-to-treat-analysis" (D9) was insufficient in most trials, resulting in a low score of these items. The overall Cohen's κ was 0.785, corresponding with high agreement between both reviewers (Table 4).

Data extraction and analysis

Primary outcome

Only nine trials stated a primary outcome. A symptom/medication score[23,27,28,31,33,36], the development of asthma[3,30] or a combined patient-doctor evaluation[17] was used to evaluate efficacy (Table 5).

Demonstrated efficacy

In four cases our conclusion on the effects of the intervention differed from the original conclusion. Ippoliti et al.[34] concluded that rhinitis symptom scores decreased significantly. However, only intra-group p-values for the symptom scores were presented. No measure of variability was stated, making it impossible to calculate the inter-group p-value (SLIT vs. placebo) and to draw a conclusion on the effect of the intervention. Vourdas et al.[33] concluded that SLIT had a positive effect on the conjunctivitis score, but we classified the results as "not stated" because only one of the three eye symptoms was presented. Dreborg et al.[14] reported a significant difference in rescue medication scores. As this difference was found in two weeks of the 10-week evaluation period only, we concluded that there was no positive effect present. Rolinck-Werninghaus et al.[28] calculated the difference in rescue medication scores after one and three years of treatment and compared these differences between both treatment groups. They concluded that a significant difference was found between the treatment groups regarding medication scores. However, the medication scores of the SLIT-group after one year of treatment were much higher than the scores of the placebo group and a correction for this difference between both groups should have been incorporated in the analysis. We analysed the difference in medication scores after three years of treatment and found no statistically significant difference between both treatment groups (p=0.72) (Table 6).

Level of evidence

The SCIT and LNIT subgroups contained only one high-quality RCT[11,19], therefore all trials were considered. The findings within the SCIT and OIT groups were not consistent, resulting in conflicting evidence. Although ≥75% of the trials demonstrated efficacy in the LNIT group, the level of evidence was considered moderate because only one high-quality trial was available. In 83% of the high-quality trials within the SLIT group efficacy was not demonstrated[27,32,33,35,36], resulting in no evidence of effect. Only for the perennial

Table 2. Treatment characteristics

	ARTICLE	ALLERGEN	CONTROL GROUP	CUM DOSE IN MCG	DURATION	TIME	EXPOSURE MEASURED
SCIT	Sanders 1966 [11]	grass pollen	placebo	NS	NS	pre-seasonal	yes
	Möller 2002 [3]	grass pollen and/or birch pollen	symptomatic	NS	36 months	all year	no
	Fontana 1966 [12]	ragweed pollen	placebo	NS	±6 months **	pre-seasonal	yes
	Weisnagel 1979 [13]	ragweed pollen	placebo	NS	1 month	pre-seasonal	yes
	Dreborg 1986 [14]	mould (*Cladosporium herbarum*)	placebo	NS	10 months	pre-, co- and post-seasonal	yes
	Urbanek 1991 [15]	house dust mite	symptomatic	NS	12 months	all year	no
LNIT	Bardare 1996 [16]	grass pollen	placebo	NS	3 months *	pre-seasonal	no
	Mehta 1975 [17]	grass pollen	placebo	NS	±3 months	pre-seasonal	yes
	Cserháti 1997 [18]	group 1 grass pollen group 2 ragweed pollen	symptomatic	NS	12 months **	pre-seasonal	no
	Marcucci 2002 [19]	house dust mite	placebo	NS	18 months	all year	no
OIT	Caffarelli 2000 [20]	grass pollen	placebo	NS	3 months	pre-seasonal	yes
	Urbanek 1990 [21]	grass pollen #	SCIT	NS	24 months	all year	yes
	Cooper 1984 [22]	grass pollen	placebo	NS	at least 3.5 months	pre-seasonal and early co-seasonal	yes
	Reinert 1983 [23]	pollen (grass/rye)	placebo	NS	24 months	all year	no
	Möller 1986 [24]	birch pollen	placebo	NS	10 months	pre- and postseasonal	yes
	Giovane 1994 [25]	house dust mite	placebo	NS	36 months	all year	no
	Urbanek 1982 [26]	house dust mite	placebo	NS	10.5 months	NS	no

Table 2. (*continued*)

	ARTICLE	ALLERGEN	CONTROL GROUP	CUM DOSE IN MCG	DURATION	TIME	EXPOSURE MEASURED
SLIT	Bufe 2004 [27]	grass pollen	placebo	9600 (Phl p 5)	12 months *	all year	yes
	Rolinck-W. 2004 [28]	grass pollen	placebo	188 (major grass pollen allergen)	32 months	all year	yes
	Yuksel 1999 [29]	grass pollen	placebo	NS	12 months	all year	no
	Novembre 2004 [30]	grass pollen	symptomatic	120 (major allergen group 5)	4 months/yr during 3 years	co-seasonal	yes
	La Rosa 1999 [31]	parietaria pollen	placebo	52500 (Par j1)	24 months	all year	yes
	Pajno 2003 [32 $]	parietaria pollen	placebo	20.3 (Par j1)	13 months	all year	yes
	Vourdas 1998 [33]	olive pollen	placebo	8100 (Ole e1)	6 months/yr during 2 years	pre- and co-seasonal	yes
	Ippoliti 2003 [34]	house dust mite	placebo	90 (60 Der p1 / 30 Der p2)	6 months	NS	no
	Bahçeciler 2001 [35]	house dust mite	placebo	1540 (560 D.pter / 980 D.far)	6 months	NS	no
	Hirsch 1997 [36]	house dust mite	placebo	570 (Der p1)	12 months	all year	yes
	Tari 1990 [37]	house dust mite	placebo	NS	18 months	all year	yes

SCIT, subcutaneous immunotherapy; LNIT, local nasal immunotherapy; OIT, oral immunotherapy; SLIT, sublingual immunotherapy; NS, not stated

* only the first year of the study was double-blind placebo-controlled; ** sufficient data are only presented for the first year; $ both the intervention and the control group used inhaled fluticasone; # group 1 low dose - group 2 high dose

Table 3. Participant characteristics (demographic data of analysed population unless otherwise stated)

	ARTICLE	N INCLUDED verum/control	% DROP-OUT	N ANALYSED verum/control	AGE - RANGE verum/control	% MALE verum/control	RHINITIS (Rh), ASTHMA (A) verum/control
SCIT	Sanders 1966 [11]	32 (16/16)	0%	32 (16/16)	4-15 yr *	78% *	Rh and/or A 16/16
	Möller 2002 [3]	205 (103/102)	7%	191 (97/94)	6-15 yr *	67% *	Rh 163 / Rh+A 42 *
	Fontana 1966 [12]	101 (51/50)	9%	91 (47/44)	6-17 yr ****	64% *	Rh and/or A 47/44
	Weisnagel 1979 [13]	34 (17/17)	21%	23 (8/15)	NS	71 / 71% **	Rh 12/15; Rh+A 5/2 **
	Dreborg 1986 [14]	30 (16/14)	0%	30 (16/14)	5-17 yr *	47% *	Rh and/or A 16/14
	Urbanek 1991 [15]	57 (26/31)	14%	49 (24/25)	NS	NS	Rh or A 24/25
LNIT	Bardare 1996 [16]	40 (NS/NS)	3%	39 (NS/NS)	5-15 yr *	70% *	Rh 40; A 0 *
	Mehta 1975 [17]	46 (23/23)	9%	42 (21/21)	6-18+ yr #	NS	Rh 21/21; A NS ##
	Cserháti 1997 [18]	36 (grass 12/ragweed 12/control 12)	0%	36 (12/12/12)	4-18 yr ****	50 / 83 / 75% **	Rh 12/12/12; A 0/0/0
	Marcucci 2002 [19]	32 (16/16)	19%	26 (12/14)	5-14 / 4-14 yr *	63 / 44% *	Rh 16/16; A 0/0 **
OIT	Caffarelli 2000 [20]	48 (24/24)	8%	44 (24/20)	4-14 yr *	50 / 65%	Rh 1/0; Rh+A 16/17; A 7/3
	Urbanek 1990 [21]	60 (low NS/high NS/control NS)	25%	45 (16/14/15)	6-15 yr ***	31% ***	Rh 60; A NS *
	Cooper 1984 [22]	54 (28/26)	13%	47 (25/22)	5-15 yr ****	NS	Rh 28; Rh+A 16; A 3 ***
	Reinert 1983 [23]	20 (10/10)	30%	14 (7/7)	4-11 yr *	NS	Rh 14; Rh+A 6 *
	Möller 1986 [24]	30 (14/16)	0%	30 (14/16)	8-16 / 10-15 yr **	57 / 50% **	Rh 14/16; A 0/0
	Giovane 1994 [25]	18 (10/8)	0%	18 (10/8)	4-11 / 3-9 **	70 / 88%	Rh+A 10/8
	Urbanek 1982 [26]	32 (17/15)	16%	27 (15/12)	4-13 yr ***	NS	Rh and/or A 17/15 **

Table 3. (*continued*)

	ARTICLE	N INCLUDED verum/control	% DROP-OUT	N ANALYSED verum/control	AGE - RANGE verum/control	% MALE verum/control	RHINITIS (Rh), ASTHMA (A) verum/control
SLIT	Bufe 2004[27]	161 (83/78)	18%	161 (83/78)	NS	NS	Rh 79/76; A 33/35
	Rolinck-W. 2004[28]	97 (49/48)	23%	77 (39/38)	3-14 yr *	61 / 73%	Rh 29/29; Rh+A 20/19 **
	Yuksel 1999[29]	39 (21/18)	0%	39 (21/18)	6-15 / 8-15 yr	NS	Rh 10/10; A 11/8
	Novembre 2004[30]	113 (54/59)	14%	97 (47/50)	5-14 / 4-16 yr	70 / 70%	Rh 47/50; A 0/0
	La Rosa 1999[31]	41 (20/21)	20%	33 (16/17)	6-14 / 7-13 yr **	65 / 57% **	Rh±A 16/17
	Pajno 2003[32]	30 (15/15)	10%	27 (14/13)	8-14 / 8-14 yr **	47 / 40% **	Rh+A 14/13
	Vourdas 1998[33]	66 (34/32)	3%	64 (33/31)	8-17 / 7-17 yr **	74 / 75% **	Rh 1/3; Rh+A 33/29 **
	Ippoliti 2003[34]	86 (47/39)	0%	86 (47/39)	5-12 / 7-11 yr	60 / 56%	Rh+A 18/15; A 29/24
	Bahçeciler 2001[35]	15 (8/7)	0%	15 (8/7)	7-18 / 7-15 yr	50 / 57%	Rh+A 8/7
	Hirsch 1997[36]	30 (15/15)	3%	30 (15/15)	6-15 / 6-14 yr	67 / 67%	Rh 3/5; Rh+A 9/5; A 3/5
	Tari 1990[37]	66 (34/32)	12%	58 (30/28)	5-12 yr ****	64% *	Rh+A 30/28

SCIT, subcutaneous immunotherapy; LNIT, local nasal immunotherapy; OIT, oral immunotherapy; SLIT, sublingual immunotherapy; NS, not stated

* total included participants; ** included participants; *** total analysed participants; **** inclusion criterium

2 participants were 18+; ## the exact number was not stated, however, a comment was made that most patients had some degree of seasonal asthma

Table 4. Results of the methodological quality assessment

	ARTICLE	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	Total Delphi*	Cohen's kappa**
SCIT	Sanders 1966 [11]	1	0	0	0	1	1	1	1	1	1	1	**8**	0.744
	Möller 2002 [3]	1	0	0	1	0	0	0	1	0	1	1	5	0.814
	Fontana 1966 [12]	1	1	0	0	0	0	0	0	0	0	0	2	0.744
	Weisnagel 1979 [13]	1	1	0	0	1	0	1	1	0	0	0	5	0.814
	Dreborg 1986 [14]	1	0	0	1	0	0	1	0	0	1	1	5	0.814
	Urbanek 1991 [15]	1	0	0	0	0	0	0	1	0	1	0	3	1.000
LNIT	Bardare 1996 [16]	1	0	0	0	0	0	1	0	0	1	1	4	0.814
	Mehta 1975 [17]	1	0	0	0	0	0	0	0	0	1	1	3	0.792
	Cserháti 1997 [18]	1	0	0	1	0	0	0	0	0	1	1	4	0.792
	Marcucci 2002 [19]	1	0	1	1	0	0	1	1	0	1	1	**7**	1.000
OIT	Caffarelli 2000 [20]	1	0	0	1	1	1	1	1	0	1	0	**7**	0.298
	Urbanek 1990 [21]	1	0	0	0	0	0	0	1	0	0	0	2	0.744
	Cooper 1984 [22]	1	0	0	1	0	0	1	0	0	1	1	5	1.000
	Reinert 1983 [23]	1	0	0	0	1	1	1	1	0	0	0	5	0.645
	Möller 1986 [24]	1	0	0	1	0	0	1	0	0	1	1	5	0.814
	Giovane 1994 [25]	1	1	1	0	1	1	1	0	1	1	1	**9**	0.744
	Urbanek 1982 [26]	1	0	0	0	0	0	0	0	0	1	1	3	0.421
SLIT	Bufe 2004 [27]	1	0	0	1	0	0	0	1	1	1	1	**6**	0.814
	Rolinck-W. 2004 [28]	1	0	1	1	0	0	0	1	0	0	0	4	0.621
	Yuksel 1999 [29]	1	0	0	0	0	0	1	1	0	0	0	3	0.560
	Novembre 2004 [30]	1	0	1	1	0	0	0	1	0	1	1	**6**	0.633
	La Rosa 1999 [31]	1	0	0	1	0	0	1	1	0	0	0	4	0.792
	Pajno 2003 [32]	1	1	0	1	1	1	1	1	0	1	1	**9**	0.621
	Vourdas 1998 [33]	1	0	1	0	0	0	1	1	0	1	1	**6**	0.633
	Ippoliti 2003 [34]	1	0	1	1	0	0	0	1	1	1	1	**7**	0.814
	Bahçeciler 2001 [35]	1	0	1	0	0	0	0	1	1	1	1	**6**	0.814
	Hirsch 1997 [36]	1	1	1	1	1	1	1	0	0	1	1	**9**	0.560
	Tari 1990 [37]	1	0	0	1	0	0	0	1	0	1	1	5	1.000
Total item		28	5	8	15	7	6	15	18	5	21	19		**overall kappa**
Cohen's kappa**		1.000	0.661	0.578	0.469	0.909	0.900	0.858	0.643	0.700	0.909	0.673		**0.785**

SCIT, subcutaneous immunotherapy; LNIT, local nasal immunotherapy; OIT, oral immunotherapy; SLIT, sublingual immunotherapy; 0, no or unclear; 1, yes

* Total Delphi (range 0-11): total score ≥6 = high quality; <6 = low quality

** Cohen's kappa (range 0-1): ≥0.7 = high agreement, 0.5-0.7 = moderate agreement, <0.5 = low agreement between reviewers

Table 5. Data extraction

	ARTICLE	PRIMARY OUTCOME STATED	SYMPTOMS RECORDED	RESCUE MEDICATION RECORDED	POSITIVE EFFECT ON SYMPTOMS	POSITIVE EFFECT ON RESCUE MEDICATION	POSITIVE EFFECT ON SYMPTOM-MEDICATION SCORE
SCIT	Sanders 1966 [11]	no	ND	no	yes	NA	no *
	Möller 2002 [3]	yes	N + O[$]	no	yes	NA	NS
	Fontana 1966 [12]	no	N + O[$]	yes	no	no	NS
	Weisnagel 1979 [13]	no	N + O[$]	yes	no	no	no
	Dreborg 1986 [14]	no	N-O-B (total)	yes	no	no #	NS
	Urbanek 1991 [15]	no	ND	yes	no	NS	NS
LNIT	Bardare 1996 [16]	no	N + O[$]	yes	yes	yes	NS
	Mehta 1975 [17]	yes	ND	no	NS	NA	yes *
	Cserháti 1997 [18]	no	N + O[$]	yes	grass-yes; ragweed-no	no	NS
	Marcucci 2002 [19]	no	N + O[$]	yes	N-yes/ O-NS	yes	NS
OIT	Caffarelli 2000 [20]	no	N + O[$]	yes	no	no	no
	Urbanek 1990 [21]	no	N + O[$]	yes	no	no	no
	Cooper 1984 [22]	no	N + O[$]	yes	no	no	no
	Reinert 1983 [23]	yes	ND	no	no	NA	no *
	Möller 1986 [24]	no	N + O[$]	yes	no	no	yes
	Giovane 1994 [25]	no	N + O[$]	yes	NS	NS	N-yes / O-NS
	Urbanek 1982 [26]	no	ND	yes	NS	NS	no *

Table 5. (*continued*)

	ARTICLE	PRIMARY OUTCOME STATED	SYMPTOMS RECORDED	RESCUE MEDICATION RECORDED	POSITIVE EFFECT ON SYMPTOMS	POSITIVE EFFECT ON RESCUE MEDICATION	POSITIVE EFFECT ON SYMPTOM-MEDICATION SCORE
SLIT	Bufe 2004 [27]	yes	N-O-B (total)	yes	NS	NS	no
	Rolinck-W. 2004 [28]	yes	N + O[$]	yes	no	no #	no #
	Yuksel 1999 [29]	no	N	yes	yes	yes	NS
	Novembre 2004 [30]	yes	N-O-B (total)	yes	no	yes	no
	La Rosa 1999 [31]	yes	N + O[$]	yes	N-yes / O-NS	no	NS
	Pajno 2003 [32]	no	N + O[$]	yes	no	no	NS
	Vourdas 1998 [33]	yes	N + O[$]	yes	N-no/ O-NS #	no	no *
	Ippoliti 2003 [34]	no	N	no	NS #	NA	NS
	Bahçeciler 2001 [35]	no	N	yes	no	no	NS
	Hirsch 1997 [36]	yes	N + O[$]	yes	N-no / O-NS	no	no *
	Tari 1990 [37]	no	N + O[$]	yes	yes	NS	NS

SCIT, subcutaneous immunotherapy; LNIT, local nasal immunotherapy; OIT, oral immunotherapy; SLIT, sublingual immunotherapy; N, nasal; O, ocular; B, bronchial; NA, not applicable; ND, not defined; NS, not stated

[#] revised conclusion, differs from conclusion authors, see text for details; * only an overall evaluation (benefit-no benefit) was available; [$] seperate scores

allergen trials within the SCIT subgroup and the seasonal allergen trials within the OIT subgroup the conclusion differed from the overall conclusion for the administration form subgroup. In both cases the evidence changes from conflicting evidence to no evidence of effect (Table 6).

In consideration of the data, we decided to perform three sensitivity analyses. First, we included the trial by Caferelli et al.[20] in the SLIT subgroup, as several systematic reviews classified this trial as SLIT instead of OIT[38-42]. Inclusion of this trial in the SLIT subgroup, however, did not change the conclusion on the demonstrated efficacy for the SLIT and OIT subgroups. Secondly, we reanalysed all subgroups using the original conclusions stated by the authors. Compared to our initial analyses, the only difference was found in the SLIT subgroup, changing the conclusion from no evidence of effect to conflicting evidence. Thirdly, we excluded all trials sponsored by a pharmaceutical company. As a result the conclusion for the SCIT subgroup changed from conflicting evidence to no evidence of effect.

Secondary outcomes

Quality of life was not investigated in any of the trials included in this review.

Data on adverse events were presented in 86% of the trials.[11-14,16-25,27-28,30-36] The definition of an adverse event was often unclear. For instance, in one trial the authors stated that no side-effects occurred, but some patients suffered from nasal complaints after the LNIT application.[18] Local side-effects were more frequently reported in the intervention groups. Systemic side-effects (i.e. asthma, urticaria) were rare and mild. One SLIT trial reported a serious adverse event, an acute asthma exacerbation requiring hospitalisation, in the active group.[28] Although the same event was described in the placebo group in another SLIT trial, the authors concluded that no major adverse events were reported.[31] Systemic anaphylactic reactions did not occur.

It was often unclear whether results on compliance represented compliance to medication intake or to the study protocol. Only one trial pre-defined compliance to medication intake.[19] Some trials presented the results only in the form of general comments like "compliance was good".[16,20,34] Others reported the number of participants excluded because of "poor compliance" or "irregular intake".[23,27,28,30] Four trials mentioned the number of participants reaching the maximal dose.[12,14,19,33] Two studies gave a detailed description of the medication intake. In the trial of Hirsch et al.[36] 53% and 67% of the participants in the SLIT and placebo group respectively reported complete regular intake. Weisnagel[13] described that 59% of the participants in the intervention and 100% of the participants in the placebo group received all injections.

Table 6. Level of evidence

	ARTICLE	EFFICACY DEMONSTRATED	METHODOLOGICAL QUALITY	LEVEL OF EVIDENCE
SCIT	Sanders 1966 [11]	yes	H *	**conflicting evidence**
	Möller 2002 [3]	yes	L *	
	Fontana 1966 [12]	no	L *	
	Weisnagel 1979 [13]	no	L *	
	Dreborg 1986 [14]	no	L *	
	Urbanek 1991 [15]	no	L *	
LNIT	Bardare 1996 [16]	yes	L *	**moderate evidence for effect**
	Mehta 1975 [17]	yes	L *	
	Cserháti 1997 [18]	grass-yes; ragweed-no	L *	
	Marcucci 2002 [19]	yes	H *	
OIT	Caffarelli 2000 [20]	no	H *	**conflicting evidence**
	Urbanek 1990 [21]	no	L	
	Cooper 1984 [22]	no	L	
	Reinert 1983 [23]	no	L	
	Möller 1986 [24]	yes	L	
	Giovane 1994 [25]	yes	H *	
	Urbanek 1982 [26]	no	L	
SLIT	Bufe 2004 [27]	no	H *	**no evidence for effect**
	Rolinck-W. 2004 [28]	no	L	
	Yuksel 1999 [29]	yes	L	
	Novembre 2004 [30]	yes	H *	
	La Rosa 1999 [31]	yes	L	
	Pajno 2003 [32]	no	H *	
	Vourdas 1998 [33]	no	H *	
	Ippoliti 2003 [34]	insufficient data $	H	
	Bahçeciler 2001 [35]	no	H *	
	Hirsch 1997 [36]	no	H *	
	Tari 1990 [37]	yes	L	

SCIT, subcutaneous immunotherapy; LNIT, local nasal immunotherapy; OIT, oral immunotherapy; SLIT, sublingual immunotherapy; H, high quality RCT; L, low quality RCT

* trials providing the evidence; $ insufficient data to draw conclusion

DISCUSSION

Methodological quality

The overall quality of the trials was low. Even in studies published after the introduction of the CONSORT statement in 1996[43] one of the most important items for the assessment

of quality, i.e. concealment of treatment allocation, was often insufficiently described. As the lack of adequate allocation concealment is associated with exaggerated estimates of treatment effect and bias[44,45], inadequate description of this item hampers the interpretation of the results.

Demonstrated efficacy

We did not find conclusive evidence of the efficacy of immunotherapy in any administration form. Several explanations for this finding can be discussed.

First, although only two trials presented a power calculation[28,36], the power in most trials seems to be insufficient to detect a statistical significant difference[38]. A clinical relevant difference might be present, but because of inadequate description of the data we could only assess whether a statistical significant difference between treatment groups was found.

Secondly, efficacy could vary for different allergens. Summarising the efficacy for an administration form could conceal such differences. Although numbers were small, the sensitivity analyses did not show convincing evidence of efficacy for the seasonal and perennial allergen trials within each administration form subgroup.

Thirdly, the dosages used may have been suboptimal. Unfortunately, there are no placebo-controlled dose-finding studies in children available for SCIT, LNIT and OIT. After the inclusion of the trials a placebo-controlled dose-response study with SLIT was published, showing dose-dependent effects.[46]

Furthermore, the duration of treatment might have been too short in some trials. It has been reported for SLIT that trials with a duration <18 months did not show a significant effect on symptom scores.[42]

Lastly, compliance to medication intake is important for efficacy to emerge. Only a few trials presented data on compliance making it difficult to interpret the influence of this factor.

To our knowledge, there are no systematic reviews on the efficacy of SCIT, LNIT and OIT in children available. SCIT has been used for a long time and probably because of the fact that positive effects were found in studies performed in adults, trials in children were no longer performed. LNIT and OIT were never used on a large scale, mainly due to side effects.

Several overviews on the efficacy of SLIT have been published. However, the majority are not systematic reviews and many are written by the same authors. Taking only systematic reviews for allergic rhinitis that included children into account[38-42,47-49], these reviews differed from our overview on several points. Some only selected English publications in peer-reviewed journals.[38,40] Others restricted the selection to placebo-controlled studies[38,42,47,48] or excluded children >14 years[48] or with other clinically relevant sensibilisations[47,48]. In several reviews[38-42] the study by Cafferelli et al.[20] was classified as

a SLIT instead of an OIT trial. Our definition of SLIT was that a sublingual phase should be present. The authors described the administration form used as oromucosal-swallow immunotherapy (OSI) and the tablets were held in the mouth and not under the tongue. Inclusion of this trial in the SLIT subgroup did not alter the conclusion in our analysis. Other reviews[41,42,48,49] included the trial by Wüthrich et al. This trial was excluded from our review because the children were not reported to be randomised. The trial by Yuksel et al.[29] was only considered in one review[41], probably because the focus was on immunological parameters. However, symptom and medication scores were also reported.

The methodological quality of the included trials was assessed in three reviews, using different validated lists.[39,42,47] All lists included the important quality item "concealment of treatment allocation", but the assessment of this item varied. Wilson et al.[47] concluded that concealment of treatment allocation was adequate in all studies, but Sopo et al.[39] and Penagos et al.[42] stated that the randomisation method was not always reported.

A meta-analysis was performed in three reviews.[42,47,48] Pooling of the data, however, seems inappropriate. All meta-analyses showed a substantial statistical heterogeneity among trials. If statistical heterogeneity is present and can be explained, meta-analysis - and consequently random effect meta-analysis - should not be considered[45] and as stated, the heterogeneity among SLIT trials can be explained by the clinical diversity (variability in participants, intervention and outcomes) and methodological differences (variability in trial design and quality) presented in the trials.

Although the conclusion on the efficacy of SLIT in these previous systematic reviews varied, the authors were unanimous in their recommendation that more adequately designed studies were needed.[38-42,47-49]

Strengths and weaknesses

We had to exclude a few manuscripts because of language barriers and we cannot rule out that possible relevant trials were not included in this review. As trials from 1966 onward were selected, we decided not to collect additional information from the authors in case of insufficient or missing data. It is conceivable that as data from older studies could probably not be provided, a selection would be created. Besides being unable to pool the data, we were also unable to perform all intended analyses, because of insufficient description of the data. As it is suggested that multi-sensitised patients might not benefit from specific immunotherapy as much as mono-sensitised patients, our intention was to perform a sensitivity analysis. However, it was often unclear which allergens were tested to exclude multi-sensitisation and/or if additional sensitisations were clinically relevant, making it difficult to classify the included population as mono- or multi-sensitised.

So far, this is the only review presenting an overview on the efficacy of immunotherapy in all its administration forms in children. Furthermore, we included the methodological quality of the trials in the overall evaluation of efficacy.

Interpretation

In spite of the accepted position of immunotherapy in guidelines[50] there is at present insufficient evidence that immunotherapy in any administration form has a positive effect on symptoms and/or medication use in children and adolescents with rhinoconjunctivitis.

Future research

As only SCIT and SLIT are used in clinical practice, future research should focus on these administration forms. First, the optimal but still safe dose needs to be determined, using randomised placebo-controlled dose-finding studies. Subsequently, trials with adequate design (randomised, placebo-controlled, double-blind, sufficient number of participants, adequate duration of treatment, etc) should establish efficacy. These trials should also include assessment of quality of life and compliance to medication intake. If efficacy is demonstrated cost-effectiveness ought to be assessed.

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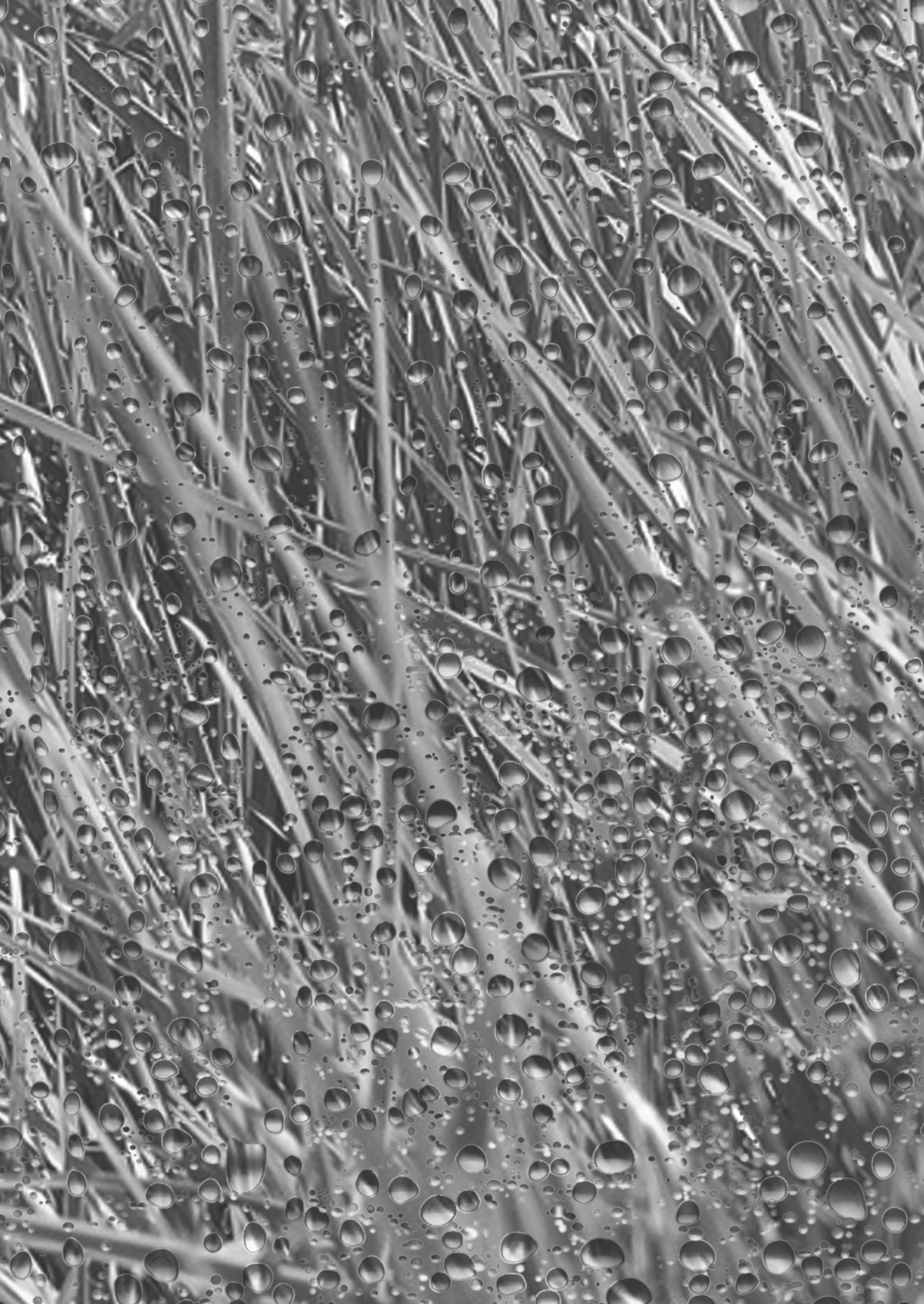

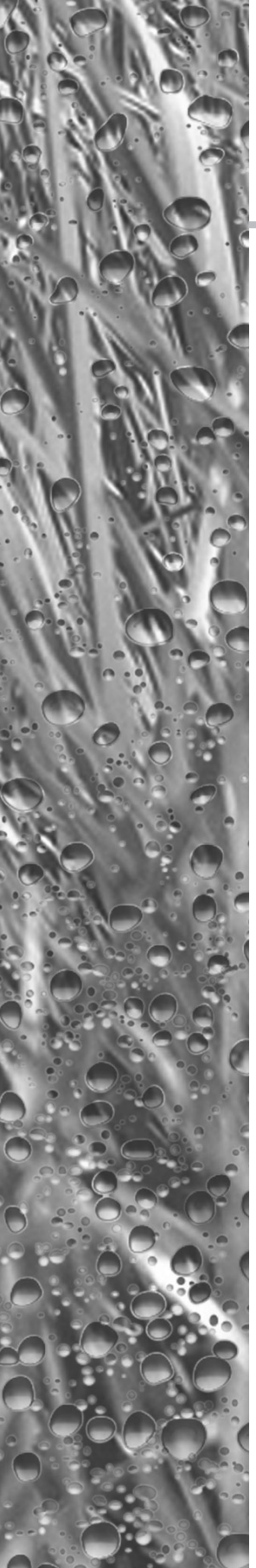

Chapter 3

Sublingual immunotherapy with grass pollen is not effective in symptomatic youngsters in primary care

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ABSTRACT

Background: Sublingual immunotherapy (SLIT) is considered safer and more convenient than subcutaneous therapy and therefore has been proposed as especially suitable for children and in primary care. Most efficacy studies in children lack power to be conclusive, and all have been performed in referral centres.

Objective: To investigate the efficacy of SLIT with grass pollen allergen in children and adolescents with rhinoconjunctivitis in a primary care setting.

Methods: Youngsters aged 6-18 years with hay fever were enrolled from general practices and randomly assigned to receive placebo or grass pollen mix for 2 years. The primary outcome was the mean daily total symptom score (scale 0-15) comprising sneezing, itching nose, watery running nose, nasal blockage, and itching eyes during the months May-August of the second treatment year.

Results: Out of 204 youngsters randomised, 168 entered the intention-to-treat analysis (91 verum, 77 placebo). The mean daily total symptom score did not differ between participants allocated to verum and those allocated to placebo (difference for verum minus placebo: –0.08; 95% CI –0.66 to 0.50; p=0.78). No differences were found for rescue medication-free days, disease-specific quality of life, and overall evaluation of the treatment effect. Local side effects were more frequent in the verum group (39% vs. 17% of participants; p=0.001).

Conclusion: Sublingual immunotherapy with grass pollen in a primary care setting is not effective in children and adolescents.

Clinical implications: Currently, SLIT cannot be recommended for general practitioners as a therapeutic modality in youngsters with grass pollen allergy.

INTRODUCTION

In recent years sublingual immunotherapy (SLIT) has been proposed as an alternative to subcutaneous immunotherapy (SCIT). Owing to a convenient administration form and good safety profile[1,2] SLIT is particularly suitable for children. The evidence on the efficacy of SLIT in children is still inconclusive. Recent meta-analyses showed conflicting results, hampered by the significant heterogeneity in allergens, duration of treatment, and outcome measures of the included studies.[3-5] The absence of serious side effects enables the administration of SLIT in primary care settings. Prescription of immunotherapy by general practitioners has the additional advantage of favouring introduction at an earlier stage of the disease, thereby potentially preventing the onset of asthma and the development of new sensitisations.[6,7] From that perspective, SLIT is ideal to treat children seen in primary care. However, until now all clinical trials involving children have been performed in referral centres only.

We therefore designed a large randomised, double-blind, placebo-controlled trial in a primary care setting to evaluate the efficacy and safety of SLIT in children and adolescents with a grass pollen-induced allergic rhinoconjunctivitis.

METHODS

Design

Using a randomised, double-blind, placebo-controlled trial design participants entered the trial and started treatment after the grass pollen season either in September-October 2001 or in September-October 2002. At the end of the trial 2 years later (in 2003 and 2004, respectively) data were pooled. The ethics committee of the Dutch health authorities and the Erasmus MC-University Medical Centre approved the study protocol. Written informed consents were obtained.

Participants

Youngsters aged 6-18 years with an International Classification of Primary Care code of R97 (hay fever/allergic rhinitis)[8] were invited by their general practitioner and screened by a research assistant. Inclusion criteria were IgE antibodies to grass pollen ≥0.7 kU/L and a history of rhinoconjunctivitis, assessed by a retrospective symptom score: participants scored 5 symptoms (sneezing, itching nose, watery running nose, nasal blockage, and itching eyes) during the previous grass pollen season (May-August) on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe; maximum total score=15). Participants with a retrospective total symptom score ≥5 were included. Exclusion criteria included the use of daily pulmonary inhaled glucocorticoids during ≥3 months in the preceding

year, immunotherapy in the preceding 3 years, sensitisation to pets in the family home (specific IgE ≥0.7 kU/L), nasal abnormalities requiring surgery, and contra-indications for immunotherapy.[9]

Additionally, IgE antibodies to house dust mite, birch, and cat were determined to assess possible multisensitisation. Specific questions on wheezing and dry cough at night from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire[10] established the presence of lower airway symptoms during the last 12 months.

Intervention

Participants underwent verum treatment with a mixture of aqueous extracts of 5 grass pollen species (*Lolium perenne, Phleum pratense, Dactylis glomeratein, Anthoxantum odoratum, and Holcus lanatus*; Oralgen Grass Pollen; Artu Biologicals, Lelystad, The Netherlands) in a glycerinated isotonic phosphate-buffered solution. Placebo treatment consisted of the solvent. Treatment starting with a single drop containing 475 biological units (BU) of allergen was increased with 1 drop daily until day 20. The maintenance dose was 20 drops (9,500 BU; 21 µg equivalent *Lol p 5*) twice weekly for 2 years, resulting in a mean cumulative dose of 1,976,000 BU (4.5 mg equivalent *Lol p 5*). The drops were administered sublingually and kept there for at least 1 minute before being swallowed. A research assistant instructed the participants and provided written instructions. A pharmacist allocated medication in accordance with a computer–generated randomisation list stratifying for symptom score and participating general practice. Participants, parents, investigators and caregivers were unaware of the group assignment and could not make a distinction between verum and placebo treatment.

Outcome measures

The primary outcome in this study was the mean daily total symptom score comprising sneezing, itching nose, watery running nose, nasal blockage, and itching eyes in the second treatment year. The symptoms were scored on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe) and recorded on diary cards during the period May 1-August 31. Beforehand, several measures were taken to ensure that days with sufficient exposure to grass pollen would be analysed (see Statistical Analysis). Daily pollen counts were obtained from the pollen-monitoring station in Leiden (Burkard pollen trap, Leiden University Medical Centre). These counts represented the pollen exposure in the region where the participants were recruited and evaluated.

Secondary outcomes were the percentage symptom-free days, the percentage rescue medication-free days, the type of rescue medication used, disease-specific quality of life, overall evaluation of the treatment effect, and safety.

Rescue medication was recorded on diary cards during the period May 1-August 31. Participants were provided with free cetirizine tablets, xylometazoline nose spray, and

levocabastine eye drops. The use of other antiallergic drugs was allowed. Rescue medication was categorised as follows: cetirizine, xylometazoline, levocabastine eye drops, other oral anti-histamines, nasal inhaled glucocorticoids, other nasal sprays, and other eye drops. Those days eligible for symptom score analysis were eligible for evaluation of rescue medication as well.

Rhinoconjunctivitis-specific quality of life was assessed with the validated Paediatric (6-11 years) and Adolescent (12-17 years) Rhinoconjunctivitis Quality of Life Questionnaire (PRQLQ and AdolRQLQ, respectively)[11,12] at baseline and in June during the peak of the grass pollen season. The mean overall score and the mean score for the domains separately were calculated (scale 0-6, higher score represents lower quality of life).

At the end of the study, both participants and their parents evaluated the overall effect of the treatment on a 6-point scale (1=much worse, 2=worse, 3=unchanged, 4=better, 5=much better, 6=no complaints any more).

To evaluate side effects, participants recorded all complaints irrespective of the relationship with the study medication. The complaints were grouped as follows: oral pharyngeal irritation/swelling, gastrointestinal complaints, rhinitis, conjunctivitis, shortness of breath/cough, eczema/itch/rash, allergy (not specified), and other.

Compliance was determined by weighing the returned study medication and calculating the medication intake during the study period. The participant was considered compliant if the medication intake was ≥80% of prescribed.

A research assistant contacted the participants every 6 weeks during the 2-year follow-up either by visits (March-November) or by telephone.

Statistical analysis

The primary outcome was the mean daily total symptom score during the months May-August of the second treatment year. The sample size was based on an earlier trial among adults.[13] A difference in primary outcome between treatment groups of at least 30% was considered to be the minimal clinical important difference.[14] To detect a difference of 30%, 70 participants were required in each treatment group (2-sided alpha=0.05, power=90%). To allow for dropouts, we aimed to randomise 100 participants to each group.

The protocol incorporated several measures to ensure that only days with sufficient exposure to grass pollen were analysed. First, a minimum mean seasonal grass pollen count of 20-30 pollen grains/m^3 was required for the efficacy of grass pollen immunotherapy to emerge.[15] Therefore, if the mean daily pollen count was less than 25 pollen grains/m^3 during the period of May 15-June 30 of a particular year, that year was considered a lost season and would not be evaluated. Secondly, only those days that exceeded the median pollen count of that year were considered as pollen-relevant days and consequently evaluated. Finally, an often-used and straightforward intention-to-

treat procedure, the last observation carry-forward (LOCF) method, was used to impute missing data. Thus, if a participant left the study before the second year or the diary card assessed during the second year was incomplete (i.e. <50% of pollen-relevant days were filled out), the first year would be analysed, provided that the first year diary was sufficiently complete (i.e. ≥50% of pollen-relevant days were filled out).

Univariate comparison of the primary outcome, all secondary outcomes, and compliance was done by the Mann-Whitney test or Chi-square test in case of percentages.

The main evaluation of the primary end point was done using analysis of covariance (ANCOVA) with the *a priori* defined covariates "age", "sensitisation to house dust mite", "sensitisation to birch pollen" and "retrospective total symptom score" included in the model. "Gender" was also included as a covariate, because the girls/boys ratio at baseline differed between both groups. Appropriate interaction terms were used to investigate whether these baseline variables modified the treatment effect.

To estimate the maximal effect of the intervention in this population, the 95% confidence interval for the ratio (verum/placebo) of the adjusted mean of the daily total symptom score was determined using the bootstrap method.

Preplanned ANCOVA analysis with the covariate "use of nasal inhaled glucocorticoids in the month prior to the evaluated season" and with the covariate "cohort" (i.e. the year of inclusion) also included were performed.

Preplanned ANCOVA subgroup analyses were performed according to age (< or ≥ the median) and the retrospective total symptom score (≤ or > the median).

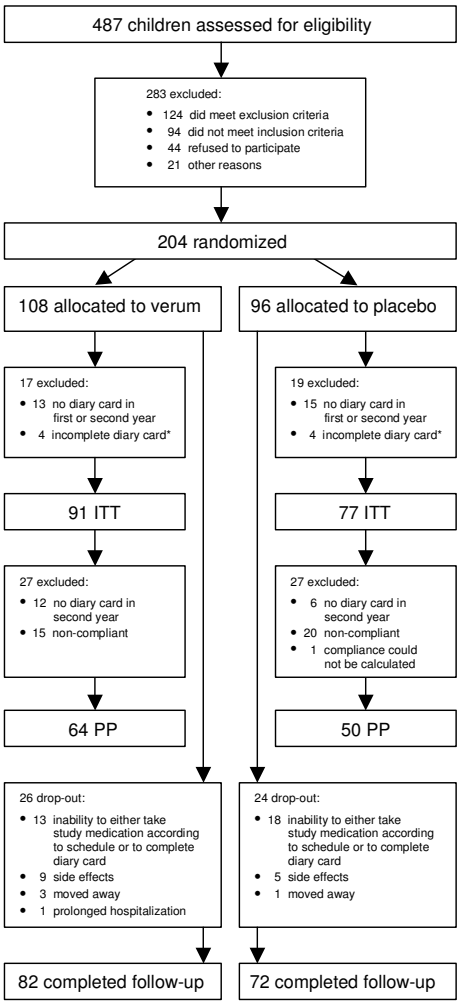
All analyses were done according to the intention-to-treat principle. A per-protocol analysis of the primary outcome was also performed, excluding participants with either an incomplete or a missing diary card in the second year and participants who were noncompliant (i.e. medication intake <80% of prescribed).

The limit of significance in all analyses was considered p=0.05 (2-sided).

RESULTS

From 64 general practices, 1,590 children and adolescents received written invitation to participate. 829 youngsters (52%) responded, of which 384 were not interested in participating, mainly because of a lack of symptoms (n=117). After adding 42 extra youngsters (29 from a pilot study and 13 siblings), 487 participants entered the screening procedure (Figure 1). Two hundred eighty-three children (58%) were excluded, mainly because of the use of daily pulmonary inhaled glucocorticoids during ≥3 months in the preceding year (n=63) and IgE antibodies to grass pollen <0.7 kU/L (n=54). Finally, 204 youngsters were randomly assigned to verum (n=108) or placebo (n=96) treatment. All participants started treatment. Fifty participants (25%) did not complete the 2-year follow-up; 26 of

Figure 1. Trial profile



In the per-protocol analysis, participants with a diary card in the first year only and participants who were noncompliant (i.e. medication intake <80% of prescribed) were excluded

* incomplete diary card: <50% of the evaluated days were filled out.

ITT, intention to treat analysis; PP, per protocol analysis

them withdrew before the first grass pollen season. No statistically significant or clinically relevant difference between groups was found for the number of drop-outs and the reasons for discontinuation. The main reason for discontinuation was the inability either to take the study medication according to schedule or to complete the diary card (n=31).

One hundred sixty-eight participants were included in the intention-to-treat analysis (91 verum, 77 placebo). The baseline characteristics of the intention-to-treat population

Table 1. Baseline characteristics of the intention-to-treat population

	Verum n = 91	**Placebo n = 77**
Age (y)		
mean (SD)	12.9 (2.6)	12.5 (2.9)
median (range)	13.0 (7-17)	13.0 (6-17)
Gender		
number of male participants	61 (67%)	34 (44%)
Retrospective symptom score		
total [mean(SD)]*	8.7 (2.6)	9.0 (2.4)
nose [mean(SD)]**	6.7 (2.2)	7.0 (2.1)
eye [mean(SD)]***	2.0 (0.8)	2.0 (0.9)
Specific IgE grass pollen (kU/L)		
median-range	75.8 (0.8 to >100)	76.8 (1.6 to >100)
No. of participants with multisensitisation (%)#	69 (76%)	57 (74%)
No. of participants with lower airway symptoms (%)$	52 (57%)	46 (60%)

* scale 0-15 ** scale 0-12 *** scale 0-3

sensitisation (IgE antibodies ≥0.7 kU/l) to house dust mite and/or birch pollen and/or cat

$ in the last 12 months (ISAAC questionnaire)

are shown in Table 1. The treatment groups were comparable for age, severity of symptoms, specific IgE, multisensitisation, and lower airway symptoms. By chance, relatively more boys were randomised to verum treatment. The same distribution was observed in the intention-to-treat and per-protocol population.

All seasons could be evaluated, because the prespecified limits of the grass pollen counts were met. During each pollen season of 123 days, 61 days met the criterion of a pollen count above the median. A diary card could be evaluated if at least 50% (i.e. 31 days) was filled out properly. The average evaluated days (SD) was 55 (7) and 54 (8) days in the verum and placebo group, respectively (p=0.45).

The mean daily total symptom score (SEM) in the verum group was 3.1 (0.2) compared with 3.4 (0.2) in the placebo group (p=0.398; Table 2). Analysis of covariance (ANCOVA), allowing for the prespecified covariates plus "gender", was performed with the mean daily total symptom score as outcome parameter (Table 3; total group). The adjusted difference in mean daily total symptom score was small and equalled 0.1 on a scale of 15, in favour of the verum group. The resulting adjusted mean daily total symptom scores for verum and placebo were 3.2 and 3.3, respectively. The ratio of both means (verum/placebo) equalled 0.97 (95% CI, 0.82-1.16). The covariates "gender" and "retrospective total symptom score" had a significant effect on the outcome parameter. Further analysis showed that none of the baseline variables affected the treatment effect. Inclusion of the covariate "cohort" (i.e. the year of inclusion) or "use of nasal inhaled glucocorticoids in the month before the evaluated season" (3 verum, 5 placebo) did not affect the outcomes of the ANCOVA. Age (<13 years and ≥13 years) and the retrospective total

Table 2. Results of the univariate analyses of the primary and secondary outcome measures (intention-to-treat analysis)

	Verum n = 91	Placebo n = 77	P-value
Mean daily total symptom score [0-15]	3.1 (0.2)	3.4 (0.2)	0.398
Percentage symptom-free days [0-100]	27.7 (3.0)	24.1 (3.0)	0.398
Percentage rescue medication free days [0-100]	69.3 (3.4)	74.2 (3.2)	0.674
Overall evaluation [1-6]			
participant	3.9 (0.1)	3.8 (0.1)	0.143
parent[#]	3.7 (0.1)	3.7 (0.1)	0.643
PRQLQ (6-11 y)	n=30	n=26	
total score [0-6]	1.7 (0.2)	1.4 (0.1)	0.799
AdolRQLQ (12-17 y)	n=56	n=47	
total score [0-6]	1.7 (0.2)	2.1 (0.2)	0.272

PRQLQ, paediatric rhinoconjunctivitis quality of life questionnaire; AdolRQLQ, adolescent rhinoconjunctivitis quality of life questionnaire.

All values are mean (SEMs). Data between brackets represent the scales of the various items

PRQLQ-AdolRQLQ: numbers do not add up to 91 and 77, because of occasional missing data (verum 5/91, placebo 4/77)

[#] missing evaluation parent: verum 2, placebo 3

Table 3. Results of the analysis of covariance of the mean daily total symptom score (intention to treat analysis)

Total group - all covariates		**Effect[#]**	**95% CI**	**P-value**
Treatment	(verum minus placebo)	−0.08	−0.66 to 0.50	0.778
Age	(per year)	0.01	−0.10 to 0.11	0.927
Gender	(female minus male)	0.76	0.17 to 1.35	0.012
Sensitisation to house dust mite	(yes minus no)	−0.54	−1.11 to 0.03	0.062
Sensitisation to birch pollen	(yes minus no)	0.35	−0.22 to 0.91	0.230
Retrospective total symptom score	(per point)*	0.14	0.03 to 0.26	0.014
Subgroups - covariate treatment	**N (verum/placebo)**	**Treatment effect[#]**	**95% CI**	**P-value**
Age				
<13 y	36/38	0.12	−0.62 to 0.87	0.744
≥13 y	55/39	−0.02	0.92 to 0.88	0.963
Retrospective total symptom score*				
≤8	47/38	0.03	−0.77 to 0.83	0.934
>8	44/39	−0.14	−1.05 to 0.76	0.756

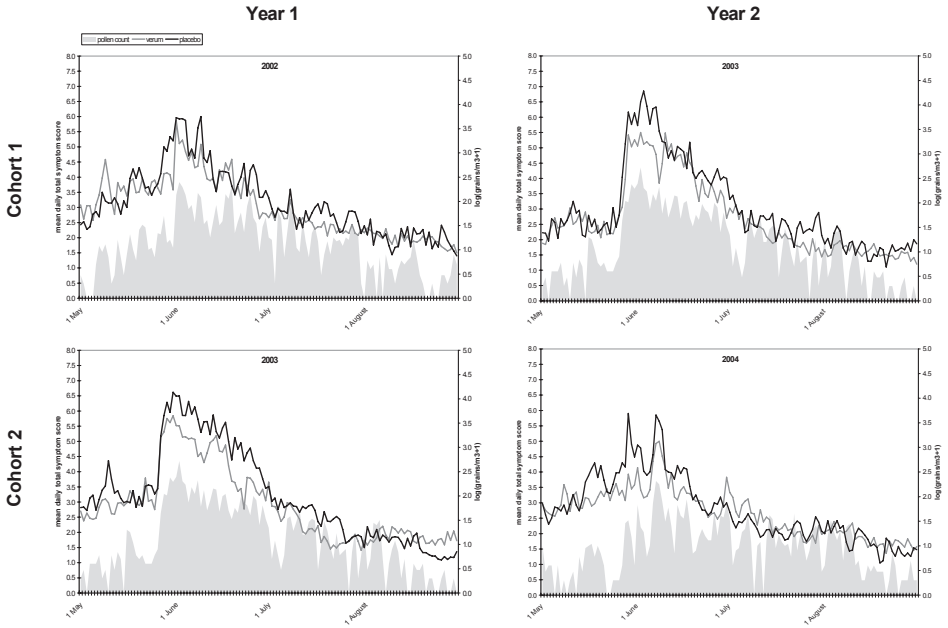
[#] difference in mean daily total symptom score (verum minus placebo)

* scale 0-15

symptom score (≤8 and>8) categories equally affected the adjusted difference of the means (Table 3; subgroups).

Per-protocol analysis of the primary end point also did not reveal a significant difference between both treatment groups.

Figure 2. Mean daily total symptom scores and grass pollen counts during the first and second year of treatment according to cohort



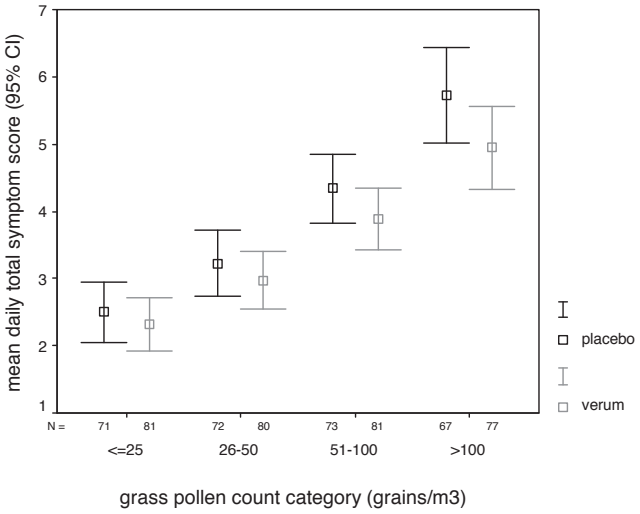
As shown in Figure 2, symptoms scores and pollen counts in both treatment groups parallel each other closely. Study days of the second year were grouped according to the daily pollen counts (<25, 25-50, 51-100, >100 grains/m^3), and within each category the mean daily total symptom score was determined for each participant. Figure 3 clearly shows an increase in mean daily total symptom scores with increasing pollen count. No significant differences between treatments within pollen count categories were observed (all 4: p-values ≥0.30).

In line with these findings, analyses of the secondary outcome measures shown in Table 2 did not reveal statistically significant differences between treatment groups. No significant difference was found for the total days on which rescue medication was used. Analysing categories of rescue medication, no difference between verum and placebo group regarding the mean percentage of days with cetirizine (18.9% vs. 17.2%), levocabastine eye drops (8.6% vs. 6.9%), and nasal inhaled glucocorticoids (6.0 vs. 4.0%) use was observed. Only the use of xylometazoline differed significantly between treatment groups (verum 3.4% vs. placebo 5.9% of days; p=0.022).

Separate PRQLQ and AdolRQLQ domains also did not differ between treatment groups.

Regarding possible side effects, only local oral pharyngeal reactions were more frequently recorded in the verum group (verum 39% vs. placebo 17% of participants; p=0.001; Table 4). These reactions were mild. Systemic anaphylactic reactions did not

Figure 3. Mean daily total symptom scores (with 95% CI) according to grass pollen count category (grains/m^3) and treatment. Data represent outcomes for the second year of treatment



N, number of participants

Table 4. Adverse events

	Verum n = 108	Placebo n = 96	P-value*
Local reactions			
Oral pharyngeal irritation/swelling	42 (39%)	16 (17%)	0.001
Gastrointestinal complaints	80 (74%)	70 (73%)	0.978
General reactions			
Rhinitis	89 (82%)	76 (79%)	0.682
Conjunctivitis	53 (49%)	54 (56%)	0.377
Shortness of breath/cough	29 (27%)	28 (29%)	0.833
Eczema/itch/rash	42 (39%)	34 (35%)	0.714
Allergy (not specified)	10 (9%)	9 (9%)	1.0
Other	102 (94%)	90 (94%)	1.0

Data represent number (percentage) of participants

* Chi-square test

occur. Hospital admissions (none related to study medication) were noted in 13 children (9 verum, 4 placebo).

The percentage of compliant participants did not differ significantly between both groups (80% [73/91] verum vs. 71% [55/77] placebo; p=0.312).

DISCUSSION

This is, at present, the largest study with SLIT in children and the first performed in a setting that is considered ideal for the use of SLIT. In this study we demonstrated that, when used in a primary care setting, SLIT with grass pollen is not effective in reducing symptoms and medication use in youngsters with rhinoconjunctivitis. Until now, all trials have been performed in referral centres and the evidence resulting from these relatively small and heterogeneous studies is conflicting.[16-25] The 2 large randomised, double-blind, placebo-controlled trials using grass pollen allergen in children with rhinoconjunctivitis demonstrated moderate effects only. Bufe et al.[16], using a composite symptom-medication score, showed no difference between active and placebo treatment after one year follow-up. After another 2 years in an open setting, in which all children received SLIT, post hoc analysis showed a favourable effect of SLIT in a subgroup of patients with severe symptoms.[16] Rolinck-Werninghaus et al.[17] demonstrated an effect on medication scores only, without apparent influence on symptoms. In contrast, the present study showed no effect of SLIT. Moreover, we did not identify subgroups that might benefit from this treatment. The lower limit of the ratio (verum/placebo) of the mean daily total symptom scores amounted to 0.82, representing at most an 18% reduction in symptom scores. Therefore, we could not reach the minimal clinical acceptable improvement of 30%.[14] Before accepting these results, we have to address several issues.

The present large trial -the only one in children in a primary care setting- has the advantage that results are relevant and applicable for children with hay fever presenting to general practitioners. An issue to be addressed is whether efficacy could be demonstrated in these patients from a primary care setting. Two large studies showed that approximately 90% of the patients consulting general practitioners have moderate/severe rhinitis.[26,27] Apart from the retrospective symptom score, we do not have an indication of symptom severity before the study. However, as the quality-of-life data obtained during the trial demonstrated that participants experienced practical problems and daily life activity impairments, rhinitis severity could be labelled at least as moderate[28], whereas the percentage of days with symptoms (verum 72.3% and placebo 75.9%) refers to persistent rhinitis[28]. Moreover, patients were independently selected by researchers from the University Hospital according to predefined criteria taking the degree of grass pollen sensitisation and the level of symptom severity into account. Therefore, the study population should be appropriately composed to detect treatment effects. Especially in a paediatric population, there is reluctance to use (long-term) pharmacological treatment. Not only are such patients good candidates for immunotherapy according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines[28], SLIT with its good safety profile might be particularly tailored to the needs of these patients.

To include participants with sufficient symptoms and to ensure stratification of the treatment groups as to disease severity, we used a post-season retrospective instead of a run-in season symptom score. Although a retrospective score cannot be considered a substitute for baseline evaluation, Bodtger et al.[29] showed that even though post-season assessment using a 4-point scale tends to overrate average severity it could be used as the basis of randomisation. We could exclude a possible effect of overrating, because participants with a retrospective total symptom score higher than the median did not show significant treatment effect.

It is often suggested that multisensitised patients might not benefit from specific immunotherapy as much as monosensitised patients, but, as indicated in the ARIA guidelines, further research on this subject is needed.[28] In our study we included both mono-and multisensitised participants. About 75% of the participants were multisensitised, possibly representing the average allergic population seen both in general and in hospital-based practice. Analysis of the subgroup of monosensitised participants showed no significant difference between the verum and the placebo group (data not shown). In line with this observation, we did not see any effect from co-sensitisation in the ANCOVA.

To assess efficacy, only days with sufficient pollen counts were analysed. Symptoms fluctuated with the level of exposure, and even on days with high exposure and therefore higher symptom scores there was no significant difference between the treatment groups. It is not very likely that rescue medication, in particular nasal steroids, have masked efficacy, because the use of concomitant medication was low and well balanced between groups. According to the protocol, all days regardless of the pollen count were analysed as well (data not shown). In that analysis we also could not demonstrate an effect of SLIT on the primary outcome or on medication use. Although participants were enrolled in 2 consecutive years, analysis showed that this did not affect the outcome.

One of the great advantages of SLIT is a good safety profile. In our study, minor side effects only were reported in both treatment groups. Not unexpectedly, significantly more local reactions in the oral pharyngeal cavity were reported in the verum group. In a study among adults enrolled from general practices, common but well tolerated side effects led to the conclusion that SLIT is safe to use in a community setting.[30]

Data on compliance with SLIT are seldom reported.[16-18,23,24] Those studies do not predefine compliance, and it is often unclear whether the results represent compliance to medication intake or to the study protocol. Moreover, they lack information on data collection or use patient reports instead of investigator-verified medication intake. The overall compliance to medication intake in our study was good, especially considering the age of the participants and the duration of the study. In daily clinical practice where patients are not intensively supervised, compliance might drop.

The issue of optimal dosage has not been solved yet. The wide range of allergen preparations used and a lack of information concerning the dose expressed in micrograms of major allergen make it difficult to compare studies. In the present study, the total dose was 4.5 mg equivalent *Lol p 5* given over 24 months. The only 2 other studies in children using grass pollen allergen and reporting the dose of major allergen in micrograms used different dosages: 0.188 mg major grass pollen allergen given over 32 months[17] and 9.6 mg equivalent *Phl p 5* given over 36 months[16]. Nevertheless, it cannot be excluded that a higher dosage may lead to better results. A recent study with grass pollen SLIT in adults suggested a dose-related response. Treatment with the highest dose of 1.9 mg equivalent *Phl p 5* given over 4.5 months, however, did not lead to a clinically relevant reduction in symptom scores.[31]

In conclusion, in this large randomised, double-blind, placebo-controlled study in a primary care setting, SLIT with grass pollen was not effective in children and adolescents with seasonal allergic rhinoconjunctivitis. Currently, SLIT cannot be recommended for general practitioners as a therapeutic modality in children or adolescents with grass pollen allergy.

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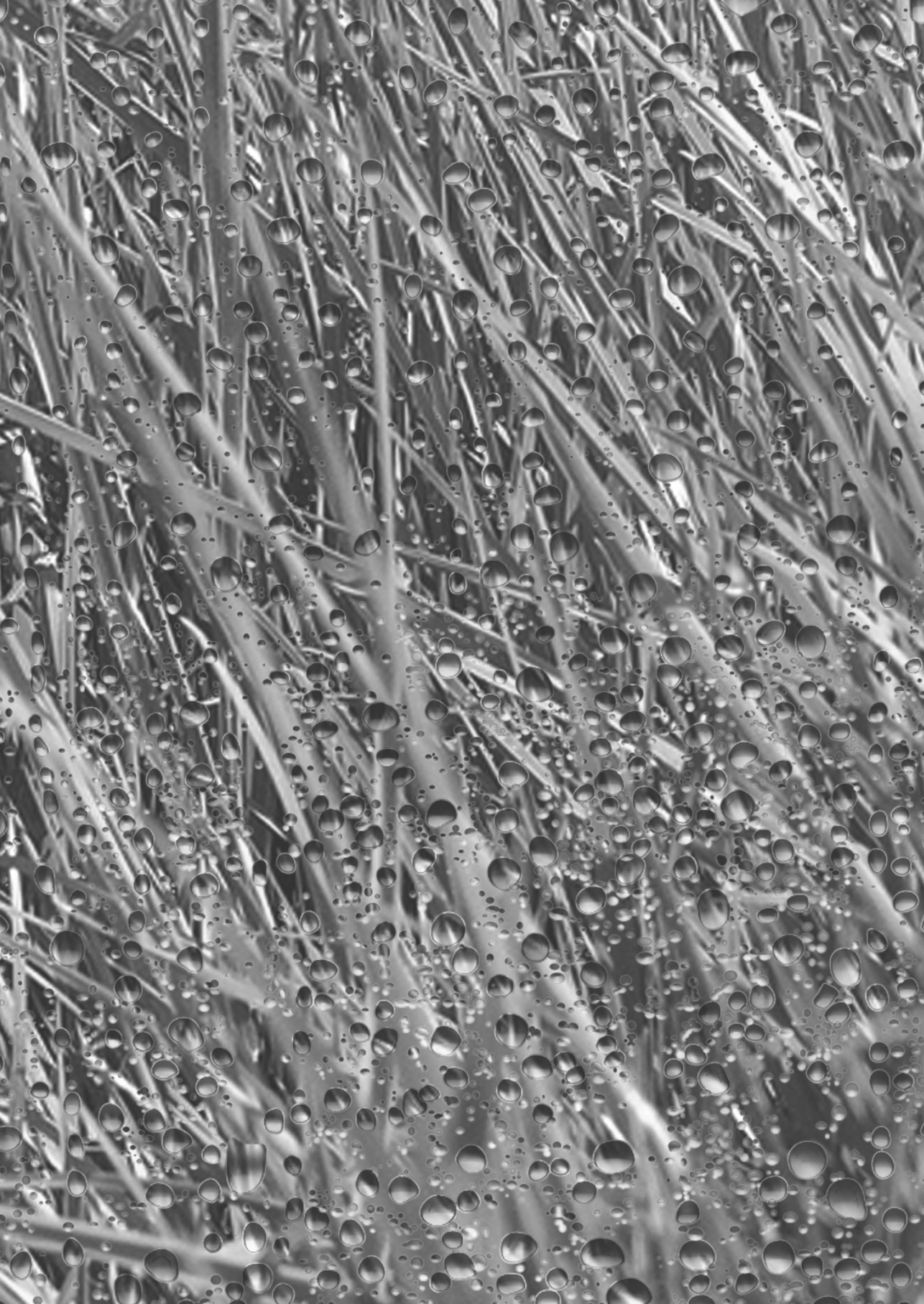

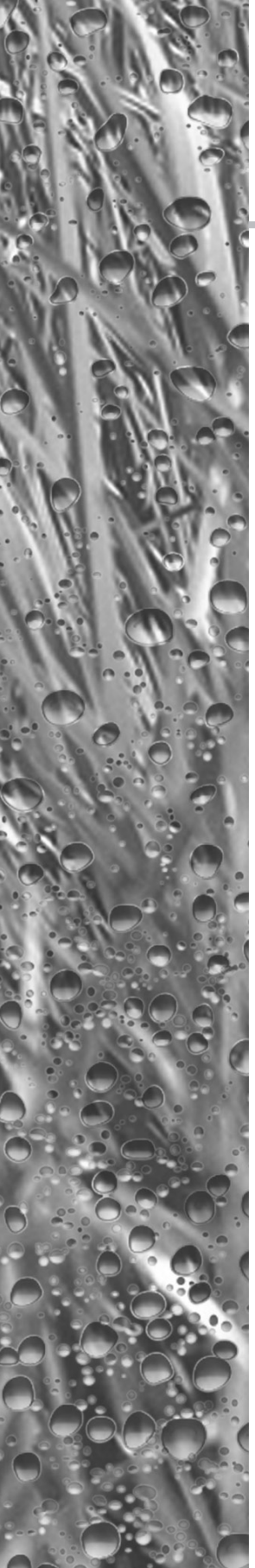

Chapter 4

Sublingual immunotherapy in youngsters: adherence in a randomised clinical trial

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ABSTRACT

Background: Adherence is essential for effective treatment. Although several trials on the efficacy of sublingual immunotherapy (SLIT) in youngsters have been published, few contain data on medication intake.

Objective: We aimed to quantify adherence both to study protocol and medication intake as well as to identify factors that may influence adherence to SLIT in youngsters with rhinoconjunctivitis.

Methods: 204 youngsters (6-18 years) with hay fever participated in a randomised controlled trial and used grass pollen extract or placebo for 2 years. The primary outcome of the trial was the mean daily total rhinoconjunctivitis symptom score in the second grass pollen season. Participants having completed the follow-up were considered adherent to the study protocol. Adherence to medication intake was assessed by weighing the study medication. Participants who completed the follow-up and used ≥80% of the prescribed medication were considered adherent to medication intake. Patient-, disease- and treatment-related factors were analysed.

Results: 154 youngsters completed the study. Main reason for discontinuation was the inability to take medication according to schedule. Drop-outs were older, had more difficulty following the medication instructions and their overall evaluation of the treatment effect was lower. The number and reasons for drop-out did not differ between treatment groups. In total 77% of the participants was adherent to medication intake. Self-reported adherence was 99%. Non-adherent participants experienced more severe symptoms before the trial. Symptom scores did not differ between adherent and non-adherent participants. In adherent as well as non-adherent participants, no difference was found between verum and placebo group with respect to symptom scores.

Conclusion: Adherence to both study protocol and medication intake was good. Drop-out was affected by age, evaluation of the treatment effect and medication instructions. Non-adherence to medication intake was influenced by the severity of the disease before the trial. The ineffectiveness of SLIT could not be explained by non-adherence.

INTRODUCTION

The efficacy of sublingual immunotherapy (SLIT) in youngsters with rhinoconjunctivitis is still subject of debate. An important issue in any discussion on the efficacy of a drug is adherence.

Adherence (or compliance) is generally defined as the extent to which patients use their medication as prescribed. Adherence is essential for the efficacy of a treatment to emerge. Medication intake can be assessed using direct or indirect methods. Direct methods measure the concentration of a drug or its metabolite in blood or urine. Questionnaires and counting left-over tablets are examples of indirect methods. Age, severity of the disease and adverse events are a few factors that influence adherence.[1]

The good safety profile and route of administration give SLIT the advantage of administration at home, taking away an important reason for non-adherence with the subcutaneous form.[2-4] On the other hand, self-administration and consequently lack of supervision might lead to incorrect or irregular use. Although several randomised placebo-controlled trials on the efficacy of SLIT in youngsters with rhinoconjunctivitis have been published[5-15], few contain data on medication intake. In some articles compliance is mentioned, but the results do not refer to medication intake, but to the number of drop-outs[11-13] or it is not clear what the results represent[15]. Furthermore, where medication intake was registered[6,10,14], the results are not always presented[10] or are difficult to interpret, because compliance is not defined[14]. Only one study describes medication intake in detail.[6] Factors that could influence adherence have never been investigated.

As part of a randomised placebo-controlled trial on the efficacy of SLIT with grass pollen allergen in youngsters with rhinoconjunctivitis, we aimed to quantify adherence both to the study protocol and medication intake, as well as to identify factors that may influence adherence.

METHODS

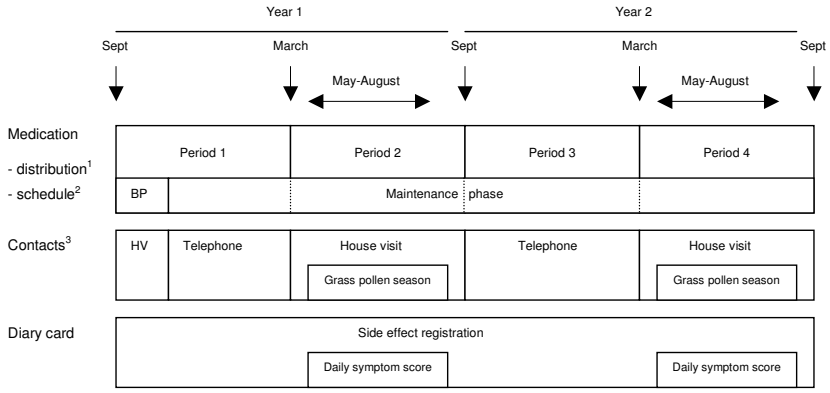
Participants and study design

A detailed description of the randomised, double-blind, placebo-controlled trial has been reported elsewhere.[16] The population and study design (Figure 1) are summarised below.

Participants

204 youngsters aged 6-18 years (114 boys/90 girls; mean age [SD] 12.9 [2.8] years) with hay fever were enrolled from general family practices in The Netherlands and randomly assigned to verum (n=108) or placebo treatment (n=96). All participants had

Figure 1. Study design



[1] At the beginning of each period 4 bottles were handed out; at the end of the period the medication was handed in and weighed

[2] Build-up phase (BP): 20 days, daily intake, increasing with 1 drop; Maintenance phase: twice weekly intake, 20 drops

[3] Every six weeks, either by house visit (HV) or telephone

IgE antibodies to grass pollen ≥0.7 kU/l (Phadia) and a history of rhinoconjunctivitis. The latter was assessed by a retrospective symptoms score: participants scored 5 symptoms (sneezing, itching nose, watery running nose, nasal blockage and itching eyes) during the previous grass pollen season (May-August) on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe; maximum total score=15). Participants with a retrospective total symptom score ≥5 were included. Participants with daily pulmonary inhaled glucocorticoids during ≥3 months in the preceding year, or immunotherapy in the preceding 3 years were excluded. Other exclusion criteria were sensitisation (specific IgE ≥0.7 kU/l) to pets present in the family home, nasal abnormalities requiring surgery and general contra-indications for immunotherapy.[17]

Study design

Participants entered the trial and started treatment after the grass pollen season, either in September-October 2001 or in September-October 2002. Both cohorts ended the trial two years later (in 2003 and 2004, respectively). Data were pooled at the end of the study.

The primary outcome of our trial was the mean daily total symptom score comprising sneezing, itching nose, watery running nose, nasal blockage and itching eyes in the second treatment year. The symptoms were scored on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe) and recorded on diary cards during the period May 1-August 31. To evaluate side effects participants recorded all complaints irrespective of the relationship with the study medication on a diary card.

Participants, parents, investigators and caregivers were unaware of the group assignment. Verum treatment consisted of a mixture of aqueous extracts of 5 grass pollen species (*Lolium perenne, Phleum pratense, Dactylis glomeratein, Anthoxantum odoratum, Holcus lanatus*; Oralgen Grass Pollen; Artu Biologicals, Lelystad, The Netherlands) in a glycerinated isotonic phosphate-buffered solution. Placebo treatment consisting of the solvent was delivered in such a way that neither participants, parents, investigators, nor caregivers could make a distinction between verum and placebo. Treatment started with a single drop and was increased with one drop daily until day 20. The maintenance dose was 20 drops twice weekly for 2 years. The drops were administered sublingually before breakfast (on an empty stomach) and kept there for at least 1 minute before being swallowed.

A research assistant instructed the youngsters and their parents in the use of the study medication. Participants received written instructions, a schedule to record medication intake and an hourglass for timing the sublingual phase. They were contacted by a research assistant every 6 weeks during the 2-year follow-up, either by visits (March-October) or by telephone. The first follow-up contact took place in the final stage of the build-up phase. Medication use was checked during each contact and when necessary questions were answered and/or instructions repeated.

Adherence

Adherence to study protocol, i.e. drop-outs

Participants having completed the study were considered adherent to the study protocol. Drop-outs were asked for the reason of discontinuing their participation in the study.

Adherence to medication intake

Because no methods for measuring blood- or urine-levels are available, medication intake was measured by weighing the study medication.

Every six months, the participants received a standard amount of study medication (4 bottles). The study medication was handed in at the end of every 6-month period and the bottles were weighed. The participants were unaware of the fact that the study medication was being weighed. The second and fourth period included the grass pollen season.

Adherence was determined for the total 2-year study period and for each of the four 6-month periods separately. In the absence of a clear-cut definition of adherence, we applied a cut-off level of ≥80%.[18] First, the weight of the drops that were prescribed was calculated (prescribed). Secondly, the weight of the returned bottles was subtracted from the weight of the full bottles and the weight of the consumed drops computed

(consumed). Finally, if the participant completed the follow-up and the amount of consumed study medication was ≥80% of the amount that was prescribed [(consumed/prescribed) x 100%] the participant was considered adherent to medication intake. Adherence in a certain period could only be calculated if a participant returned all bottles. Only those periods in which all bottles were returned were considered eligible for the determination of the adherence in the total study period.

Additionally, the youngsters were asked during each contact to report the number of drops they forgot to take since the last contact to determine the self-reported adherence for the total study period and for each period separately. If a participant stated that ≥80% of the amount that was prescribed was taken, the participant was considered self-reported adherent.

Factors influencing adherence

All data, with the exception of multisensitisation, were collected using structured questionnaires.

1 Patient-related factors
   a) Age
   b) Gender
   c) Expected treatment allocation: at the end of the study the participants were asked if they thought they received the grass pollen extract or placebo
   d) Overall evaluation treatment effect: at the end of the study, the youngsters judged the overall effect of the treatment on a 6-point scale (1=much worse, 2=worse, 3=unchanged, 4=better, 5=much better, 6=no complaints any more).
2 Disease-related factors
   a) Duration of disease: parents reported the age at which symptoms first occurred
   b) Severity of the disease: retrospective total symptom score (as described in the Method-section)
   c) Multisensitisation: IgE antibodies ≥0.7 kU/l to house dust mite and/or birch pollen and/or cat
3 Treatment-related factors
   a) Complexity of the instructions: participants were asked if they considered the following instructions difficult (yes/no): time of day (before breakfast, on an empty stomach); sublingual application (getting and keeping the drops under the tongue); counting drops; build-up phase (daily intake, increasing with one drop); maintenance-phase (twice-weekly intake of 20 drops); keeping medication refrigerated. Also, the total number of instructions that was considered difficult was calculated.
   b) Local side effects: the presence of oral pharyngeal irritation/swelling.

Additionally, at the end of the study, the participants were asked if they had suggestions to enhance adherence in future users.

Statistical analysis

Univariate comparison was done by the Mann-Whitney test or Chi-square test in case of percentages. P=0.05 (two-sided) was considered the limit of significance.

RESULTS

Adherence to the study protocol, i.e. drop-outs

Fifty participants (25%; 26 verum, 24 placebo) did not complete the 2-year follow-up, 26 (52%) of them withdrew before the first grass pollen season. The mean duration of participation of the drop-outs was 231 days (SD=183). The main reason for discontinuation was the inability to take the study medication according to schedule (n=26; Table 1). Neither the number of drop-outs nor the reasons for discontinuation for the total study period as well as for the periods separately differed between youngsters allocated to verum and those allocated to placebo.

Participants who discontinued the study, compared to participants having completed the study were slightly older, had more difficulty in following the instructions and their overall evaluation of the treatment effect was lower (Table 2a).

80% (24/30) of the drop-outs reported forgetting drops during the build-up phase compared to 37% (55/150) of the youngsters who completed the follow-up period ($p<0.0001$). No difference was found between groups considering forgetting drops during the maintenance phase, e.g. 72% (110/152) complete follow-up vs. 73% (22/30) drop-outs.

Table 1. Adherence to study protocol: drop-out according to reason and time

	Period 1	Period 2	Period 3	Period 4	Total
Inability to take study medication according to schedule	8 (16%)	1 (2%)	3 (6%)	1 (2%)	13 (26%)
Inability to complete the diary cards	0 (0%)	4 (8%)	1 (2%)	0 (0%)	5 (10%)
Inability to take study medication according to schedule and to complete the diary cards	4 (8%)	5 (10%)	3 (6%)	1 (2%)	13 (26%)
Side effects	12 (24%)	1 (2%)	0 (0%)	1 (2%)	14 (28%)
Prolonged hospitalisation	0 (0%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)
Moved away	2 (4%)	1 (2%)	1 (2%)	0 (0%)	4 (8%)
Total	26 (52%)	12 (24%)	8 (16%)	4 (8%)	50 (100%)

Number of participants (percentage of total number of drop-outs, n=50)

Period 2 and 4 included the grass pollen season

Table 2. Factors influencing adherence

A Total population: complete follow-up vs. drop-outs	Complete follow-up n=154	Drop-out n=50	P-value
Patient			
Age (mean-SD)	12.6 (2.7)	13.7 (2.8)	p<0.001
Gender (N male participants)	88 (57%)	26 (52%)	ns
Expected treatment allocation (N verum)	64 (43%)**	16 (52%)[#]	ns
Overall evaluation of treatment effect (mean-SD) [1-6]	3.9 (0.9)	3.2 (1.1)***	p<0.0001
Disease (baseline)			
Duration (yr; mean-SD)	5.9 (3.5)	6.3 (3.7)	ns
Severity (mean-SD) [0-15]	8.8 (2.5)	9.2 (2.4)	ns
Multisensitisation[$] (N)	115 (75%)	33 (66%)	ns
Treatment			
Verum (N)	82 (53%)	26 (52%)	ns
Instructions considered difficult[#]			
time of day (N)	61 (40%) *	23 (74%)	p<0.001
sublingual application (N)	64 (42%)	16 (52%)	ns
counting drops (N)	20 (13%)	7 (23%)	ns
build-up phase (N)	56 (36%)	18 (58%)	p<0.05
maintenance-phase (N)	24 (16%)	14 (47%)*	p<0.001
keeping medication refrigerated (N)	25 (16%)	7 (23%)	ns
total number (mean-SD) [0-6]	1.6 (1.3) *	2.8 (1.2)*	p<0.0001
Local side effects (N)	45 (29%)	13 (26%)	ns

B Complete follow-up[##]: adherent vs. non-adherent	Adherent n=118	Non-adherent n=35	P-value
Patient			
Age (mean-SD)	12.5 (2.9)	13.0 (1.9)	ns
Gender (N-male participants)	70 (59%)	17 (49%)	ns
Expected treatment allocation (N-verum)	49 (43%)**	15 (43%)	ns
Overall evaluation of treatment effect (mean-SD) [1-6]	3.9 (0.9)	3.8 (1.0)	ns
Disease (baseline)			
Duration (yr; mean-SD)	6.0 (3.6)	5.7 (3.2)	ns
Severity (mean-SD) [0-15]	8.6 (2.6)	9.4 (2.0)	p<0.05
Multisensitisation[$] (N)	88 (75%)	26 (74%)	ns
Treatment			
Verum (N)	67 (57%)	15 (43%)	ns
Instructions considered difficult			
time of day (N)	43 (37%)*	18 (51%)	ns
sublingual application (N)	49 (42%)	14 (40%)	ns
counting drops (N)	16 (14%)	4 (11%)	ns
build-up phase (N)	42 (36%)	14 (40%)	ns
maintenance-phase (N)	19 (16%)	5 (14%)	ns
keeping medication refrigerated (N)	18 (15%)	7 (20%)	ns
total number (mean-SD) [0-6]	1.6 (1.3)*	1.8 (1.3)	ns
Local side effects (N)	36 (31%)	9 (26%)	ns

N, number of participants

[$] additional sensitisation (specific IgE ≥0.7 kU/l) to house dust mite, birch pollen and/or cat

[#] drop-out n=31, 19 drop-outs refused to fill out the questionnaire

[##] adherence could not be calculated for 1 participant

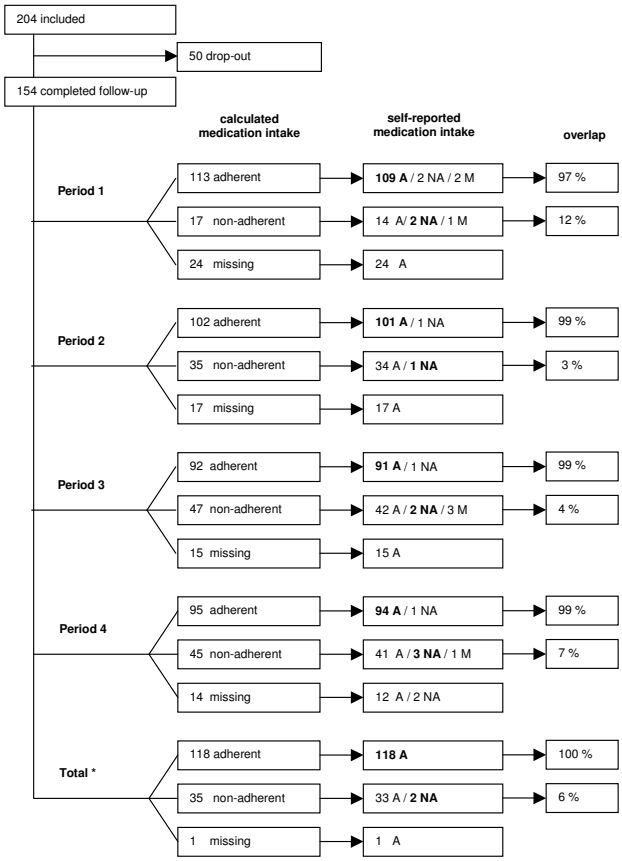
*1 missing, ** 5 missing, ***6 missing

Adherence to medication intake

154 youngsters completed the study (82 verum, 72 placebo). 101 participants returned all bottles in 4 periods, 40 participants in 3 periods, 10 participants in 2 periods and 2 participants in 1 period. One participant failed to hand in a complete set of study medication in all periods and therefore adherence could not be calculated. Disposal of an empty or broken bottle was the main reason for not returning study medication. The average weight of the provided medication (in 4 full bottles) and the prescribed medication was 94.5 and 57.5 g, respectively.

77% (118/153) of the participants were considered adherent in the 2-year follow-up (Figure 2 and 3). These adherent participants used on average 100% (SD=15) of the

Figure 2. Calculated and self-reported adherence to medication intake

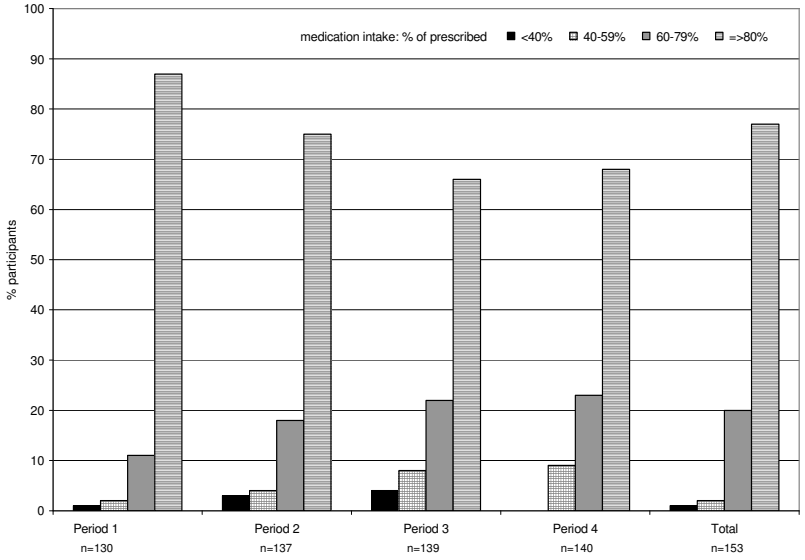


Adherent (A), medication intake ≥ 80% of prescribed; Non-adherent (NA), medication intake < 80% of prescribed; Missing (M, ≥1 bottle not returned (calculated intake) or missing data (self-reported intake).

* For the total study period only those periods in which all bottles were returned (calculated intake) or all data were available (self-reported intake) were considered.

Period 2 and 4 included the grass pollen season

Figure 3. Adherence to medication intake in participants having completed the follow-up period



Period 2 and 4 included the grass pollen season

prescribed study medication. The percentage adherent participants did not differ significantly between both treatment groups (82% [67/82] verum vs. 72% [51/71] placebo). Adherence in the first, second, third and fourth 6-month period was 87%, 74%, 66% and 68% respectively (Figure 2 and 3). Considering the 101 participants who returned all 16 bottles, 78% of these youngsters was adherent for the total study period. They used on average 94% (SD=20) of the prescribed medication. The non-adherent participants used on average 68% (SD=11) of the prescribed study medication.

The self-reported adherence was 99% (151/153) in the total study period and 95%, 99%, 96% and 96% in the first, second, third and fourth period, respectively (Figure 2). The overlap between calculated (non-)adherence and self-reported (non)-adherence was 78% (120/153) in the total study period and 85%, 74%, 67% and 69% in the first, second, third and fourth period respectively (Figure 2). In adherent participants the overlap varied from 97-100%, whereas the overlap in non-adherent participants ranged from 3-12% (Figure 2). Similar results were found for the 101 participants returning all bottles in all periods. Adherent and non-adherent youngsters reported comparable results on forgetting drops during the build-up phase (37% [42/115] adherent vs. 35% [12/34] non-adherent) or during the maintenance phase (72% [83/116] adherent vs. 74% [26/35] non-adherent). Adherent participants reported to miss 0.03% of the intakes, whereas non-adherent participants missed 0.06%. Most participants were supervised by their parent(s), only 8% (10/118) of the adherent and 11% (4/35) of the non-adherent youngsters were solely responsible for medication intake.

Adherent participants could be distinguished from non-adherent participants in severity of the disease prior to the study only. Non-adherent youngsters experienced more severe symptoms in the season preceding the trial (Table 2b).

The mean daily total rhinoconjunctivitis symptom score (SD), the primary outcome of our study, did not differ between adherent and non-adherent participants (3.2 [1.8] vs. 3.5 [2.3]). If considered separately, in adherent as well as non-adherent participants, no difference was found between the verum and placebo group with respect to the symptom scores.

DISCUSSION

Adherence to the study protocol

The drop-out rate in our trial (25%) was comparable to other trials using pollen extracts. Seasonal allergen trials[8,9,12-15] (all year treatment) seem to have higher drop-out rates (mean 14%; range 0-23%) compared to trials using house dust mite extracts[5,6,10,11] (mean 4%, range 0-12%). Possible explanations could be that in general seasonal allergen trials last longer than trials using a perennial allergen and that house dust mite patients have complaints throughout the year, which might keep them motivated.

The number and reasons for drop-out did not differ between treatment groups. Therefore, it is unlikely that drop-out influenced the results on efficacy.

The main reason for discontinuing the study was difficulty in following the treatment schedule. Most drop-outs already forgot to take their medication in the first weeks of treatment. Several preparations from various manufacturers are available on the market. These preparations differ in administration form (drops/vials/tablets) and instructions. However, most preparations have one instruction in common, that being the medication has to be taken before breakfast, an instruction that was considered difficult by 74% of the drop-outs.

All trials on the efficacy of SLIT in youngsters report the number of drop-outs for each treatment group and the reasons for discontinuation are in general clearly stated. No data are available from other trials on factors influencing drop-out. In one observational study only, Pajno et al. investigated the drop-out rate and the reasons for stopping immunotherapy in children (6-15 years) using subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT) or local nasal immunotherapy (LNIT). The drop-out rates after 3 years were 11%, 21,5% and 73% for SCIT, SLIT and LNIT respectively.[19] Most participants discontinued SCIT because they considered the treatment expensive or time-consuming. High costs, followed by the ineffectiveness of the treatment, were the main reason for discontinuing SLIT. More than 50% of the youngsters using LNIT stopped treatment due to the unpleasant nasal reactions. The authors concluded that

SCIT is the most suitable immunotherapy form and route of administration in children and adolescents. It is, however, difficult to compare these data to our study as our study was placebo-controlled, the participants were more frequently supervised and the study medication was free of charge.

Adherence to medication intake

The overall adherence to medication intake in our trial was good. Previously reported average adherence to treatment by children and adolescents was 58%.[1] In our study 97% (148/153) of the youngsters used more than 58% of the prescribed medication. Self-reported medication intake was overestimated, as has previously been shown for inhaled steroids in children with asthma, also a long-term preventive therapy.[20] Medication intake decreased gradually, but appeared to stabilise after 1 year. Grass pollen exposure and concomitant symptoms did not influence medication intake. Only one difference between adherent and non-adherent participants could be found. Non-adherent youngsters experienced more severe symptoms in the season preceding the trial. A possible explanation could be that youngsters with more severe symptoms might have had more symptoms because they were also non-adherent to pharmacotherapy in previous seasons. Another reason could be that these youngsters had higher expectations of the treatment.

Adherence and efficacy are inter-related. Non-adherence compromises efficacy and inefficacy may lead to non-adherence. However, in our study SLIT was ineffective, but the adherence to medication intake was good and comparable in both intervention groups. Therefore, the ineffectiveness of SLIT could not be explained by non-adherence.

In three randomised placebo-controlled trials in youngsters with allergic rhinitis, participants recorded their medication intake on a diary card.[6,10,14] Bahçeciler et al.[10] did not present the results. Rolinck-Werninghaus et al.[14] reported the number of drop-outs due to non-compliance, but it was not clear how compliance was defined. Hirsch et al.[6] presented a detailed report of the medication intake and showed that 53% (8/15) and 67% (10/15) of the participants (6-15 years) in the SLIT and placebo group, respectively, reported complete regular intake.

In a non-controlled study, participants were called unannounced after 3 and 6 months of treatment and asked to count the left-over mono-dose vials. Passalacqua et al.[21] reported that 69% (49/71) and 66% (37/56) of the participants (2-13 years) used more than 90% of prescribed after 3 and 6 months, respectively.

Indirect methods (e.g. weighing, counting, diary card) have been used in all studies to measure adherence. A major disadvantage of these indirect methods is that there is no guarantee that the medication was indeed consumed. For instance, in our trial, the liquid could have been thrown away. Moreover, the average rate of adherence in clinical trials is higher than in daily practice, due to the selection of participants and the atten-

tion participants receive during the study. Therefore, the actual percentage of adherent participants in daily clinical practice will be lower. Although measuring medication intake in a trial may overrate adherence, the results are essential for the interpretation of the (in)efficacy of a treatment.

Enhancing adherence

We received several suggestions from the youngsters to enhance adherence. One of the major problems was taking the medication before breakfast, the busiest time of the day according to the youngsters. Participants indicated that it would be easier if the medication could be used during the day. Most participants used a reminder. They placed the schedule on a visible location (e.g. on the refrigerator), put the hourglass on their breakfast plate or set an alarm. Some youngsters preferred to receive a message on their mobile phone on the day of medication intake. Counting drops was not a major problem, but a large number of participants considered it difficult to get and keep the drops under their tongue. Several youngsters suggested the use of a tablet. Keeping medication refrigerated was not considered very difficult, however, participants reported that keeping the medication cool when travelling and on holiday was problematic. Although other preparations can be kept at room temperature (15-25 °C), one still has to take measures to keep the medication at this temperature during the summer and on holiday.

Practical implications

In our study, adequate adherence was obtained. The procedures and results from this study could be used in daily clinical practice. Before starting treatment, the instructions and preventive nature of the treatment (no instant relief of symptoms) should be explained to create realistic expectations. Measures to enhance adherence may include the use of reminders and attention to the timing of intake. Adherence is a dynamic process and therefore patients should be monitored regularly from the beginning to the end of treatment to check medication intake, and evaluate symptoms and side effects. Although serious systemic side effects have never been reported, incorrect use is a potentially life-threatening risk. One should be aware that self-reported medication intake over-estimates adherence. Special attention should be paid to patients with serious complaints before treatment (as they might have been non-adherent to therapy before), the first months of treatment (as the chance of drop-out is highest then) and to the holiday season (as logistic problems may appear and patients might want to discontinue treatment for a while).

Future research

Adherence affects the effectiveness of a treatment. Therefore, every randomised clinical trial investigating the efficacy of a drug should not only report the number and reason for drop-out, but also assess (either by questionnaire and/or more objective means) and report on medication intake.

Adherence is influenced by a large interactive complex of factors. Our study is the first to investigate factors that affect adherence to SLIT in youngsters. More research is needed to gain more insight in the influence of other factors, for instance, socio-economic status and the doctor-patient relationship, to optimise adherence.

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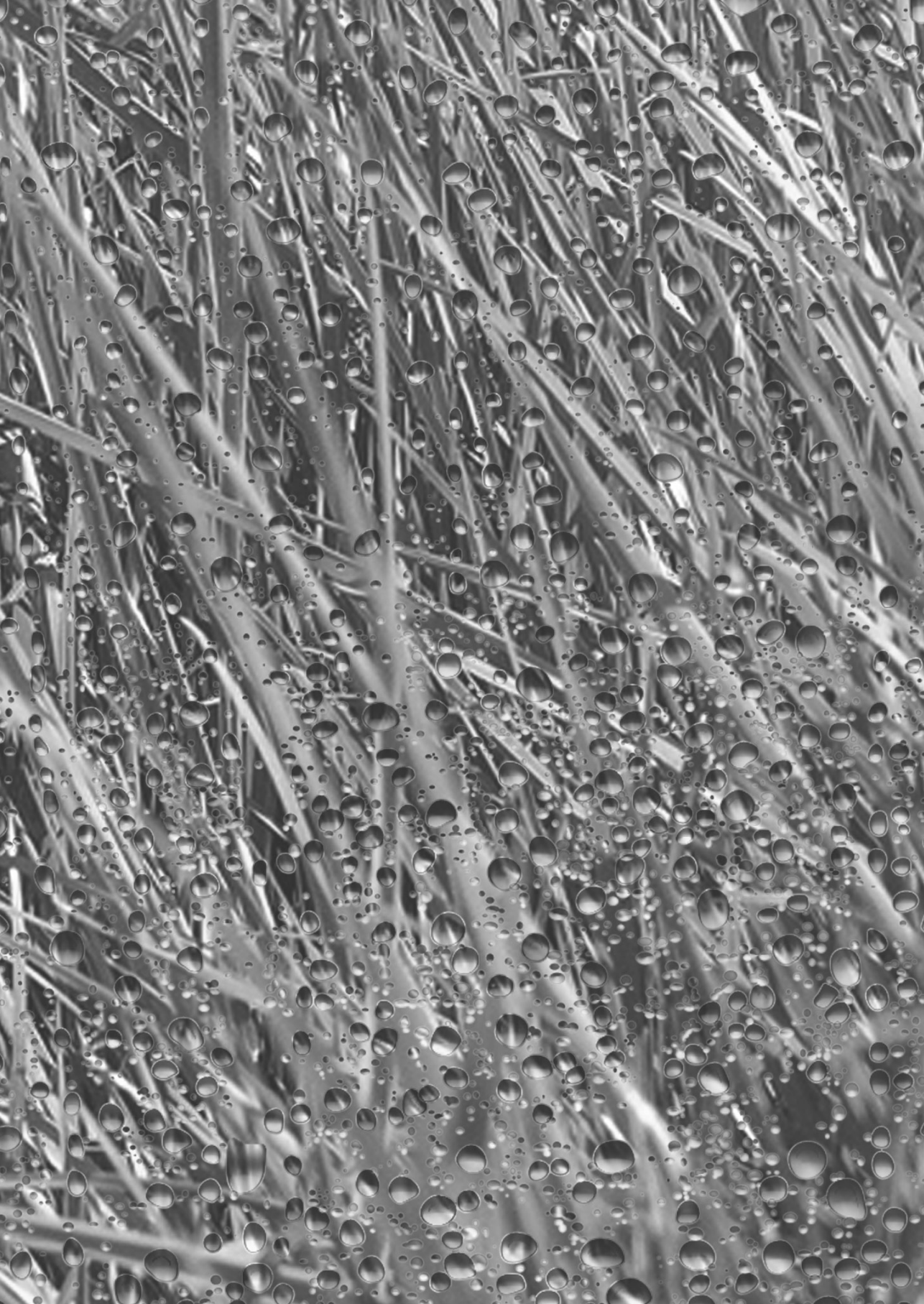

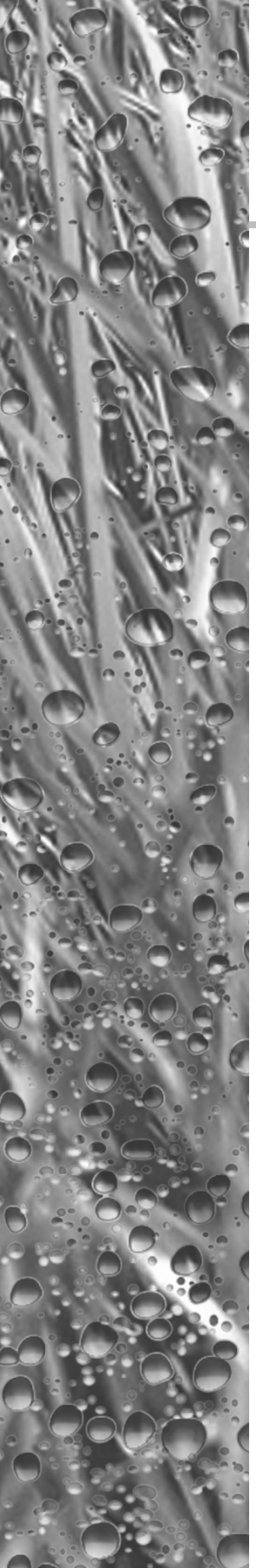

Chapter 5

The relevance of patient-reported outcomes in a grass pollen immunotherapy trial in youngsters with rhinoconjunctivitis

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Submitted

ABSTRACT

Patient-reported outcomes (PROs) are the only instruments available to assess the efficacy of an intervention in patients with allergic rhinoconjunctivitis. As allergic rhinoconjunctivitis is a systemic disease, it is now recommended to use not only PROs focusing at classical symptoms, but also health related quality of life (HRQL) instruments in immunotherapy trials. A previously published immunotherapy trial in youngsters (6-18 years) with hay fever provided us with data to assess the relevance of two of these additional outcome measures, the disease-specific Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) and the generic COOP/WONCA-charts (CWC). We hypothesised that a relevant PRO would have to be responsive to pollen exposure and would at least have a moderate correlation with the classical symptoms of allergic rhinoconjunctivitis. Furthermore, we evaluated a post-season PRO, i.e. a global assessment of symptoms (GAS). This assessment is used in clinical trials as a tool for selecting participants with sufficient symptoms and in daily practice to evaluate the patient's complaints during the preceding season. We assessed the correlation of this retrospective score with the actual symptoms during the previous pollen season. Data from 36 children and 63 adolescents were analysed. Based on the total scores of the paediatric and adolescent version of the RQLQ, both questionnaires were considered relevant, as they were responsive to exposure and showed a moderate to strong correlation with the rhinoconjunctivitis symptoms. However, in both children and adolescents 40% of the RQLQ items were not relevant according to our definition. The CWC as a whole and the separate charts appear less relevant because of the weak correlations with the daily symptom score from the diary. The correlation between our post-season GAS and the in-season daily symptom score was weak. In conclusion, the paediatric and adolescent RQLQ are relevant, but could be shortened as they contain a substantial number of irrelevant items. The CWC are not relevant in the monitoring of youngsters with allergic rhinoconjunctivitis due to grass pollen. The retrospective GAS does not sufficiently reflect the actual symptoms during the preceding season.

INTRODUCTION

Allergy is a systemic disease. Patients with allergic rhinoconjunctivitis do not only suffer from nose and eye symptoms, but also from general complaints such as fatigue, sleeping problems and difficulty concentrating.[1] As a result, allergic rhinoconjunctivitis interferes with many aspects of daily life.[2] Focusing on symptoms alone might therefore not fully reflect the impact of this allergic disease. To estimate the burden of a disease as perceived by the patient, health related quality of life (HRQL) can be assessed. Nowadays, the demonstration of the effects on quality of life in immunotherapy trials is recommended by the World Allergy Organisation (WAO) and the Global Allergy and Asthma European Network (GA²LEN).[3,4] So far, quality of life outcome measures were included in only a few immunotherapy trials with inhalant allergens in youngsters.[5-7] Although well accepted, it is not known how relevant the inclusion of these HRQL instruments is. A previously published immunotherapy trial in youngsters (6-18 years) with hay fever provided us with data to investigate the relevance of a disease-specific and a general HRQL measure. For the assessment of the disease-specific quality of life, the most widely used and validated paediatric and adolescent version of the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) was used.[8,9] Several generic quality of life questionnaires for youngsters have been developed. However, most lists are long (up to 153 items), the applicability in different cultures is often unknown and none of the lists are widely used.[10] We explored the use of the COOP/WONCA-charts (CWC)[11-13] in our population, in spite of the fact that this instrument has been validated for adults only and not for children or adolescents. The CWC measure the functional health status and have three major advantages. First, it is a short questionnaire consisting of six questions. Secondly, the answers are illustrated with a simple drawing, which might facilitate the use in our age group. Thirdly, the CWC appear to have low susceptibility to cultural differences.[13]

In addition to these two in-season patient-reported outcomes (PROs), we also asked the participants to complete a global assessment of symptoms after the grass pollen season to evaluate their complaints in the preceding months. This assessment is often used as a tool for selecting participants with sufficient symptoms in clinical trials. In daily clinical practice physicians often rely on such retrospective statements from their patients to evaluate symptoms and treatment effects during the previous season.

In this study, we first aimed to assess the relevance of the HRQL PROs by studying the influence of pollen exposure and the relationship with the classical features of allergic rhinoconjunctivitis. We hypothesised that a relevant PRO would be responsive to exposure and would have at least a moderate correlation with the daily symptom score. Secondly, we were also interested in the most important impairments as perceived by the participants in a low and a high pollen period as well as possible differences between periods. Finally, we investigated how well the retrospective global assessment of

symptoms represented the actual complaints during the season. We hypothesised that the retrospective score would sufficiently reflect the preceding season if the correlation with the daily symptom score was strong.

METHODS

Participants

A detailed description of the randomised double-blind placebo-controlled trial has been reported elsewhere.[5] A total of 204 children and adolescents aged 6 to 18 years (mean age [SD] 12.9 [2.8] years; 114 boys/90 girls) with hay fever were enrolled from general family practices in The Netherlands. All participants had IgE antibodies to grass pollen ≥0.7 kU/l (Phadia) and a history of rhinoconjunctivitis. The latter was assessed by a retrospective symptom score: participants scored 5 symptoms (sneezing, itching nose, watery running nose, nasal blockage and itching eyes) during the previous grass pollen season (May-August) on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe; maximum total score=15). Participants with a score ≥5 were included.

Participants with daily pulmonary inhaled glucocorticoids during ≥3 months in the preceding year, or immunotherapy in the preceding 3 years were excluded. Other exclusion criteria were sensitisation (specific IgE ≥0.7 kU/l; Phadia) to pets present in the family home, nasal abnormalities requiring surgery and general contra-indications for immunotherapy.[14] The participants were allowed to be sensitised to birch pollen and house dust mite. In The Netherlands, the birch pollen season precedes the grass pollen season, whereas the peak of the house dust mite season follows the grass pollen season.

Participants were included in two consecutive years. Both cohorts entered the trial and started treatment after the grass pollen season, in September-October, and participated for two years. Data from the overlapping year (i.e. the second year of the first cohort and the first year of the second cohort) were selected for the present analysis. Most importantly, data from both treatment groups were pooled, as there were no differences between treatment groups in the primary and secondary outcome measures of the trial, including symptom score and disease specific quality of life.[5]

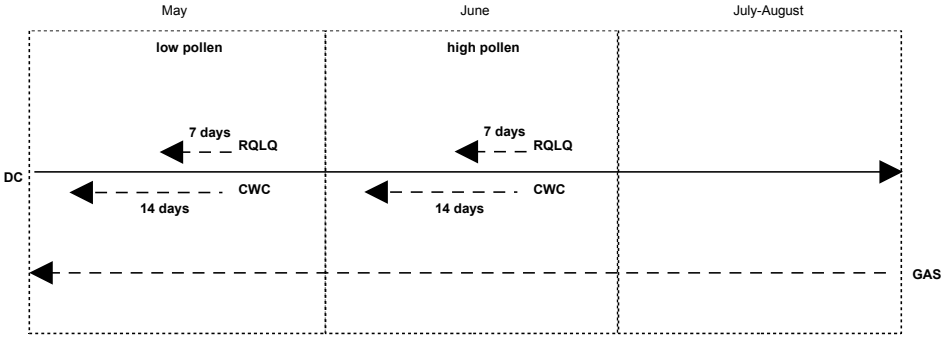
Exposure

Daily pollen counts were obtained from the pollen monitoring station in Leiden (Burkard pollen trap, Leiden University Medical Centre, The Netherlands). These counts represent the pollen exposure in the region where the participants were recruited and evaluated. In The Netherlands, the grass pollen season starts in May, with low pollen counts, and the highest pollen counts are recorded in June.

Patient-reported outcomes

In general, the PROs were interview-administered in children and self-administered in adolescents. A parent or research assistant was allowed to assist, provided that they would not influence the response of the participant. The study design is presented in Figure 1.

Figure 1. Study design



CWC, COOP/WONCA-charts; DC, Diary Card; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire; GAS, Global assessment of symptoms.

Rhinoconjunctivitis specific quality of life questionnaire (RQLQ)

The disease specific quality of life was assessed using the validated paediatric (6-11 years; PRQLQ)[8] and adolescent (12-17 years; AdolRQLQ)[9] rhinoconjunctivitis quality of life questionnaire. The PRQLQ consists of 23 items in 5 domains: "nose symptoms", "eye symptoms", "practical problems", "activities" and "other symptoms". The AdolRQLQ consist of 25 items in 6 domains: "nose symptoms", "eye symptoms", "practical problems", "activities", "emotional symptoms" and "nonhayfever symptoms". The youngsters were asked to recall their experiences during the previous seven days. Each item is scored on a 7-point ordinal scale ranging from 0 ("no impairment") to 6 ("maximum impairment"). The RQLQs were completed during two house visits: one in a period with low exposure (May) and the other in a period with high pollen counts (June). Only complete questionnaires were analysed. The mean overall score and the mean score for each domain separately in the low and high pollen period were calculated (range 0-6).

COOP/WONCA-charts (CWC)

The CWC measure 6 core aspects of functional status: "physical fitness", "feelings", "daily activities", "social activities", "change in health" and "overall health". Each domain is rated on a 5-point ordinal scale ranging from 1 ("no limitation at all") to 5 ("severely limited"); for the domain "change in health" score 1 indicates "much better" and score 5 "much worse". The recall period is fourteen days.[13] The questionnaire was completed during the same visits as the RQLQ and only complete questionnaires were analysed. The mean

overall score and the mean score for each domain separately in the low and high pollen period were calculated (range 1-5).

Diary card (DC)

Participants scored five symptoms - sneezing, itching nose, watery running nose, nasal blockage and itching eyes - on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe) on diary cards during the period May 1-August 31. For the comparison with the RQLQ, the seven days in the diary card that corresponded with the recall period of the RQLQ were analysed, provided that on at least four days all five symptoms were recorded. For the comparison with the CWC, the fourteen days in the diary card that corresponded with the recall period of the CWC were analysed, provided that on at least eight days all five symptoms were recorded. The mean total symptom score (range 0-15), the mean total nose symptom score (range 0-12) and the mean total eye symptom score (range 0-3) during the low and high pollen period were calculated. Also, the mean total symptom score for the whole season (May-August) was calculated (range 0-15). For the latter analysis only pollen relevant days were analysed (i.e. days with a pollen count that exceeded the median pollen count of that season), provided that on at least 50% of the days all five symptoms were recorded.

Global assessment of symptoms (GAS)

After the grass pollen season the participants evaluated their complaints during the previous season (May-August) by scoring 5 symptoms - sneezing, itching nose, watery running nose, nasal blockage and itching eyes on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe). The mean total score was calculated (range 0-15).

Statistical analysis

Only participants with an analysable RQLQ, CWC and DC in both the RQLQ-week and CWC-weeks, were analysed. Children (6-11 years) and adolescents (12-17 years) were analysed separately, because the PRQLQ and AdolRQLQ contain different items and therefore cannot be combined.

Comparison of the scores between the low and high pollen period was done using the paired Wilcoxon test. The limit of significance in these comparisons was considered p=0.05 (two-sided).

The minimal important difference (MID) for the RQLQ is 0.5. Changes larger than 1.0 and 1.5 are considered moderate and large differences, respectively.[15] The MID for the CWC is unknown.

The correlation between the different PROs was analysed using Spearman correlation. In view of the multitude of correlations analysed we set the level of significance at p=0.01 (two-sided) in these analyses. A correlation coefficient (r) $\geq$0.7 is generally considered to

represent a strong correlation, a coefficient between 0.5 and 0.7 a moderate correlation, between 0.3 and 0.5 a weak correlation and below 0.3 as little if any correlation.[16]

In order to identify the most important impairments as perceived by the patients in both periods, the items and domains of the HRQL questionnaires were ranked according to severity (i.e. from highest to lowest mean score).

Determination of relevance

The overall RQLQ and CWC were considered relevant if the following conditions were met:

1 the total score was responsive to exposure: the score in the high pollen period was higher compared to the score in the low pollen period and this difference was statistically significant and, in case a minimal important difference (MID) is known, also clinically relevant.
2 the total score correlated with the rhinoconjunctivitis symptoms: the score in the high pollen period had at least a statistically significant moderate correlation with the DC total score in the same period and the change in the HRQL total score had at least a statistically significant moderate correlation with the change in DC total score (changes in outcomes are represented by the symbol "Δ" in text and tables).

The same conditions were used for the determination of the relevance of the separate RQLQ and CWC domains. Only condition 1 was considered for the RQLQ items.

The retrospective GAS was considered a relevant representative of the actual complaints during the season if the correlation with the mean total symptom score of the DC for the whole season was at least high.

RESULTS

At the beginning of the grass pollen season, 55 children and 116 adolescents were participating in the study. Due to drop-out and incomplete diary cards and/or questionnaires, 18 children and 53 adolescents could not reliably be evaluated and were excluded from the analyses. The data from one child were not analysed because both house visits took place in a high pollen period. In total, 36 children (mean age [SD] 9.4 [1.3] years; 25 boys/11 girls) and 63 adolescents (mean age [SD] 14.0 [1.7] years; 33 boys/30 girls) were analysed. In both age groups there were no significant differences with respect to age, gender and the retrospective symptom score at the start of the trial between participants who were included in the analyses compared to those who were excluded.

Relevance of the RQLQ and CWC

For all youngsters the mean grass pollen count in the evaluated RQLQ-week and CWC-weeks was higher in the high pollen period. The total scores of all PROs (RQLQ, CWC and related DCs) were significantly higher in the high pollen period in both age groups (Table 1 and 2).

The change in total scores (Δ) of the PRQLQ and AdolRQLQ reached the MID limit. The total scores of the RQLQs in the high pollen period showed a strong correlation with the DC total scores and the changes in scores of both RQLQs showed a moderate correlation with the changes in DC total scores (Table 3, column "DC-total"). Consequently, the overall PRQLQ and AdolRQLQ are considered relevant.

The PRQLQ domain "other symptoms" and the AdolRQLQ domains "nonhayfever symptoms" and "emotional symptoms" did not reach the MID threshold, nor did they fulfil our criteria for the correlations. Therefore, those domains are not considered relevant. We found strong associations ($r \geq 0.70$) between the DC and the RQLQ domains covering nasal or eye symptoms. If RQLQ total scores are calculated without the nose and eye symptom domains, the changes in both adjusted total scores were significant ($p \leq 0.01$). The change in the adjusted total score of the PRQLQ was 0.7 and the difference in the adjusted AdolRQLQ total score just failed to reach the MID limit. The correlations with the DC total scores were also lower, but still significant (Children: low pollen r=0.44 / high pollen r=0.65 / Δ r=0.60. Adolescents: low pollen r=0.67 / high pollen r=0.68 / Δ r=0.57. All p-values ≤0.01).

As stated before, the CWC total scores were responsive to exposure. This was also the case for the domain "overall health" in both age groups and the domain "change in health" in children and "daily activities" in adolescents (Table 2). Analysis of the relationship between the CWC and DC yielded mainly weak correlations ($r < 0.50$; Table 4, column "DC-total"). Therefore, the overall CWC and its separate domains cannot be considered relevant in both age groups, although in children the CWC total score and the domain "overall health" just failed to meet the conditions concerning the correlation with symptoms.

We also investigated the correlation between the separate DC domains "nose symptoms" and "eye symptom" and the total and domain scores of both HRQLs (Table 3 and 4, column "DC-nose" and "DC-eye"). The results of these analyses correspond with the results of the correlations between the DC total score and the HRQLs.

All RQLQ items had a higher mean score in the high pollen period, except the item "headache" in the adolescent group. Changes in 4 out of 23 PRQLQ items and 10 out of 25 AdolRQLQ items were below the MID-limit of 0.5. In children, 7 items of various domains fulfilled the criterion of a moderate change, whereas in adolescents only the item "red eyes" reached this limit. The change in PRQLQ item "take medications" was the only large difference. For an item to be considered relevant, the change in scores had to

reach the MID-limit and be statistically significant. In children, 13 out of the 23 items met these criteria, and in adolescents 15 out of 25.

Most important impairments

In both age groups and both pollen periods, the RQLQ domain "nose symptoms" had the highest score followed by the practical problems domain in children and the activity domain in adolescents (Table 1).

Looking at the RQLQ items, there were no major differences between the low and high pollen period. The PRQLQ and AdolRQLQ items with the highest mean scores were the five nose and eye symptoms that were also recorded in the DC, practical problems (like "rub eyes/nose" and "blow nose") and activities. Notable is the high score for "thirst" in children, rank 3 in both periods.

In both age groups and in both pollen periods, the CWC-domains "change in health" and "overall health" are the items with the highest mean scores (Table 2).

Relevance of the GAS

The mean GAS in children and adolescents was 6.5 (range 1-12) and 6.8 (range 0-13) respectively. The mean DC total symptom score in children and adolescents was 3.7 (range 0.6-8.2) and 3.8 (range 0.3-9.8) respectively. The correlation between the GAS and the DC total symptom score of the whole season was 0.42 both in children and adolescents ($p \leq 0.01$). The correlation between the GAS and other in-season PRO total scores (RQLQ, CWC and the corresponding DCs in both low and high pollen period) was lower, except for the RQLQ total score in the low and high pollen period in adolescents ($r=0.49$ and $r=0.53$ respectively; $p<0.001$).

DISCUSSION

Patient-reported outcomes, which are subjective measures, are the only outcomes available to evaluate the effects of medication or other interventions in patients with allergic rhinoconjunctivitis. Objective instruments focusing at nasal function (e.g. peak nasal inspiratory flow) may at best confirm the PROs. In the past, available PROs comprised the scoring of nasal symptoms and medication only. In the last two decades, however, the awareness of the impact of allergic rhinoconjunctivitis increased. It has now been acknowledged that patients also suffer from general complaints like fatigue, sleeping problems and difficulty concentrating and as a consequence allergic rhinoconjunctivitis can significantly influence the patient's quality of life due to its impact on daily activities, school- and work performance.[1] This awareness led to the development of disease-specific questionnaires to measure the burden of illness as

Table 1. Paediatric and Adolescent Rhinoconjunctivitis Quality of Life Questionnaire: Relevance and most important impairments

A. 6-11 years

			Low pollen		**High pollen**		**Δ**		
Pollen			median (range)		median (range)				
			3.0 (2.4 to 24.7)		93.7 (57.0 to 239.3)				
DC			mean (range)		mean (range)		mean (range)	P	
total			2.5 (0 to 9.6)		4.3 (0 to 10.6)		1.8 (-6.0 to 10.4)	#	
RQLQ			mean (range)	rank	mean (range)	rank	mean (range)	P	
total			1.1 (0.3 to 2.8)		1.8 (0.1 to 5.4)		0.8 (-0.9 to 4.6)	#	R
domain	nose symptoms		1.9 (0.5 to 4.0)	1	2.8 (0.3 to 6.0)	1	0.9 (-1.8 to 4.3)	#	R
	practical problems		1.2 (0 to 3.8)	2	2.0 (0 to 5.8)	2	0.8 (-1.4 to 5.2)	#	R
	other symptoms		0.9 (0 to 2.2)	3	1.3 (0 to 4.0)	5	0.4 (-0.8 to 3.0)	#	-
	eye symptoms		0.8 (0 to 3.3)	4	1.9 (0 to 5.5)	3	1.0 (-1.3 to 5.0)	#	R
	activities		0.6 (0 to 3.3)	5	1.4 (0 to 6.0)	4	0.8 (-1.8 to 6.0)	#	R
item^	sneezing *	N	2.7 (0 to 5)	1	3.3 (1 to 6)	2	0.6 (-4 to 4)	ns	-
	rub nose/eyes	PR	2.4 (0 to 6)	2	3.4 (0 to 6)	1	1.1 (-4 to 6)	$	R
	thirst	O	2.4 (0 to 6)	3	3.0 (0 to 6)	3	0.7 (-3 to 5)	ns	-
	itchy eyes *	E	2.0 (0 to 6)	4	2.9 (0 to 6)	4	0.9 (-5 to 4)	#	R
	itchy nose *	N	1.8 (0 to 5)	5	2.6 (0 to 6)	6	0.8 (-2 to 4)	$	R
	blow nose	PR	1.8 (0 to 6)	6	2.5 (0 to 6)	8	0.7 (-5 to 5)	ns	-
	stuffy/blocked nose *	N	1.7 (0 to 5)	7	2.9 (0 to 6)	5	1.2 (-2 to 5)	#	R
	playing outdoors	A	1.3 (0 to 6)	8	2.5 (0 to 6)	7	1.2 (-2 to 6)	#	R
	runny nose *	N	1.2 (0 to 6)	9	2.4 (0 to 6)	9	1.2 (-3 to 5)	#	R
	take medications	PR	0.9 (0 to 5)	10	2.4 (0 to 6)	10	1.5 (-3 to 6)	#	R
	headache	O	0.9 (0 to 5)	11	1.0 (0 to 4)	17	0.1 (-2 to 4)	ns	-

Table 1. (*continued*)

		Low pollen		High pollen		Δ		
carry kleenex	PR	0.8 (0 to 6)	12	1.5 (0 to 6)	12	0.8 (-3 to 6)	$	R
scratchy/itchy throat	O	0.7 (0 to 6)	13	1.3 (0 to 6)	15	0.6 (-3 to 6)	ns	-
watery eyes	E	0.6 (0 to 5)	14	1.8 (0 to 6)	11	1.3 (-1 to 5)	#	R
sore eyes	E	0.5 (0 to 3)	15	1.2 (0 to 6)	16	0.7 (-2 to 6)	$	R
hard to get to sleep	A	0.5 (0 to 2)	16	1.3 (0 to 6)	14	0.8 (-2 to 6)	#	R
tired	O	0.4 (0 to 2)	17	0.9 (0 to 5)	18	0.5 (-2 to 4)	$	R
don't feel well all over	O	0.4 (0 to 2)	18	0.7 (0 to 6)	22	0.3 (-1 to 6)	ns	-
hard to pay attention	A	0.4 (0 to 2)	19	0.9 (0 to 6)	19	0.5 (-2 to 6)	ns	-
irritable	O	0.3 (0 to 3)	20	0.8 (0 to 5)	21	0.4 (-1 to 4)	$	-
wake up during night	A	0.3 (0 to 3)	21	0.9 (0 to 6)	20	0.5 (-2 to 6)	ns	-
swollen/puffy eyes	E	0.3 (0 to 2)	22	1.5 (0 to 5)	13	1.2 (-1 to 5)	#	R
feel embarrassed	PR	0.1 (0 to 2)	23	0.3 (0 to 5)	23	0.1 (-1 to 5)	ns	-

N=36; Low pollen, May; High pollen, June; Δ, Difference: High pollen minus Low pollen

DC, Diary Card (scale 0-15). RQLQ, Paediatric Rhinoconjunctivitis Quality of Life Questionnaire (scale 0-6, a higher score indicates a lower quality of life)

* symptoms also recorded in the diary card

^ corresponding domains: A=Activities; E=Eye symptoms; N=Nose symptoms; O=Other symptoms; PR=Practical problems

P, p-value: #, p≤0.01; $, p≤0.05

R, relevant; for the total and domain scores the results from the correlation-analyses (see Table 3) were also incorporated

Table 1. Paediatric and Adolescent Rhinoconjunctivitis Quality of Life Questionnaire: Relevance and most important impairments

B. 12-17 years

			Low pollen		High pollen		Δ		
Pollen			median (range)		median (range)				
			3.0 (2.4 to 24.7)		87.0 (46.3 to 239.3)				
DC			mean (range)		mean (range)		mean (range)	P	
total			3.2 (0 to 10.0)		4.7 (0 to 12.0)		1.5 (-3.6 to 7.9)	#	
RQLQ			mean (range)	rank	mean (range)	rank	mean (range)	P	R
total			1.3 (0 to 4.7)		1.8 (0 to 4.5)		0.5 (-1.5 to 3.2)	#	R
domain	nose symptoms		2.2 (0 to 6.0)	1	2.8 (0 to 6.0)	1	0.7 (-2.5 to 4.8)	#	R
	activities		1.9 (0 to 5.0)	2	2.4 (0 to 6.0)	2	0.5 (-2.0 to 4.7)	#	R
	eye symptoms		1.4 (0 to 5.5)	3	2.3 (0 to 6.0)	3	0.9 (-2.8 to 5.8)	#	R
	practical problems		1.3 (0 to 5.4)	4	2.0 (0 to 5.4)	4	0.7 (-2.0 to 3.6)	#	R
	nonhayfever symptoms		0.8 (0 to 5.0)	5	1.1 (0 to 5.0)	5	0.3 (-3.0 to 2.4)	#	-
	emotional symptoms		0.4 (0 to 3.5)	6	0.6 (0 to 4.3)	6	0.2 (-1.3 to 3.0)	$	-
item^	rub nose/eyes	PR	2.7 (0 to 6)	1	3.4 (0 to 6)	1	0.7 (-3 to 5)	#	R
	sneezing *	N	2.5 (0 to 6)	2	3.1 (0 to 6)	2	0.5 (-2 to 4)	#	R
	itchy eyes *	E	2.2 (0 to 6)	3	3.0 (0 to 6)	3	0.8 (-4 to 6)	#	R
	stuffy/blocked nose *	N	2.1 (0 to 6)	4	2.9 (0 to 6)	4	0.8 (-3 to 6)	$	R
	itchy nose *	N	2.1 (0 to 6)	5	2.8 (0 to 6)	5	0.7 (-3 to 6)	#	R
	activity 2	A	2.0 (0 to 6)	6	2.5 (0 to 6)	8	0.5 (-3 to 6)	ns	-
	runny nose *	N	1.9 (0 to 6)	7	2.5 (0 to 6)	6	0.6 (-5 to 6)	$	R
	activity 1	A	1.9 (0 to 6)	8	2.3 (0 to 6)	12	0.4 (-3 to 5)	$	-
	activity 3	A	1.7 (0 to 6)	9	2.3 (0 to 6)	11	0.6 (-3 to 6)	#	R
	blow nose	PR	1.7 (0 to 6)	10	2.5 (0 to 6)	7	0.8 (-4 to 5)	#	R
	watery eyes	E	1.6 (0 to 6)	11	2.4 (0 to 6)	9	0.8 (-4 to 6)	#	R

Table 1. (*continued*)

		Low pollen		High pollen		Δ		
carry kleenex	PR	1.4 (0 to 6)	12	2.1 (0 to 6)	13	0.7 (-4 to 6)	#	R
tired/worn out	NH	1.2 (0 to 6)	13	1.3 (0 to 5)	17	0.1 (-4 to 4)	ns	-
red eyes	E	1.2 (0 to 5)	14	2.4 (0 to 6)	10	1.2 (-5 to 6)	#	R
thirst	NH	1.1 (0 to 6)	15	1.6 (0 to 6)	14	0.5 (-2 to 3)	#	R
headache	NH	0.8 (0 to 5)	16	0.8 (0 to 5)	22	–0.1 (-3 to 3)	ns	-
irritable	F	0.7 (0 to 5)	17	1.0 (0 to 6)	18	0.3 (-4 to 5)	ns	-
swollen eyes	E	0.7 (0 to 5)	18	1.4 (0 to 6)	15	0.8 (-5 to 6)	#	R
lack of good night's sleep	PR	0.5 (0 to 6)	19	1.4 (0 to 6)	16	0.9 (-4 to 5)	#	R
generally don't feel well	NH	0.5 (0 to 5)	20	0.9 (0 to 5)	20	0.4 (-3 to 5)	$	-
(school) work [1]	PR	0.5 (0 to 6)	21	0.8 (0 to 5)	21	0.3 (-6 to 5)	$	-
can't concentrate	NH	0.4 (0 to 6)	22	1.0 (0 to 5)	19	0.5 (-6 to 4)	#	R
frustrated	F	0.4 (0 to 5)	23	0.4 (0 to 5)	24	0.0 (-3 to 2)	ns	-
restless	F	0.3 (0 to 2)	24	0.7 (0 to 5)	23	0.4 (-2 to 5)	$	-
upset/embarrassed [2]	F	0.2 (0 to 2)	25	0.3 (0 to 6)	25	0.1 (-2 to 6)	ns	-

N=63; Low pollen, May; High pollen, June; Δ, Difference: High pollen minus Low pollen

DC, Diary Card (scale 0-15); RQLQ, Adolescent Rhinoconjunctivitis Quality of Life Questionnaire (scale 0-6, a higher score indicates a lower quality of life)

* symptoms also recorded in the diary card

^ corresponding domains: A=Activities; E=Eye symptoms; F=Emotional symptoms; N=Nose symptoms; NH=Nonhayfever symptoms; PR=Practical problems

[1] complete description: unable to do (school) work as well as usual

[2] complete description: upset/embarrassed by other's response to your hay fever symptoms

P, p-value: #, p≤0.01; $, p≤0.05

R, relevant; for the total and domain scores the results from the correlation-analyses (see Table 3) were also incorporated

Table 2. COOP/WONCA charts: Relevance and most important impairments

A. 6-11 years

		Low pollen		**High pollen**		**Δ**		
Pollen		median (range)		median (range)				
		2.6 (1.9 to 16.6)		102.6 (61.1 to 175.4)				
DC		mean (range)		mean (range)		mean (range)	P	
total		2.4 (0 to 8.5)		4.5 (0.1 to 12.5)		2.1 (-3.2 to 12.3)	#	
CWC		mean (range)	rank	mean (range)	rank	mean (range)	P	R
total		1.6 (1.0 to 2.7)		1.8 (1.2 to 4.2)		0.2 (-0.8 to 2.3)	$	-
domain	change in health	2.3 (1 to 4)	1	2.8 (1 to 5)	1	0.6 (-1 to 4)	#	-
	overall health	1.8 (1 to 3)	2	2.3 (1 to 5)	2	0.5 (-2 to 4)	$	-
	feelings	1.6 (1 to 5)	3	1.6 (1 to 5)	3	0.0 (-3 to 4)	ns	-
	physical fitness	1.5 (1 to 3)	4	1.5 (1 to 4)	4	0.0 (-1 to 1)	ns	-
	daily activities	1.3 (1 to 3)	5	1.4 (1 to 4)	5	0.2 (-2 to 3)	ns	-
	social activities	1.2 (1 to 4)	6	1.1 (1 to 3)	6	-0.1 (-2 to 1)	ns	-

N=36

Low pollen, May; High pollen, June; Δ, Difference: High pollen minus Low pollen

CWC, COOP/WONCA-charts (scale 1-5, a higher score indicates a lower quality of life); DC, Diary Card (scale 0-15)

P, p-value: #, p≤0.01; $, p≤0.05

R, relevant; for the total and domain scores the results from the correlation-analyses (see Table 4) were also incorporated

Table 2. (*continued*)

B. 12-17 years

		Low pollen		High pollen		Δ		
Pollen		median (range)		median (range)				
		2.6 (1.9 to 16.6)		99.7 (51.6 to 79.6)				
DC		mean (range)		mean (range)		mean (range)	P	
total		3.2 (0 to 8.4)		4.8 (0 to 10.3)		1.6 (-2.4 to 7.0)	#	
CWC		mean (range)	rank	mean (range)	rank	mean (range)	P	R
total		1.8 (1.0 to 3.7)		1.9 (1.2 to 3.5)		0.2 (-1.2 to 1.8)	$	-
domain	change in health	2.7 (1 to 4)	1	2.8 (1 to 5)	1	0.1 (-3 to 3)	ns	-
	overall health	2.3 (1 to 5)	2	2.6 (1 to 5)	2	0.3 (-2 to 3)	$	-
	feelings	1.8 (1 to 5)	3	1.7 (1 to 4)	4	-0.1 (-3 to 2)	ns	-
	daily activities	1.5 (1 to 4)	4	1.9 (1 to 4)	3	0.4 (-2 to 3)	#	-
	physical fitness	1.4 (1 to 3)	5	1.5 (1 to 3)	5	0.1 (-1 to 2)	ns	-
	social activities	1.1 (1 to 4)	6	1.2 (1 to 4)	6	0.1 (-2 to 3)	ns	-

N=63

Low pollen, May; High pollen, June; Δ, Difference: High pollen minus Low pollen

CWC, COOP/WONCA-charts (scale 1-5, a higher score indicates a lower quality of life); DC, Diary Card (scale 0-15)

P, p-value: #, p≤0.01; $, p≤0.05

R, relevant; for the total and domain scores the results from the correlation-analyses (see Table 4) were also incorporated

Table 3. Correlation between Diary card and Paediatric and Adolescent Rhinoconjunctivitis Quality of Life Questionnaire

				DC - Total	DC - Nose	DC - Eye
RQLQ	6-11 years	Total	low pollen	0.64	0.62	-
			high pollen	0.74	0.72	0.67
			Δ	0.64	0.60	0.52
		Nose	low pollen	0.64	0.67	-
			high pollen	0.74	0.75	0.57
			Δ	0.68	0.67	0.43
		Eye	low pollen	0.51	-	0.75
			high pollen	0.64	0.57	0.74
			Δ	0.60	0.49	0.68
		Activities	low pollen	-	-	-
			high pollen	0.58	0.58	0.50
			Δ	0.61	0.59	0.50
		Practical	low pollen	-	-	-
			high pollen	0.67	0.65	0.62
			Δ	0.67	0.60	0.56
		Other	low pollen	-	-	-
			high pollen	0.52	0.51	0.49
			Δ	-	-	-
	12-17 years	Total	low pollen	0.77	0.75	0.51
			high pollen	0.77	0.74	0.71
			Δ	0.68	0.68	0.54
		Nose	low pollen	0.80	0.82	0.40
			high pollen	0.78	0.77	0.61
			Δ	0.67	0.71	0.44
		Eye	low pollen	0.63	0.52	0.76
			high pollen	0.70	0.64	0.79
			Δ	0.69	0.62	0.70
		Activities	low pollen	0.61	0.59	0.45
			high pollen	0.56	0.52	0.56
			Δ	0.55	0.52	0.53
		Practical	low pollen	0.68	0.69	0.34
			high pollen	0.69	0.68	0.55
			Δ	0.55	0.59	0.33
		Emotional	low pollen	0.40	0.40	-
			high pollen	0.49	0.48	0.37
			Δ	-	-	-
		Non hay fever	low pollen	0.41	0.39	-
			high pollen	0.61	0.58	0.58
			Δ	0.35	0.40	-

6-11 years n=36; 12-17 years n=63

DC, Diary card; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire

In the rows labelled "low pollen" and " high pollen" the correlations are shown for the scores in May and June respectively.

In the rows labelled "Δ" the correlations are shown for the changes (high pollen minus low pollen) of both scores.

Spearman correlation; only significant correlations ($p \leq 0.01$) are shown

Table 4. Correlation between Diary card and COOP/WONCA-charts

				DC - Total	DC - Nose	DC - Eye
CWC	6-11 years	Total	low pollen	-	-	-
			high pollen	0.47	0.47	-
			Δ	0.50	0.54	-
		Physical fitness	low pollen	-	-	-
			high pollen	-	-	0.48
			Δ	0.56	0.56	0.54
		Feelings	low pollen	-	-	-
			high pollen	-	-	-
			Δ	-	-	-
		Daily activities	low pollen	-	-	-
			high pollen	-	-	-
			Δ	-	-	-
		Social activities	low pollen	-0.43	-0.45	-
			high pollen	-	-	-
			Δ	-	-	-
		Change in health	low pollen	-	-	-
			high pollen	-	-	-
			Δ	-	-	-
		Overall health	low pollen	-	-	-
			high pollen	0.49	0.47	-
			Δ	0.52	0.54	-
	12-17 years	Total	low pollen	0.35	0.35	-
			high pollen	0.38	0.40	-
			Δ	-	-	-
		Physical fitness	low pollen	-	-	-
			high pollen	-	-	-
			Δ	-	-	-
		Feelings	low pollen	-	-	-
			high pollen	0.31	0.29	0.32
			Δ	-	-	-
		Daily activities	low pollen	-	-	-
			high pollen	0.38	0.39	0.33
			Δ	0.35	0.35	-
		Social activities	low pollen	-	-	-
			high pollen	0.35	0.34	-
			Δ	-	-	-
		Change in health	low pollen	-	-	-
			high pollen	-	-	-
			Δ	-	-	-
		Overall health	low pollen	0.39	0.39	-
			high pollen	-	-	-
			Δ	0.47	0.43	0.48

6-11 years n=36; 12-17 years n=63

DC, Diary card; CWC, COOP/WONCA-charts.

In the rows labelled "low pollen" and " high pollen" the correlations are shown for the scores in May and June respectively.

In the rows labelled "Δ" the correlations are shown for the changes (high pollen minus low pollen) of both scores.

Spearman correlation; only significant correlations ($p \leq 0.01$) are shown

perceived by patients. In 1991, the RQLQ designed for adults was introduced as a new instrument to measure health status for clinical trials in rhinoconjunctivitis.[17] In 1994 and 1998, instruments designed for adolescents and children became available.[8,9] The items of the RQLQs reflect the most important impairments as reported by patients in each specific age group. Nowadays, assessment of disease-specific quality of life is an essential part of randomised controlled trials (RCTs) in allergic rhinoconjunctivitis. On the other hand, generic quality of life instruments are not commonly used in these RCTs. Generic HRQL measures are less sensitive to capture small but important changes that may occur in the course of a disease, for instance before and after treatment. On the other hand, they can be used to compare the impairments caused by different diseases and some generic HRQL measures are used in cost-effectiveness analyses. In contrast to the disease-specific RQLQ, no widely accepted generic list is available for children. We wished to explore the properties of the CWC being a simple and short instrument. The CWC were developed as a screening instrument in general practice. If the responses indicate a decrease in health status, a longer and more sophisticated instrument can be used to get more precise information.[11-13] As the CWC are validated for adults only, we did not incorporate the CWC in our analysis of the efficacy of sublingual immunotherapy in youngsters. However, the immunotherapy trial provided us with the opportunity to test the responsiveness to pollen exposure and to correlate the outcome of the CWC with rhinoconjunctivitis symptoms. In case of responsiveness and moderate correlation, a formal validation would be the next step.

In this study we looked at the relevance of the RQLQ and CWC in an immunotherapy trial in youngsters with hay fever. First, we evaluated the outcome measures in terms of responsiveness to pollen exposure. In our opinion, symptoms or problems not responding to increased pollen exposure are irrelevant in the context of well-established pollen allergy. All PROs appeared to be responsive to higher pollen exposure. The clinical significance of the observed changes of the CWC cannot be estimated as information about the MID of this instrument is not available. The RQLQs showed heterogeneity in responsiveness. Although changes in total scores and most domains reached the limit of 0.5, one sixth of differences in the PRQLQ items and almost one third of the changes in AdolRQLQ items remained under the level of clinical significance.

Secondly, we expected a relationship with the classical symptoms of rhinoconjunctivitis as recorded with diary cards. It does not make sense to assess symptoms or problems that are apparently not related to the features of nasal disease. The PRQLQ and the AdolRQLQ both performed better than the CWC, with respect to the total scores as well as to the separate domains. The CWC showed predominantly weak correlations or no significant correlations with daily symptoms at all. The analysis of the different domains of both RQLQs however revealed again domains that did not fulfil the correlation criteria.

In a clinical trial, participants should not be burdened with questions that address the same aspects of a disease. In our study, we found high correlations between the nasal and eye domains of the DC and the RQLQ. This is not unexpected, as answers to questions about severity, frequency and impact of symptoms - although differently phrased - will be inter-related, in particular if they are put to patients in the same time frame. When the nose and eye domains were removed from the RQLQs, the PRQLQ was still responsive to exposure, but the AdolRQLQ just failed to reach the MID limit. As anticipated, the correlations with the DC were somewhat lower, but still highly significant. It would be interesting to investigate if an adjusted version of the RQLQ without nose and eye symptoms might be a relevant addition to the DC in clinical trials. Another option to minimise overlap is replacing the diary card with the RQLQ and using the RQLQ as a primary outcome. In a GA^{2}LEN paper on the conduct of immunotherapy trials it was stated that HRQL measures may soon become primary outcomes.[18] In contrast, the WAO stated on the same issue, that this is not possible, partly because there is no accepted way of correcting the use of rescue medication.[3] However, this is also true for the classical symptom scores as still no widely used standardised method of combining symptom severity and medication use is available. Recently three interesting articles were published addressing these issues. Häfner et al. as well as Grouin et al. validated a new combined symptom-medication score.[19,20] Franzke et al. on the other hand, focussed on the patient's needs and benefits and developed a new instrument for the assessment of patient-defined benefit that can be used for the evaluation of allergic rhinitis treatments.[21] Besides addressing the same issues in different questionnaires, asking questions that are not relevant should be avoided. In both age groups, 40% of the RQLQ items were not relevant. Removing these items from the questionnaires results in shorter lists with mere relevant questions, which will enhance compliance. For adults such a shortened version of the RQLQ, the mini-RQLQ, is already available.[22] Furthermore, both PRQLQ and AdolRQLQ are developed by and validated in youngsters with pollen induced allergic rhinitis. It has been suggested that the lists might be missing some important items for patients with persistent rhinitis caused by exposure to indoor allergens, such as snoring and mouth breathing.[23] Therefore, it would be interesting to assess the relevance of the complete and/or shortened questionnaires in for instance house dust mite allergic youngsters.

Clinically relevant differences in symptom or HRQL scores can only be detected if patients with sufficient symptoms are included. To select such patients a baseline period (i.e. observation during the season before randomisation) could be used. In grass pollen immunotherapy trials a baseline season is not mandatory, because of the variability in exposure between seasons.[23] In some trials participants are selected only on the basis of having experienced rhinoconjunctivitis symptoms during the previous year(s).[24,25] Others also assess the severity of symptoms by using a retrospective assessment of

symptoms during the previous season to select patients with sufficient symptoms.[26] In our study, the correlation between the GAS and the actual symptoms during the season was statistically significant, but not strong. This result resembles the outcome of a study performed in adults where the retrospective assessment also overrated the severity of and only had a fair to moderate agreement with the in-season assessment.[27] The phrasing of the GAS is not the same in all trials. For instance, Wahn et al. asked the patients to assess the worst symptoms during the season and not evaluate the symptoms during the whole of the season.[26] Further research is needed to determine if rephrasing the questions might improve the correlation of this retrospective assessment with the in-season symptom scores. Our findings on the GAS have implications going beyond RCTs. Physicians often see patients after the season and rely on the severity of symptoms and the effect of treatment as perceived retrospectively by patients. Our results point at the imprecision of such statements.

Another result that can be helpful for physicians when treating children and adolescents with hay fever is the analysis of the separate items of the RQLQs, as this analysis may give valuable information on the problems these youngsters experience. In general, children and adolescents are bothered by the same issues. In daily practice, evaluation of symptoms, practical problems (like rubbing nose/eyes) and impairment in activities will give a good impression of the impact of the disease. It appears that children also perceive thirst as an important issue, a complaint that is not spontaneously brought to the physician's attention. In children special attention should also be paid to medication use. When pollen exposure rises, taking their medication becomes much more bothersome to children, which might lead to non-compliance and consequently more symptoms. As the pollen season progresses, both adolescents and children experience a substantial impact from eye symptoms. Emotional problems, such as embarrassment and frustration, are considered least important in both age groups.

This study has a few limitations. First, the study is mainly exploratory and derived from a dataset from a RCT. However, as the instruments are designed to be used in clinical trials, this study population is appropriately composed to evaluate the properties of the instruments. Secondly, the numbers of participants are small, however they appeared to be sufficient for the analyses we made. Larger study groups and an extension to adults may however strengthen our findings. Thirdly, the relevance of the HRQL questionnaires was based on assumptions about the meaning of the correlations. Such assumptions are helpful in deciding what is important, but they have to be used with caution.

In conclusion, we demonstrated that the paediatric and adolescent RQLQ are relevant as they both are responsive to exposure and correlate well with rhinoconjunctivitis symptoms in pollen seasons. However, both RQLQs contain a substantial number of

irrelevant items and therefore both questionnaires could be shortened. Furthermore, our data showed that the CWC are not relevant in the monitoring of youngsters with allergic rhinoconjunctivitis. In this study, the retrospective GAS was not a relevant representative of the actual symptoms during the previous season. Because the GAS is used as an evaluation tool in research as well as in daily practice, further research is needed to determine if for instance rephrasing the questions, e.g. focusing on days with severe symptoms, might improve this retrospective assessment.

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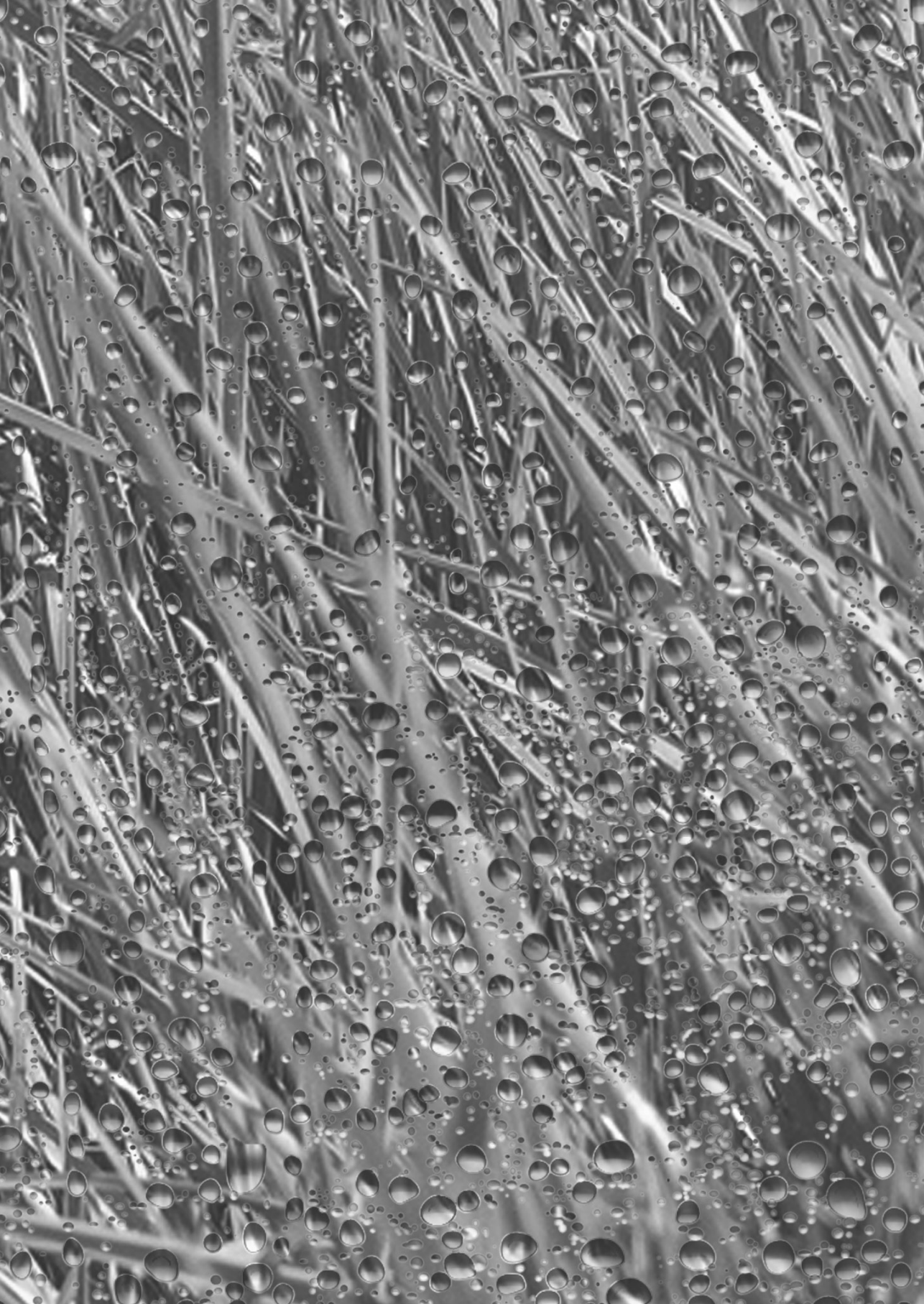

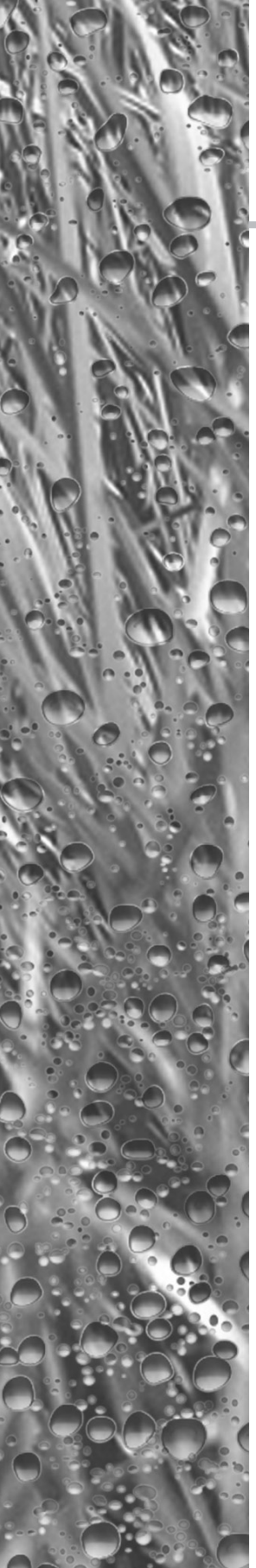

Chapter 6

Soluble biological markers during sublingual immunotherapy with grass pollen allergen in youngsters with rhinoconjunctivitis

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Submitted

ABSTRACT

Sublingual immunotherapy (SLIT) is an attractive treatment option for children, because of its disease-modifying qualities. In this study, our main aim was to assess the effect of SLIT on the immune system and ideally search for markers of clinical efficacy. We used the level of soluble biological markers (SBMs) in serum as read-out parameters of immunological reactivity. Serum samples were collected from 203 youngsters (6-18 years) participating in a randomised placebo-controlled trial with grass pollen SLIT. The samples were collected at 5 time points during the 2-year treatment period and analysed for their IL-10, IL-12, IL-13, IFN-γ, TNF-α, sIL-2Receptor, sICAM-1 and sE-selectin content. To overcome changes in assays and the presence of a substantial number of non-detects for some SBMs in the dataset, the data were analysed using quantile regression. SLIT with grass pollen did not induce changes in serum levels of SBMs in youngsters with allergic rhinoconjunctivitis. These findings are in line with the negative clinical outcomes of the study.

INTRODUCTION

Various drugs available for the treatment of allergic rhinoconjunctivitis merely ameliorate symptoms. Only allergen-specific immunotherapy offers a disease-modifying and long-term beneficial approach.[1] Allergen-specific immunotherapy (SIT) consists of repeated administration of the allergen to which the patient is allergic, with the intention to induce specific immune tolerance. SIT via the conventional subcutaneous route is effective, but the risk of severe systemic side effects and the use of injections limit its application, especially in children. A patient-friendlier and safer alternative is sublingual immunotherapy, where the allergen is administered under the tongue in drops or tablets.[2]

In recent years much attention has been paid to T-cell dynamics in allergic disease and SIT. It is well recognised that T-cell activation and alteration of related cytokine levels are involved in the pathogenesis of allergic rhinitis and asthma. Various T-cell subsets (including Th1, Th2 and regulatory T-cells) are involved in the induction and maintenance of allergic inflammation in the respiratory mucosa.[3,4] The presence of inflammation in target organs and the expression of Th2 responses to allergen exposure are the hallmarks of allergic diseases at all ages. Immunological studies monitoring the efficacy of SIT have focused on the role of Th1 (induced by IL-12 and characterised by the signature cytokine IFN-γ), Th2 (with the signature cytokines IL-4 and IL-13) and regulatory T-cells (with the signature cytokine IL-10).[3,4] These activated T-cell subsets will release soluble forms of the IL-2 Receptor (sIL-2R), which is implicated as a clinical marker in monitoring allergic disease.[5] Innate cytokines, like pro-inflammatory TNF-α, are also implicated during immunotherapy, particularly for their role in up-regulating the expression of adhesion molecules like sE-selectin and sICAM-1 for the homing of Th1 and regulatory T-cells.[6,7] The levels of cytokines, soluble adhesion molecules and activation markers - constituting soluble biological markers (SBMs) - reflect the degree of systemic allergic inflammation. The exact dynamics of these molecules in the pathogenesis of allergic disease and their evolution during immunotherapy treatment remains to be established. Additional mechanisms that may explain the efficacy of systemic immunotherapy are based on the induction of IgG antibodies that interfere with IgE facilitated antigen presentation[8] and the generation of IgA antibodies[9,10].

With the increasing knowledge about the immunological parameters involved in the modulation of the allergic reaction by immunotherapy, the question arises whether these parameters may be used as objective measure for the clinical response to immunotherapy. As part of a randomised clinical trial on the efficacy of sublingual immunotherapy (SLIT) with grass pollen among 204 youngsters with hay fever[11] we aimed to assess the effect of SLIT and other factors on the immune system and ideally search for markers of clinical efficacy. In this large trial, we limited our analyses to SBMs in serum,

because those markers could be easily obtained and assessed in order to identify markers that might be relevant to apply in clinical practice. Therefore, we excluded cellular analyses. We also did not measure serum IgE, IgG or IgG4, as changes in these markers are not clearly associated with clinical benefit.[12]

During the trial, which took several years, we were facing changes in assays and consequently changes in detection limits. Furthermore, for some SBMs a substantial proportion of the data consisted of non-detects, i.e. values below the detection limit. Therefore, we were not able to perform conventional statistical analyses on this dataset and we had to find an innovative way of analysis to overcome these unexpected barriers.

MATERIALS AND METHODS

Participants, treatment and clinical outcomes

A detailed description of the randomised double-blind placebo-controlled trial is reported elsewhere.[11,13]

A total of 204 youngsters aged 6-18 years (114 boys/90 girls; mean age [SD] 12.9 [2.8] years) with hay fever were recruited from general practices by the investigators and randomly assigned to verum (n=108) or placebo treatment (n=96). All participants had IgE antibodies to grass pollen ≥0.7 kU/l (Phadia) and a history of rhinoconjunctivitis. The latter was assessed by a retrospective symptom score: participants scored 5 symptoms (sneezing, itching nose, watery running nose, nasal blockage, and itching eyes) during the previous grass pollen season (May-August) on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe; maximum total score=15). Participants with a retrospective total symptom score ≥5 were included. Additionally, IgE antibodies to house dust mite, birch pollen and cat were determined to assess possible multisensitisation. Participants were included in two consecutive years. Both cohorts entered the trial and started treatment after the grass pollen season, in September-October, and participated for two years. Data were pooled at the end of the study.

Verum treatment consisted of a mixture of aqueous extracts of five grass pollen species (*Lolium perenne, Phleum pratense, Dactylis glomeratein, Anthoxantum odoratum, Holcus lanatus*; Oralgen Grass Pollen; Artu Biologicals, Lelystad, The Netherlands) in a glycerinated isotonic phosphate buffered solution. The mean cumulative monthly dose was 76,000 BU corresponding with 168 µg equivalent *Lol p 5*. The mean cumulative dose over the 2-year treatment period was 1,976,000 BU corresponding with 4.5 mg equivalent *Lol p 5*. Placebo treatment consisted of the solvent. The treatment schedule and instructions have been described elsewhere.[11,13]

The clinical part of the study included as primary outcome a mean daily total symptom score comprising sneezing, itching nose, watery running nose, nasal blockage and itch-

ing eyes. Secondary outcomes comprised rescue medication free days, disease specific quality of life and overall evaluation of the treatment effect. Neither the primary nor the secondary outcomes differed between participants allocated to verum and those allocated to placebo.

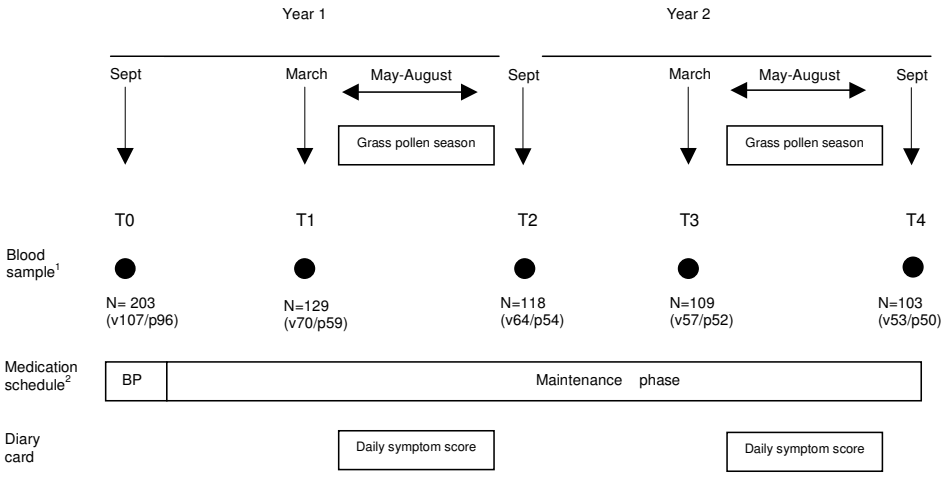
The ethics committee of the Dutch health authorities and the Erasmus MC-University Medical Centre approved the study protocol. Written informed consent was obtained from all participants.

Soluble biological markers (SBMs)

At baseline, i.e. before the start of treatment - after the pollen season (T0), a venous blood sample for specific-IgE and SBM analysis was taken from all participants. After inclusion, the participants were asked to give informed consent for four follow-up blood samples for SBM analysis: after 6 months - before the first pollen season (T1); after 12 months - after the first pollen season (T2); after 18 months - before the second pollen season (T3); after 24 months - after the second pollen season (T4) (Figure 1). Serum samples were collected, stored at -80 ºC and analysed for their IL-10, IL-12, IL-13, IFN-γ, TNF-α, sIL-2Receptor, sICAM-1 and sE-selectin content.

Initially, for the time points T0, T1 and T2, the production of these SBMs was detected with Enzyme-Linked Immunosorbent Assay (ELISA), using commercially available ELISA kits from Immunotech (Luminy, Marseille, France) and Biosource (Cytosets, Biosource Europe SA, Nivelles, Belgium). Nunc Maxisorp 96-wells plates (Sanbio, Uden, The Netherlands) were coated overnight at 4 °C. The concentrations of the coating antibodies used

Figure 1. Study design



[1] v, verum; p, placebo

[2] Build-up phase (BP): 20 days, daily intake, increasing with 1 drop; Maintenance phase: twice weekly intake, 20 drops

were 1 µg/ml for IFN-γ, IL-10, IL-12, IL-13 and sIL-2Receptor; 2 µg/ml for TNF-α; 3 µg/ml for sICAM-1, and 10 µg/ml for sE-selectin. The coating antibodies were diluted in PBS (150 mM NaCl, 8 mM $Na_2HPO_4 \cdot 2H_2O$, 1.5 mM KH_2PO_4, 2.7 mM KCl, pH 7.4). Each well was incubated for 2 hrs at room temperature on a plate shaker with 200 µl blocking solution (5% [w/v] BSA in PBS, pH 7.4). Plates were washed five times with 250 µl PBS-Tween (0.1% Tween-20 in PBS, pH 7.4) and 100 µl of sample or standard was added to duplicate wells. Samples and standards were diluted in assay buffer (blocking buffer with 0.1% Tween-20). Biotinylated detection antibodies were diluted in assay buffer to the final concentration of 0.2 µg/ml for IL-13 and IL-12, 0.4 µg/ml for IL10 and IFN-γ and 0.8 µg/ml for TNF-α. For sE-selectin, sIL-2Receptor, and for sICAM-1 10 µg/ml of biotinylated detection antibody was used. Fifty µl biotinylated mAb was added to the samples and standards. The plates were incubated for 2 hrs and washed as before. One hundred µl streptavidin poly-horse radish peroxidase (Sanquin, Amsterdam, The Netherlands; 1 mg/ml) diluted 1:10,000 in assay buffer was added to each well and the plates were incubated for 30 min at RT while shaking, and then washed as before. Thereafter 100 µl/well of the chromogen tetramethylbenzidine (TMB) was added and incubated for 30 min in the dark (RT); 100 µg 1M H_3PO_4 was finally added per well to stop the reaction. The optical densities (OD) at 450 nm and 690 nm were measured in an ELISA Multiskan MS reader (Merlin Diagnostic systems BV, Breda, The Netherlands). The sensitivity limits for quantitative determinations, according to the various manufacturers, were 1.19 pg/ml (IFN-γ), 1.15 pg/ml (IL-10), 7.85 pg/ml (IL-12), 5.21 pg/ml (IL-13), 8.81 pg/ml (TNF-α), 13.40 pg/ml (sIL-2R), 0.11 ng/ml (sE-selectin), and 1.43 ng/ml (sICAM-1).

For the later time points T3 and T4, the SBM production was measured with Cytometric Bead Assay Flex sets (CBA; BD, Pharmingen, San Diego, USA). All buffers used in this protocol were obtained from the BD CBA Soluble Protein Master Buffer Kit (BD, Pharmingen, San Diego, USA). The procedure was performed according to the manufacturer's protocol. The samples were measured on the FACSArray, using the FCAP software (BD Biosciences). The sensitivity limits for quantitative determinations were 0.3 pg/ml (IFN-γ), 2.3 pg/ml (IL-10), 2.2 pg/ml (IL-12), 1.6 pg/ml (IL-13), 0.7 pg/ml (TNF-α), 12.5 pg/ml (sIL-2R), 5 pg/ml (sE-selectin), and 0.23 ng/ml (sICAM-1).

The measurements above the detection limit were not affected by the change in assays.

The values were expressed as pg/ml (average of duplicate wells ± SD) or ng/ml (for sICAM-1 and sE-selectin) deduced from the OD of the standard curve after subtracting the blanks, reference values and the spontaneous secretion of unstimulated cells.

Statistical analysis

Univariate comparison of the group of youngsters that gave informed consent for the additional blood samples and the group that did not - with respect to treatment

group, age, gender, severity of symptoms before the trial, specific IgE to grass pollen, multisensitisation, and lower airway symptoms - was done by the Mann-Whitney test or Chi-square test in case of percentages. The same methods were used to compare the baseline characteristics of the treatment groups. P=0.05 (two-sided) was considered the limit of significance.

As described above, the serum samples were analysed in two parts (T0-2 and T3-4), because we had to present an interim-analysis to our sponsor. As a consequence, two different assays with different detection limits were used. We chose to apply the detection limits of one method to all data. The detection limits of the first method (ELISA) were used, because these limits were higher than those of the second method (CBA) for all SBMs except IL-10. For IL-10 the detection limits of both methods were used. All values below the detection limit were replaced with the value between 0 and the detection limit (i.e. half of the detection limit).

Our initial plan was to analyse the data using linear regression to evaluate the influence of several variables – treatment, age, gender, cohort (i.e. first or second year of inclusion), time point and co-sensitisation to birch pollen and house dust mite – on SBM levels. However, when exploring the data, it became clear that at certain time points for some SBMs more than half of the data consisted of non-detects, i.e. values below the detection limit. This makes the use of standard statistical methods unreliable. Therefore, we used an advanced statistical method called quantile regression.[14] This method has already been used in the fields of genetics and ecology, where non-detects are also a well-known problem. A detailed non-technical description of this method, as applied to immunological data, will be published elsewhere[15]; here we only sketch how it works.

Standard statistical methods use the principle of least squares. A model delivers fitted values and their differences with the observed values are called residuals. Model parameters are chosen in such a way that the sum of the squares of the residuals is minimised. A simple example is the arithmetic mean: it is the number that minimises the sum of the differences (of the mean) to the observations. Simple linear regression is a more advanced example, but the principle is the same: the sum of the squares of the distances from the observations to the regression line is minimised.

An alternative approach is to minimise not the sum of squares, but that of absolute values, of the residuals. Using this as a principle, one finds that the mean is replaced by the median. A key fact is that only the sign of a residual matters, not its size. In other words: only the fact that an observation is above or below the median is being used to compute the median.

Percentiles are generalisations of the median. P75, the 75th percentile, is the number below which 75 per cent of the observations lie. It is also the number that minimises a weighted sum of absolute values of residuals, if weight 0.75 is given to the observations above P75 and weight 0.25 to those below it. Again, the only information that is being

used is whether observations are below or above the percentile to be computed, not how far removed they are.

We can extend these concepts to regression, so-called quantile regression, estimating a model that gives us the expected P75 for given values of covariates of our choice. Only the information that observations are above or below the expected P75 is being used for estimating the model. Hence it does not matter what values non-detects really have: as long as they are below the expected P75 any nominal value can be filled in.

With quantile regression it is not possible to get p-values for model coefficients like slope and intercept; instead 95% confidence intervals are presented.

In this study we used P75 in our analyses, because, except for IL-12 at one time point, the percentage of non-detects did not exceed 75%. For the analysis and presentation of the data we use either the concentrations themselves or the logarithms (to base 10) of concentrations. In case the levels of SBMs are plotted against for instance age, the logarithms (to base 10) of concentrations are used to plot the data appoints and to compute intervals and regression lines (as shown in Figure 3). Otherwise only the observations of a few large concentrations will be visible in isolation, while all other observations will cluster near zero. Furthermore, the data points are "jittered" in the horizontal direction, again for better visibility. Plotting for instance age and (logs of) concentrations directly will cause many data points to overlap perfectly, giving a wrong impression of the distribution of the observations. Jittering adds a small (between -0.2 and 0.2) random number to age, shifting the dots. For the statistical analysis the concentrations themselves are used, not their logarithms (as depicted in Figure 2 and Table 2). Quantile regression essentially is sensitive to the ranks of observations, not their size. Hence the effect of transformations is much smaller than in the world of least squares. The advantage of working with the concentrations is that regression coefficients can be interpreted easily. Take age as an example: its coefficient tells us how much a concentration (in pg/ml or ng/ml) changes in one year.

Quantile regression is easily and freely available through the package *quantreg* for the Open Source statistical system R.[16]

RESULTS

Participant characteristics

The analysed population consisted of 203 participants, because from one of the randomised children not enough material was available for SBM analysis. One hundred and forty youngsters gave informed consent for follow-up measurements. The group of youngsters that gave informed consent for the additional blood samples did not differ from the group that did not, with respect to treatment group, age, gender, severity of

Table 1. Baseline characteristics of the analysed population

	Verum n=107	Placebo n=96	Total n=203[@]
Age (y)			
mean (SD)	13.0 (2.6)	12.8 (3.0)	12.9 (2.8)
median (range)	13 (7-17)	13 (6-17)	13 (6-17)
Gender			
number of male participants (%)	71 (66%)	43 (45%)	114 (56%)
Retrospective symptom score			
total [mean (SD)] *	8.7 (2.5)	9.0 (2.4)	8.9 (2.5)
nose [mean (SD)] **	6.7 (2.2)	7.1 (2.1)	6.9 (2.2)
eye [mean (SD)] ***	2.0 (0.8)	1.9 (0.9)	2.0 (0.9)
Specific IgE grass pollen (kU/L)			
median (range)	75.9 (0.8 to >100)	74.2 (1.6 to >100)	75.8 (0.8 to >100)
No. of participants with multisensitisation (%)[#]	79 (74%)	68 (71%)	147 (72%)
No. of participants with lower airway symptoms (%)[$]	62 (58%)	61 (64%)	123 (61%)

[@] from one of the included participants not enough material was available for cytokine analysis

* scale 0-15; ** scale 0-12; *** scale 0-3

[#] Multisensitisation: IgE antibodies ≥0.7 kU/L to house dust mite and/or birch pollen and/or cat

[$] in the 12 months before inclusion; ISAAC questionnaire

symptoms prior to the trial, multisensitisation, and lower airway symptoms. The group that did not give informed consent had significantly higher IgE levels (median 90.5; range 1.6 to >100 kU/l) compared to the group that did (median 73.1; range 0.8 to >100 kU/l; p=0.02). Due to drop-out, a follow-up measurement was available for 129, 118, 109 and 103 participants at T1, T2, T3 and T4, respectively (Figure 1). Drop-out did not differ between both treatment groups.

The baseline characteristics of the analysed population are presented in Table 1. The treatment groups were comparable for age, severity of symptoms, specific IgE to grass pollen, multisensitisation, and lower airway symptoms. By chance, relatively more boys were randomised to verum treatment.

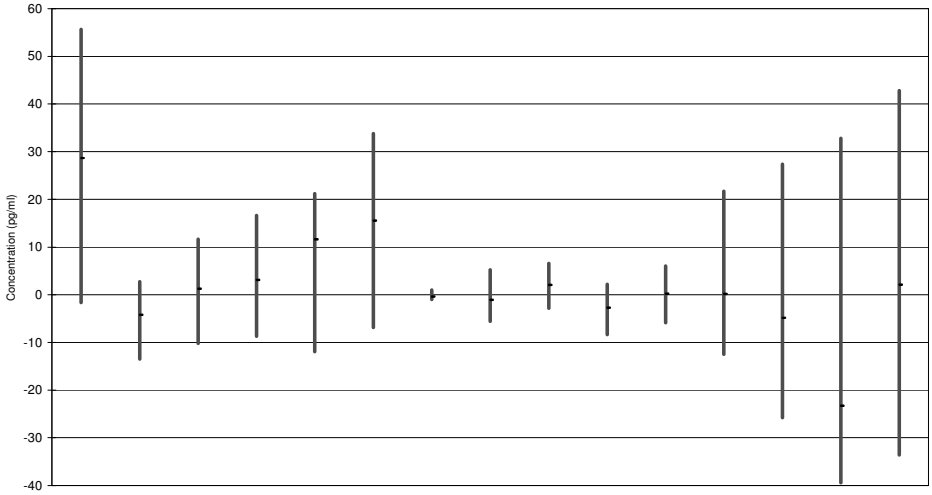
About two thirds of the youngsters had specific IgE to grass pollen ≥50 kU/l, i.e. RAST-class ≥5. These 129 youngsters were equally distributed between the low (≤8) and high (>8) retrospective total symptom score groups.

Almost 75% of the participants were multisensitised. The distribution of the number of additional sensitisation and the type of allergen(s) did not differ between both treatment groups. The most prevalent co-sensitisations were sensitisations to birch pollen and house dust mite.

Factors influencing SBM levels

The influence of the different factors on the level of IL-10, the cytokine most likely to be affected by successful immunotherapy, is shown in Figure 2. The results for the other

Figure 2. The influence of treatment and other factors on the 75th percentile (P75) of IL-10



	(Intercept)	treat-T0	treat-T1	treat-T2	treat-T3	treat-T4	age	gender	birch	hdm	cohort	T1	T2	T3	T4
C	28,6	-4,2	1,2	3,1	11,6	15,5	-0,4	-1,1	2,0	-2,8	0,2	0,1	-4,9	-23,3	2,1
LB	-1,6	-13,5	-10,2	-8,7	-12,0	-6,9	-1,0	-5,6	-2,9	-8,4	-5,9	-12,5	-25,8	-39,4	-33,6
UB	55,6	2,7	11,7	16,6	21,2	33,8	1,0	5,2	6,5	2,2	6,0	21,7	27,4	32,8	42,8

Quantile regression coefficients are presented as midpoint and lower and upper bound of their 95% confidence interval. The intervals are not symmetric around the midpoint.

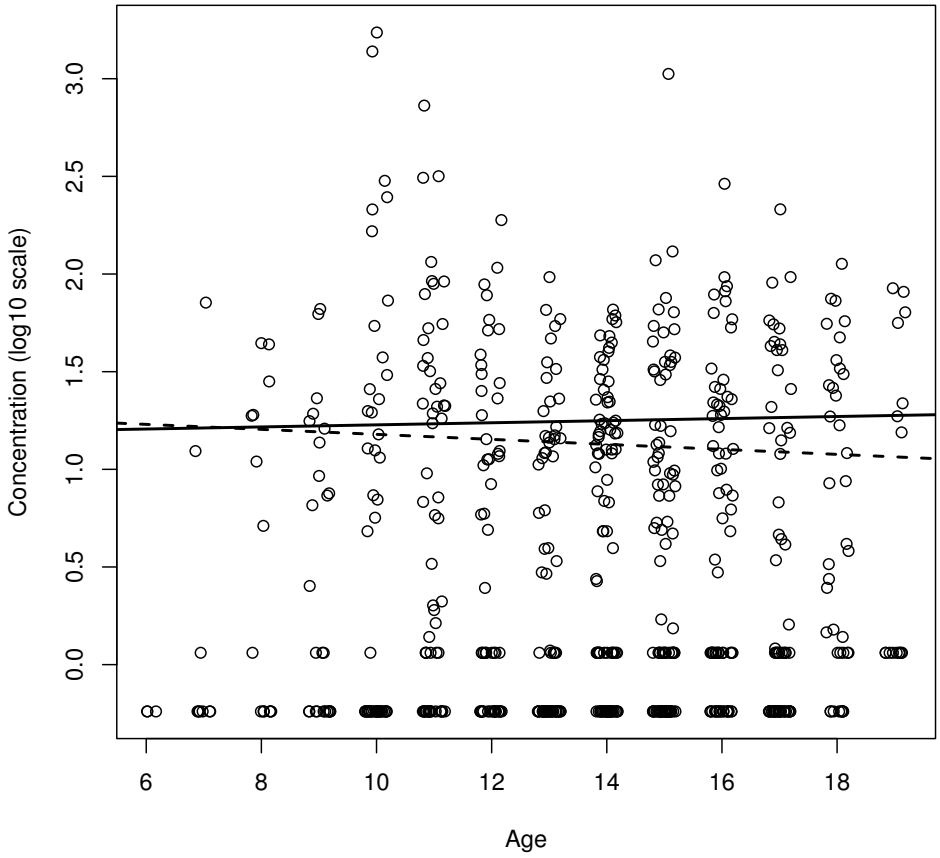
C, coefficient; LB, lower bound, UB, upper bound. Treat, treatment: SLIT with grass pollen extract vs. placebo. Gender, girls vs. boys. Birch, co-sensitisation to birch pollen. Hdm, co-sensitisation to house dust mite. Cohort, second year vs. first year of inclusion. T0, baseline. T1-2-3-4, follow-up after 6-12-18-24 months respectively.

SBMs are presented in Table 2. For the greater part the confidence intervals included zero. When this was not the case, the confidence intervals were wide and therefore the results were considered not to be relevant. The only exceptions to this pattern were the observation points T1 and T2 for sICAM-1. Consequently, the 75th percentiles (P75) of all SBMs did not differ between participants allocated to verum and those allocated to placebo, at baseline and the four follow-up time points. Age, gender, "sensitisation to house dust mite", "sensitisation to birch pollen" and cohort also did not influence the SBM levels. Only for sICAM-1 a possible time trend was seen. Replacement of the factors "sensitisation to house dust mite" and "sensitisation to birch pollen" by the factor "multisensitisation" did not change the results. Furthermore, in the subgroups of youngsters with severe symptoms before the trial (i.e. a retrospective total symptom score >8; n=104), or a high level of specific IgE to grass pollen (i.e. RAST class ≥5; n=129) or both (n=67), treatment did not influence the levels of the SBMs during the trial.

The effect of age on SBM levels

Besides the effect of SLIT on the SBM levels, we were also especially interested in the influence of the factor "age" and therefore we determined regression lines for P75 on age, using quantile regression.[15] Figure 3 shows an example, again for IL-10. Age was rounded to integer years. Two lines are shown in Figure 3. The full line is the result of quantile regression on age only. The broken line adds the factor "time point" as an explanatory variable. A quantile regression line with only "time point" as an explanatory variable showed a slightly increasing trend. Therefore, in the analysis on the effect of age, "time point" was incorporated as an additional factor (i.e. with a separate coefficient for each time point). The level of IL-10 did not change with age. The same result was found for the other SBMs (data not shown).

Figure 3. Trend for the 75th percentile (P75) of IL-10 with age



The data points have been "jittered" (a random horizontal shift between -0.2 and 0.2) for better visibility. The full line is obtained by quantile regression on age only, the broken line with the observation time point as an additional factor.

Table 2. The influence of treatment and other factors on the 75th percentile (P75) of IL-10, IL-12, IL-13, IFN-g, TNF-α, sIL-2Receptor, sICAM-1 and sE-selectin

		C	LB	UB			C	LB	UB
IL-10	(Intercept)	28.6	-1.6	55.6	**TNF-α**	(Intercept)	52.5	30.0	68.2
	treatment-T0	-4.2	-13.5	2.7		treatment-T0	-7.3	-15.4	6.0
	treatment-T1	1.2	-10.2	11.7		treatment-T1	4.6	-6.0	13.8
	treatment-T2	3.1	-8.7	16.6		treatment-T2	12.6	-4.9	25.7
	treatment-T3	11.6	-12.0	21.2		treatment-T3	9.6	-2.4	20.9
	treatment-T4	15.5	-6.9	33.8		treatment-T4	14.5	-2.0	30.4
	age	-0.4	-1.0	1.0		age	-0.3	-1.0	0.0
	gender	-1.1	-5.6	5.2		gender	0.0	-3.5	3.7
	birch	2.0	-2.9	6.5		birch	-2.7	-6.1	2.3
	hdm	-2.8	-8.4	2.2		hdm	1.6	-2.4	5.2
	cohort	0.2	-5.9	6.0		cohort	-4.7	-7.6	1.3
	T1	0.1	-12.5	21.7		T1	-15.8	-31.8	3.4
	T2	-4.9	-25.8	27.4		T2	-22.5	-43.4	7.4
	T3	-23.3	-39.4	32.8		T3	-21.6	-37.9	0.3
	T4	2.1	-33.6	42.8		T4	-21.5	-42.3	11.2
IL-12	(Intercept)	106.8	54.7	128.0	**sIL-2R**	(Intercept)	476.8	348.5	634.2
	treatment-T0	-29.3	-42.8	3.9		treatment-T0	-18.0	-91.6	29.4
	treatment-T1	20.1	-16.0	36.5		treatment-T1	-24.1	-71.6	60.2
	treatment-T2	28.5	-0.1	38.0		treatment-T2	-7.9	-66.7	49.1
	treatment-T3	18.8	-31.8	40.7		treatment-T3	-139.9	-188.4	38.1
	treatment-T4	13.3	-26.0	40.9		treatment-T4	153.7	4.7	364.7
	age	-0.8	-2.0	0.0		age	-10.8	-15.3	-4.8
	gender	0.8	-2.3	6.6		gender	33.8	5.4	54.4
	birch	0.6	-3.0	6.1		birch	26.2	-12.2	47.6
	hdm	0.4	-3.3	4.3		hdm	-12.3	-39.0	19.4
	cohort	-0.7	-7.8	2.8		cohort	-6.3	-38.5	28.7
	T1	-69.3	-98.4	0.9		T1	51.6	-164.3	160.3
	T2	-90.2	-104.8	-41.0		T2	38.7	-65.0	128.8
	T3	37.1	-0.4	164.4		T3	204.0	7.5	336.7
	T4	20.2	-21.9	103.2		T4	149.9	-191.1	366.8

Quantile regression coefficients are presented as midpoint and the lower and upper bound of their 95% confidence interval (pg/ml or ng/ml for sICAM-1 and sE-selectin). The intervals are not symmetric around the midpoint.
C, coefficient; LB, lower bound, UB, upper bound.
Treatment: SLIT with grass pollen extract vs. placebo. Gender, girls vs. boys. Birch, co-sensitisation to birch pollen. Hdm, co-sensitisation to house dust mite. Cohort, second vs. first year of inclusion.
T0, baseline. T1-2-3-4, follow-up after 6-12-18-24 months respectively.

Table 2. (*continued*)

		C	LB	UB			C	LB	UB
IL-13	(Intercept)	9.4	-20.1	32.9	**sICAM-1**	(Intercept)	593.5	571.4	631.2
	treatment-T0	1.7	-12.3	18.0		treatment-T0	-0.3	-11.0	9.6
	treatment-T1	-8.5	-22.4	2.7		treatment-T1	7.0	-6.9	16.6
	treatment-T2	-1.7	-13.6	8.6		treatment-T2	6.7	-16.3	14.5
	treatment-T3	-13.2	-45.9	2.2		treatment-T3	6.5	-62.3	145.6
	treatment-T4	2.6	-21.4	15.4		treatment-T4	206.2	-180.2	417.6
	age	0.0	-0.9	0.7		age	-1.3	-3.3	-0.5
	gender	5.7	0.4	9.4		gender	-2.7	-9.9	5.5
	birch	4.8	0.4	8.5		birch	1.2	-5.1	9.1
	hdm	0.0	-3.1	4.3		hdm	-2.6	-9.6	4.3
	cohort	-0.4	-7.1	2.3		cohort	-0.2	-8.9	8.7
	T1	-0.6	-17.8	22.0		T1	-188.3	-205.9	-166.3
	T2	-16.4	-26.5	4.5		T2	-148.5	-166.5	-117.2
	T3	41.6	15.6	113.3		T3	91.2	-98.3	240.0
	T4	48.0	25.1	84.1		T4	840.1	554.9	1279.7
IFN-γ	(Intercept)	77.0	10.4	125.4	**sE-selectin**	(Intercept)	90.8	74.1	101.0
	treatment-T0	-12.8	-48.3	22.9		treatment-T0	-1.2	-7.1	7.0
	treatment-T1	11.2	-17.0	43.9		treatment-T1	2.2	-7.0	12.0
	treatment-T2	13.9	-19.0	54.5		treatment-T2	-2.8	-8.6	4.4
	treatment-T3	-29.1	-119.2	103.0		treatment-T3	2.4	-8.1	11.8
	treatment-T4	-41.3	-136.2	37.0		treatment-T4	5.1	-14.2	20.7
	age	0.4	-0.5	1.9		age	-1.9	-2.4	-1.3
	gender	5.0	-0.6	10.5		gender	4.7	0.8	6.9
	birch	2.0	-3.4	10.8		birch	0.2	-2.0	3.8
	hdm	-3.4	-9.8	1.9		hdm	-3.8	-5.8	0.2
	cohort	21.6	12.8	34.6		cohort	2.0	-2.0	3.9
	T1	-105.7	-154.5	-55.3		T1	-14.2	-29.2	-1.6
	T2	-79.1	-144.3	-25.1		T2	-28.5	-40.6	-9.1
	T3	103.6	-88.7	262.7		T3	-42.5	-54.6	-27.4
	T4	153.1	36.7	268.3		T4	8.1	-26.1	33.5

DISCUSSION

The aim of the current study was to assess the effect of SLIT on SBM levels in a large group of children and to identify markers that could be applied in clinical practice as determinants of clinical efficacy. During the two years of monitoring, we were not able to detect any treatment effect on SBM levels. Particularly, we did not find an effect on IL-

10 levels, the cytokine that should have been affected by successful immunotherapy.[3,4] These findings mirror the negative results that were found for the clinical efficacy. Furthermore, we did not identify other factors, especially age, which influenced the level of SBMs.

One might argue that, instead of using serological data, it would have been more appropriate to study T-cell responses and cytokine production by examining the white blood cell fraction (PBMC, peripheral blood mononuclear cell fraction). In our study, analyses at serological level were more feasible than analyses at a cellular level, because the participants were monitored during house visits and lived at various distances of our centre. Moreover, assays for analyses at cellular level are complex and difficult to standardise between laboratories, making them less suitable for the monitoring of patients.

The SBMs measured in this study were chosen because of their relationship with T-cell activation and allergic inflammation. At the time of performing the study, data could be derived from SCIT studies only. Recent studies suggest that the same mechanisms observed in SCIT seem to be applicable to SLIT.[3,4,17] Nowadays, the effects of immunotherapy are attributed to stimulation of Th1 cells, suppression of Th2 cells, induction of regulatory T-cells and subsequent reduction of inflammation. Following these concepts, we evaluated the Th1/Th2 shift by measuring the Th1 cytokines IFN-γ and IL-12 and the Th2 cytokine IL-13, whereas IL-10 was used as signature cytokine for the induction of regulatory T-cells.[3,4,17] In addition we measured the soluble form of the IL-2 receptor (sIL-2R) as a product of T-cells and we monitored inflammation by determining the adhesion molecules sICAM-1 and sE-selectin and the pro-inflammatory cytokine TNF-α. The choice for the selected biomarkers also emerged from previous observations that the level of some of these SBMs correlated with airway symptoms and the severity of atopic dermatitis.[18,19]

Up to today few SBMs in serum have been investigated as potential markers for monitoring the clinical efficacy of immunotherapy. In an open study in atopic children sensitised to either grass pollen, birch pollen or house dust mite, conflicting results were found as the significant decrease in sICAM-1, sIL-2R and sE-selectin levels after 1 year of SLIT was not consistent among the three allergen groups. In the same study, no difference was observed in IL-12 expression.[5] A significant reduction in sICAM-1 after 1 year of successful grass pollen SLIT was reported in another open study.[7] In a 10-year follow-up study in adult patients with allergic rhinitis due to house dust mite, IL-4, sIL-2R and sICAM-1 levels progressively decreased in the SCIT group, whereas no changes were observed in the untreated group.[20]

We chose not to monitor participants by determining specific IgE or IgG. It has been shown that changes in allergen-specific IgE levels do not correlate with the clinical status.[12] Conflicting results were found when the use of the serum-specific IgE/total IgE ratio as a biomarker for effective immunotherapy was evaluated.[21,22] Serum IgG levels, especially IgG4, merely reflect the exposure to the allergen, but do not have a connec-

tion with clinical improvement.[23] In recent years it became clear that the inhibitory activity of specific IgG antibodies correlates better with the clinical improvement of SIT, even after discontinuation of treatment.[23] Recent large SLIT trials in adults[24] and children[25] included the assessment of these blocking antibodies. In both studies active treatment was characterised by a rise in blocking antibody activity. These studies suggest that measurement of blocking antibody activity has the potential to be used as a surrogate marker for clinical efficacy in the individual patient.[3,24]

Although the results from these studies look promising, unfortunately, efficient and easily available surrogate markers are still not at hand in clinical practice.[12]

It cannot be ruled out that the use of symptomatic medication, such as oral antihistamines, influenced the levels of some SBMs.[4] However, especially in the beginning of immunotherapy treatment, most patients will need symptomatic medication to relief their symptoms. Therefore, a relevant marker has to be relatively unaffected by the influence of such co-medication.

Because of the absence of clinical efficacy we were not able to identify potential SBMs useful for monitoring the outcome of SLIT. It is a matter of debate as to whether the absence of a treatment effect can be attributed to an ineffective extract used in this trial. In recent studies, showing effectiveness of SLIT with grass pollen allergen in children, higher dosages were used.[25,26] Although it is still difficult to compare the dosage of various extracts, it is possible that the dosage of the extract used in our trial was too low to evoke an immune response. In retrospect, IgG4 measurements could have clarified this issue.

Initially, the analysis of the data was hampered by two well-known problems in the field of immunology, changes in assays and the presence of non-detects.[27] By using the advanced statistical method of quantile regression we were able to overcome these difficulties and reliably answer our research questions.

Conclusion

In this large study among children with allergic rhinoconjunctivitis, we did not find an effect of SLIT with grass pollen on soluble serum markers. These findings are in line with the negative clinical outcomes of the study. Moreover, we were not able to identify other factors, including age, which might have an important effect on soluble biomarkers as measured outside the pollen season. Finally, by using the advanced statistical approach quantile regression we were able to analyse a large dataset hampered by changes in assays and the presence of substantial numbers of non-detects.

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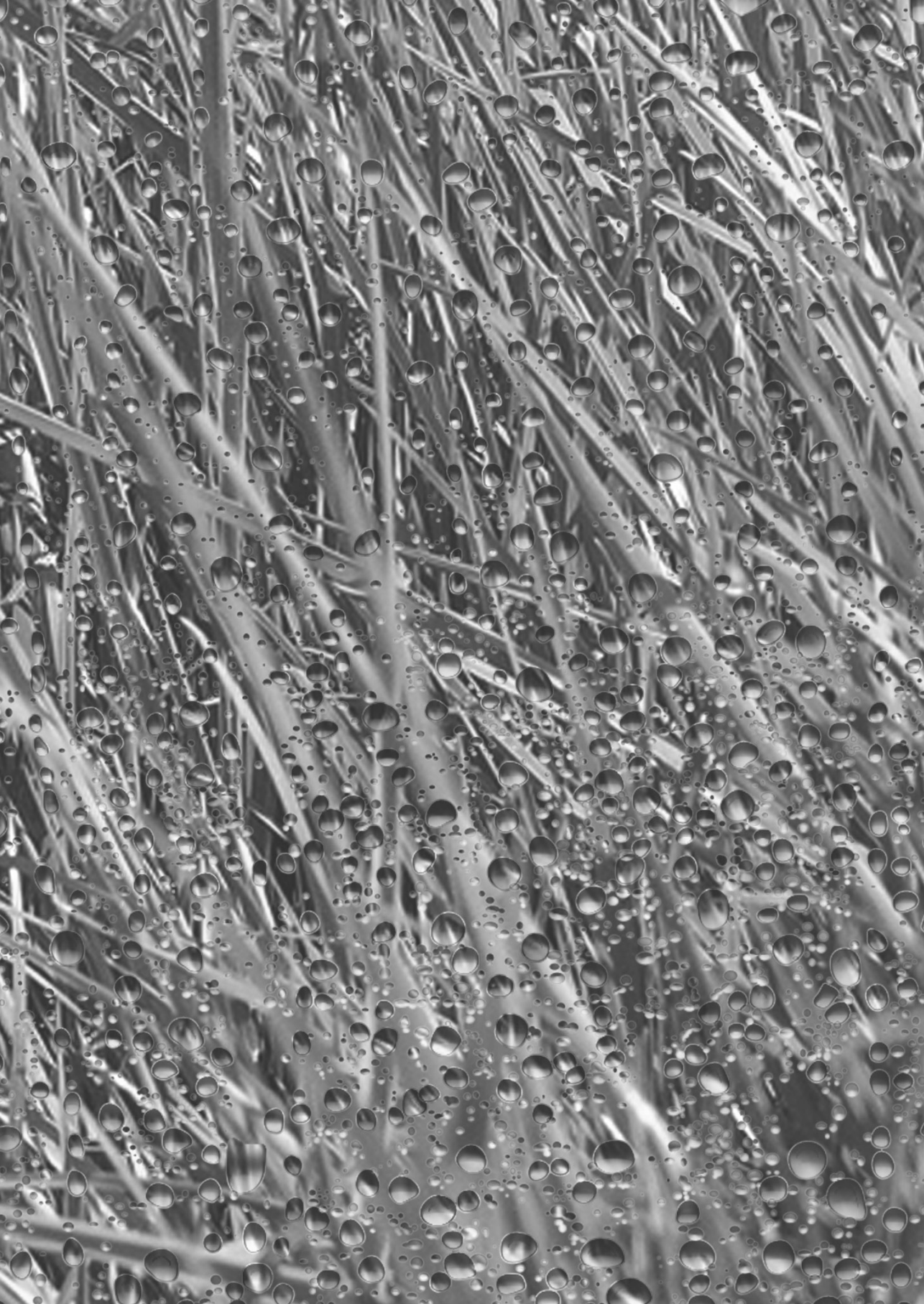

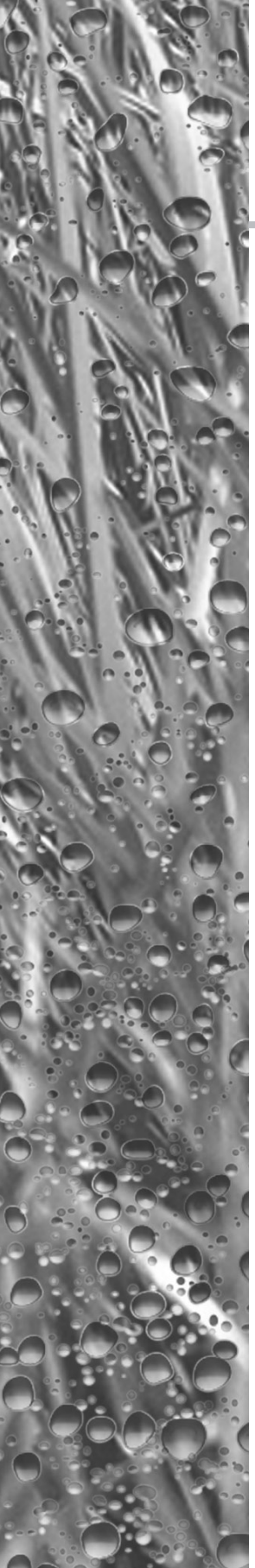

Chapter 7

Quantile regression for the statistical analysis of immunological data with many non-detects

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Submitted

ABSTRACT

Background: Immunological parameters are hard to measure. A well-known problem is the occurrence of values below the detection limit, the non-detects. Non-detects are a nuisance, because classical statistical analyses, like ANOVA and regression, cannot be applied. The more advanced statistical techniques currently available for the analysis of datasets with non-detects can only be used if a small percentage of the data are non-detects.

Methods and Results: Quantile regression, a generalisation of percentiles to regression models, models the median or higher percentiles and tolerates very high numbers of non-detects. We present a non-technical introduction and illustrate it with an implementation to real data from a clinical trial. We show that by using quantile regression, groups can be compared and that meaningful linear trends can be computed, even if more than half of the data consists of non-detects.

Conclusion: Quantile regression is a valuable addition to the statistical methods that can be used for the analysis of immunological datasets with non-detects.

INTRODUCTION

Immunological parameters are hard to measure. A well-known problem is the occurrence of values below the detection limit, the non-detects.[1] In a project that we will use as an example in this paper, depending on the parameter, more than half of the data, concentrations of soluble biological markers in human blood, consists of non-detects.

Non-detects (NDs) are a nuisance in statistical analysis. An ad-hoc solution is to fill in values for the NDs, e.g. one half of the detection limit. This may be acceptable if only a few per cent of the observations are NDs. If there are many of them, estimated values of means, standard errors and trend lines will be unreliable and conclusions may be wrong.

NDs occur in many places in science and technology. They have received a lot of attention in the work of Helsel.[2,3] Although NDs are extremely common in immunology, the literature about them is not very extensive. An exception is the paper by Uh et al. that studies a number of approaches to analyse datasets with NDs.[1] In that paper quantile regression was not considered. We believe it to be a very useful tool, and like to share our experiences in this expository paper.

Most statistical methods develop a model for the expected values of the observations. In an analysis of variance (ANOVA) these will be the mean values for different groups. In the case of the regression line $y = ax + b$, the parameters a and b allow us to compute the expected value of an observation y for every x, which might be age or time or another covariate, that we are interested in. In addition, we can compute prediction intervals, in which a new observation will lie with a specified probability. This type of model belongs to the standard toolbox that most applied scientists learn these days in their statistics lessons. Modern statistical packages make it very easy to use them in practice.

Regression and ANOVA (which is a special case of regression), use the so-called principle of least squares: parameters like a and b in the example above, are computed in such a way that the sum of the squares of the residuals is minimised. The residuals are the differences between expected values, according to the model, and the observations. If a part of the observations is wrong, because of many NDs, the parameter estimates will be (very) wrong.

In this paper we propose to use quantile regression instead of the usual linear regression models. A simple example is provided by ANOVA. Instead of computing means per groups, one could compute the medians, also known as P50, the 50th percentile. A familiar recipe for computing the median of a set of numbers is to sort them from low to high and pick the middle number in the sorted list. Half of the data will be below this number and the other half will be above it. The key point is that the actual values of the lowest observations play no role: what matters is that they are lower than the median. So if we would have 30% NDs and gave them small values, the computed median would still be the same.

If more than 50% of the observations are NDs, but less than 75%, we are still able to compute the P75, the number below which 75% of the data are found. In ANOVA we can still compare P75 in the different groups and look for interesting differences.

For a regression line, the sorting recipe will not work. However, in the last two decades a very useful generalisation of regression modelling has become available, quantile regression. With this method we can estimate regression lines, which allow us to compute for *y* a percentile of our choice for any value of *x*. The only condition is that all NDs lie below the line. With many NDs, as in our example data set, this means that it is not possible to compute a line for the median, but that the P75 is sufficient.

The outline of the paper is as follows. First, we introduce quantile regression. We have tried to limit the amount of technical material, keeping in mind the expected statistical level of our audience. We also show in this section how the required computations can be done relatively easily with the R system and the package *quantreg*.[4] Then we apply quantile regression to a real data set, with an extremely high number of NDs. The paper ends with a short discussion.

QUANTILE REGRESSION

In this paper the words quantiles and percentiles will be used repeatedly. To avoid confusion we first make their meaning precise. The 90th percentile is the number below which 90% of the data lie. It is also the 0.9 quantile. So, when we use percentages we talk of percentiles, and when we use fractions we talk of quantiles.

In the Introduction we described the familiar sorting algorithm for computing percentiles. It has a strong intuitive appeal, and it is easy to implement, or even to do by hand. However, it cannot be generalised to the case of a regression line or more complicated models. Fortunately there exists another, more flexible approach, based on optimisation.

The mean of n observations, y_1 to y_n, is computed as $\bar{y} = \sum_i y_i / n$. Averaging is a familiar process that one usually does not give much thought to the fact that the sum of squares

$$S_2 = \sum_i (y_i - \mu)^2$$

is minimised when $\mu = \bar{y}$, the mean of *y*. The sum of squares is stated explicitly in more complicated models like a linear regression line and it leads to explicit expressions for optimal values of the parameters in the model. This is an extremely powerful statistical tool.

For percentiles we can also introduce a function that has to be minimised, in such a way that the desired percentile minimises it. Compared to the sum of squares, two changes are needed:

1) replace the squares by absolute values, and 2) give different weights to positive and negative residuals. The residuals are the differences between the observations and the percentile that is being computed. As a formula: minimise

$$S_1 = \sum_i w_i(p) \mid y_i - q(p)|.$$

Here $q(p)$ is the p-quantile (the $100p$ percentile) for a chosen value p (with $0 < p < 1$) and $w_i(p)$ is the weight of observation i, computed as

$$w_i(p) = p \qquad \text{if} \qquad y_i > q(p) \qquad (1)$$

$$w_i(p) = 1 - p \qquad \text{if} \qquad y_i \leq q(p) \qquad (2)$$

So, in the case of the 0.9-quantile, the positive residuals get a nine times larger weight (i.e. 0.9) than the negative ones (i.e. 0.1). It is not directly obvious why this procedure leads to the desired quantile, but after some mathematical adjustments one finds indeed that 90% of the observations have to be below $q_{0.9}$ to minimise S_1.

Now that we have an optimisation criterion, it is very easy to extend the quantile idea to more complicated models. In the case of a linear regression line, the function to be minimised is

$$S_1 = \sum_i w_i(p) \mid y_i - a(p) - b(p)x_i \mid.$$

It will be clear that we can generalise this to more complicated models. Notice that generally the values of *a(p),* the intercept, and *b(p),* the slope, change with *p*.

It is easy to state the function that has to be minimised, but computing the solution is harder than for classical models (based on least squares). Excellent software is available, free of charges. It is an extension, a so-called package (named *quantreg*), for the statistical software system R.[4] Fitting a linear regression line for the 90th percentile is as simple as writing *model = rq(ỹx, tau = 0.9)*. The parameter *tau* corresponds to *p* in our formulas. We did our computations with this package.

With quantile regression it is not possible to get *p*-values for model coefficients like slope and intercept; instead the *quantreg* package delivers 95% confidence intervals (which actually are more useful).

For those interested in statistical backgrounds of quantile regression, we can recommend a paper by Koenker and Portnoy[5] and a book by Koenker[6]. An interesting paper from an applied point of view (i.e. that of ecologists) is the one by Cade and Noon.[7]

AN IMPLEMENTATION

To illustrate the use of quantile regression in immunology, we use data from a randomised placebo-controlled trial in 204 youngsters (6-18 years) with hay fever. A detailed description can be found in Röder et al.[8] The main aim of the study was to determine the effect of sublingual immunotherapy (SLIT) with grass pollen allergen on nose and eye symptoms (e.g. sneezing and itchy eyes). Allergen-specific immunotherapy consists of the repeated administration of the allergen to which the patient is allergic, with the intention to modulate the response of the immune system to the allergen.[9] In the case of SLIT, the allergen is administered under the tongue by drops or tablets. In a sub-study, the effect of SLIT and other factors on the immune system was assessed by measuring the levels of soluble biological markers (SBMs) in serum during the trial. Serum samples were collected at five time points during the two-year treatment period: baseline (T0), after 6 months (T1), after 12 months (T2), after 18 months (T3) and after 24 months (T4). The samples were analysed for their IL-10, IL-12, IL-13, IFN-γ, TNF-α, sIL-2Receptor, sICAM-1 and sE-selectin content. The following factors were studied: treatment, age, gender, cohort (i.e. year of inclusion), time points and co-sensitisation to birch pollen and house dust mite.

Out of the 203 youngsters included in this sub-study, 103 subjects were observed all 5 times and 74 only once. The 26 remaining subjects were observed 2 to 4 times.

We start by presenting histograms of the measurements, emphasising the need for a statistical method that can handle a large proportion of NDs. Then we compute trends with age using quantile regression.

Distributions and non-detects

The samples were analysed in two parts, because an interim-analysis had to be presented to our sponsor. As a consequence, two different assays with different detection limits were used.

Initially, for the time points T0, T1 and T2, the production of the SBMs was detected with Enzyme-Linked Immunosorbent Assay (ELISA). The sensitivity limits for quantitative determinations were 1.19 pg/ml (IFN-γ), 1.15 pg/ml (IL-10), 7.85 pg/ml (IL-12), 5.21 pg/ml (IL-13), 8.81 pg/ml (TNF-α), 13.40 pg/ml (sIL-2R), 0.11 ng/ml (sE-selectin), and 1.43 ng/ml (sICAM-1). For the later time points T3 and T4, the SBM production was measured with Cytometric Bead Assay Flex sets (CBA). The sensitivity limits for quantitative determinations were 0.3 pg/ml (IFN-γ), 2.3 pg/ml (IL-10), 2.2 pg/ml (IL-12), 1.6 pg/ml (IL-13), 0.7 pg/ml (TNF-α), 12.5 pg/ml (sIL-2R), 5 pg/ml (sE-selectin), and 0.23 ng/ml (sICAM-1). The measurements above the detection limits were not affected by the change in assays

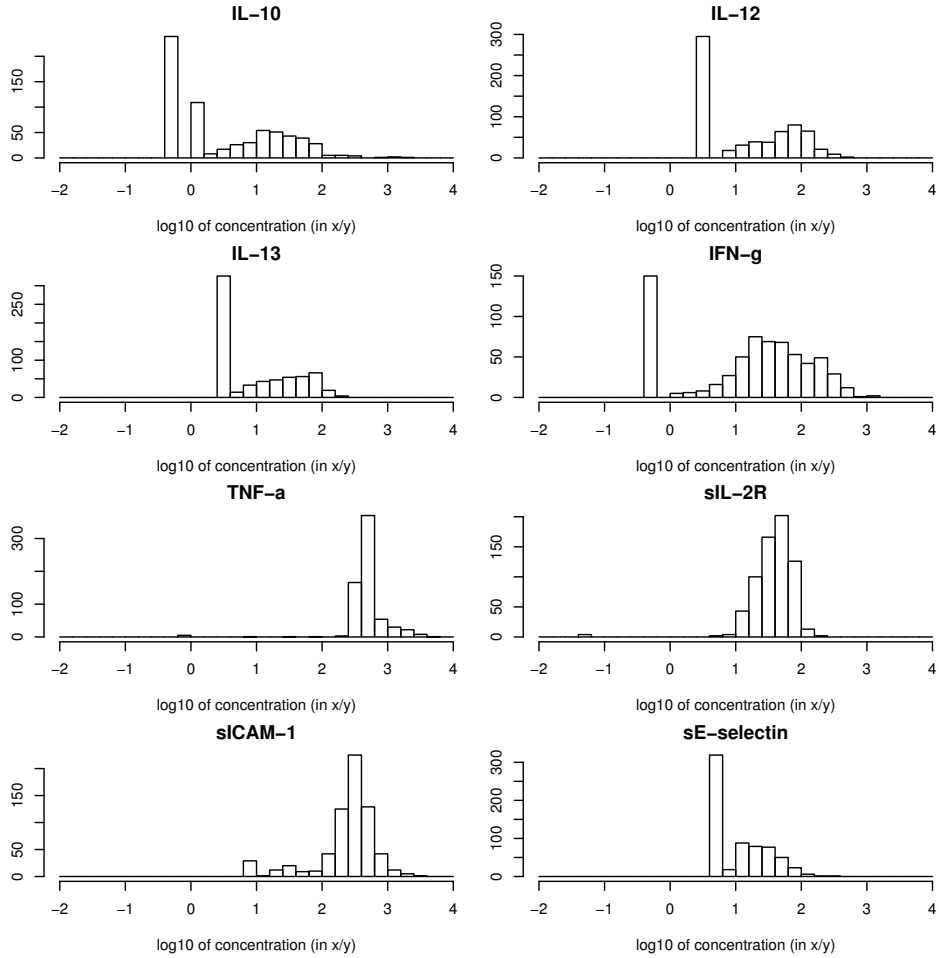
We chose to apply the detection limits of one method to all data. The detection limits of the first method (ELISA) were used because these limits were higher than those of

the second method (CBA) for all SBMs except IL-10. For IL-10 the detection limits of both methods were used. All values below the detection limit were replaced with the value between 0 and the detection limit.

For the analysis and presentation of the data in this implementation we use the logarithms (to base 10) of concentrations. The largest concentrations measured were around 5000 pg/ml, the smallest were always at the detection limit. Because of the enormous range of the concentrations, the highest ones being more than a 1000 times higher than the smallest ones, we work exclusively on the logarithmic scale.

Figure 1 shows histograms of the logarithms (expressed as pg/ml or ng/ml; to base 10) of the concentrations of the eight SBMs. For IL-10, IL-12, IL13, IFN-γ, TNF-α and sIL-2R the percentage of NDs ranged from 4% up to 52%. The NDs clearly stick out as isolated bars

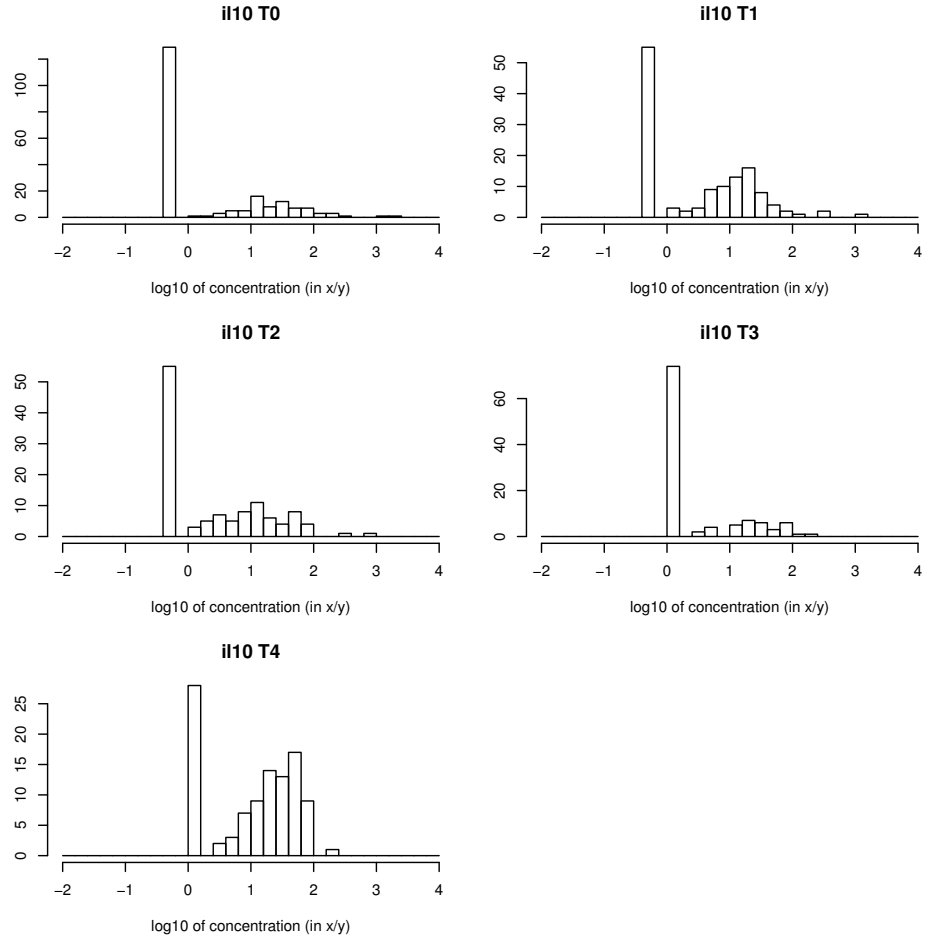
Figure 1. Histograms of (logarithms) of SBM concentrations, all five time points combined.



at the left side of the histograms and are relatively close to the rest of the distribution. For sICAM-1 and sE-selectin they are small and at a large distance. For those SBMs the fraction of NDs was below 1%. In fact one could well apply a classical statistical analysis to these SBMs after discarding the few NDs. The number of NDs also varied between time points, as demonstrated for IL-10 in Figure 2. Also visible in this figure is the change in detection limit between T2 and T3. Except for IL-12 at one time point, the percentage of non-detects did not exceed 75%.

Summarising, due to changes in detection limits and the presence of a substantial number of NDs for some SBMs, classical statistical analyses, like ANOVA and regression, could not be applied to this dataset.

Figure 2. Histograms of (logarithms) of IL-10 concentrations, for each of the five time points.

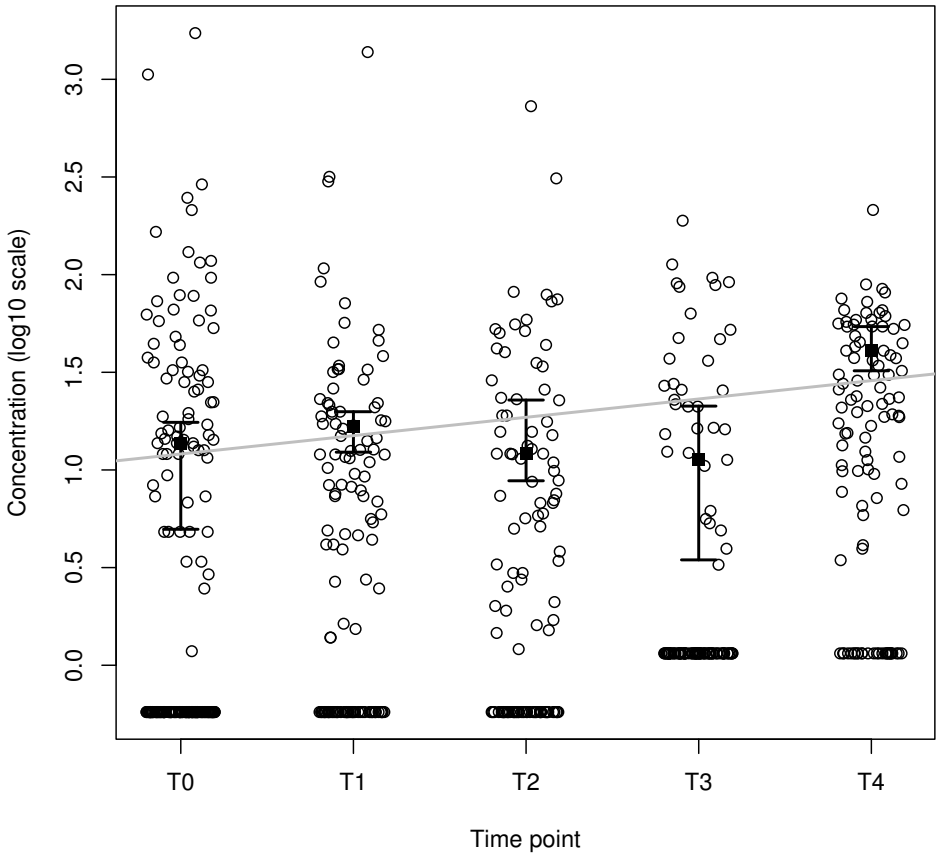


T0, baseline. T1-2-3-4, follow-up after 6-12-18-24 months respectively.

Trends and quantile regression

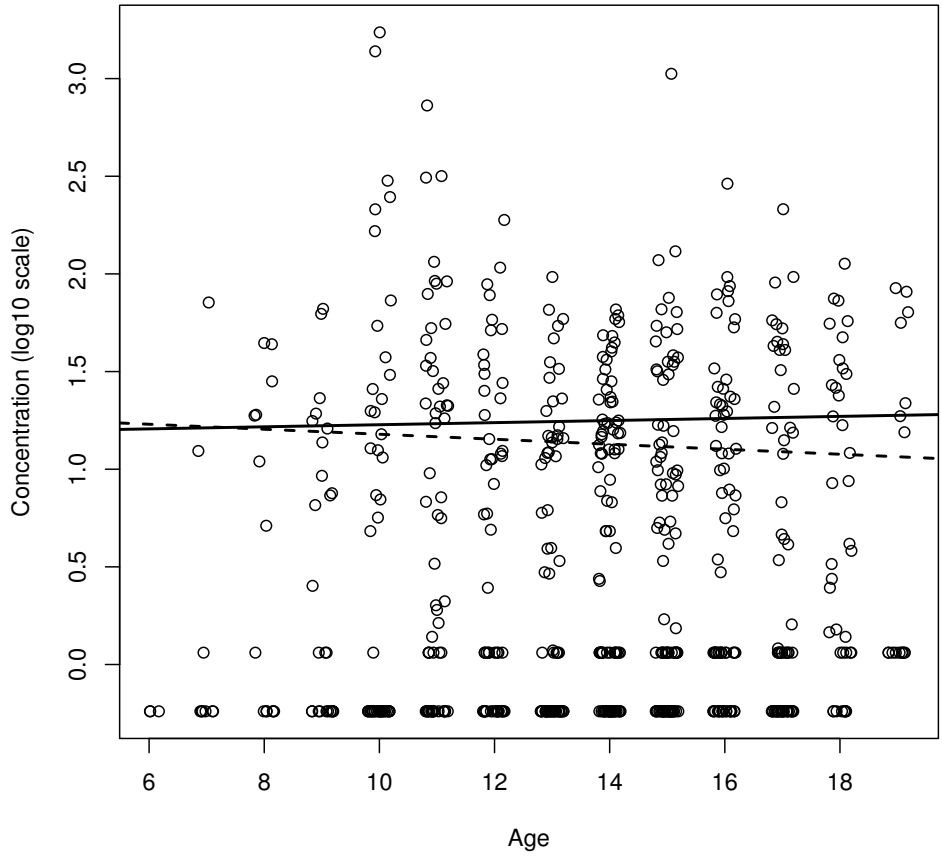
One of the research questions was to determine whether concentrations of SBMs change with age. We use quantile regression for P75, the 75th percentile. Before a quantile regression line on age only was calculated, the influence of the variable "time point" was explored. Figure 3 shows an example, again for IL-10. The data points have been "jittered" in the horizontal direction for better visibility. Plotting the time points and (logs of) concentrations directly would cause many data points to overlap perfectly, giving a wrong impression of the distribution of the observations. Jittering adds a small (between -0.2 and 0.2) random number to the time point, shifting the dots. For each time point the P75 midpoint with its 95% CI is presented. The line represents the P75 quantile regression line with only "time point" as an explanatory variable. Because this

Figure 3. The five observation time points of IL-10.



The data points have been "jittered" (a random horizontal shift between -0.2 and 0.2) for better visibility.
For each time point the midpoint (coefficient) with 95% confidence interval are shown. The intervals are not symmetric around the midpoint. The line represents the P75 quantile regression line for the logarithm of the concentration.
T0, baseline. T1-2-3-4, follow-up after 6-12-18-24 months respectively.

Figure 4. Trend for the 75th percentile (P75) of IL-10 with age



The data points have been "jittered" (a random horizontal shift between -0.2 and 0.2) for better visibility. The full line is obtained by quantile regression on age only, the broken line with the observation time point as an additional factor.

line showed a slightly increasing trend, "time point" was incorporated as an additional factor (i.e. with a separate coefficient for each time point) in the analysis on the effect of age. Also visible in Figure 3 is the change in the analytical procedure between T2 and T3, leading to an increase in detection limits. As stated before, the measurements above the detection limits were not affected by the change in assays. Figure 4 shows the results of the analysis on the influence of age on the IL-10 levels. Age was rounded to integer years and again the data points have been "jittered" in the horizontal direction for better visibility. Two lines are presented in Figure 4. The full line is the result of quantile regression on age only. The broken line adds the factor "time point" as an explanatory variable. Thus, age appears not to have an effect on this SBM.

DISCUSSION

Immunological datasets often contain many non-detects. When a signal produced by the stimulant is too small for the instrumentation to discriminate the signal from the background noise, a value cannot be determined precisely. Values below a given detection limit are called non-detects (NDs). The presence of NDs will cause the data to be left-censored and special attention should be paid to selecting the appropriate statistical method to analyse such a censored dataset.

Several statistical methods are being used to deal with NDs. Uh et al. evaluated the performance of several commonly used methods in immunology and more advanced methods used in other fields such as environmetrics and econometrics via simulation studies.[1] Two often-used approaches, deletion or single value substitution followed by linear regression, did not perform well. Because NDs are not missing at random, bias can be expected when dropping NDs. Uh et al. showed that even with a ND proportion of 10%, the bias was unacceptable. Substitution of NDs with 0, half of the detection limit or the detection limit itself, followed by linear regression, underestimated the variance. Two more sophisticated methods, the TOBIT method and the multiple imputation technique, performed well but only when the proportion of NDs was less than 30% and 50%, respectively. In our dataset, for some markers the percentage of NDs was higher. Furthermore, a condition of the TOBIT method is that the normality assumption is met. Like in our dataset, immunological measurements are often positively skewed and even after logarithmic transformation normality cannot always be achieved. Therefore, we had to seek for a method that could handle large proportions of NDs with no assumptions on the underlying distribution. We explored the use of quantile regression, a generalisation of percentiles to regression models. Like for the computation of simple percentiles, the only information that is being used is whether observations are below or above estimated model values. If the number of NDs is not too large, one can estimate models for P50, the median. But in extreme cases, like for some immunological markers in the data set we used as an example, it is necessary to go to higher percentiles. In fact we chose P75.

We illustrated quantile regression with data from a clinical trial in youngsters with hay fever, in which the effect of immunotherapy treatment and other factors on the immune system was evaluated by measuring levels of soluble biological markers (SBMs). We showed that groups can be compared and that meaningful linear trends can be computed, even with very large fractions of NDs. The slope of the regression line for a percentile is the same as that for the mean in the case of a linear relationship plus errors with a constant variance, the common default assumption in linear regression. That means that the estimated slope for the P75 is also a very good estimate for the usual regression slope that would be obtained if NDs did not occur.

We have not discussed efficiency. It is true that quantile regression uses less information, that is, only the signs of residuals, disregarding their size, leading to wider confidence intervals and consequently loss of power. This means that if data are complete (no NDs), estimated classical regression coefficients have more narrow confidence intervals than those obtained from quantile regression. But this does not help us much if we have many NDs. When analysing our data set, we chose one percentile level, 75% for all variables. In principle it could vary with the fraction of NDs. For some variables P50 could have been chosen. We felt that this would have made the interpretation more complicated.

A limitation might be that the data have been analysed as 662 independent observations. This is not correct, as 558 observations represent multiple observations on 129 participants. In the world of standard least squares statistical methods, one would use repeated measure ANOVA or a mixed model for a proper analysis. Unfortunately similar technology is not yet developed enough for quantile regression, although research is ongoing. NDs can, however, generate unpleasant complications when using mixed models. It might happen that all or most measurements of some of the subjects are NDs. Consequently mixed models, which rely on fitting (restricted) individual coefficients to subjects, might be difficult to use. As far as we know, no statistical technology is yet available to handle mixed models with NDs.

Conclusion

Quantile regression is a valuable addition to the statistical methods that can be used for the analysis of immunological datasets with non-detects.

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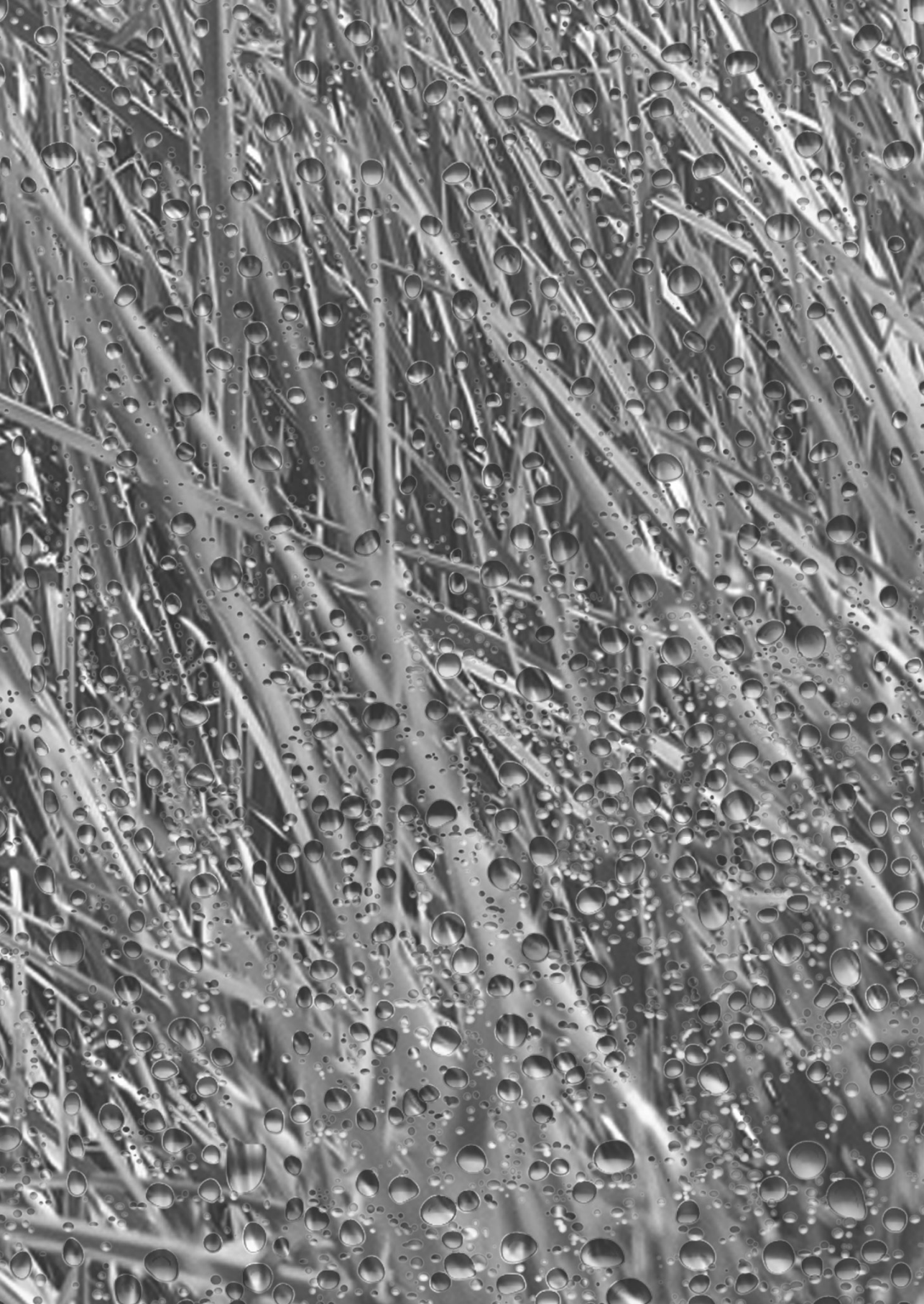

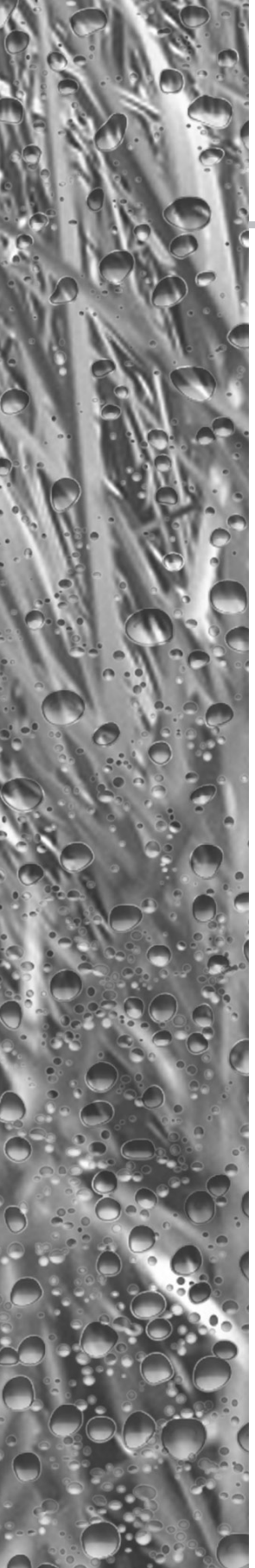

Chapter 8

General discussion

INTRODUCTION

Allergic rhinitis can have a serious impact on the daily life of children, because it interferes with their social activities, hobbies, schoolwork and sleep. An effective and safe treatment will therefore improve the quality of life and development of these children. Immunotherapy is an attractive treatment option due to its disease-modifying and possible preventive qualities. In the last century, many clinical immunotherapy trials have been performed, mainly in adult populations. In general, the assumption was made that the results from these adult studies could be extrapolated to children and as a result children are now treated with subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) in daily clinical practice. Scientific support for this assumption, however, did not exist. The first aim of this thesis was to evaluate the evidence for the efficacy of immunotherapy with inhalant allergens in all its different administration forms in youngsters with allergic rhinitis. Before the results of this evaluation were known, it was already evident that there was a need for large well-designed trials with SLIT in youngsters, studying not only symptoms and medication use, but also quality of life, adherence and immunological effects. The second aim of this thesis was to meet this demand and therefore the STARDROP-study was designed to investigate the efficacy of SLIT with grass pollen allergen in children and adolescents with rhinoconjunctivitis.

EFFICACY OF IMMUNOTHERAPY IN CHILDREN

To evaluate the existing evidence for the efficacy of immunotherapy in children and adolescents (0-18 years) with allergic rhinitis, a systematic review of the literature was performed. Our review showed that there was no evidence that immunotherapy with inhalant allergens in any administration form had a positive effect on symptoms and/or medication use in this population.

While analysing, we encountered two major problems that hampered our evaluation. The first problem was the low quality of the studies, both in design as well as in the description of the results. We had to incorporate low-quality studies in our analysis, since there were not enough high-quality studies available in some of the administration form subgroups. The majority of the low-quality studies were older studies, published before the introduction of the Consolidated Standards of Reporting Trials (CONSORT) in 1996.[1] However, only 2 out of the 14 trials published after 1996 described the concealment of the treatment allocation. In recent years, the lack of high-quality studies has been acknowledged and several guidelines by WAO [2], ARIA [3] and GAL^{2}EN [3,4] have been published with the intention to improve the design, evaluation and report of immunotherapy trials.

The second problem was the heterogeneity among studies with respect to participants (number and type of disease, e.g. concomitant asthma), allergen (type and dose), treatment schedule, duration of treatment, and outcomes. Because of the heterogeneity among the studies, we followed the Cochrane guideline[5] and did not perform a meta-analysis (MA). As Malling et al. showed, heterogeneity can impair the results of a MA, even if it is conducted in accordance with the Cochrane statistical guidelines. Malling et al. recommended using influential statistics to identify extreme outliers and reasons for heterogeneity and interpreted the results with caution.[6] This prudential approach to MAs did not discourage authors to perform MAs on the efficacy of SLIT and many MAs, of varying methodological quality[7], have been published in the past decade. Even two meta-analyses of the meta-analyses have been performed.[8,9] Nieto et al. conscientiously examined five MAs on SLIT.[8] All MAs applied different inclusion and exclusion criteria and even within a MA the selection of studies was not always performed according to a uniform approach. Discrepancies were found among the MAs in the data reported from the same original studies: the MAs reported different estimates for the same outcome or the same estimates for different outcomes. Correction for possible publication bias (e.g. studies with a negative outcome that are not published) in the MAs led to a decrease in several effect estimates and even to a loss of statistical significance in some of the previously significant outcomes. Furthermore, the conclusions of the MAs on the effect of SLIT on symptoms and medication use in general as well as for the different age groups, diseases and allergens in specific, were inconsistent. Because of the discrepancies, lack of robustness and inconsistencies, the authors concluded that the MAs did not provide enough evidence to support the current use of SLIT in patients with allergic rhinitis. Compalati et al. however, came to the conclusion, after analysing the same MAs, that SLIT is effective and can be recommended as a treatment option for patients with allergic rhinitis.[9]

Due to the differences in methodological quality of the individual studies and meta-analyses, as well as the widely acknowledged heterogeneity among trials and the inconsistencies of the MAs, no definite conclusion on the efficacy of SLIT can be drawn. As the characteristics of individual SLIT products might also contribute to the differences in clinical outcomes of the SLIT trials[6], it has now been suggested to judge each product separately on its efficacy and safety. This is only possible when the evidence comes from large and well-designed studies.

SUBLINGUAL IMMUNOTHERAPY TRIALS IN CHILDREN

Sublingual immunotherapy seems the ideal administration form in children, when considering convenience and side effects. As stated before, large and well-designed

trials need to be performed to establish the efficacy and safety of this treatment. In this thesis the results of a randomised controlled trial with SLIT with a grass pollen extract in children with hay fever are described. When the results were published in 2007, this trial was the largest one in children and the only one performed in a primary care setting. We showed that SLIT with the grass pollen extract used in this trial was not effective. In the following section several aspects related to the results of our trial will be discussed.

Population

As is often the case with negative studies, our trial underwent an extra thorough evaluation[10] and this resulted in interesting discussions, especially about the setting of our trial.

A much discussed issue was the fact that our participants were recruited from general practices. It was suggested that primary care patients suffer from less severe allergic rhinitis than patients seen in referral centres and therefore have no indication for immunotherapy. This suggestion arose from, in the eyes of some, low diary card symptom scores reported in our trial. However, these symptom scores were comparable to those reported in two grass pollen SLIT trials that included children from referral centers.[11,12] Moreover, participants in our study experienced symptoms on approximately 75% of the days, corresponding to persistent rhinitis.[13] Quality of life data indicated that, because the participants encountered practical problems and daily life activity impairments, they suffered from at least moderate rhinitis.[13] Furthermore, two large studies showed that around 90% of the patients consulting a general practitioner have moderate to severe rhinitis.[14,15] It is therefore unlikely that the negative outcome of our trial can be explained by the inclusion of participants with insufficient symptoms to detect a treatment effect.

Another point of discussion related to the primary care setting of our trial, was based on the misconception that the general practitioners assessed the indication for immunotherapy and supervised treatment. Actually, the patients in our study were invited by their general practitioner to participate, but researchers from the University Medical Centre assessed the eligibility for immunotherapy using pre-defined criteria, and monitored the patients. This misconception, however, led to interesting discussions about the role of the general practitioner in immunotherapy treatment. In The Netherlands, the majority of patients with allergic rhinitis are treated by their general practitioner. Subcutaneous immunotherapy is not often applied in primary care, because of the risk of serious adverse events. Sublingual immunotherapy, with its favourable safety profile, is marketed by manufacturers as an ideal treatment option for general practitioners. As a result, an increase in the use of SLIT in primary care is inevitable. Therefore, it is important that general practitioners are trained for the diagnosis, management and treatment of allergic rhinitis as well as all aspects related to the treatment with SLIT.[16]

The fact that 75% of our study population was multisensitised was also debated, because it has been suggested that multisensitised patients might not benefit from

immunotherapy as much as monosensitised patients.[13] However, in two trials demonstrating a reduction of symptoms and medication use by grass pollen SLIT in children with rhinitis, 60% and 82% of the participants were multisensitised.[11,12] Furthermore, as was reported in a large study amongst adolescents and adults from an outpatient clinic, more than 80% of patients in daily practice is multisensitised.[17] Most trials include multisensitised patients, with the restriction that the most relevant sensitisation is the one treated with SLIT and symptoms related to the additional sensitisations did not occur in the period analysed. This seems a logical choice, since the study population should ideally represent the patients you would treat in daily practice.

Dosage

An important issue in the discussion about the efficacy of SLIT is dosage. It would be interesting to compare the dosage of the extract used in our trial with that of other marketed products. Unfortunately, it is not easy to compare extracts. The comparison is hampered by the way the manufacturers label the potency of their extracts. This labelling is based on skin tests.[18] The number of patients tested is relatively small and the sensitivity of the population chosen as well as the methodology can differ.[18-20] Furthermore, each manufacturer uses its own allergen specific units, resulting in a variety of unrelated unit names. Examples of allergen units that are being used are IU (international unit), AU (allergy unit), BAU (biological allergy unit), BU (biological unit) and IR (index of reactivity). Lately, manufacturers are urged to present the content of representative major allergens (i.e. the protein that causes sensitisation in the majority of grass pollen allergic patients) in their products in units of mass (mg/ml). However, due to differences in analytic materials and methods (i.e. quantification technique, reference extracts and antibodies), comparison is still hindered.[20-22] Ideally, manufacturers should use standardised assays to analyse the content of major allergen in their extracts.

In the case of grass pollen, comparison of dosages is even more complicated, as an extract may contain one or a mixture of grass pollen species. The reported dosage generally refers to the content of a major allergen of one grass pollen specie, which can differ between extracts. In addition, extracts may vary in biological potency in spite of containing major allergens from the same group (e.g. group 5 major allergen) with the same amount of major allergen in micrograms. The discussion about the optimal number of species needed in an effective extract is still ongoing. The advocates of a single specie extract claim that using one specie is just as effective as using a mixture, but with the advantage of batch-to-batch consistency, optimal efficacy and minimisation of the risk of side effects.[23] The supporters of a mixture, on the other hand, say that a multiple specie extract better reflects the natural exposure and therefore will be more effective.[24]

In our study a mixed pollen extract was used and the mean cumulative monthly dose was 76,000 BU corresponding with 168 µg equivalent *Lol p 5* (group 5 major allergen of

Lolium perenne or Perennial ryegrass). In two paediatric studies with grass pollen allergen tablets that did show a positive effect on symptoms and medication use, higher dosages were reported.[11,12] In the study by Bufe et al. the mean monthly dose of their single allergen tablet was 450 µg *Phl p 5* (group 5 major allergen of *Phleum pratense* or Common timothy).[11] The mean monthly dose of the multiple allergen tablet in the study of Wahn et al. was 600 µg group 5 major allergen.[12] When we disregard all the impediments described above and compare these dosages with our monthly maintenance dose, then the dosage used in our study appears relatively low, and it is therefore possible that an insufficient dosage is the reason why our study had a negative outcome.

Adherence

Another factor that influences efficacy is adherence. Efficacy and adherence are interrelated. Non-adherence compromises efficacy and inefficacy may lead to non-adherence. Therefore, in a randomised clinical trial investigating the efficacy of immunotherapy adherence should be assessed. In the literature the term adherence is used to describe medication intake (i.e. the extent to which a patient follows treatment instructions) as well as the continuation of treatment (i.e. the duration of time from initiation to discontinuation of therapy). The latter actually refers to medication persistence.[25] Medication adherence and persistence are determined by the interaction of patient-, treatment-, disease-, health system- and social-economic factors. In a clinical trial, the research setting serves as an additional factor. Usually, participants in a trial are monitored more frequently, which might have a positive effect on medication adherence and persistence. On the other hand, having to fill in questionnaires and undergo procedures such as lung functions may be a reason for a participant to withdraw from the trial and stop treatment.

In our trial, we investigated adherence to the study protocol and medication intake as well as factors that may influence adherence. Participants having completed the follow-up were considered adherent to the study protocol and 75% of our participants met that definition. Although there are no defined rates for drop-out that are associated with quality, some authors suggest that the drop-out rate should be less than 25%.[26] Therefore, it was suggested that we might not have been able to detect a treatment effect, because the drop-out rate of 25% in our trial had a negative impact on the power of the trial. However, we included more participants in the analysis on the efficacy than needed according to our pre-specified power calculation. Furthermore, the number and reasons for drop-out did not differ between treatment groups. As a consequence, the results on the efficacy in our study were not affected by the drop-out. The main reason for discontinuing the study was the inability to take the medication according to schedule. The fact that the study medication had to be taken before breakfast, the busiest time of the day according to the youngsters, was considered difficult by almost 75% of the drop-outs.

The persistence with SLIT in "real-life" has been investigated in several studies. Unfortunately, in most studies the follow-up is less than a year, whereas the minimum required treatment period is three years. Three studies that did investigate persistence after three years showed inconsistent results. In a large observational study in children, the persistence rate was 79% after 3 years.[27] In contrast, two studies that calculated the rate of spontaneous discontinuations of SLIT by assessing the sales data of manufacturers, reported that only around 15% of the patients used SLIT for at least three years.[28,29] If we rely on the latter studies, it is an understatement to say there is room for improvement and thus studies investigating factors that could improve persistence are needed. Two recent studies showed the influence of the frequency of control visits and patient education on the persistence with SLIT. In a paediatric study, 82% of the children who received a clinical monitoring visit four times a year still used SLIT after 2 years, compared to 30% of the children who were monitored only once a year.[30] More frequent control visits, therefore, appear to improve persistence. The impact of patient education on the persistence with SLIT was investigated in a small randomised study in adults, in which one group received standard verbal instructions and the other a complete 3 hour education programme and written instructions on allergic rhinitis and SLIT. The most interesting result was that in the group who received standard verbal information more patients discontinued treatment (mostly during the updosing phase) because of local side effects.[31] Since local side effects can be managed, patient education might prevent unnecessary withdrawals from SLIT.

Even when a patient continues treatment for at least 3 years, efficacy can be compromised by non-adherence to medication intake. It is unknown how much deviation from the treatment schedule is allowed in order for immunotherapy to be still effective. We used an often-applied but arbitrary limit of 80% and defined adherence to medication intake as using 80% or more of the prescribed study medication for 2 years. According to that definition, 77% of the participants were adherent to medication intake and therefore the ineffectiveness of SLIT could not be explained by non-adherence. An interesting finding in our trial was that the self-reported adherence was 99% and thus gravely overestimated the actual medication intake. Two paediatric studies investigating the efficacy of a grass pollen SLIT tablet after a pre-coseasonal treatment period of approximately 6 months, reported adherence rates of around 94%.[11,12] However, one trial did not state how adherence was defined[11] and the other did not report the method used to assess adherence[12]. The only factor that could be identified to influence the adherence to medication intake in our trial, was the patient's perception of their symptoms during the preceding season. At the start of the treatment, non-adherent youngsters reported more severe symptoms in the preceding grass pollen season. A possible explanation could be that youngsters with more severe symptoms might also have been non-adherent to pharmacotherapy. Another reason could be that these

youngsters had higher expectations of the treatment. The expectations and knowledge of immunotherapy was evaluated in a real-life study among patients with allergic rhinitis receiving SCIT in allergy clinics. This study revealed a serious lack of knowledge and numerous misconceptions. For example, patients anticipated improvement within days or weeks from the initiation of treatment, failed to identify at least one of the allergens they were receiving and were not aware that immunotherapy might have some potential risk or adverse effects. Patients who filled in the questionnaire in the first 6 months of treatment had more knowledge, underlining the importance of repeating the information.[32] Thus, as for medication persistence with SLIT, it appears that educating the patient before treatment and regular control visits could also improve medication adherence.

Safety

Adverse reactions to immunotherapy are divided into local and systemic reactions. Local reactions occur at or near the administration site. Itching and swelling of the lips and in the mouth are, just like in our study, the most frequent reported local reactions to SLIT. These reactions are usually mild and of a temporary nature.[33] Systemic reactions can manifest themselves in various organ systems and become evident as for instance severe rhinitis, asthma exacerbation, generalised urticaria/angioedema and life-threatening anaphylaxis. In our study no serious systemic side effects were reported.

Since no universal definition and classification of local side effects related to SLIT and systemic side effects of immunotherapy in general are available, interpreting data on these reactions is difficult. For systemic reactions related to immunotherapy, a standardised method for reporting these reactions is now being developed that can be used in both research and clinical practice. In research, this method will make it possible to compare outcomes of different clinical trials on various allergen extracts and administration forms. Additionally, the collection of surveillance data will be improved. In clinical practice the standardised method will facilitate the diagnosis and treatment of these reactions.[34]

Even though comparing data at this moment is still difficult, the number of reported systemic side effects related to SLIT is substantially lower than in SCIT. Fatalities related to SCIT have been reported and the administration of the injections is therefore restricted to experienced physicians or specialised centres. Because of the good safety profile, SLIT can be administered at home. Recent publications showed, however, that serious adverse events can occur. So far, 8 cases of anaphylaxis to SLIT have been reported.[35-40] Several lessons can be learned from these case reports.

Previously, SLIT was recommended as an alternative for patients who suffered from severe adverse reactions to the subcutaneous form.[13] However, Cochard and de Groot both reported two anaphylactic reactions related to SLIT using standardised extracts

from different manufacturers (Stalloral 300® - Stallergenes and Grazax® - ALK-Abelló respectively).[37,40] All four patients (3 adolescents and 1 adult) discontinued SCIT in the past because of side effects. This resulted in the recommendation not to switch to SLIT when a patient experiences systemic side effects with SCIT. Furthermore, since two patients experienced a reaction after the first dose, it is now advised to always take the first dose at the doctor's office followed by a 30-minute observation period. This advice is now stated in the package label of both registered high-dose sublingual tablets available in The Netherlands (Oralair® - Stallergenes and Grazax® - ALK-Abelló).

The case report by Blazowski shows the importance of continuous counselling of patients from the beginning to the end of treatment.[36] In this case, the anaphylactic reaction occurred after three years of treatment due to an overdose.

Although serious side effects related to SLIT are rare, physicians and other health care personnel involved in treating patients with immunotherapy should be aware of the possibility and know how to adequately treat serious side effects. Patients should be monitored regularly and be informed about the signs of possible side effects and the steps to take when they occur. Besides educating doctors and patients about the side effects of existing forms of immunotherapy, novel extracts with reduced allergenicity (e.g. peptide and recombinant allergen-based immunotherapy) and even a new administration form (i.e. epicutaneous immunotherapy) are being studied with the goal to improve the safety of immunotherapy.

Relevance of patient-reported outcomes

Patient-reported outcomes (PROs), which are subjective measures, are the only outcomes available to evaluate the effects of medication or other interventions in patients with allergic rhinitis. Objective instruments focusing at nasal function (e.g. peak nasal inspiratory flow) may at best confirm the PROs. Different categories of PROs are available for immunotherapy trials.

The first category consists of symptom and medication scores. According to the World Allergy Organisation (WAO) and the European Medicines Agency (EMA) a combined symptom-medication score is the preferred primary endpoint in immunotherapy trials, because the severity and frequency of symptoms and the use of rescue medication are interdependent.[2,41] Remarkably, a validated method for calculating such a score did not exist until recently, when two new methods were published which look promising.[42,43] Because no validated method was available at the time, we reported symptom scores and medication use separately. Calculating symptom and medication scores is not as straightforward as might be expected. As our review already showed, several methods for calculating symptom and medication scores are being used, which hamper the comparability between studies. With regard to the symptom score, usually a rhinitis total symptom score (RTSS) is computed, which besides nasal symptoms often includes

ocular symptoms as well. The type and number of symptoms that are included in the RTSS vary between studies. Medication scores are often calculated by assigning points to every medication intake (e.g. two points for one antihistamine tablet) and computing a total medication score. However, the absolute points assigned to the same type of medication, the relative weight that is applied between different types of medication, as well as the method for calculating a total score differ between trials. Because no widely accepted method was available for calculating a medication score, we used an often-applied procedure and reported the percentage of days on which rescue medication was used. Another complicating factor in the analysis of symptom and medication scores, especially in trials evaluating a pollen extract, is the level of exposure. Exposure varies from day-to-day and when an average symptom/medication score is calculated for the entire season (i.e. analysing days with high and low exposure) this will result in planation of the scores. These low scores will make it more difficult to demonstrate clinical relevant differences. In most trials, like ours, only days and/or periods with a certain level of exposure are analysed. Another option is to focus on days with severe symptoms, because those are the days that will have a significant impact on the patient's daily life.[44] An interesting recent development to overcome problems related to exposure is the use of allergen challenge chambers, where exposure can be controlled.[45]

The second category of PROs comprises quality of life questionnaires. Since it has been acknowledged that allergic rhinitis can have a significant negative impact on the patient's daily life, the assessment of quality of life has been incorporated in a growing number of trials. Nowadays, in paediatric trials the inclusion of the disease-specific paediatric and adolescent version of the rhinoconjunctivitis quality of life questionnaire (PRQLQ and AdolRQLQ) as a secondary outcome measure is made obligatory by the EMA.[46] The analyses on the relevance of the PRQLQ and AdolRQLQ in our trial showed that these questionnaires as a whole can be considered relevant as they were responsive to exposure and correlated well with the nose and eye symptoms reported in the diary cards. However, there are several indications that these questionnaires could be revised. First, our analyses revealed that both questionnaires contain a substantial number of irrelevant items. Secondly, we found a strong correlation between the nose and eye symptom domains in the RQLQs and the symptom scores recorded in the diary cards. This indicates that when both RQLQ and diary card are used in a trial, participants are burdened with questions that address the same aspects of a disease. Furthermore, both PRQLQ and AdolRQLQ are developed by and validated in youngsters with pollen induced allergic rhinitis and might be missing some important items for patients with persistent rhinitis caused by exposure to indoor allergens, such as snoring and mouth breathing.[3] Besides disease-specific questionnaires, generic quality of life instruments are available. The advantage of these questionnaires is that they enable the comparison of impairments caused by different diseases and some can be used in cost-effectiveness

analyses. Because generic questionnaires are less sensitive to capture small but important changes that may occur in the course of a disease, for instance before and after treatment, they are not commonly used in immunotherapy trials. For children, no widely accepted generic tool is available. The questionnaire evaluated in our study, the COOP/WONCA charts, appeared not to be relevant in the monitoring of youngsters with allergic rhinitis.

The third category comprises the assessment of the patient-relevant treatment benefit. This method includes the effect of a treatment on symptoms and quality of life, as well as treatment satisfaction and treatment burden. Recently, a new instrument for the assessment of patient-defined benefit was developed that can be used for the evaluation of allergic rhinitis treatments.[47] In this instrument the patient's needs (e.g. the need to be able to stay outdoors without symptoms) and benefits (e.g. having an easily applicable treatment) are combined.

The fourth category consists of the retrospective global assessment of symptoms (GAS). This PRO is assessed after the grass pollen season and evaluates the patient's complaints in the preceding months. In clinical trials, the GAS is usually not used as a secondary outcome measure, but as a tool for selecting participants with sufficient symptoms. Clinically relevant differences in symptom scores can only be detected if patients with sufficient symptoms are included. In grass pollen immunotherapy trials a baseline season (i.e. observation during the season before randomisation) is not mandatory, because of the variability in grass pollen exposure between seasons.[3] When the STARDROP-study was designed, we assumed that the GAS would sufficiently reflect the disease severity and therefore the GAS was used to include patients with sufficient symptoms. However, analyses of data collected during the trial showed that the correlation between the GAS and the symptoms scored during the preceding season was statistically significant, but not strong.

Although several PROs are available for immunotherapy trials, they all have limitations. Therefore, it is not surprising that in the latest guidelines on how to design and evaluate immunotherapy trials, defining and validating PROs for all age groups, including the assessment of the clinical minimal difference, was stated as an important and urgent research need.[3]

Analysis of immunological data

Besides the effect on clinical parameters, we were also interested in the influence of SLIT on the immune system. Additionally, we aimed to identify immunological markers that may be used as an objective measure for the clinical response to immunotherapy. Such objective markers of clinical efficacy might not only enable the monitoring of a treatment effect, but also improve the selection of patients who are likely to respond to immunotherapy. In our trial, the levels of several soluble biological markers (SBMs),

consisting of cytokines, soluble adhesion molecules and activation markers, in serum were used as read-out parameters of immunological reactivity. We limited our analyses to serological markers, because those markers can be easily obtained and assessed and therefore might be relevant to apply in clinical practice.

Collecting samples and measuring immunological data is time-consuming and expensive. Subsequently analysing the data with the wrong statistical method is a waste of time and money. Immunological datasets and the corresponding research questions can be very complex and in general more advanced statistical methods are needed to extract the maximum amount of information from the data and to get the right answers. Such sophisticated methods are used in many other scientific disciplines, but immunological studies in general still use simple statistical techniques for data analysis.[48] Finding the right method, however, is not easy and depends on several factors, such as the research question (e.g. identify associations between immunological and clinical outcomes), sample size, number of groups and variables, type of data (e.g. continuous, categorical), and the assumptions required for a method to be used (e.g. normal distribution).[48] Two very helpful articles in this matter are the ones by Genser et al. and Uh et al., describing the use of advanced statistical approaches in immunology.[48,49] The article by Uh et al. was of special interest to us, because it focused on datasets with non-detects, i.e. values below the detection limit.[49] When we explored our dataset we found that for some SBMs more than half of the data consisted of non-detects. Furthermore, as often seen in immunological measurements, the data were positively skewed and normality could not be achieved for all SBMs, even after logarithmic transformation. Therefore, we had to seek for a method that can handle large proportions of non-detects and assumes no underlying distribution. Unfortunately, our dataset did not meet the conditions for the advanced approached described by Uh et al. and thus we explored another advanced method, called quantile regression. Quantile regression is a generalisation of percentiles to regression models and has already been used in the fields of genetics and ecology, where non-detects are also a well-known problem.[50] By using quantile regression we were able to compare groups and compute meaningful linear trends, even if more than half of the data consists of non-detects. The results of our analysis showed that treatment with SLIT did not influence the level of the measured SBMs and relevant markers of clinical efficacy could not be determined. This result was in line with the clinical outcome of the trial. Quantile regression proved to be a valuable addition to the statistical methods that can be used for the analysis of immunological datasets with non-detects.

FINAL NOTE

This discussion only focussed on issues relevant to the content of this thesis. There are, however, many more interesting aspects of immunotherapy and consequently even more research needs than discussed here. The new developments in the immunotherapy field are highly interesting and dynamic and hopefully (despite the cut down on budgets) will eventually lead to a curative and safe treatment for allergic diseases in children and adolescents.

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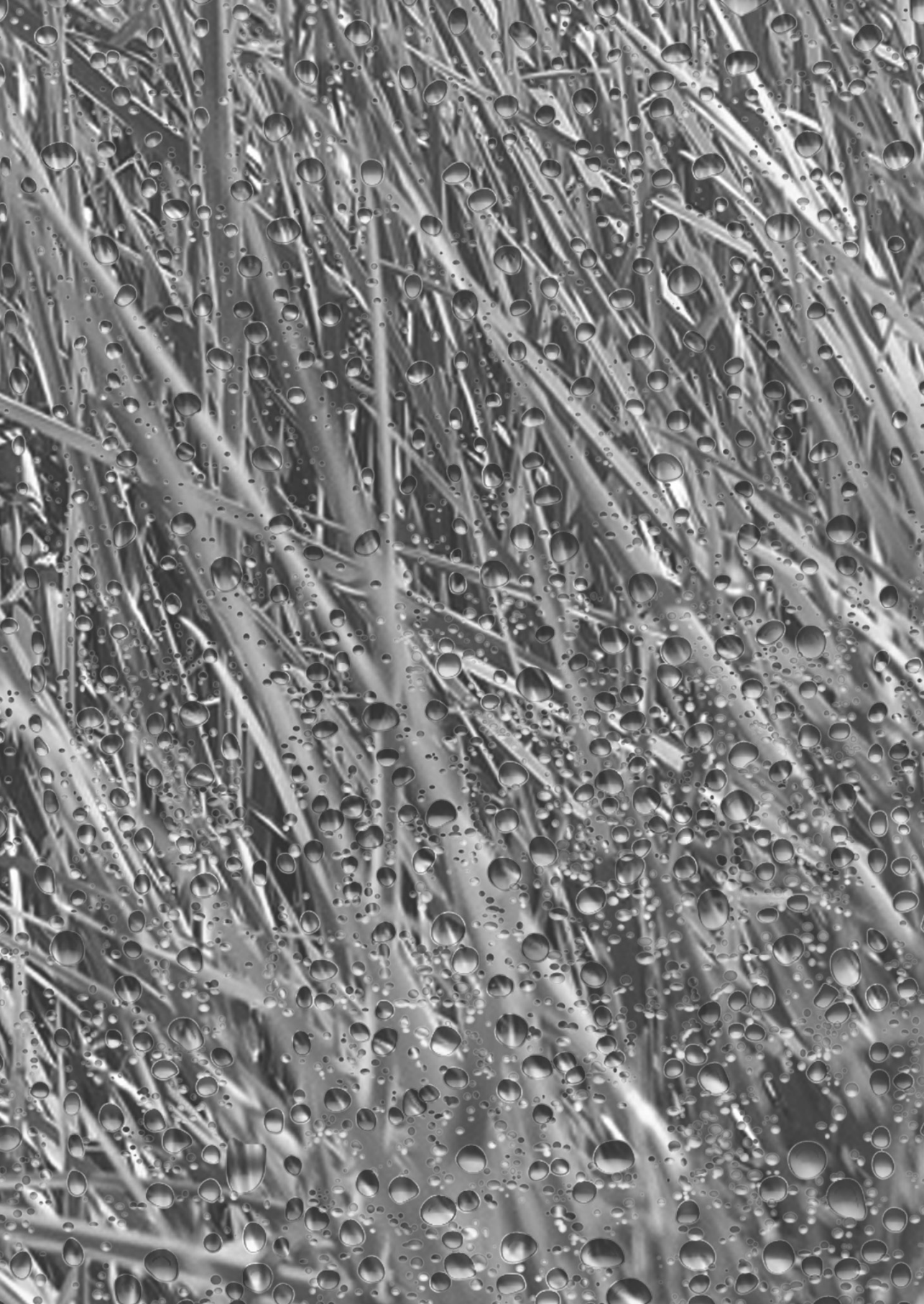
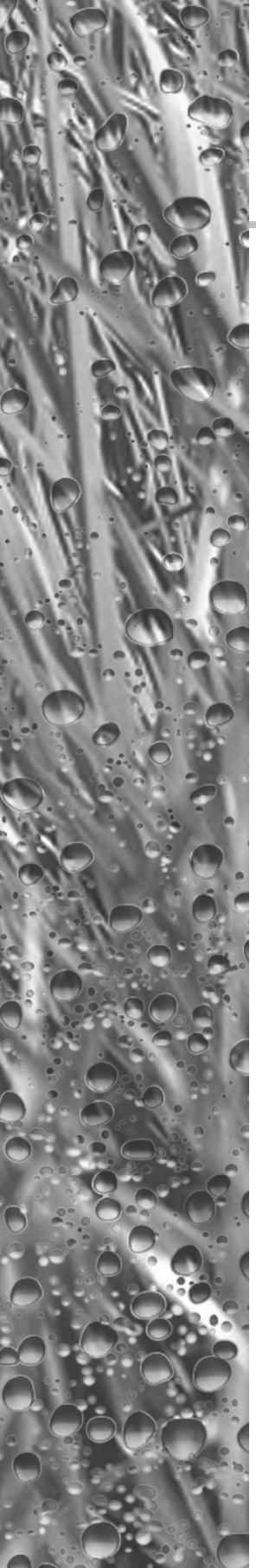




Chapter 9

Summary

Samenvatting

List of abbreviations

Summary

INTRODUCTION

Allergic rhinitis is one of the most prevalent chronic diseases in Europe. Besides nose symptoms such as sneezing and a blocked nose, patients also suffer from general complaints like fatigue, sleeping problems and difficulty concentrating. Allergic rhinitis can significantly influence the patient's quality of life due to its impact on daily activities, school- and work performance.

Allergic rhinitis is caused by an excessive inflammatory reaction of the immune system to normally harmless allergens, like grass pollen. Allergen avoidance and symptomatic treatment ameliorate symptoms, but only as long as these measures are applied. Allergen-specific immunotherapy on the other hand, targets the cause of the disease. Immunotherapy consists of the repeated administration of the allergen to which the patient is allergic for at least 3 years, either by subcutaneous injections (subcutaneous immunotherapy) or by drops/tablets under the tongue (sublingual immunotherapy). The idea behind this treatment is that due to the induction of immunological tolerance the patient will become less sensitive to the allergen. Immunotherapy reduces not only symptoms and medication requirements during treatment, but these effects also persist for several years after discontinuation of treatment. Subcutaneous immunotherapy (SCIT) is effective, but the inconvenient injections limit its application in children and the risk of serious side effects restrict its use to a hospital or primary care setting. Sublingual immunotherapy (SLIT) is not only patient-friendlier but also has a favourable safety-profile, making administration at home possible. These advantages make SLIT an ideal treatment option for children and facilitate its use in primary care.

AIMS OF THIS THESIS

Until 2001, many clinical immunotherapy trials had been performed, mainly in adult populations. In general, the assumption was made that the results from these studies could be extrapolated to children and as a result children are treated with SCIT and SLIT in daily clinical practice. Scientific evidence for this assumption, however, did not exist. The first aim of this thesis was to evaluate the evidence for the efficacy of immunotherapy with inhalant allergens in all its different administration forms in youngsters with allergic rhinitis. Before the results of this review were known, it was already evident that there was a need for large well-designed trials with SLIT in youngsters, studying

not only symptoms and medication use, but also quality of life, adherence and immunological effects. The second aim of this thesis was to meet this demand and therefore the STARDROP-study was designed to investigate the efficacy of SLIT with grass pollen allergen in children and adolescents with allergic rhinitis.

EFFICACY OF IMMUNOTHERAPY IN CHILDREN

To evaluate the evidence for the efficacy of immunotherapy with inhalant allergens in all its different administration forms in youngsters (0-18 years) with allergic rhinitis, a systematic review of the literature was performed. The results of this review are presented in **chapter 2**.

Randomised controlled trials (RCTs) comparing immunotherapy with placebo, symptomatic treatment or a different administration form of immunotherapy were included. The primary outcome of the review was the effect of immunotherapy on the symptom and/or medication scores. Methodological quality was assessed using the validated Delphi list. A method of best evidence synthesis, a rating system with levels of evidence based on the overall quality and the outcome of the trials, was used to assess the existing evidence for efficacy. Medline, EMBASE and the Cochrane Controlled Clinical Trials Register were searched, which resulted in 1629 titles and abstracts published between 1966 and June 2006. Twenty-eight publications met the predefined criteria and were included in the review: six subcutaneous (SCIT), four nasal (LNIT), seven oral (OIT) and eleven sublingual (SLIT) immunotherapy trials. Overall 1619 youngsters (3-18 years) were included. Only 39% of the trials were of high methodological quality, of which the majority was found within the SLIT subgroup. For the SCIT and OIT subgroups the level of evidence for efficacy was conflicting. Moderate evidence of effect was found for LNIT. Analysis of the SLIT subgroup showed no evidence of effect. The evidence for the perennial and seasonal allergen trials within the subgroups varied from moderate evidence of effect to no evidence of effect.

Conclusion: in 2006, there was insufficient evidence that immunotherapy in any administration form had a positive effect on symptoms and/or medication use in children and adolescents with allergic rhintis.

SUBLINGUAL IMMUNOTHERAPY IN CHILDREN WITH ALLERGIC RHINITIS: THE STARDROP-STUDY

Until 2001, most efficacy studies in children lacked power and methodological quality to be conclusive and all were performed in referral centres. There was a need for large well-

designed trials with SLIT in youngsters, studying not only symptoms and medication use, but also quality of life, adherence and immunological effects. To meet this demand, the STARDROP-study was designed. In this large randomised trial 204 youngsters aged 6 to 18 years (114 boys/90 girls; mean age 13 years), known to their general practitioner with allergic rhinitis due to a grass pollen allergy, received either grass pollen extract (GP) or placebo for two years. The mean cumulative dose was 4.5 mg equivalent *Lol p 5* (group 5 major allergen of *Lolium perenne* or Perennial ryegrass).

Efficacy and safety

Chapter 3 focuses on the primary and secondary outcomes of the study. The primary outcome was the mean daily total symptom score (scale 0-15) comprising sneezing, itching nose, watery running nose, nasal blockage and itching eyes during the months May through August of the second treatment year. Additionally, the use of symptomatic medication, overall evaluation of treatment, quality of life and side effects were evaluated. Out of the 204 randomised youngsters, 168 entered the intention-to-treat analysis (91 GP, 77 placebo). The mean daily total symptom score did not differ between participants allocated to the grass pollen extract and those allocated to placebo (difference for GP minus placebo: -0.08; 95% CI -0.66 to 0.50; p=0.78). The same result was found within the subgroups "age" (<13 and ≥13 years) and "severity of symptoms prior to study" (symptom score ≤8 and >8). Even on days with high grass pollen exposition, and therefore higher symptoms scores, no significant difference between treatment groups was observed. Furthermore, no differences were found for rescue medication free days, disease specific quality of life and overall evaluation of the treatment effect. Local side effects, like swelling and itching in the mouth, were more frequent in the intervention group (39% vs. 17% of participants; p=0.001). Systemic anaphylactic reactions were not reported.

Conclusion: SLIT with the grass pollen extract used in this study was not effective in children and adolescents recruited from primary care settings, and therefore could not be recommended for general practitioners as a therapeutic modality in youngsters with hay fever.

Adherence

Adherence (or compliance) is generally defined as the extent to which patients use their medication as prescribed. Efficacy and adherence are inter-related. Non-adherence compromises efficacy and inefficacy may lead to non-adherence. The administration at home is considered a great advantage of SLIT, because it takes away an important reason for non-adherence with the subcutaneous form. On the other hand, self-administration and consequently lack of medical supervision might lead to incorrect or irregular use. Before the STARDROP-study, several trials on the efficacy of SLIT in youngsters were

published, but only a few reported data on medication intake. Factors that could influence adherence to SLIT were never investigated. The data on adherence both to the study protocol and medication intake as well as factors that influenced adherence to SLIT in the STARDROP-study are described in **chapter 4**. Participants having completed the follow-up were considered adherent to the study protocol. Adherence to medication intake was assessed by weighing the study medication. Participants who completed the follow-up and used ≥80% of the prescribed medication were considered adherent to medication intake. Patient-, disease- and treatment-related factors were analysed. 154 youngsters completed the study. Main reason for discontinuation was the inability to take medication according to schedule. Drop-outs were older, had more difficulty following the medication instructions and their overall evaluation of the treatment effect was lower. The number and reasons for drop-out did not differ between treatment groups. In total 77% of the participants was adherent to medication intake. Self-reported adherence was 99%. Non-adherent participants experienced more severe symptoms prior to the trial. Symptom scores did not differ between compliant and non-compliant participants. In adherent as well as non-adherent participants, no difference was found between the intervention and the placebo group with respect to symptom scores.

Conclusion: adherence to both study protocol and medication intake was good. Drop-out was affected by age, evaluation of the treatment effect and medication instructions. Non-adherence to medication intake was influenced by the severity of the disease prior to the trial. The ineffectiveness of SLIT in our study could not be explained by non-adherence.

The relevance of patient-reported outcomes

Patient-reported outcomes (PROs) are the only instruments available to assess the efficacy of an intervention in patients with allergic rhinoconjunctivitis. As allergic rhinoconjunctivitis is a systemic disease, it is now recommended to use not only PROs focusing at classical symptoms, but also health related quality of life (HRQL) instruments in immunotherapy trials. Although well accepted, it is unknown how relevant the inclusion of these HRQL instruments is. In **chapter 5** we aimed to determine the relevance of two of these additional outcome measures, the disease-specific Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) and the generic COOP/WONCA-charts (CWC). We hypothesised that a relevant PRO would have to be responsive to pollen exposure and would at least have a moderate correlation with the classical symptoms of allergic rhinoconjunctivitis. Furthermore, we evaluated a post-season PRO, i.e. a global assessment of symptoms (GAS). This assessment is used in clinical trials as a tool for selecting participants with sufficient symptoms and in daily practice to evaluate the patient's complaints during the preceding season. We assessed the correlation of this retrospective score with the actual symptoms during the previous pollen season. Data from 36

children and 63 adolescents were analysed. Based on the total scores of the paediatric and adolescent version of the RQLQ, both questionnaires were considered relevant, as they were responsive to exposure and showed a moderate to strong correlation with the rhinoconjunctivitis symptoms. However, in both children and adolescents 40% of the RQLQ items were not relevant according to our definition. The CWC as a whole and the separate charts appear less relevant because of the weak correlations with the daily symptom score from the diary. The correlation between our post-season GAS and the in-season daily symptom score was weak.

Conclusion: the paediatric and adolescent RQLQ are relevant, but could be shortened as they contain a substantial number of irrelevant items. The CWC are not relevant in the monitoring of youngsters with hay fever. The retrospective GAS does not sufficiently reflect the actual symptoms during the preceding season.

Soluble biological markers

Besides the effect on clinical parameters, we were also interested in the influence of SLIT on the immune system. Additionally, we aimed to identify immunological markers that may be used as an objective measure for the clinical response to immunotherapy. Such objective markers of clinical efficacy might not only enable the monitoring of a treatment effect, but also improve the selection of patients who are likely to respond to immunotherapy. In our trial, the levels of several soluble biological markers (SBMs), consisting of cytokines, soluble adhesion molecules and activation markers, in serum were used as read-out parameters of immunological reactivity. We limited our analyses to serological markers, because those markers can be easily obtained and assessed and therefore might be relevant to apply in clinical practice.

Serum samples were collected at 5 time points during the 2-year treatment period and analysed for their IL-10, IL-12, IL-13, IFN-γ, TNF-α, sIL-2Receptor, sICAM-1 and sE-selectin content. These SBMs were chosen because of their relationship with T-cell activation and allergic inflammation. During the trial, that took several years, we were facing changes in assays and consequently changes in detection limits. Furthermore, initial analysis of the dataset revealed that for some SBMs a substantial proportion of the data consisted of non-detects, i.e. values below the detection limit. Therefore, we were not able to perform conventional statistical analyses (like ANOVA and regression) on this dataset. Because our data did not meet the conditions for the few sophisticated statistical methods currently available for the analyses of datasets with non-detects, we explored the use of the advanced method of quantile regression. Although new to the field of immunology, quantile regression has already been used in the fields of genetics and ecology, where non-detects are also a well-known problem. Quantile regression, a generalisation of percentiles to regression models, models the median or higher percentiles and tolerates very high numbers of non-detects. A detailed non-technical description of this method

with an implementation to immunological data is presented in **chapter 7**. With the use of quantile regression, we were able to compare groups and compute meaningful linear trends, even if more than half of the data consisted of non-detects. As described in **chapter 6**, the results of our analysis showed that treatment with SLIT with grass pollen did not influence the level of the measured SBMs. Particularly, we did not find an effect on IL-10 levels, the cytokine that should have been affected by successful immunotherapy. This result was in line with the negative clinical outcomes of the trial. Moreover, we were not able to identify other factors, including age, which might have an important effect on soluble biomarkers as measured outside the pollen season.

Conclusion: SLIT did not induce changes in the serum levels of the selected SBMs and relevant markers of clinical efficacy could not be determined. Quantile regression proved to be a valuable addition to the statistical methods that can be used for the analysis of immunological datasets with non-detects.

In the general discussion, **chapter 8**, the main findings of the review and the results of the STARDROP-study are reflected on.

Samenvatting

INLEIDING

Allergische rhinitis is een van de meest voorkomende chronische aandoeningen in Europa. Patiënten met allergische rhinitis hebben last van niezen, een loopneus, jeuk in neus of ogen en een verstopte neus. Daarnaast hebben ze ook algemene klachten zoals vermoeidheid, slaapproblemen en concentratiestoornissen. Allergische rhinitis kan de kwaliteit van leven van een patiënt aanzienlijk verminderen, omdat de patiënt gehinderd wordt in zijn dagelijkse activiteiten en (school) werk.

Allergische rhinitis wordt veroorzaakt door een overdreven reactie van het immuunsysteem op een allergeen (bijv. graspollen). Het vermijden van het allergeen en het gebruik van symptomatische medicatie verminderen de klachten, maar alleen zolang deze maatregelen worden toegepast. Allergeen-specifieke immunotherapie is de enige behandeling die de oorzaak van de ziekte aanpakt. Een behandeling met immunotherapie houdt in dat de patiënt gedurende minimaal 3 jaar het allergeen waar hij/zij allergisch voor is krijgt toegediend, hetzij door middel van subcutane injecties (subcutane immunotherapie) of door middel van druppels of tabletten onder de tong (sublinguale immunotherapie). Het doel van immunotherapie is de reactie van het immuunsysteem op het allergeen te veranderen zodat de patiënt minder gevoelig wordt voor dat specifieke allergeen. Immunotherapie vermindert hierdoor niet alleen de klachten gedurende de behandeling, maar dit effect houdt tenminste enkele jaren nadat de behandeling is beëindigd aan. Subcutane immunotherapie (SCIT) is effectief, maar het gebruik van injecties beperkt de toepassing bij kinderen en vanwege de kans op ernstige bijwerkingen kan SCIT alleen worden toegepast in een ziekenhuis of huisartsenpraktijk. Sublinguale immunotherapie (SLIT) is niet alleen patiëntvriendelijker, maar ook veiliger en kan daarom thuis gebruikt worden. Deze voordelen maken SLIT een ideale behandelingsmogelijkheid voor kinderen en vergemakkelijkt het gebruik van immunotherapie in de huisartspraktijk.

DOELEN VAN DIT PROEFSCHRIFT

Tot aan 2001 waren al veel onderzoeken naar de effectiviteit van immunotherapie uitgevoerd, voornamelijk bij volwassen patiënten. Aangenomen werd dat de resultaten van deze studies geëxtrapoleerd konden worden naar kinderen en dus worden in de dagelijkse praktijk kinderen al vele jaren behandeld met zowel SCIT als SLIT. Echter,

wetenschappelijk bewijs voor deze aanname bestond niet. Het eerste doel van dit proefschrift was om het bewijs voor de effectiviteit van alle toedieningsvormen van immunotherapie met inhalatieallergenen bij kinderen met allergische rhinitis te evalueren. Voordat de resultaten van deze evaluatie bekend waren, was het al duidelijk dat er behoefte was aan grote goed opgezette kinderstudies met SLIT. Het tweede doel van dit proefschrift was om in deze behoefte te voorzien en daarom werd de STARDROP-studie opgezet om de effectiviteit van SLIT met graspollen bij kinderen met allergische rhinitis te onderzoeken.

EFFECTIVITEIT VAN IMMUNOTHERAPIE BIJ KINDEREN

Het bewijs voor de effectiviteit van immunotherapie bij kinderen werd onderzocht door het maken van een systematisch overzicht van alle gepubliceerde studies die de effectiviteit van immunotherapie met inhalatieallergenen bij kinderen en adolescenten (0-18 jaar) met allergische rhinitis hadden onderzocht. Dit systematische overzicht van de opzet en de resultaten van deze studies wordt gepresenteerd in **hoofdstuk 2**.

Gerandomiseerde en gecontroleerde trials (RCTs) die immunotherapie vergeleken met placebo, symptomatische behandeling of een andere toedieningsvorm van immunotherapie werden geïncludeerd. De primaire uitkomst was het effect van immunotherapie op de symptoomscore en/of medicatiescore. De methodologische kwaliteit van de RCTs werd beoordeeld met behulp van de gevalideerde Delphi-lijst. De methode van "best evidence synthesis", een beoordelingssysteem met niveaus van bewijskracht, gebaseerd op de kwaliteit en de uitkomst van de RCTs, werd gebruikt om het bestaande bewijs voor de effectiviteit te beoordelen. Een zoekactie in de literatuurdatabases Medline, EMBASE en Cochrane Controlled Trials Register leverde 1629 artikelen op, gepubliceerd tussen 1966 en juni 2006. Hiervan voldeden zes subcutane (SCIT), vier nasale (LNIT), zeven orale (OIT) en elf sublinguale (SLIT) immunotherapie trials aan de inclusiecriteria. Het totale aantal deelnemers in deze 28 studies was 1629 (3-18 jaar). Slechts 39% van de RCTs was van hoge methodologische kwaliteit. Dit waren voornamelijk SLIT-trials. De bewijskracht voor de effectiviteit van SCIT en OIT was tegenstrijdig en voor LNIT matig. Analyse van de SLIT subgroep liet zien dat er geen bewijs voor effectiviteit was. Als binnen de verschillende toedieningsvormen gekeken werd naar de effectiviteit van immunotherapie bij seizoensgebonden allergieën (pollen) en bij niet-seizoensgebonden allergieën (huisstofmijt), dan varieerde de bewijskracht van matig tot geen bewijs voor effectiviteit.

Conclusie: in 2006 was voor geen van de toedieningsvormen van immunotherapie voldoende bewijs dat het bij kinderen en adolescenten met allergische rhinitis een positief effect op klachten en/of medicatiegebruik had.

SUBLINGUALE IMMUNOTHERAPIE BIJ KINDEREN MET ALLERGISCHE RHINITIS: DE STARDROP-STUDIE

De kinderstudies, gepubliceerd tot 2001, waren klein en over het algemeen van onvoldoende methodologische kwaliteit. Hierdoor kon op dat moment geen uitspraak worden gedaan over de effectiviteit van SLIT bij kinderen. Ook was er nog geen studie uitgevoerd met kinderen die bij hun huisarts bekend waren met allergische neusklachten, terwijl de meeste kinderen in de huisartspraktijk behandeld worden. Er was dus behoefte aan grote goed opgezette SLIT-studies bij kinderen, die niet alleen het effect van de behandeling op symptomen en gebruik van symptomatische medicatie beoordeelden, maar ook kwaliteit van leven, therapietrouw en het effect op het immuunsysteem evalueerden. Om in deze behoefte te voorzien werd de STARDROP-studie opgezet. In deze grote trial werden 204 jongeren tussen de 6 en 18 jaar (114 jongens/90 meisjes; gemiddelde leeftijd 13 jaar), die bekend waren bij hun huisarts met allergische rhinitis, gedurende 2 jaar behandeld met een graspollen extract (GP) of placebo. De gemiddelde totale dosis van het belangrijkste graspollenallergeen in het extract was 4,5 mg *Lol p 5* (*Lolium perenne* oftewel Engels raaigras).

Effectiviteit en veiligheid

Hoofdstuk 3 focust op de resultaten van de primaire en secundaire uitkomsten van de studie. De primaire uitkomst was de gemiddelde dagelijkse symptoomscore (niezen, jeukende neus, loopneus, verstopte neus, jeukende ogen; schaal 0-15) in de maanden mei t/m augustus van het tweede behandeljaar. Secundaire uitkomsten waren het percentage dagen zonder symptomatische medicatie, de beoordeling van het behandeleffect door de deelnemer, ziektespecifieke kwaliteit van leven en veiligheid.

Van de 204 gerandomiseerde jongeren konden er 168 worden geanalyseerd (91 GP, 77 placebo; intention-to-treat analyse). Er kon geen statistisch significant of klinisch relevant verschil in symptoomscores worden aangetoond tussen de groep deelnemers die het graspollenextract had gebruikt en de groep die placebo kreeg (verschil GP minus placebo: -0.08; 95% CI -0.66 tot 0.50; p=0.78). De subgroepanalyses naar leeftijd (<13 en ≥13 jaar) en ernst van de klachten in het seizoen voor de studie (symptoom score ≤8 en >8) lieten evenmin een verschil zien. Zelfs op dagen met hoge graspollenexpositie en dus hogere symptoomscores, kon geen significant verschil tussen beide behandelgroepen worden aangetoond. De analyses van de secundaire uitkomsten lieten ook geen statistisch significante verschillen zien tussen beide behandelgroepen. In totaal werden in beide groepen evenveel bijwerkingen gemeld. In de groep die het graspollenextract had gebruikt werden meer lokale bijwerkingen, zoals jeuk en zwelling in de mond-keelholte, gerapporteerd (39% vs. 17% van de deelnemers; p=0.001). Ernstige systemische reacties deden zich niet voor.

Conclusie: in onze studie was SLIT met graspollen niet effectief bij kinderen en adolescenten die gerekruteerd waren in huisartsenpraktijken. Het onderzochte extract kan daarom niet worden aanbevolen aan huisartsen als behandeling voor jongeren met hooikoorts.

Therapietrouw

Therapietrouw, oftewel de mate waarin de patiënt medicatie volgens voorschrift gebruikt, is essentieel voor de werkzaamheid van een behandeling. Anderzijds kan ineffectiviteit leiden tot therapieontrouw. Het feit dat SLIT thuis gebruikt kan worden, wordt gezien als een groot voordeel ten opzichte van SCIT. Echter, thuisgebruik en daardoor gebrek aan medische supervisie kan ook leiden tot onregelmatig of onjuist gebruik. Voor de start van de STARDROP-studie werd in de gepubliceerde studies over de effectiviteit van SLIT bij kinderen nauwelijks gegevens over therapietrouw gepresenteerd. Factoren die de therapietrouw zouden kunnen beïnvloeden waren nooit onderzocht. Wij stelden ons tot doel om de trouw aan het studieprotocol en de therapietrouw (d.w.z. medicatieinname) te kwantificeren en daarnaast factoren die deze trouw mogelijk kunnen beïnvloeden te identificeren. De resultaten worden beschreven in **hoofdstuk 4**. Deelnemers die de studie hadden voltooid werden beschouwd als trouw aan het studieprotocol. Voor het bepalen van de medicatieinname werd de ingeleverde studiemedicatie gewogen. Deelnemers die de studie hadden voltooid en 80% of meer van de voorgeschreven medicatie hadden gebruikt werden beschouwd als therapietrouw. Diverse patiënt-, ziekte- en behandelings-gerelateerde factoren werden geanalyseerd. De studie werd voltooid met 154 jongeren (75%). De voornaamste reden van uitval was het onvermogen de studiemedicatie volgens schema te gebruiken. Uitvallers waren ouder, hadden meer moeite met het opvolgen van de medicatievoorschriften en hun algemene beoordeling van het behandeleffect was lager. Het aantal uitvallers en de redenen om met het onderzoek te stoppen verschilden niet tussen beide behandelgroepen. In totaal was 77% van de deelnemers therapietrouw. Zelfgerapporteerde therapietrouw was 99%. Therapieontrouwe deelnemers gaven aan meer neus- en oogklachten te hebben gehad in het graspollenseizoen voor de studie. Symptoomscores gedurende de studie verschilden niet tussen therapietrouwe en therapieontrouwe deelnemers. Zowel in de groep therapietrouwe als in de groep therapieontrouwe deelnemers kon geen verschil worden aangetoond tussen de interventie- en de placebogroep wat betreft symptoomscores.

Conclusie: de mate van trouw aan het studieprotocol en de medicatieinname in onze studie was goed. Factoren die de uitval beïnvloedden waren leeftijd, algemene beoordeling van het behandeleffect en de medicatievoorschriften. Therapietrouw werd beïnvloed door de ernst van de klachten voorafgaand aan de studie. De ineffectiviteit van SLIT in de STARDROP-studie kon niet worden verklaard door therapieontrouw.

De relevantie van door de patiënt gerapporteerde uitkomstmaten

Door de patiënt gerapporteerde uitkomstmaten (PRO) zijn de enige instrumenten om de effectiviteit van een interventie bij patiënten met allergische rhinitis mee te kunnen bepalen. Aangezien allergische rhinitis een systemische ziekte is, wordt tegenwoordig geadviseerd om in immunotherapie-studies niet alleen het effect van de behandeling op de allergische neus- en oogklachten, maar ook het effect op gezondheidsgerelateerde kwaliteit van leven (HRQL) te onderzoeken. Het is echter onduidelijk hoe relevant de toevoeging van deze HRQL-uitkomsten is. In **hoofdstuk 5** hadden wij ons tot doel gesteld om de relevantie van twee van deze aanvullende uitkomsten, namelijk de ziekte specifieke Rhinoconjunctivitis Kwaliteit van Leven Vragenlijst (RQLQ) en de algemene kwaliteit van leven vragenlijst COOP/WONCA (CWC), te bepalen. Onze hypothese was dat een relevante PRO gevoelig moet zijn voor pollenexpositie en tenminste een matige correlatie moet hebben met de allergische neus- en oogklachten, die dagelijks gescoord werden in een dagboek. Daarnaast hebben wij de relevantie van de globale beoordeling van symptomen (GAS) geëvalueerd, een PRO die na het graspollenseizoen wordt afgenomen. Deze retrospectieve GAS wordt in klinische trials gebruikt voor de selectie van deelnemers met voldoende klachten en in de dagelijkse praktijk om de klachten van een patiënt in het voorgaande seizoen te evalueren. Wij stelden dat de GAS als relevant kon worden beschouwd als een sterke correlatie met de werkelijke symptomen in het voorafgaande graspollenseizoen kon worden aangetoond. De gegevens van 36 kinderen en 63 adolescenten werden geanalyseerd. Gebaseerd op de totaalscores van de kinder- en adolescenten-versie van de RQLQ, konden beide lijsten als relevant worden beschouwd, aangezien zij zowel gevoelig waren voor expositie als matig tot sterk correleerden met de neus- en oogklachten. Echter, 40% van de vragen van zowel de kinder- als adolescenten-versie van de RQLQ bleek niet relevant te zijn volgens de door ons gehanteerde definitie. De CWC als geheel en de afzonderlijke vragen leken minder relevant, omdat zij een zwakke correlatie met de neus- en oogklachten lieten zien. De correlatie tussen de na het seizoen afgenomen GAS en de in het seizoen gescoorde symptomen was zwak.

Conclusie: de RQLQs voor kinderen en adolescenten zijn relevant, maar zouden ingekort kunnen worden aangezien ze een aanzienlijk aantal irrelevante vragen bevatten. De CWC is niet relevant voor het monitoren van jongeren met hooikoorts. De retrospectieve GAS weerspiegelt onvoldoende de werkelijke symptomen in het voorafgaande seizoen.

Biologische markers

Naast het effect op klinische parameters, waren wij ook geïnteresseerd in de invloed van SLIT op het immuunsysteem. Daarnaast wilden we onderzoeken of we een immunologische marker konden identificeren die mogelijk als objectieve maat voor de klinische

respons op immunotherapie zou kunnen dienen. Met behulp van deze immunologische indicatoren van klinische effectiviteit zou het behandeleffect gemonitord kunnen worden en patiënten geselecteerd kunnen worden die goed op de behandeling zullen reageren. In onze studie dienden de niveaus van verscheidene oplosbare biologische markers (SBMs) in serum, bestaand uit cytokines, adhesie moleculen en activatie markers, als uitleesparameters voor immunologische reactiviteit. We hebben ons beperkt tot de analyse van serologische markers, omdat deze markers eenvoudig te verkrijgen zijn en bepaald kunnen worden, waardoor ze mogelijk relevant zijn voor gebruik in de dagelijkse klinische praktijk.

Tijdens de 2 jaar durende studie werd op 5 momenten bloed afgenomen en in het serum de hoeveelheid IL-10, IL-12, IL-13, IFN-γ, TNF-α, sIL-2Receptor, sICAM-1 en sE-selectin bepaald. Deze SBMs zijn gekozen vanwege hun relatie met T-cel activatie en de allergische ontstekingsreactie. Gedurende de trial, die meerdere jaren in beslag nam, werden we geconfronteerd met veranderingen in assays en dus ook veranderingen in detectiegrenzen. Daarbij liet een eerste analyse van de dataset zien dat voor sommige SBMs een aanzienlijk deel van de data bestond uit non-detects, d.w.z. waarden onder de detectiegrens. Hierdoor kon deze dataset niet met de conventionele statistische analysemethoden (zoals ANOVA en regressie analyse) geanalyseerd worden. Omdat onze dataset niet voldeed aan de voorwaarden van de paar geavanceerde statistische methoden die op dit moment beschikbaar zijn voor de analyse van datasets met non-detects, hebben wij een andere geavanceerde methode gebruikt, genaamd "quantile regression". Deze methode is niet eerder gebruikt in de immunologie, maar wel al in de genetica en ecologie, waar non-detects ook een welbekend probleem zijn. "Quantile regression", een generalisatie van percentielen naar regressie modellen, modelleert de mediaan of hogere percentielen en tolereert zeer hoge aantallen non-detects. Een gedetailleerde niet-technische beschrijving van deze methode met een toepassing op immunologische data wordt in **hoofdstuk 7** gepresenteerd. Door het gebruik van "quantile regression" waren we in staat om groepen te vergelijken en betekenisvolle lineaire trends te berekenen, zelfs als meer dan de helft van de data bestond uit non-detects. Zoals beschreven in **hoofdstuk 6**, lieten de resultaten van onze analyses zien dat de behandeling met SLIT met graspollen geen invloed had op het niveau van de gemeten SBMs. In het bijzonder vonden we geen effect op IL-10, de cytokine die beïnvloed zou moeten worden door succesvolle immunotherapie. Dit resultaat was in overeenstemming met de negatieve klinische uitkomsten van onze studie. We waren bovendien niet in staat om andere factoren te identificeren, waaronder leeftijd, die mogelijk een belangrijk effect hebben op (buiten het graspollenseizoen gemeten) SBMs.

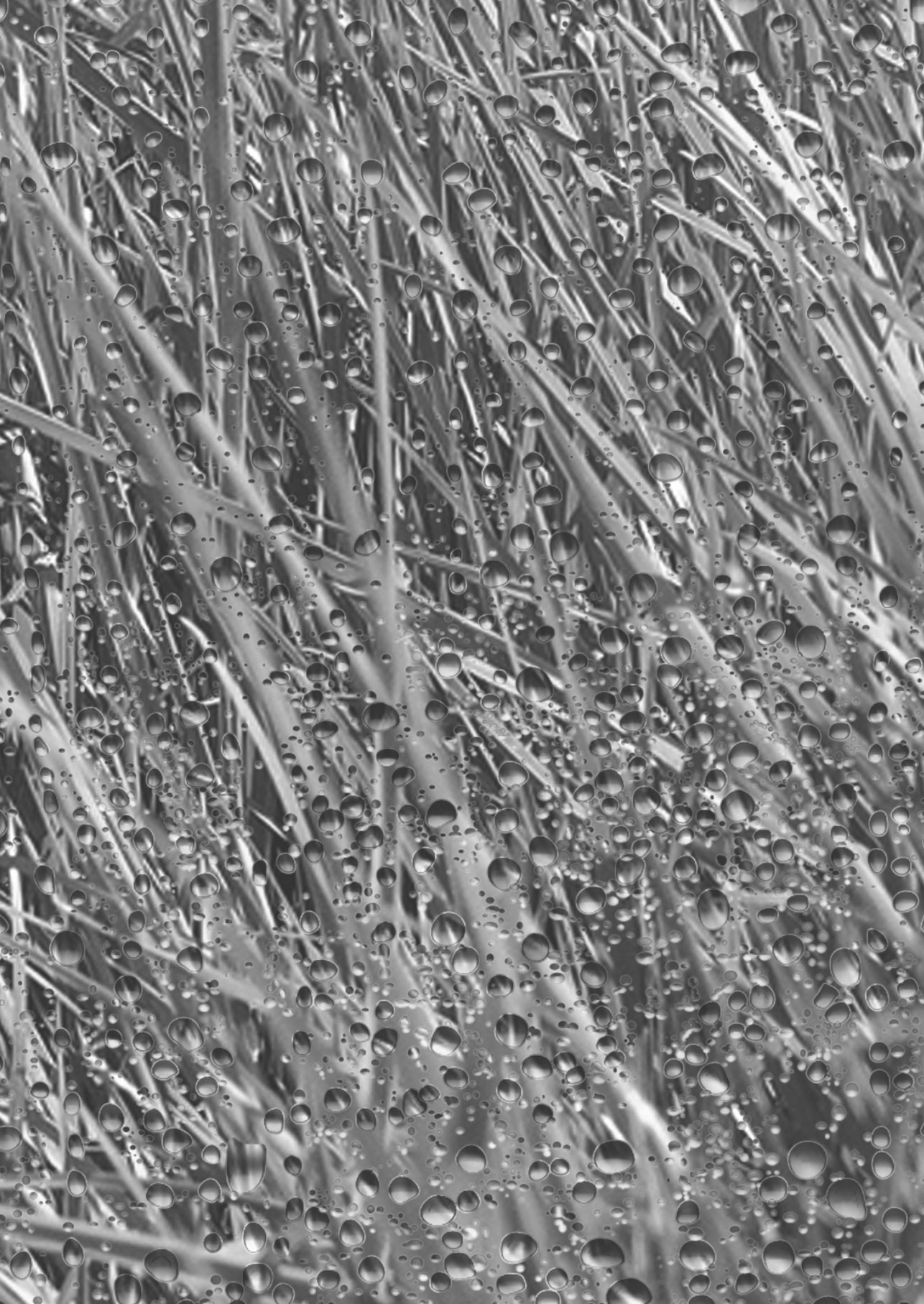
Conclusie: SLIT veranderde het niveau van de geselecteerde SBMs in serum niet en een relevante marker voor klinische effectiviteit kon niet worden vastgesteld. "Quantile

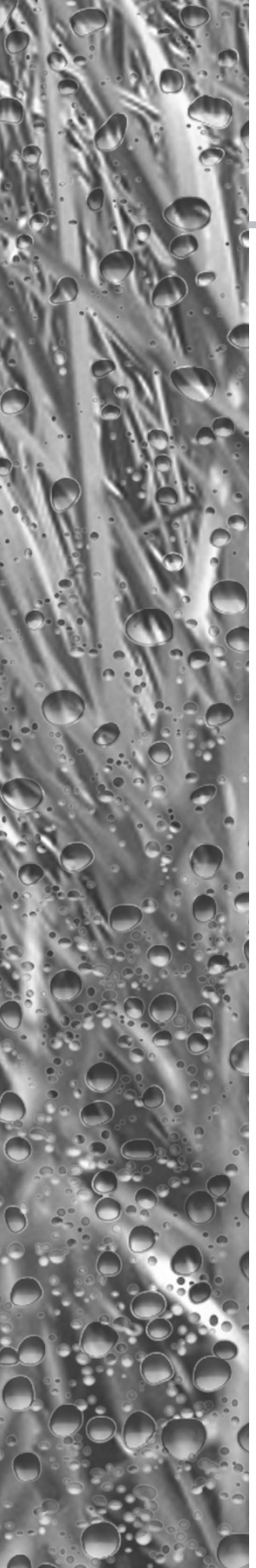
regression" bleek een waardevolle aanvulling van de statistische methoden die gebruikt kunnen worden voor de analyse van immunologische datasets met non-detects.

In **hoofdstuk 8** worden de belangrijkste bevindingen van het literatuuroverzicht en de resultaten van de STARDROP-studie bediscussieerd.

List of abbreviations

AdolRQLQ	Adolescent rhinoconjunctivitis quality of life questionnaire
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARIA	Allergic rhinitis and its impact on asthma
BU	Biological units
CBA	Cytometric bead assay
CONSORT	Consolidated standards of reporting trials
CWC	COOP/WONCA-charts
DC	Diary card
ELISA	Enzyme-linked immunosorbent assay
EMA	European medicines agency
GA^{2}LEN	Global allergy and asthma European network
GAS	Global assessment of symptoms
GP	Grass pollen
HRQL	Health related quality of life
ISAAC	International study of asthma and allergies in childhood
LNIT	Local nasal immunotherapy
MA	Meta-analysis
MID	Minimal important difference
ND	Non-detect
OIT	Oral immunotherapy
PRO	Patient-reported outcome
PRQLQ	Paediatric rhinoconjunctivitis quality of life questionnaire
RCT	Randomised controlled trial
RQLQ	Rhinoconjunctivitis quality of life questionnaire
RTSS	Rhinitis total symptom score
SBM	Soluble biological marker
SCIT	Subcutaneous immunotherapy
SIT	Allergen-specific immunotherapy
SLIT	Sublingual immunotherapy
STARDROP	Sublingual immunotherapy in youngsters with allergic rhinitis, a double-blind randomised controlled study with grass pollen allergen
WAO	World allergy organisation





Chapter 10

Dankwoord

Curriculum vitae

PhD portfolio

Dankwoord

Een Afrikaans gezegde luidt: als je snel wilt gaan, ga dan alleen. Als je ver wilt gaan, ga dan samen. Het ging niet snel, maar samen met heel veel mensen ben ik wel ver gekomen en daar ben ik ze heel dankbaar voor!

Zonder **deelnemers** geen onderzoek en daarom wil ik als eerste de kinderen, hun ouders en huisartsen bedanken voor deelname aan het onderzoek.

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Hans de Groot Jij was de stille kracht in dit project. Jouw reactiesnelheid op e-mails en neus voor ontbrekende punten zijn echt ongekend. Bedankt voor alle hulp, steun en zelfvertrouwenboosts die ik als onderzoeker en als arts-assistent van je heb gekregen.

Huub Savelkoul Jij was een van mijn grote uitdagingen in dit onderzoek, maar altijd enthousiast en een zeer prettig mens om vooruit te branden.

Wim "horizontal organizer" Hop, Roos "eyeball statistics" Bernsen en Paul "structured procrastinator" Eilers Wie denkt dat statistici saai zijn heeft jullie nog niet ontmoet! Door jullie liefde voor het vak, praktische instelling en geweldige humor was het een feest om met jullie samen te werken.

Het STARDROP-team Een groot en enthousiast team dat er voor heeft gezorgd dat het onderzoek liep als een trein. Dankjewel Mariet, Ellen, Ineke, Wilma, Caroline, Margriet, Anuschka, Gonny, Lya, Fatih, Sabri, Özlem en in het bijzonder Metthilde en Toke, die zich allebei van de eerste tot de laatste dag enorm hebben ingezet voor het onderzoek.

Metthilde, jij waakte als een moeder over "jouw kinderen" en het team. **Toke**, tegen jouw enthousiasme en overtuigingskracht waren de huisartsen en deelnemers niet opgewassen. **Kris Sieradzan** LieverT, ik mis onze 'cappo'-overleggen, dubbelzinnige gesprekken en briljante ideeën die ontstonden onder het genot van een heerlijk glas wijn of bier. Met je humor en relativerende kijk op de zaak heb je heel wat beren van mijn weg gehaald. Dziękuję! **Angélique le Grand** Een vrouw met een groot hart voor kinderen. Bedankt voor de fijne samenwerking en inspirerende gesprekken tijdens jouw monitorbezoeken. **René Suurland** Jij regelde de zakelijke kant van het onderzoek, maar had altijd oog voor het persoonlijke. Dank voor al je hulp en oprechte interesse. **Paul van der Heijden** "Onze man" bij het AKC. Vaak kwamen we op het laatste moment met een verzoek en je was altijd bereid om te kijken of je ons kon helpen. Dankjewel voor je flexibiliteit en enthousiasme.

Afdeling Huisartsgeneeskunde Ik heb tijdens mijn jaren op de afdeling op meerdere locaties en kamers gezeten, altijd met gezellige collega's, die niet alleen hun persoonlijke maar ook wetenschappelijke ervaringen deelden (ook toen ik niet meer op de afdeling werkte). Cindy, Heleen en Hans, fantastisch dat de lessen geleerd in STARDROP gebruikt konden worden in STARDROP-II en dat er zelfs een gezamenlijk artikel gaat komen. Iedereen - en in het bijzonder Rianne, Frieke, Anita, Celinde, Marienke en Rogier - ontzettend bedankt!

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Edith Heintjes Tijdens onze gezellige treinreizen tussen Leiden en Rotterdam hebben we heel wat hoogte- en dieptepunten van het doen van wetenschappelijk onderzoek gedeeld. Dankjewel voor je vriendschap. **Jiska Patiwael** Mijn "partner in crime" op allergologie- en later ook op promotiegebied, waarmee ik alles kan bespreken en die begrijpt wat ik bedoel. Ik heb veel bewondering voor de wijze waarop jij altijd bezig bent jezelf verder te ontwikkelen. Nog "even" die verdediging en dan gaan we er een mooi feestje van maken dr. Patiwael! **Miriam Monteny** Jij hebt op zoveel manieren

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Dit proefschrift draag ik op aan **mijn oma's**, twee sterke en intelligente vrouwen, die mij altijd gestimuleerd en geholpen hebben om een goede opleiding te volgen. Ik hoop dat jullie "daar boven" trots op mij zijn.

Esther

Curriculum vitae

Esther Röder werd op 6 maart 1972 geboren in Den Haag. Na het behalen van haar VWO-diploma aan het Huygens Lyceum te Voorburg is zij in 1990 geneeskunde gaan studeren aan de Universiteit van Utrecht. Tijdens haar studie werkte zij o.a. mee aan een onderzoek naar het bestaan van astma/COPD bij 1e graads familie en partners van patiënten met astma/COPD. In maart 1998 begon zij als arts-onderzoeker/arts-assistent op de afdeling Longziekten van het Diaconessenhuis Voorburg (nu onderdeel van de Reinier de Graaf Groep) en was betrokken bij diverse klinische trials en patiëntenzorg. Vervolgens deed zij vanaf december 1999 in het Amsterdams Medisch Centrum op de afdeling Klinische Farmacologie en Farmacotherapie onderzoek naar de diagnostiek en preventie van pseudoallergische reacties op geneesmiddelen. In mei 2001 startte zij met het STARDROP-onderzoek naar de effectiviteit van sublinguale immunotherapie met graspollenextract bij jongeren met hooikoorts, waaruit de artikelen in dit proefschrift voortkwamen. Dit onderzoek was een samenwerkingsverband tussen de afdelingen Allergologie en Huisartsgeneeskunde van het Erasmus MC te Rotterdam. De afgelopen jaren heeft zij de afronding van haar proefschrift gecombineerd met een baan als arts-assistent op de afdeling Allergologie van het Erasmus MC en het Maasstad Ziekenhuis in Rotterdam. Sinds januari 2009 is zij werkzaam op de afdeling Allergologie van het Erasmus MC als arts-onderzoeker en coördinator van het AIRFORCE-onderzoek naar de kosten-effectiviteit van subcutane immunotherapie bij volwassenen met allergische rhinitis.

Esther woont met Ewald Walraven en hun kinderen Joshua (2006) en Fabian (2010) in Leiden.

PhD portfolio

Courses

Basiscursus Regelgeving Klinisch Onderzoek, 2010	28 hours
Principles of Research in Medicine and Epidemiology (NIHES), 2004	15 hours
Clinical Trials and Drug Risk Assessment (NIHES), 2004	35 hours
Introduction to Data-analysis (NIHES), 2004	25 hours
Regression Analysis (NIHES), 2004	30 hours
How to write a medical article (NIHES), 2004	16 hours

Conferences

International Conference on Specific Immunotherapy 2010	16 hours
European Academy of Allergy and Clinical Immunology (EAACI) Congress 2008, poster presentation	16 hours
Dutch Society for Allergology (NVvA) Congress 2008, oral presentation	20 hours
Dutch College of General Practitioners (NHG) Congress 2006, oral presentation	20 hours
Dutch Society for Allergology (NVvA) Congress 2006, oral presentation	20 hours
Dutch Society of Pediatrics (NVK) Congress 2006, poster presentation	16 hours
World Allergy Congress (WAC) 2005, poster presentation	16 hours
European Academy of Allergy and Clinical Immunology (EAACI) Congress 2004, poster presentation	16 hours
European Academy of Allergy and Clinical Immunology (EAACI) Congress 2003, poster presentation	16 hours
Dutch Symposium of Epidemiology (WEON) 2002, oral presentation and poster presentation	20 hours

Teaching activities

Supervising medical students, 2003 and 2004	160 hours