

Efficacy, safety, and immunological effects of a 2-year immunotherapy with Depigoid[®] birch pollen extract: a randomized, double-blind, placebo-controlled study

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Summary

Background Rhinoconjunctivitis due to birch pollen sensitization is common in Northern Europe. A depigmented polymerized birch pollen extract – Depigoid[®] has been developed for immunotherapy.

Objective To evaluate its clinical efficacy, safety, and effects on immunological parameters.

Methods Sixty-one patients aged 7–69 years were included in a randomized, double-blind, placebo-controlled trial of subcutaneous immunotherapy (SCIT) using depigmented polymerized birch pollen extract. SCIT consisted of four increasing doses at 7-day intervals, followed by maintenance injections of 500 DPP (corresponding to 30 µg Bet v1 before depigmentation) at 6-week intervals for 18 months. The primary outcome was the combined symptom and medication score during the 2006 birch pollen season. The frequency of peripheral blood mononuclear cells (PBMC) producing IL-4, IL-10, IL-12, and IL-13 was assessed in a subgroup of patients by ELISPOT assay.

Results After 18 months of treatment, the median combined symptom and medication score (upper/lower quartile) of treated patients was significantly lower than those on placebo: 8.0 (5.8–10.3) and 12.6 (8.6–16.2), respectively ($P = 0.004$). Systemic reactions occurred in 29 patients (12 active, 17 placebo), were grades 1 or 2, and none required specific treatment. After 18 months of treatment, mean serum concentrations of specific IgE increased significantly in both groups ($P < 0.0001$) whereas serum concentrations of both specific IgG1 and IgG4 only increased significantly in the SCIT group ($P = 0.002$) and not in the placebo group. The seasonal increase in numbers of IL-4- and IL-13-producing PBMC was blunted by immunotherapy.

Conclusions SCIT with depigmented polymerized birch pollen extract significantly reduced symptom and medication scores when compared with the placebo, was well tolerated, and resulted in immunological changes comparable with those of native pollen extracts.

Keywords birch pollen, depigmented polymerized allergen extract, specific immunotherapy

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Introduction

In sensitized individuals, rhinoconjunctivitis and asthma are symptomatic disorders induced by allergen exposure and caused by an IgE-mediated inflammation in upper and lower airways [1, 2].

There is clear evidence that subcutaneous immunotherapy (SCIT) using inhalant allergens is clinically effective in the treatment of allergic rhinitis and asthma, induces

clinical and immunologic tolerance, has long-term efficacy, may prevent the progression of allergic disease, and also improves the quality of life (QOL) of allergic patients [3–8]. In birch pollen-allergic patients, clinical efficacy was also confirmed for SCIT with birch pollen extracts [9, 10].

The use of SCIT for treatment of rhinoconjunctivitis and asthma is limited by the risk of systemic side-effects

[11,12]. In an attempt to overcome these potential hazards, allergoids were developed by polymerizing allergen extracts with glutaraldehyde [13]. The aim of such chemical modification is to reduce specific IgE binding (which is responsible for immediate systemic reactions) while maintaining T cell reactivity (which is thought to be responsible for induction of tolerance) [14]. Allergoids should therefore be safer than unmodified vaccines while retaining clinical efficacy [15]. Allergoids have been further developed by introducing a depigmentation step, before the polymerization process. The depigmentation removes protein-bound pigments, enhances the solubility of the allergoids, and inactivates the enzymatic activity [16]. These modifications allow shortening of the build-up phase and the administration of higher doses than those used with non-modified allergens [17].

The clinical safety and efficacy of depigmented allergoids have been shown in several clinical studies in patients with pollen- and house dust mite (HDM)-induced allergic symptoms [18–23]. In most of available studies, the birch pollen allergoid is not studied and because the effect of a depigmented allergen varies with the allergen this study is of importance.

The objective of the study was to evaluate clinical efficacy and safety and effect on QOL of 18-month SCIT with standardized depigmented polymerized birch pollen extract. Additionally, the effect of treatment on immunological parameters was assessed.

Patients and methods

Patients and study design

This was a randomized, double-blind, placebo-controlled clinical study including both adults and children. Patients with a medical history of allergic rhinoconjunctivitis with or without allergic asthma due to birch pollen were recruited at nine study centres in Sweden and Germany in autumn 2004 and were randomized to receive either SCIT or placebo. Positive skin prick tests to birch pollen resulting in a weal diameter ≥ 4 mm as well as serum level of specific IgE against birch pollen ≥ 0.7 kU/L were required. Patients were excluded if they had perennial symptoms or allergic sensitization, other than to birch pollen, causing symptoms during the birch pollen season. Written informed consent was obtained from patients and/or childrens' parents or caregivers. Randomization was based on a computer-generated randomization list. Within the study centres, patients were allocated to the treatment in ascending order. The study was approved by the relevant Ethics Committees and competent authorities and was conducted in accordance with the latest version of the Declaration of Helsinki, Good Clinical Practice, and applicable local laws [24].

Immunotherapy

SCIT was performed using Depigoid® (Laboratorios LETI SL, Tres Cantos, Spain), a standardized depigmented and glutaraldehyde-polymerized birch pollen (*Betula alba*) extract adsorbed onto aluminium hydroxide. Depigoid® was provided at two different strengths: 100 DPP/mL (Vial 1) and 1000 DPP/mL (Vial 2). The biological activity unit of 1 DPP was the result of depigmenting and polymerizing one histamine equivalent prick test unit of allergen extract. (The histamine equivalent unit was 10 mg) 100 DPP corresponded to 6 µg Bet v1 of major allergen equivalent before depigmentation and polymerization. Placebo medication was identical in appearance and contained the excipients only: sodium chloride 9 mg/mL, phenol 5 mg/mL, aluminium hydroxide 1.1 mg/mL, and water for injection. There was no visible difference between Placebo and Depigoid® vials. The medication was packed by the company and labelled according to the currently valid version of International Conference on Harmonization – Good Clinical Practice (ICH-GCP) and then it was distributed via local pharmacies. Patients initially received gradually increasing doses of 0.2 and 0.5 mL of Vial 1 as well as 0.2 and 0.5 mL of Vial 2 at 7-day intervals, followed by the maintenance dose of 0.5 mL of Vial 2 at 6-week intervals for 18 months. Thus, one maintenance dose corresponded to 30 µg Bet v1 of major allergen equivalent before depigmentation and polymerization.

All patients had a 30 min waiting period in the immunotherapy unit after each injection, following the recommendations of the EAACI [25].

The placebo injections were without histamine. This would not have interfered with blinding because there were very few local reactions observed in total. Because the percentage of local reactions was slightly higher in the placebo group, adding of histamine would not have been a good way to mimic active treatment in this study (Table 1).

Table 1. Demographic baseline characteristics (mITT set, $n = 61$)

Demographic variable	Depigoid® birch pollen ($n = 31$)	Placebo ($n = 30$)
Mean age (years) (minimum–maximum)	32 (10–54)	33 (7–69)
Height (cm), mean (range)	172 (141–188)	168 (128–190)
Weight (kg), mean (range)	72 (35–107)	69.5 (26–93)
Female/male	18/13	20/10
Mean IgE (IU/mL) (minimum–maximum)	26.1 (2.23–83.70)	30 (4.65–100.01)
Skin prick test	Positive	Positive
Allergic asthma n	11	12
VAS (mm) season before inclusion (mean $\pm$ SD)	7.2 $\pm$ 2.0	6.9 $\pm$ 1.9

n , number of patients; SD, standard deviation; mITT, modified intent-to-treat.

Pollen counts

The local birch pollen seasons 2005 and 2006 were determined for each study centre by local pollen counts. The pollen season was predefined and the pollen load was considered relevant to cause symptoms in patients when the pollen count was ≥ 10 pollen/mm³/24 h in more than 3 consecutive days. When the pollen count was ≤ 10 pollen/mm³/24 h in 3 consecutive days, it indicated the end of relevant exposure. The pollen counts were registered daily during the birch pollen season using a volumetric pollen trap (Burkard Scientific Ltd, Uxbridge, UK) and expressed as numbers of grains per cubic metre of air.

Assessment of efficacy

The primary efficacy parameter was the time-weighted AUC of the combined symptom and rescue medication score [26]. Using diaries, patients recorded their individual rhinitis, conjunctivitis, and asthma symptoms on a daily basis: sneezing, nasal pruritus, rhinorrhea, nasal congestion, ocular pruritus, increased lacrimation, eye redness, chest tightness, wheezing, coughing, and difficulty breathing. Individual symptoms were scored on a scale from 0 to 3, with no symptoms = 0, mild = 1, moderate = 2, and severe = 3, respectively. The daily symptom score was calculated as the sum of all symptoms experienced on that day by each patient. Children scored their symptoms with the help of their parents. The area under the curve (AUC) for the peak pollen season defined as ± 5 days around the pollen peak for each study centre, containing the mean daily symptom score of rhinoconjunctivitis for each patient in the treatment groups, was then constructed.

Because of the size in diameter of the birch pollen, it causes mostly rhinoconjunctivitis symptoms. The patients also had to have a history of rhinoconjunctivitis to participate in the study. Only a small amount of patients would be anticipated to develop symptoms of asthma.

During each pollen season, patients were supplied with rescue medication. Each drug was scored for the dosing according to the recommendation of the manufacturer. No drugs other than the prescribed rescue medication were allowed. Each drug of rescue medication was scored as follows: 0 = no medication; 1 = topical nasal, ocular or lung treatment apart from corticosteroids; 2 = cetirizine tablet; 3 = topical or systemic corticosteroids for nose or lung. For each patient, the daily rescue medication score was defined as the highest score of the used rescue medication (0–3) [27]. Maximum daily score would be 3. The medication score of a patient was the AUC of all daily medication scores during the peak pollen season.

QOL was assessed using the Pediatric Rhinoconjunctivitis Quality of Life Questionnaire (PRQLQ) [28] or the

mini Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) [29], depending on patients' age.

Assessment of safety

Adverse events were documented throughout the study. Investigators' terms of adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA). Moreover, reactions were graded according to the EAACI classification [25].

Immunological parameters

The effects on specific IgE, IgG1, and IgG4 as well as on IL-4-, IL-10-, IL-12-, and IL-13-producing cells were studied [30].

Blood samples were collected before the start of the treatment and outside the season and during the 2006 treatment season and after 18 months of treatment. Serum concentrations of birch pollen-specific IgE were measured using ImmunoCAP and IgG1 and IgG4 using ELISA. Briefly, microtitre plates coated with recombinant Bet v1a were blocked with bovine serum albumin and then incubated with diluted patient serum samples. Monoclonal antibodies against IgG1 and IgG4 were added, followed by biotinylated rabbit anti-mouse antibody and horseradish peroxidase-conjugated streptavidine. As a substrate, tetra methyl benzidine was used.

For enzyme-linked immuno spot technique (ELISPOT), peripheral blood mononuclear cells (PBMC) were isolated on a Ficoll Paque gradient and cultured (2 million cells/mL) in the presence of Bet v1a (BIOMAY, Vienna, Austria) 10 µg/mL or anti-CD3 (MabTech, Stockholm, Sweden) 100 ng/mL. They were incubated for 4 h in 37° and 5% CO₂. After incubation, the cells were re-suspended and transferred to antibody-coated nitro-cellulose ELISPOT plates. The plates were coated with 100 µL of anti-human IL-4, IL-10, IL-12, and IL-13 antibodies (MabTech, Stockholm, Sweden) diluted to a concentration of 15 µg/mL in sterile PBS (pH 7.2). Plates were incubated overnight at 4 °C and thereafter unbound antibodies were washed away with filtered PBS. One hundred µL of the prestimulated cell suspension (200 000 cells for IL-4, IL-12, and IL-13 and 20 000 cells for IL-10) were added in triplicate and the plates were incubated for 40–42 h at 37 °C after which the cells were washed away and 100 µL of the biotinylated MoAbs (1 µg/mL) were added and incubated for 2 h at room temperature (RT). The plates were then washed and incubated for 90 min at RT with 100 µL streptavidin-alkaline phosphatase (MabTech) diluted 1/1000. Unbound conjugate was removed and 100 µL of 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium substrate solution (Bio-Rad, Richmond, CA, USA) was added and incubated at RT [30]. The spots were counted using an

Immuno Spot Analyzer (Cellular Technology, Cleveland, OH, USA).

Statistics

The primary efficacy parameter of this study was the time-weighted AUC of combined symptom and rescue medication scores during the 10 weeks around the peak of the 2006 birch pollen season in the modified intent-to-treat (mITT) population, i.e. after 18 months of treatment.

The two-sided Wilcoxon's rank-sum test was used for all between-groups comparisons and the Wilcoxon's signed-rank test for within-group comparisons, both with a significance level of $\alpha = 0.05$.

Patients eligible for the mITT population had to reach the maintenance dose after the build-up phase and receive at least one dose of study medication during the 2006 pollen season.

It should be noted that since this trial started already in 2001, it was not registered according to current ICJME standards.

Results

In total, 61 patients aged 7–69 years (five children aged 7–12 years, five adolescents aged 13–17 years, and 51 adults) were randomized to receive either SCIT with Depigoid® ($n = 31$) or placebo ($n = 30$). No significant differences were detected between the groups at baseline for clinical characteristics, allergen-specific IgE, or retrospective scoring of symptoms from the previous season (Table 1, data for statistical comparison not shown).

Three patients (SCIT: $n = 2$; placebo: $n = 1$) discontinued the study treatment prematurely due to adverse events whereas only one event (conjunctivitis) in the SCIT group was considered treatment related.

In total, 53 out of 58 patients (SCIT: $n = 27$; placebo: $n = 26$) went into the mITT analysis. One patient on placebo was not compliant and therefore excluded from the mITT. Data of 45 (SCIT: $n = 24$; placebo: $n = 21$) patients were available for the primary analysis at the end of the second year.

Efficacy

During the 2005 birch pollen season, pollen exposure was unexpectedly low (mean time-weighted AUC: 424 pollens/ m^3 /day), whereas during the 2006 pollen season, pollen exposure was within the expected range (mean time-weighted AUC: 1341 pollens/ m^3 /day). There were no statistically significant differences in pollen exposure detected between the two treatment groups in any of the two seasons.

There was a statistically significant difference for the primary efficacy parameter, median combined symptom

and medication score, throughout the birch pollen season between the two treatment groups after 18 months ($P = 0.004$), with significantly lower combined score in the SCIT group (Fig. 1a). The median (lower quartile – upper quartile) combined scores of the SCIT and placebo groups were 8.0 (5.8–10.3) and 12.6 (8.6–16.2), respectively. This was a 36.5% reduction in the SCIT group compared with the placebo.

Assessing secondary efficacy parameters the median symptom score was not significantly different between treatment groups during the 2006 pollen season ($P = 0.375$), despite a reduction of 25% in the SCIT group compared with placebo (Fig. 1b).

The median medication score was significantly different between treatment groups after 18 months ($P = 0.016$): 2.1 (0.6–3.7) in the SCIT group and 4.4 (1.9–14.0) in the placebo group. This was a 51.9% reduction in the SCIT group compared with placebo (Fig. 1c).

In contrast, during the 2005 pollen season, median combined symptom and medication scores did not differ significantly between treatment groups (6.9; $P = 0.621$).

Also, no statistically significant difference was found for the mean symptom score ($P = 0.897$) or medication score ($P = 0.864$).

The birch pollen season was predefined in the protocol based on birch pollen counts. The onset of birch pollen season is usually rapid and most of the relevant pollen exposure follows within a fairly short time. This leads to symptoms, which may endure throughout and even past the season. It is however commonly observed that almost all birch pollen-allergic patients react to alder and hazel due to cross reactivity. This might explain why some patients reported symptoms already when the season started according to our definition.

Rhinoconjunctivitis QOL assessment combined the sum scores of the PRQLQ and RQLQ. At baseline, there was a statistically significant difference between the two treatment groups in the mean RQLQ (SCIT: 25.8; placebo: 2.7; $P < 0.0001$). During the study, RQLQ improved in both groups. The change in mean RQLQ between baseline and the assessment during pollen season 2005 after 6 months of treatment was significantly different between the two treatment groups (SCIT: -3.8 ; placebo: -0.1 ; $P < 0.0001$).

Safety

In total, 29 patients (SCIT: $n = 12$, placebo: $n = 17$) developed 58 side-effects (SCIT: $n = 30$; placebo: $n = 28$). All of them were of mild or moderate intensity. Nasopharyngitis, headache, asthma, and other allergy-related symptoms were the most frequently reported events in both treatment groups. None of the events in the SCIT group were considered serious and did not require specific treatment.

Table 2 displays the patients with local or grades 1 or 2 systemic reactions according to the EAACI classification

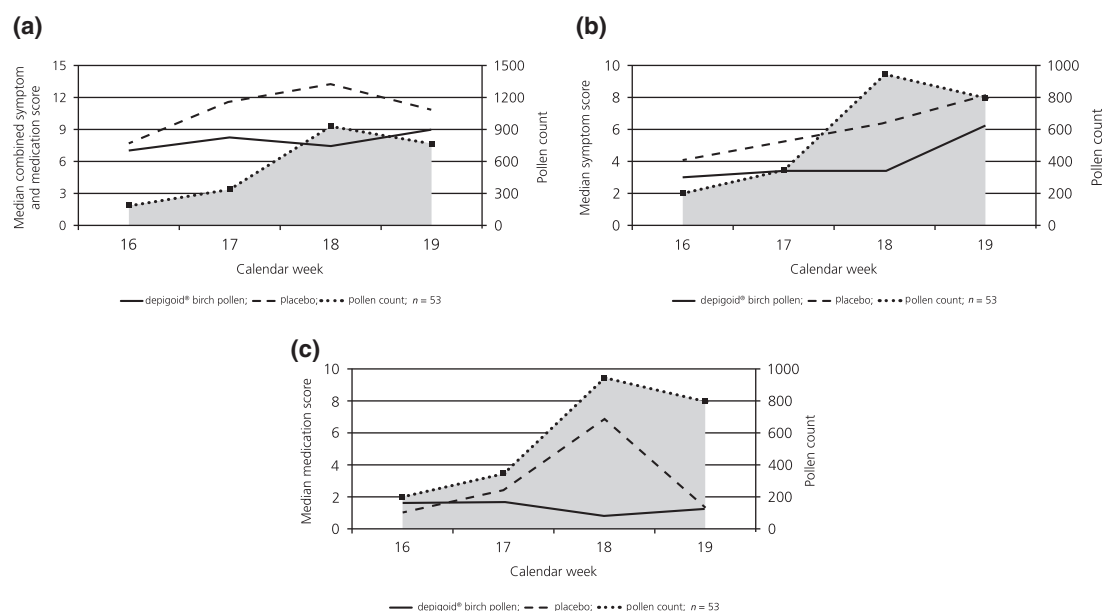


Fig. 1. (a) Combined symptom and medication score in 2006. (b) Symptom score in 2006. (c) Medication score in 2006. —, Depigoid® birch pollen; ----, placebo; pollen count; $n = 53$.

Table 2. Number of patients (%) with local or systemic reactions according to EAACI [25]

EAACI classification	MedDRA low-level term	SCIT	Placebo
		($n = 31$)	($n = 30$)
Local reaction	Injection site reaction	2 (6)	4 (13)
Systemic reactions grade 1	Headache	7 (23)	4 (13)
	Fever	3 (10)	—
Systemic reactions grade 2	Allergic asthma	3 (10)	3 (10)
	Conjunctivitis	2 (6)	10 (33)
	Allergic rhinoconjunctivitis	8 (17)	5 (27)
	Atopic eczema	4 (3)	2 (3)
	Urticaria	—	1 (3)
	Food allergy	—	3 (10)
	OAS	3 (10)	—

n , number of patients, include double entries; SCIT, subcutaneous immunotherapy.

[25]. There was no grades 3 or 4 systemic reaction reported in this study.

Immunological effects

Mean serum concentrations of birch pollen-specific IgE increased significantly in both groups ($P < 0.0001$). No difference was observed between treatment groups. Serum concentrations of both birch pollen-specific IgG1 and IgG4 increased significantly in the SCIT group ($P = 0.002$), whereas there were no significant changes in the placebo group. Between-group comparisons revealed significant differences ($P = 0.007$ and 0.003 , respectively) (Table 3a, Figs 2a and b).

The number of IL-4- and IL-13-producing cells increased significantly in samples obtained during the 2006 pollen season compared with the out of season baseline in the placebo group ($P = 0.04$ and 0.03 , respectively), whereas this seasonal increase was not seen in SCIT-treated patients. No significant differences for numbers of IL-10- and IL-12-producing cells were detected (Table 3b and Figs 3a and b). Only a subgroup of patients could participate in this part of the study due to the need for fresh blood when performing the analyses. The laboratory was located in Gothenburg and patients at other sites were therefore naturally excluded. The patients included did not in any way differ from the rest of the patients.

Discussion

In this randomized, double-blind, placebo-controlled study with birch pollen-induced allergic rhinoconjunctivitis, SCIT with depigmented polymerized birch pollen extract proved to be clinically effective and safe in both adults and children. Active treatment with depigmented polymerized birch pollen extract resulted in increased birch pollen-specific IgG1 and IgG4 and these changes were accompanied by the blunting of a seasonal increase in the frequency of PBMC producing Th2 cytokines.

During the 2005 birch pollen season, pollen exposure was unexpectedly low, and therefore, median combined symptom and medication scores in both groups were similar. In contrast, during the 2006 pollen season, pollen exposure was sufficient to cause significant allergic symptoms based on our experience. During this birch pollen season, a statistically significant difference ($P = 0.004$) and a clinically relevant magnitude of effect

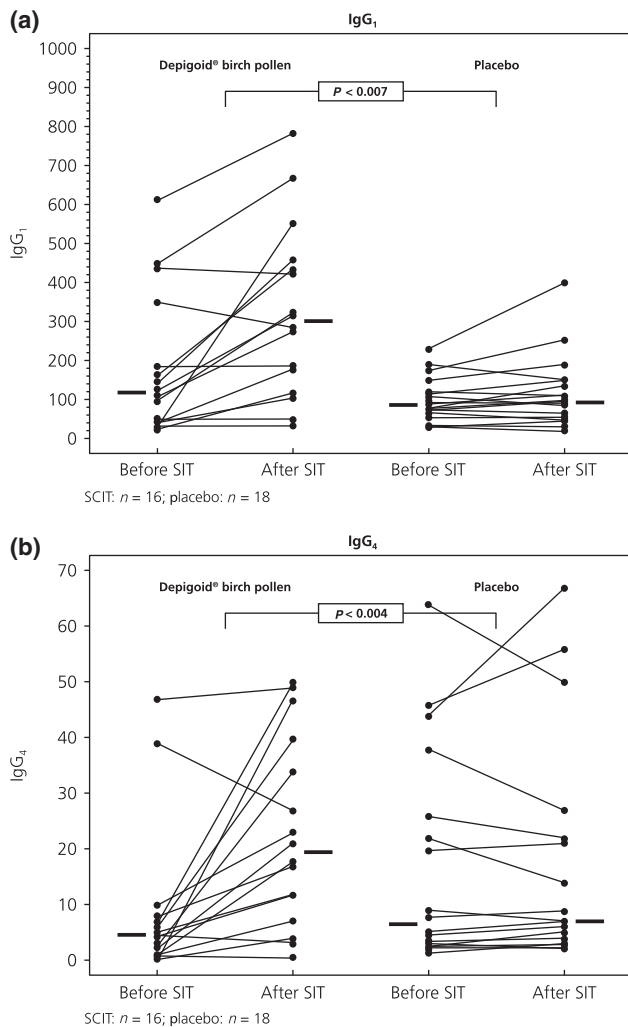


Fig. 2. (a) Changes in IgG1 serum concentrations (µg/L) in individual patients during immunotherapy. SCIT: $n = 16$; placebo: $n = 18$. (b) Changes in IgG4 serum concentrations (µg/L) in individual patients during immunotherapy. SCIT: $n = 16$; placebo: $n = 18$. SCIT, subcutaneous immunotherapy.

(−36.5%) were detected for the primary efficacy parameter, the median combined symptom and rescue medication score. Using this primary efficacy parameter was in line with current advice from regulatory bodies [31]. The size of response to treatment seen in our study is comparable with that noted in previous studies of immunotherapy with unmodified birch pollen extract [9, 10, 32, 33]. This is the first study to report improvement in rhinitis QOL scores following immunotherapy with a depigmented polymerized birch pollen extract, although this has been reported for patients sensitive to grass pollens and HDM [20, 22]. Of note, some of these studies did show significant reductions in symptom scores, which was not observed in our study. This may reflect differences in severity of disease, use of rescue medication or the inclusion of children in our study.

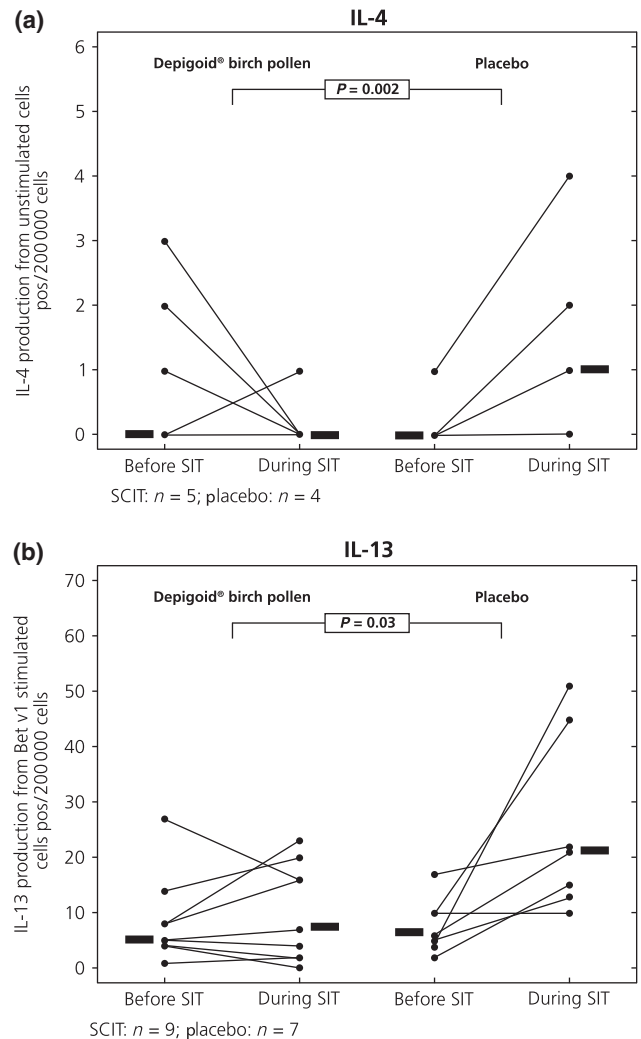


Fig. 3. (a) Changes in IL-4 production from prestimulated cell suspensions of individual patients' peripheral blood mononuclear cells during immunotherapy. SCIT: $n = 5$; placebo: $n = 4$. (b) Changes in IL-13 production from prestimulated cell suspensions of individual patients' peripheral blood mononuclear cells during immunotherapy. SCIT: $n = 9$; placebo: $n = 7$. SCIT, subcutaneous immunotherapy.

We predefined the period of the season to be used for the primary outcome variable. Although combined symptom and medication scores are recommended as the primary outcome measure for immunotherapy studies, there is as yet no validated way of combining these scores. Of note, the median combined scores were already measurable at the start of this period. Many birch pollen allergic patients react to alder and hazel due to cross reactivity. However, these values rose from median values of 2 at the start of the observation period to over 8 at the peak of the season (Fig. 1). The onset of the birch pollen season is commonly rapid and most of the relevant pollen exposure follows within a fairly short time. This leads to symptoms of rhinoconjunctivitis that may endure throughout and past the season.

Table 3a. Differences in birch pollen-specific antibody serum concentrations from before to after immunotherapy during the 2006 birch pollen season

	SCIT			Placebo			
	<i>n</i>	Median (minimum–maximum) difference	<i>P</i> -value*	<i>N</i>	Median (minimum–maximum) difference	<i>P</i> -value*	<i>P</i> -value†
IgE (kU/L)	27	8.4 (–6.3–79)	<0.0001	25	15.8 (–445–580)	<0.0001	NS
IgG1 (µg/L)	16	152.5 (–65–520)	0.002	18	–25 (–35–170)	NS	0.003
IgG4 (µg/L)	16	8.2 (–12–46.6)	0.002	18	0.45 (–14–23)	NS	0.007

*Wilcoxon's signed-rank test for within-group comparisons.

†Wilcoxon's rank-sum test for between-group comparisons.

n, number of patients; SCIT, subcutaneous immunotherapy; SD, standard deviation.**Table 3b.** Differences in the number of Bet v 1 prestimulated IL-4-, -10-, -12-, and -13-producing cells per 200 000 peripheral blood mononuclear cells from before to after immunotherapy during the 2006 birch pollen season

	SCIT (<i>n</i> = 9)		Placebo (<i>n</i> = 7)		
	Median (minimum–maximum) difference	<i>P</i> -value*	Median (minimum–maximum) difference	<i>P</i> -value*	<i>P</i> -value†
IL-4	4 (–23–11)	NS	15 (–72–4)	0.04	0.07
IL-13	1 (–15–11)	NS	13 (–47–0)	0.03	0.03
IL-10	37 (295–639)	NS	62 (–198–203)	NS	NS
IL-12	6 (–117–278)	NS	46 (–120–75)	NS	NS

*Wilcoxon's signed-rank test for within-group comparisons.

†Wilcoxon's rank-sum test for between-group comparisons.

n, number of patients; SCIT, subcutaneous immunotherapy; SD, standard deviation; NS, not significant ($P > 0.05$).

Our study was not a head-to-head study with allergoid, native extract and placebo but a comparison between placebo and allergoid. In a non-clinical trial by Henmar et al. [34], a comparison was made of allergenicity and immunogenicity between three intact allergen vaccines and four allergoid vaccines for subcutaneous grass pollen immunotherapy. The authors of this study conclude that there is a variation in laboratory measurements related to each product and emphasize the need of justification of every individual product. The encouraging safety profile seen in our study (no grades 3 or 4 systemic reactions), is in line with some studies using unmodified allergen birch pollen extract. As shown by Winther et al. [35] in a prospective multi-centre study assessing safety in SCIT, there is a difference in the incidence and severity of side-effects depending on the allergen administered with native birch pollen extract rarely causing severe side-effects. A difference in potency between extracts and the composition of the allergen extracts (minor/major allergens) have been proposed as part of the explanation. However one report documented five grade-3 reactions and one grade-4 in 24 patients treated with unmodified birch pollen extract by SCIT [32]. Larger studies are needed to make more definitive statements on safety. Similar favourable safety profiles were demonstrated for depigmented glutaraldehyde-polymerized allergen extracts in children and adults with allergic rhinoconjuncti-

vitis and/or asthma due to HDMs and pollen [19, 22, 23, 36–39]. In two prospective, non-interventional studies including 175 and 766 patients who received a variety of different depigmented glutaraldehyde-polymerized allergen extracts, no grades-3 or -4 systemic reactions were reported [23, 40]. It is of note that the reduction in IgE binding resulting from depigmentation and polymerization allows a convenient rapid up dosing schedule for immunotherapy using four up-dosing injections over 3 weeks as in this study or even a rush protocol achieving maintenance dose at the first visit with two injections with a low rate of mild systemic reactions [21].

As only five patients younger than 13 years and five younger than 18 years were included in this study, a separate analysis of efficacy and safety could not be performed. Nevertheless, only two children of the SCIT group and all five of the placebo group developed mild systemic reactions during the course of the study, which supports the former results in HDM allergic children. Local reactions were not more frequent in children compared with adults.

The immunological changes seen in this study are in line with previous reports [41]. SCIT with unmodified allergen extracts has been associated with increases in allergen-specific IgG1 and IgG4 [41]. Indeed, it has been suggested that part of the mechanism of immunotherapy may involve induction of IgG antibodies that block

interaction of allergen and IgE with effector cells [41]. Here, we confirm that depigmented polymerized birch pollen extract immunotherapy also induced allergen-specific IgG1 and IgG4 confirming the retained immunogenicity of this product. Another immunological effect of immunotherapy is the down-regulation of Th2 cytokine production induced by either allergen challenge or seasonal allergen exposure [30, 41]. Previous reports have demonstrated this effect in nasal biopsies [42]. Here, using ELISPOT, we show a seasonal increase in the frequency of IL-4- and IL-13-producing cells in peripheral blood of placebo patients, and for the first time an abrogation of this increase in patients treated with Depigoid®, which is similar with results in PBMC obtained from patients treated with unmodified extracts [30].

These data likely explain the widely reported blunting of seasonal increases in allergen-specific IgE seen with immunotherapy because both IL-4 and IL-13 drive IgE class switching and production from B cells [41]. Interestingly, these reductions in frequencies of IL-4- and IL-13-producing cells were not accompanied by changes in numbers of cells producing IL-10 or IL-12 although these did not change with season in placebo patients. Further studies will be required to determine whether depigmented allergoids induce IL-10-producing regulatory T cells or favour a T-helper type 1 (Th1) switch through IL-12 or other cytokines.

In conclusion, SCIT with standardized depigmented polymerized birch pollen extract proved to be clinically effective, was well tolerated, and resulted in immunological changes comparable with those of native birch pollen extracts, i.e. increased specific IgG1 and IgG4 antibody production and down-regulation of IL-4- and IL-13-producing cells in the peripheral blood of treated patients. The safety profile of standardized depigmented polymerized birch pollen extract may be superior to those seen with native birch pollen extracts.

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Conflict of interest: Dr A. Sager and Dr D. Robinson are employed by the LETI pharmaceutical company. Professor S. Rak participates in several multicentre clinical trials. Dr V. Strand participates in several multicentre clinical trials. Dr A. Höiby participates in multicentre clinical trials.

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