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# Immunotherapy with a modified birch pollen extract in allergic rhinoconjunctivitis: clinical and immunological effects

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**Summary**

*Background* Modification of allergens by glutaraldehyde in extracts used for immunotherapy reduces the risk for side-effects, but the therapeutic efficacy of such extracts still requires further evaluation. The aim of this study was to show the efficacy and safety of immunotherapy with a single-strength glutaraldehyde-modified aluminium hydroxide-adsorbed extract of birch pollen.

*Methods* In a multi-centre, randomized, placebo-controlled double-blind setting, starting in 2001 between 1 August and 15 December, birch pollen-allergic subjects ($n = 62$) were injected subcutaneously with increasing doses of the allergen extract or placebo at weekly intervals over a 6-week period (or longer if adverse reactions occurred). Maintenance dose was given monthly for at least 18 months till June 2003. Efficacy was evaluated on the basis of the clinical index score (CIS), a combined symptom and medication score.

*Results* Fifty-eight patients could be evaluated for clinical efficacy. Treatment with the birch pollen extract resulted in a lower CIS for the eye and nose during the peak birch pollen season of 2003, compared with placebo (reductions of 42% and 31%, respectively) ($P = 0.017$ and 0.039). Active treatment induced IgG and IgG4 antibodies reacting with Bet v 1 ($P < 0.001$). Sera from treated patients had a blocking effect on Bet v 1-induced basophil activation ($P < 0.04$). No major adverse reactions occurred, and local reactions, if occurring, were mild.

*Conclusion* Immunotherapy with a modified slow-release birch pollen extract, administered in a single-strength preparation with a rapid dose increase, is safe and efficacious. IgG and IgG4 antibodies against native Bet v 1 are induced, which block basophil activation.

**Keywords** allergoid, birch pollen allergy, immunotherapy, modified allergens, SCIT
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## Introduction

It is now well established that subcutaneous immunotherapy (SCIT) with allergen extracts of pollen, cat dander or house dust mites is beneficial in patients with allergic rhinitis (AR) and asthma [1–3]. Immunotherapy is the only therapeutic approach in respiratory allergy that sometimes can modify the natural evolution and progression of atopic diseases [4], influence the predisposition of AR patients to develop asthma [5, 6] and that has long-lasting effects after stopping the treatment [7]. In view of the rising prevalence of respiratory allergy [8, 9], there is an increasing need for efficacious and easy-to-perform immunotherapy. Unfortunately, immunotherapy also implies some risks, especially that of anaphylactic reactions to the injected allergens. Such risks can be decreased by adsorption of allergens on aluminium hydroxide, and by chemical modification of the allergens (hence called allergoids) with glutaraldehyde or formaldehyde in order to reduce the IgE-binding capacity. The mast cell-triggering capacity of a glutaraldehyde-modified mite extract has been compared with a non-modified extract in a group of highly sensitized patients [10]. A 44 times diluted extract of the non-modified preparation (containing 150 μg/mL of Der p 2) gave the same skin reaction as the modified formulation (containing 6629 μg/mL of Der p 2

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assessed before modification). This is attributed to a lower capacity to bind and cross-link IgE, thus improving the safety of these extracts for therapy, as also shown in clinical studies [11]. Several studies have shown that clinical efficacy is maintained when using modified extracts [12–15].

We present the results of a double-blind placebo-controlled, randomized, multi-centre trial with birch pollen extract, adsorbed on aluminium hydroxide after modification with glutaraldehyde. The trial was designed to demonstrate the efficacy, safety and immunological effects of this preparation in patients with birch pollen allergy. Treatment was started in the second half of 2001, and continued for at least 18 months. The primary objective was to investigate whether there was a difference between study groups (placebo or verum treated) during the birch pollen season of 2003, using a combined symptom and medication score for evaluation of clinical efficacy.

## Materials and methods

### *Investigational preparations*

A glutaraldehyde-modified birch pollen extract adsorbed onto aluminium hydroxide (produced and marketed as PURETHAL® Birch, Leiden, the Netherlands) was provided by HAL Allergy BV (the Netherlands). The placebo preparation, prepared by HAL Allergy BV, contained all the ingredients of the verum suspension, except for the modified birch pollen extract. Only one strength of study preparation was used. One millilitre contains 500 μg of modified birch pollen allergens. The Bet v 1 content was 52 μg. The other ingredients were aluminium hydroxide (1.1 mg), sodium chloride (9.0 mg), phenol (5.0 mg) and water for injection (1.0 mL).

### *Study design*

The study was designed as a double-blind, randomized, placebo-controlled multi-centre study. The treatment period was at least 18 months. Based on symptom scores in other studies, an improvement of about 1 point of the CIS with a standard deviation of 1–1.5 was expected. With a level of significance of $\alpha = 5\%$, a power of 80% and assuming a dropout rate of 10–15%, the number of patients to be included as appropriate to conduct the study was calculated to be $2 \times 40$.

Protocol and amendments were approved by the Independent Ethics Committees of the Medical Faculty of the U.Z. Gasthuisberg in Leuven, of the University Hospital of Antwerp and of Ziekenhuis Leyenburg in The Hague. Inclusion required a history of seasonal rhinoconjunctivitis of at least 2 years' duration, and no or only mild asthma ($FEV_1$ above 70% of predicted). Allergy to birch pollen was confirmed with a positive skin prick test (SPT) and a specific serum anti-birch IgE (Phadia, Uppsala, Sweden) concentration of at least 1 U/mL. Only patients mono-sensitized to tree pollen (or sensitized to tree and grass pollen but without clinical symptoms in the grass pollen season) were included. Patients sensitized to weed pollen, mites, animal dander or mould spores were excluded. Other exclusion criteria were pregnancy, autoimmune or inflammatory diseases, intake of β-blockers or ACE-inhibitors and previous immunotherapy. Patients were informed about the nature and the conduct of the study and written consent was obtained before their study participation. Patients were recruited at eight different allergy centres in Northern Belgium (Flanders) and in the Netherlands, both areas with a similar climate and similar birch pollen counts in early spring (March/April). Patients aged between 18 and 65 were recruited from August 2001 till 15 December 2001, and treated till June 2003. Birch pollen extract was used rather than mixed tree pollen extract because clinical results with both types of extracts have been shown to be similar [16]. Verum or placebo preparations were injected subcutaneously. Initially, incremental doses of 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mL were given at 1-week intervals, followed by three additional doses of 0.5 mL at intervals of 2 weeks. When adverse reactions did occur, the same or a lower dose was repeated until well tolerated. The maintenance dose of 0.5 mL was given at monthly intervals.

Patients were allowed to take rescue medication during the pollen season. For nose symptoms, they were advised to take an antihistamine nose spray on demand as a first step, oral antihistamines on demand as a second step, topical steroid sprays on a regular basis as a third step and oral corticosteroids if severe symptoms occurred. For eye symptoms, the first treatment steps were topical antihistamines on demand, the second oral antihistamines on demand, and the third oral corticosteroids. For asthma treatment, the use of short-acting $\beta_2$-sympathomimetics on demand was advised first, thereafter inhaled corticosteroids on a regular basis, and finally long-acting $\beta_2$-sympathomimetics in combination with inhaled steroids.

### *Efficacy and safety evaluation*

Diaries were completed by the patients in March until May (2002 and 2003). Clinical efficacy was evaluated during the peak of the birch pollen season of 2002 and of 2003 on the basis of the clinical index score (CIS, as described by Bufe) [17], a combined score of symptoms and medication use. For eyes, nose and lower airways, symptoms were scored using four questions and a visual analogue scale, resulting in a value between 0 (no complaints) and 3 (severe complaints). The mean symptom score is calculated per organ, and a calculated value is added depending on the type of medication used. The combination of symptom and medication score can never exceed three, and is calculated for each organ ($CIS_{eye}$, $CIS_{nose}$ and $CIS_{lower\ airways}$). By definition, the highest score of a set of three corresponding values is the total CIS.

As symptoms depend on the presence of a minimal amount of pollen in the air (which was not the case during the whole period during which the diary was kept), it was decided to restrict the period for analysis to a specific 2-week period with maximal exposure and maximal symptoms. This period was defined before unblinding, and the CIS for the whole study population was used to avoid any bias. Thus, the period from 14 until 25 April 2003 has been selected. During this period, an increase in tree pollen counts was observed and the mean CIS values (blinded data, i.e. for the whole study population) were constantly above 0.8.

For safety assessments, local and systemic reactions both immediately and within the 24 h after injection were recorded as specific parameters. Adverse event (AE) data were collected at each visit for the whole period since the preceding visit. Immediate local reactions at the injection site were documented in the CRF according to a predefined format. AE were documented in the CRF on the basis of both descriptive and predefined text formats. The possible relationship between AE and treatment was assessed by the physician/investigator.

### *Skin prick testing*

SPTs were performed with allergen extracts in 50% glycerol from HAL Allergy BV, using a separate sterile lancet for each allergen. Histamine (1%) was used as a control. A weal diameter of at least 3 mm greater than the control was considered as positive [18].

For patients included in the study, a quantitative SPT with a 1/10 dilution series of the birch pollen extract skin test preparation (10 000 AU/mL) was performed [19].

### *Detection of antibodies*

Immunologic parameters (allergen-specific IgE and IgG antibodies) were determined at the start and after (June) the first (2002) and second (2003) pollen season. IgE, IgG and IgG4 antibody levels to natural Bet v 1, were measured by an immunoassay at Sanquin (Immunopathology, Amsterdam, the Netherlands). IgE and IgG4 were determined as described [20] while for IgG anti-Bet v 1 a simular procedure was used with Protein A Sepharose.

In a subgroup of patients (those recruited at one particular centre), the inhibitory effect of pre- and post-treatment sera on allergen-mediated basophil activation was compared *in vitro*. Degranulation of basophilic granulocytes in heparinized human whole blood in response to allergen triggering was quantitatively determined by means of FACScan flow cytometric analysis of CD63 expression on IgE-bearing cells (Becton-Dickinson, Erembodegem, Belgium). The standard method described by the manufacturer was followed. To measure the blocking effect of IgG antibodies in sera from treated patients, sera from study subjects (before and after treatment) were pre-incubated with varying concentrations of rBet v 1 (Biomay, Vienna, Austria). Basophils obtained from a birch-allergic donor (who was not treated with birch immunotherapy) were incubated with these mixtures. The percentage of CD63-expressing basophils in each test condition was determined.

### *Statistics*

Statistical analysis with non-parametric testing was performed with the help of the SPSS version 15 statistical program. In most cases, the result values were not normally distributed. Differences between treatment groups were evaluated by the Mann–Whitney *U*-test. Changes within each treatment group were evaluated using the Wilcoxon signed rank test.

## Results

In total, 62 patients (27 males and 35 females) were recruited. The mean age was 46 ($\pm$8) years. Height, weight, age distribution and sex ratio were not different between both groups. Seventy-one percent of the verum group and 74% of the placebo group reported oral allergy syndrome to fruits. Retrospectively, baseline (2001) symptom and medication scores (i.e. during the pollen season preceding the study recruitment) were similar in both groups (data not shown).

Of the 62 recruited patients, 28 and 30 of the verum and placebo group, respectively, could be evaluated in April 2003. The reasons for dropout were absence of symptoms during the pollen season before inclusion ($n = 1$, active treatment), or premature stop, either because of an unknown reason ($n = 1$, active treatment) or because of AEs possibly related to the study drug (one placebo and one active treatment patient).

*Clinical efficacy* was evaluated in the birch pollen seasons. For the season of 2002, no differences between verum and placebo groups were observed in any of the primary and the secondary efficacy parameters (data not shown). In 2003, the total CIS (which was the primary end-point in the study design) was reduced in the verum group by 24% as compared with placebo, with a *P*-value close to significance ($P = 0.055$) (Table 1). When the CIS for eye/nose symptoms (excluding lower airway symptoms) was compared, a 32% difference was found ($P < 0.04$). Analysis of the CIS per organ also revealed significant improvements in the PURETHAL treatment group compared with placebo (Table 2) for eye as well as for nose symptoms (42% and 31% improvement, respectively). CIS values for lower airways in the total group were not significantly different. However, when effects on lower airways were evaluated in the subgroup of 25 patients with a history of lower airway symptoms during the pre-treatment pollen season of 2001, the $CIS_{lower\ airways}$ in 2003 in verum-treated patients [median

**Table 1.** CIS during birch pollen season 2003

| | CIS | *n* |
|---|---|---|
| Placebo | 1.0322* (0.5882–1.6080)† | 30 |
| Verum | 0.5721* (0.2759–1.2830)† | 28 |
| Significance | $P = 0.055$‡ | |

*Median.
†Inter-quartile range.
‡Placebo vs. verum.
CIS, clinical index score.

**Table 2.** Organ specific CIS during birch pollen season 2003

| | $CIS_{eye}$ | $CIS_{nose}$ | $CIS_{lower\ airways}$ |
|---|---|---|---|
| Placebo ($n = 30$) | 0.6073* (0.2893–1.0338)† | 0.9258* (0.5658–1.3498)† | 0.3151* (0.0669–0.6639)† |
| Verum ($n = 28$) | 0.2440* (0.0586–0.5436)† | 0.5721* (0.1445–1.2198)† | 0.0881* (0.0116–0.3118)† |
| Significance | $P = 0.017$‡ | $P = 0.039$‡ | $P = 0.065$‡ |

*Median.
†Inter-quartile range.
‡Placebo vs. verum.
CIS, clinical index score.

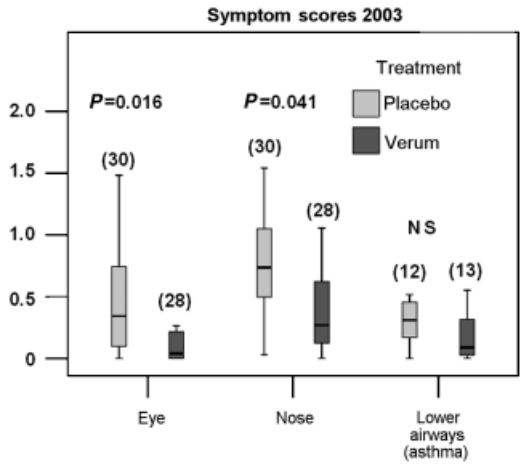


**Fig. 1.** Symptom scores (medians with inter-quartile and P5–P95 ranges) for the eye, nose and lower airways in the birch pollen season of 2003, for birch pollen extract-treated and placebo-treated patients (within parentheses: numbers of patients). For the lower airways, only patients who experienced lower airway symptoms in the pre-treatment season have been included ($n = 25$).

0.0896, inter-quartile range (IQR) ±0.4117, $n = 12$] was significantly lower ($P = 0.03$) than in placebo-treated patients (median 0.4215, IQR ±0.5148, $n = 13$).

Comparison of symptom scores also revealed significant differences for the nose and eyes (Fig. 1). No significant differences were noted on separate analyses of the medication scores (data not shown) ($P = 0.155$).

The mean total volumes of the study drug injected per patient were 11.02 and 10.62 mL in the verum and placebo group, respectively ($P = 0.227$). The mean total numbers of injections were 28.07 in the verum and 26.77 in the placebo group ($P = 0.137$).

Local reactions were reported 27 times in the verum vs. two times in the placebo group. Maintenance volume (0.5 mL) was reached after a mean of 6.86 injections in the verum group and after a mean of 6.1 injections in the placebo group ($P < 0.003$), the difference reflecting the higher incidence of local reactions in the verum group. A total of 12 systemic adverse effects (considered as being at least possibly related to study drug) occurred in the verum treatment group and 16 systemic adverse effects occurred in the placebo group. In the active treatment group, one patient reported dizziness and tachycardia several hours after an injection, not requiring treatment. This patient stopped with the trial, and the investigator considered these effects as likely related to the study drug. In the placebo group, one patient had an exacerbation of asthma, and withdrew from the study. Eye or nose symptoms after an injection were reported in 13 patients of the placebo group and in seven patients of the active treatment group (NS). Headache was reported more frequently (10 vs. four cases) in the active treatment group, but none of these was considered to be related to the study drug vs. one case in the placebo group.

*Immunologic assessments* of immunotherapy were performed by quantitative SPT and measurement of IgE, IgG and IgG4 antibodies to Bet v 1. For quantitative skin testing, no significant differences were observed between groups, only a tendency towards reduced skin reactivity in the active treatment group (not shown).

The increase in IgE antibodies, which reflects boosting by natural airway exposure (Fig. 2a), was not significantly different between treatment groups. Verum treatment induced a significant increase in IgG and IgG4 antibodies to Bet v 1, which was not observed in the placebo group (Figs 2b and c). IgG and IgG4 anti-Bet v 1 antibody levels at the end of the study (June 2003) and also after the birch pollen season of 2002 were significantly higher in the verum vs. the placebo group ($P = 0.004$, $P < 0.001$).

Functional effects of induced IgG antibodies were confirmed by studying serum blocking activity on Bet v 1-induced basophil activation. As shown in Fig. 3a, incubation of basophils from a birch pollen-allergic subject (who was not a study participant) with rBet v 1 induced membrane CD63 expression in a rBet v 1 concentration-dependent way. Sera obtained after active immunotherapy, as compared with pre-treatment sera, shifted the dose–response curve to the right, indicating that a higher level of Bet v 1 was needed to activate the basophils. This was not the case for sera obtained after placebo treatment (Fig. 3b). The area under the curve (AUC) for each dose–response curve was calculated. The changes in AUC for paired sera

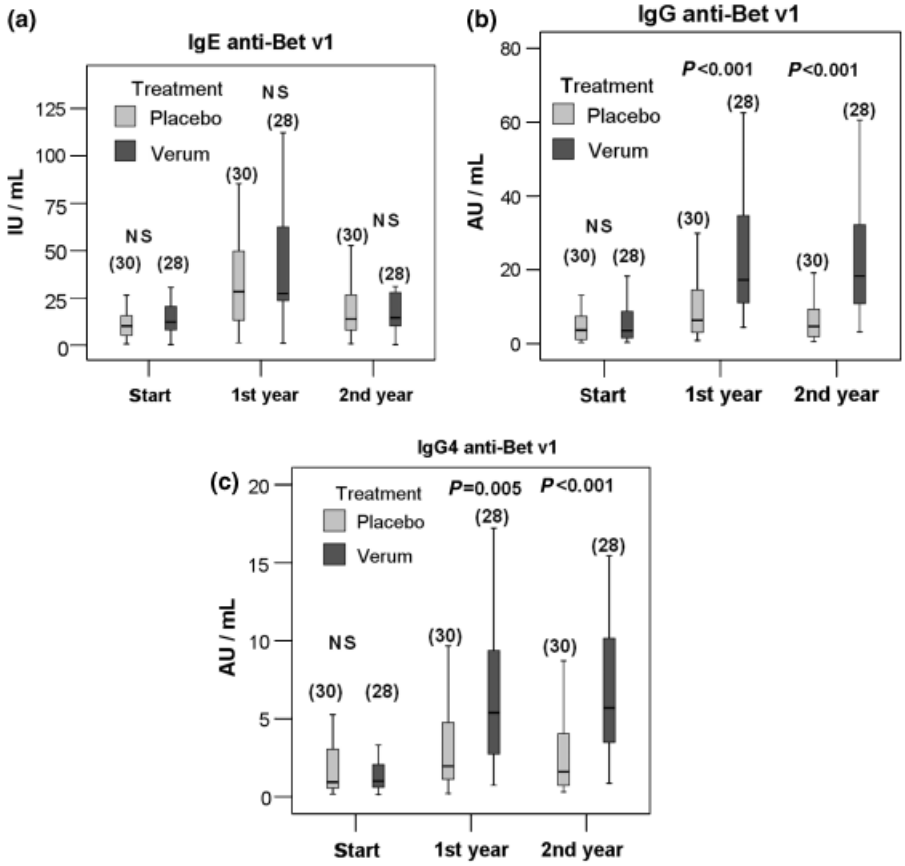


Fig. 2. Antibody levels to Bet v 1 at the start of treatment and after the first (2002) and the second (2003) birch pollen season in verum-treated (birch pollen extract) or placebo-treated patients. (a) IgE, (b) IgG, (c) IgG4. Results are presented as medians with inter-quartile and P5–P95 ranges (within parentheses: numbers of patients).

(obtained before and after treatment) show a significant inhibition of Bet v 1 activity by post-treatment sera from patients on verum treatment and not from placebo-treated patients (Fig. 3c). Similar effects were found when the mean fluorescence intensity of the CD63 staining was used instead of the percentage of CD63-positive cells (data not shown).

## Discussion

In this study, we report on the efficacy and safety of an aluminium hydroxide-absorbed allergoid preparation of birch pollen allergens, for the preventive treatment of nose and eye symptoms in birch pollen-allergic patients. For the primary efficacy parameter, the total CIS, which is a combined score of all symptoms (eye, nose and bronchi combined) and medication use, a reduction of 24% was observed in the verum-treated group. The *P*-value for this effect, however, was only 0.055. One could, however, question whether the total CIS, which also includes lower airways-related complaints, is the better parameter compared with observations for the eye and nose only. The study was indeed designed primarily for birch pollen-allergic subjects with nose and eye complaints. It turned out that only a minority of evaluable patients ($n = 25$) had a history of lower airway symptoms in the previous birch pollen seasons. The level of complaints for lower airways, including the use of medication, was low as compared with eye and nose complaints, even if only patients with a history of previous lower airway symptoms were considered (Table 2 and Fig. 1). Separate analysis of $CIS_{eye}$, $CIS_{nose}$ and $CIS_{lower\ airways}$ (this latter calculated on the subgroup with previous lower airway symptoms) all revealed highly significant improvements. Alternatively, it should also be considered that the study was underpowered. Originally, it was designed to include 80 patients. Time restrictions made it impossible to include this number of patients before 15 December.

The predominant effects were thus observed for the eyes and nose (reductions of CIS with 42%, $P = 0.017$ and 31%, $P = 0.039$, respectively, as compared with placebo). The clinical relevance of a specific improvement percentage in immunotherapy studies has been discussed by Malling [21, 22]. In a first paper, a percentage below 30% was proposed to indicate no efficacy, and values between 30% and 45% as low efficacy [21]. In a more recent paper focused on sublingual immunotherapy [22], a 20%

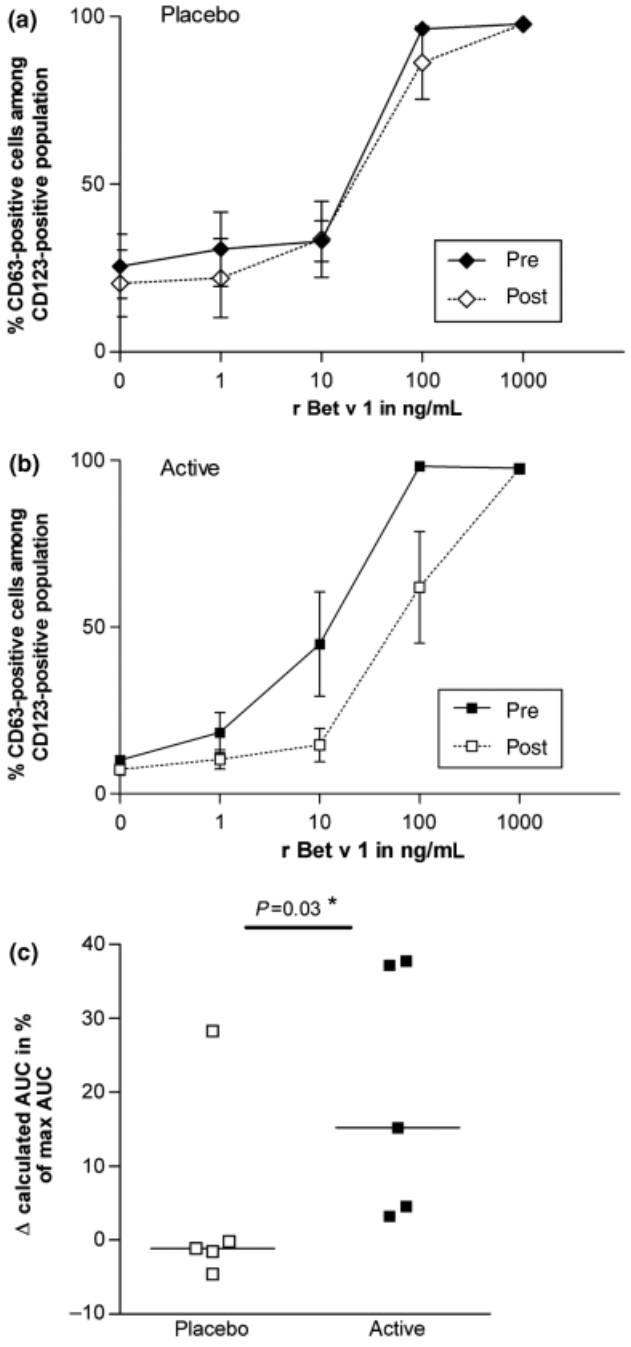


Fig. 3. Dose–response curves for Bet v 1-induced basophil activation (evaluated by CD63 expression) in the presence of serum obtained before and after treatment [(a) placebo, $n = 5$; (b) active, $n = 5$]. (c) The areas under the curve (AUCs) were calculated for each dose/response experiment, and the differences between corresponding AUCs in serum obtained before or after the treatment period were calculated.

reduction was proposed as the minimal improvement for efficacy (with reference to the effect of antihistamines).

We further show that injection of these modified allergens induced antibodies of the IgG class and IgG4 subclass that reacted with the native allergens (Bet v 1) in immunoassay tests. Such antibodies were shown, in this study and also by others [23], to block activation of IgE-loaded basophils after incubation with rBet v 1. Other potentially relevant effects of such antibodies are a reduction of allergen uptake and of presentation to T cells by dendritic cells [24], and inhibition of mast cell activation [25]. Whether these blocking antibodies are the key players for clinical efficacy remains controversial and requires further investigation. Indeed, in patients treated by insect venom immunotherapy for a long period, interruption of this treatment is usually associated with a prolonged efficacy, even when specific IgG antibodies are no longer present (reviewed in Müller and Mosbech [26]).

Despite the rapid dosage increase, no major adverse effects occurred, and local reactions were very mild. This excellent tolerability can be explained by the chemical modification of the extract with glutaraldehyde [10–15]. The safety and efficacy of this allergoid preparation makes it a very attractive tool for injection immunotherapy. Furthermore, the major advantages of this modified allergen preparation are the low number of injections required to reach the maximal allergen dose (six injections in the majority of patients), and the fact that only one single strength of allergen concentration is prepared, thus avoiding inadvertent overdoses. High allergen doses can be given without an increase in adverse reactions. As clinical efficacy is related to high-dose immunotherapy [27], this dose effect may also contribute to a more efficaceous therapy. On the basis of these results, we suggest that this type of preparation is a good alternative for the currently used preparations in SCIT.

## References

1 Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev* 2007.

2 Malling HJ. Immunotherapy for allergic rhinoconjunctivitis. *Clin Allergy Immunol* 2004; **18**:495–509.

3 Frew AJ, Powell RJ, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment of resistant seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006; **117**:319–25.

4 Bousquet J, Van Cauwenberge P, Khaltaev N, Aria Workshop Group, World Health Organization. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001; **108** (Suppl. 5): 147–334.

5 Des Roches A, Paradis L, Menardo JL, Bouges S, Daures JP, Bousquet J. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract: VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J Allergy Clin Immunol* 1997; **99**:450–3.

6 Moller C, Dreborg S, Ferdousi HA *et al*. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002; **109**:251–6.

7 Durham SR, Walker SM, Varga EM *et al*. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; **341**:468–75.
8 Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jørgensen T. Increasing prevalence of specific IgE to aeroallergens in an adult population: two cross-sectional surveys 8 years apart. The Copenhagen Allergy Study. *J Allergy Clin Immunol* 2000; **106**:247–52.
9 Linneberg A, Gislum M, Johansen N, Hisemoen LL, Jørgensen T. Temporal trends of aeroallergen sensitization over twenty-five years. *Clin Exp Allergy* 2007; **37**:1137–42.
10 Van der Zee JS, Kleinjans HAJ. Safety study with a new house dust mite (*D. pteronissinus*) allergoid. *Allergy* 2003; **58** (Suppl. 74):219, poster abstract 754.
11 Casanovas M, Martin R, Jimenez C, Caballero R, Fernandez-Caldas E. Safety of immunotherapy with therapeutic vaccines containing depigmented and polymerized allergen extracts. *Clin Exp Allergy* 2007; **37**:434–40.
12 Ariano R, Kroon AM, Augeri G, Canonica GW, Passalacqua G. Long-term treatment with allergoid immunotherapy with Parietaria. Clinical and immunologic effects in a randomized, controlled trial. *Allergy* 1999; **54**:313–9.
13 Brewczynski PZ, Kroon AM. Wirksamkeit und Verträglichkeit einer Immunotherapie mit modifizierten Gräserpollenallergenen. Ergebnisse einer plazebokontrollierten Studie. *Allergologie* 1999; **22**:S411–20.
14 Ameal A, Vega-Chicote JM, Fernandez S *et al*. Double-blind and placebo-controlled study to assess efficacy and safety of a modified allergen extract of *Dermatophagoides pteronyssinus* in allergic asthma. *Allergy* 2005; **60**:1178–83.
15 Garcia-Robaina JC, Sanchez I, de la Torre F, Fernandez-Caldas E, Casanovas M. Successful management of mite-allergic asthma with modified extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farina* in a double-blind placebo controlled study. *J Allergy Clin Immunol* 2006; **118**:1026–32.
16 Petersen BN, Janniche H, Munch EP *et al*. Immunotherapy with partially purified and standardized tree pollen extracts. I. Clinical results from a three-year double-blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel. *Allergy* 1988; **43**:353–62.
17 Bufe A, Ziegler-Kirbach E, Stoeckmann E *et al*. Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: a double-blind placebo-controlled study. *Allergy* 2004; **59**:498–504.
18 Malling HJ. Methods of skin testing. *Allergy* 1993; **48**(Suppl. 14):55–6.
19 Malling HJ. Reproducibility of skin sensitivity using a quantitative skin prick test. *Allergy* 1985; **40**:400–4.
20 Bolhaar STHP, Tiemessen MM, Zuidmeer L *et al*. Efficay of birch-pollen immunotherapy on cross-reactive food allergy confirmed by skin tests and double-blind food challenges. *Clin Exp Allergy* 2004; **34**:761–9.
21 Malling HJ. Immunotherapy as an effective tool in allergy treatment. *Allergy* 1998; **53**:461–72.
22 Malling HJ. Sublingual immunotherapy: efficacy – methodology and outcome of clinical trials. *Allergy* 2006; **61** (Suppl. 81):24–8.
23 Niederberger V, Horak F, Vrtala S *et al*. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA* 2004; **101** (Suppl. 2):14677–82.
24 Van Neerven RJJ, Wikborg T, Lund G *et al*. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4 T cells by inhibiting serum-IgE facilitated allergen presentation. *J Immunol* 1999; **163**:2944–52.
25 Larché M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006; **6**:761–71.
26 Müller U, Mosbech H. EAACI position paper: immunotherapy with Hymenoptera venoms. *Allergy* 1993; **48** (Suppl. 14):34–46.
27 Haugaard L, Dahl R, Jacobsen L. A controlled dose–response study of immunotherapy with standardised, partially purified extract of house dust mite: clinical efficacy and side effects. *J Allergy Clin Immunol* 1993; **91**:709–22.

## References

1 Chowdhary A, Agarwal K, Randhawa HS *et al.* A rare case of allergic bronchopulmonary mycosis caused by *Alternaria alternata*. *Med Mycol* 2012; PMID: 22563857.

2 Kuiper L, Ruijgrok EJ. A review on the clinical use of inhaled amphotericin B. *J Aerosol Med Pulm Drug Deliv* 2009; **22**:213–27.

3 Tolman JA, Wiederhold NP, McConville JT *et al.* Inhaled voriconazole for prevention of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 2009; **53**:2613–5.

4 Morio F, Aubin GG, Danner-Boucher I *et al.* High prevalence of triazole resistance in Aspergillus fumigatus, especially mediated by TR/L98H, in a French cohort of patients with cystic fibrosis. *J Antimicrob Chemother* 2012; **67**:1870–3.

5 Ward GW Jr, Woodfolk JA, Hayden ML, Jackson S, Platts-Mills TA. Treatment of late-onset asthma with fluconazole. *J Allergy Clin Immunol* 1999; **104**:541–6.

6 Stevens DA, Schwartz HJ, Lee JY *et al.* A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med* 2000; **342**:756–62.

7 Denning DW, O'Driscoll BR, Powell G *et al.* Randomized controlled trial of oral antifungal treatment for severe asthma with fungal sensitization: The Fungal Asthma Sensitization Trial (FAST) study. *Am J Respir Crit Care Med* 2009; **179**:11–8.

8 Platts-Mills TA, Woodfolk JA. Trichophyton asthma. *Chest* 2009; **135**:887–8.


# ERRATUM

## Immunotherapy with a modified birch pollen extract in allergic rhinoconjunctivitis: clinical and immunological effects

Ceuppens, J. L., Bullens, D., Kleinjans, H., Van Der Werf, J. and the PURETHAL Birch Efficacy Study Group (2009), Immunotherapy with a modified birch pollen extract in allergic rhinoconjunctivitis: clinical and immunological effects. *Clin Exp Allergy* 2009; 39: 1903–9.

In this study, patients were evaluated during the pollen season by a combined symptom and medication score (CIS), and medians of the CIS values are presented (tables 1 and 2, and text) after treatment with verum or placebo.

In the text, percentages are given for the differences between groups (verum treatment compared to placebo), referring to the tables. The percentages were calculated on the basis of mean CIS values (as originally presented to the journal), instead of the medians, added during editorial review of the manuscript.

Therefore the following corrections should be applied:

| | presented: | replace by: |
|---|---|---|
| Reduction CIS | 24% | 45% |
| Reduction $CIS_{eye}$ | 42% | 60% |
| Reduction $CIS_{nose}$ | 31% | 38% |
| Reduction $CIS_{eye/nose}$ | 32% | 45% |