

Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: A double-blind, placebo-controlled dose escalation study

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Background: Epicutaneous allergen administration using a patch may be an alternative to subcutaneous or sublingual immunotherapy.

Objective: To optimize treatment dose and to demonstrate the efficacy and safety of epicutaneous immunotherapy.

Methods: This monocentric, placebo-controlled, double-blind trial included 132 patients with grass pollen-induced rhinoconjunctivitis. In February 2008, patients were randomly allocated to receive placebo or 3 different doses of allergen. Before and during the pollen season 2008, patients received 6 weekly patches. Efficacy was assessed 4 to 5 months later (n = 110) and during the pollen season of the treatment-free follow-up year in 2009 (n = 93). The primary outcome was patient-reported changes in hay fever symptoms assessed by a visual analog scale. Secondary outcome measures were weekly visual analog scale symptom scores during pollen season, use of rescue medication, changes in conjunctival and skin reactivity, as well as safety.

Results: Hay fever symptoms during the pollen season were reduced by more than 30% in 2008 and by 24% in 2009 in the high-dose group as compared with that in the placebo group, and the alleviation of symptoms in the follow-up year was dependent on the treatment dose. Higher allergen doses were associated with drug-related adverse events (AEs), predominantly manifested by pruritus, erythema, wheal, or eczema. Eleven systemic AEs of grades 1 to 2 required treatment and led to study exclusion. The dropout rate due to AEs was 8.3%. No drug-related serious AE was recorded.

Conclusion: Epicutaneous immunotherapy is safe and efficacious in a dose-dependent manner after 6 patches only.

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Key words: Epicutaneous allergen-specific immunotherapy, epicutaneous immunotherapy, patch immunization

Despite insufficient symptom control with pharmacotherapy,¹ only 5% of patients suffering from allergic rhinoconjunctivitis undergo allergen-specific immunotherapy (SIT),^{2,3} which is the only disease-modifying treatment.^{4,5} The requirement for numerous visits to a medical office and allergen-associated adverse events (AEs) limit the broad application of subcutaneous allergen-specific immunotherapy. Tremendous effort has been put into developing self-administrable and more patient-convenient treatment routes.⁶⁻⁸

With the consensus approval of sublingual allergen-specific immunotherapy in 1998, a new area was initiated.⁹ Thanks to the excellent safety profile, sublingual allergen-specific immunotherapy can be self-administered and represents an increasing percentage of SIT treatments in Europe.¹⁰ However, up to 75% of patients report oral and gastrointestinal AEs.² Treatment duration is not reduced but may require daily application of high-allergen doses for several years, with considerable costs.² Also, treatment compliance with sublingual allergen-specific immunotherapy is low.¹¹

The high density and immune-surveillance function of antigen-presenting Langerhans cells in the epidermis¹² makes the skin an attractive route for the administration of SIT. Also, the epidermis lacks vascularization, thus minimizing the risk for inadvertent intravascular allergen injection and systemic allergic side effects. We therefore proposed epicutaneous allergen-specific immunotherapy (EPIT) as a needle-free and potentially self-administrable treatment route for SIT using a patch to apply allergen to the skin, and efficacy was demonstrated in a double-blind, placebo-controlled pilot trial.¹³ The earliest reports of EPIT date back to Besredka in 1917 and then Patrizel and Blamoutier in the 1950s who introduced a scarification method for SIT (as reviewed in Senti et al).¹⁴ We replaced scarification with adhesive tape stripping, physical removal of stratum corneum being important not only to enhance antigen penetration but also to activate keratinocytes that interact with Langerhans cells.^{12,15} In the present study, we tested the effect of EPIT on hay fever symptoms in patients with grass pollen allergy in a double-blind, placebo-controlled dose escalation study. The results implicate that EPIT is safe, convenient, and efficacious.

METHODS

Trial population

In total 157 persons were screened, and 132 patients aged between 18 and 65 years with a history of grass pollen allergic rhinoconjunctivitis and with

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Abbreviations used

AE: Adverse event
 CPT: Conjunctival provocation test
 EPIT: Epicutaneous allergen-specific immunotherapy
 HEP: Histamine equivalent prick
 SIT: Allergen-specific immunotherapy
 SPT: Skin prick test
 VAS: Visual analog scale

positive skin prick test (SPT) and conjunctival provocation test (CPT) results signed informed consent. Exclusion criteria were eczema on upper arms, perennial allergic rhinitis, infectious rhinitis within the last 14 days, surgery within the last 30 days, pregnancy or nursing, hypertension, history of HIV/AIDS, mastocytosis, malignancy, active infectious disease, significant cardiovascular, pulmonary, renal, hepatic, hematologic, autoimmune, neurological, or psychiatric disease, moderate to severe asthma, intake of antihistamines with long half-life within the last 2 weeks or corticosteroids within the last 5 days, intake of beta-blockers, angiotensin-converting enzyme/angiotensin II receptor antagonists, or tricyclic antidepressants, and participation in another clinical trial within the last 60 days. The study was approved by the institutional review board and the Swiss authorization agency Swissmedic (ClinicalTrials.gov NCT00719511).

Clinical trial design and study procedures

This single-center phase I/II randomized, placebo-controlled, double-blind study was designed to test the effective dose range, tolerability, safety, and the sustained treatment effect of EPIT. On the basis of a random-number table, 132 patients with grass pollen allergy were allocated to the placebo group ($n = 33$), the low-dose treatment group (10 histamine equivalent prick [HEP], $n = 33$), the medium-dose treatment group (50HEP, $n = 33$), or the high-dose grass-extract treatment group (100HEP, $n = 33$).

The study was performed between February 2008 and November 2009 at the University Hospital Zurich. The trial design is outlined in Table I. At the screening visit, consenting patients received a brief physical examination and SPT and CPT were performed. At least 4 weeks before start of the 2008 pollen season, patients received the first of 6 patches. The application was prepared by tape stripping a site on the upper arm 6 times with scotch tape (3M Company, St Paul, Minn). Then, the patch was applied and the patient was observed for 30 minutes. All patients received an emergency set containing corticosteroids (prednisone [Streuli, Uznach, Switzerland]) and antihistamines (Semprex [GlaxoSmithKline, Brentford, United Kingdom]). After 8 hours, the patch was removed by the patient. To assess local reactions, all patients were contacted by phone 48 hours after the application of the first patch. With the start of the pollen season, the 5 following patches were administered in weekly intervals (allowed range 5 days to 2 weeks). Each patch was administered at a different location on the upper extremity. If a local reaction occurred, patients were asked to take a photograph of the site and to contact the study nurse or the clinician. During the entire pollen season, the severity of hay fever symptoms was recorded weekly and the use of rescue medication was assessed daily. This study was conducted according to International Conference on Harmonisation guidelines on Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki.

Allergen patch system

The patches were prepared with the grass-allergen extract derived from the pollen of *Holcus lanatus*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, and *Festuca elatior* (MG51 Mezcal 6 Graminees, Immunotek, Madrid, Spain). Patches with different biological activity (10 HEP: total protein content using Bradford method was 76 μ g, major allergen content was 3 μ g Phl p 5 equivalents; 50 HEP: 378 μ g protein, 15 μ g Phl p 5 equivalents; 100 HEP: 757 μ g protein, 30 μ g Phl p 5 equivalents) were produced by the Cantonal Pharmacy, Canton of Zurich (Zurich, Switzerland). The allergen

TABLE I. Study outline

Visit/time	Intervention/analysis
February/March 2008	Newspaper advertisement and telephone recruitment
Screening (March-April 2008)	Medical history and examination, CPT, SPT, severity assessment of rhinoconjunctivitis with VAS
Visit 1 (March-April 2008)	First patch application Follow-up call after 48 h for record of local AEs
Visit 2 (April-May 2008)	Second patch application, AE records
Visits 3-6 (May-July 2008)	(Bi-) weekly patch application, AE records, weekly VAS symptom diary, medication diary
Visit 7 (August-September 2008)	Medical examination, CPT, SPT, AE records, recording of improvement/deterioration VAS
Visit 8 (March-May 2009)	AE records
Pollen season 2009	Weekly VAS symptom diary, medication diary
Visit 9 (September-November 2009)	Medical examination, CPT, SPT, AE records, recording of improvement/deterioration on VAS

grass-extract was dissolved in glycerol (50%), phenol (4 mg/mL), NaCl (0.15 mol/L), and phosphate buffer (10 mmol/L), at pH 7.2, and integrated into a patch system provided by Medanz Medical GmbH (Starnberg, Germany). The patch was 95 mm $\times$ 95 mm in size containing a centered 50 mm $\times$ 30 mm polyester web with the adsorbed allergen extract.

Patient-reported treatment outcome: Hay fever symptoms on visual analog scales

After the pollen seasons of 2008 (visit 7) and 2009 (visit 9), patients were asked to compare the seasonal hay fever symptoms to the symptoms experienced in previous years and to rate the general improvement or deterioration on a 200-mm visual analog scale (VAS) ranging from -100 (worst conceivable symptom deterioration) over 0 (unchanged symptoms) to +100 (total symptom relief).

Symptom and medication diary

The patients were asked to record the severity of nasal itching, sneezing, nasal obstruction, rhinorrhea, itchy eyes, lacrimation, itchy ears, itchy palate, and lung symptoms during the pollen seasons of 2008 and 2009 on a 100-mm VAS ranging from 0 (no symptoms) to 100 (most severe symptoms) on a weekly basis. To assess the average weekly symptom scores, the sum of all symptoms (except lung symptoms) was calculated and divided by the total number of symptoms. Concomitantly, the daily use of provided rescue medication was assessed: desloratadine tablets (Aerius 5 mg from Essex, Luzern, Switzerland), olopatadin eye drops (Opatanol 1 mg/mL from Alcon, Hünenberg, Switzerland), mometasone furoate nasal corticosteroid (Nasonex 50 μ g per dose from Essex), and budesonide inhalation corticosteroids (Symbicort 200 μ g from AstraZeneca, Zug, Switzerland). The start and end of the grass pollen season (April 28 to July 29, 2008 and May 4 to July 5, 2009, 9 weeks each) was determined according to the seasonal development of the pollen load as measured by the Swiss federal office for meteorology and climatology MeteoSwiss.

Conjunctival provocation test

Reactivity in CPT was tested at baseline and after the pollen seasons of 2008 (visit 7) and 2009 (visit 9). Patients were adapted to room temperature for

10 minutes. Unspecific hyperresponsiveness was excluded by the administration of a 50- μ L diluent. Subsequently, 50 μ L of increasing concentrations of the grass pollen extract (MG 51 6 Graminees, Immunotheek, Spain, Madrid) was administered into the lower conjunctival sac every 10 minutes, alternating between the 2 eyes. The CPT was stopped when the total symptom score reached 3 or more. Conjunctival redness, tearing, itching, burning, and swelling of the eyelids were assessed and graded according to Abelson et al.¹⁶

Skin prick test

Reactivity in SPT was tested at baseline and after the pollen seasons of 2008 (visit 7) and 2009 (visit 9). The patients were pricked with different concentrations of the grass pollen extract—0.05HEP, 0.5HEP, 5HEP, and 50HEP (MG51 Mezcal 6 Graminees, Immunotek)—on the volar forearm. At the screening visit, polysensitization was tested by performing SPT for mugwort, hazel, birch, alder, ash, cat hair, dog hair, *Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae* (Stallergènes, Antony, France). Histamine hydrochloride and normal saline (Stallergènes) were used as positive and negative control, respectively. SPT results were assessed according to the Joint Task Force on Practice Parameters, considering a wheal of diameter at least 3 mm more than that of the diluent control as a positive reaction.¹⁷

Safety assessment

AEs and serious AEs were defined according to the ICH-GCP guidelines. Systemic side effects related to EPIT were graded according to the guideline of the World Allergy Organization (WAO).¹⁸ Grade 1: symptoms of 1 organ system (cutaneous, upper respiratory tract, conjunctival, other); grade 2: symptoms of more than 1 organ system present or lower respiratory tract (<40% drop in peak expiratory flow [PEF] or FEV₁), gastrointestinal; grade 3: lower respiratory tract (40% drop in PEF or FEV₁), upper respiratory tract (laryngeal, tongue edema); grade 4: respiratory or cardiovascular failure.

Statistical analysis

Sample size calculation was performed assuming a mean self-reported improvement of 29% (SD = 27%) in the placebo group and a mean self-reported improvement of 52% (SD = 27%) in at least 1 treatment arm. Thus, to yield a statistically significant result with a power of 82% (2-sided analysis with $\alpha = 0.025$) and an expected dropout rate of 10%, a sample size of 33 per arm (total, 132) was calculated.

Scores from the VAS for the improvement or deterioration in hay fever symptoms were analyzed using Mann-Whitney multiple testing with Hochberg procedure adjustment. For the evaluation of seasonal VAS symptom scores, the area under the curve, assessed out of the average weekly VAS scores, was calculated for each patient. Simultaneous upper 1-sided 97.5% confidence limits for the ratios to placebo were calculated to identify the minimum effective dose to achieve a 30% improvement (ratio = 0.7) in the average VAS symptom score compared with placebo. For clinically beneficial values, the upper 1-sided limits should thus be smaller than 1. Daily medication use was compared between the groups by using the Kruskal-Wallis test. Equal distribution of local side effects at the patch application site with increasing patch number was tested by using the χ^2 test. All patients with at least 1 application of the patch were evaluated for efficacy in an intention-to-treat analysis. Similarly, all subjects receiving at least 1 patch were included in the safety analysis.

RESULTS

Patient flow and characteristics

An overview of the participant flow is provided in Fig 1. From February to March 2008, 157 persons were screened for eligibility and 132 patients with allergy to grass pollen were enrolled in the study and signed informed consent. Three patients with wheal diameters of only 2.5 mm more than those of diluent controls were also enrolled (inclusion criteria ≥ 3 mm). Thirty-three patients

were randomly allocated to each of the 4 treatment arms. A total of 124 patients were analyzed for efficacy, and out of these, 111 patients (89.5%) received all 6 patches. The mean treatment duration including the 4 weeks from the initial to the second patch and including the 13 patients who did not receive all 6 patches was 74 ± 13 days. In the placebo group, 30 patients received at least 1 patch during the pollen season 2008 and were therefore included in the intention-to-treat analysis. Similarly, 31 patients each of the 10HEP and 100HEP and 32 patients of the 50HEP group received treatment during 2008 and were analyzed for treatment efficacy (2008, $n = 110$ in total). Several patients did not attend the follow-up visits in 2009: 1 patient in the 10HEP group was excluded for the use of prednisone against asthma, and 1 patient in the 100HEP group was excluded for starting antihypertensive treatment with an angiotensin-converting enzyme inhibitor. Thus, a total of 22 patients each of the placebo group and the 10HEP treatment group as well as a total of 25 and 24 patients of the 50HEP group and the 100HEP group, respectively, were included in the efficacy analysis for the evaluation of sustained treatment effect in 2009 ($n = 93$ in total).

At baseline, the demographic- as well as the disease-specific characteristics were similar between the 4 treatment groups with regard to disease duration, allergic asthma, and cosensitization to other allergens. Also, the disease severity between the groups was comparable at baseline, all patients having a history of moderate to severe rhinitis according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines as assessed by VAS¹⁹ (Table II).

Efficacy

Patient-reported treatment outcome. As primary end-point we assessed the general impact of EPIT on grass pollen allergy by determination of the subjectively experienced improvement or deterioration in hay fever symptom severity by VAS. In August/September 2008, 5 months after the application of the first patch, the patients reported an improvement in seasonal hay fever symptoms in all treatment groups, including the placebo group (Fig 2). One year later, after having received no further immunotherapy, the original placebo effect vanished, and the results revealed a clear dose-response relationship. Median improvement in the placebo group and the 10HEP group was 30.73, whereas an improvement of 53.13 and 69.79 was recorded for the treatment groups 50HEP and 100HEP, respectively (Fig 2). Symptom alleviation for the high-dose treatment group was significantly different from that in the placebo group (placebo-10HEP: $P = .532$; placebo-50HEP: $P = .141$; placebo-100-HEP: $P = .017$).

Weekly seasonal symptom and medication score.

Treatment with 100HEP reduced the average weekly rhinoconjunctivitis symptom score by 32% (estimate = 0.68, upper 97.5% CI = 1.09) in 2008 and as compared to placebo (Fig 3). After the treatment-free follow-up year, the 100HEP-treated patients still experienced 24% reduction in the average seasonal symptom score (estimate = 0.77, upper 97.5% CI = 1.60). A dose-response relationship was observed for the reduction in seasonal symptom scores in 2009. The use of rescue medication did not significantly differ between groups within the pollen seasons of 2008 and 2009.

Conjunctival provocation test and skin prick test. No statistically significant difference in the degree of improvement was observed in conjunctival (CPT) or skin (SPT) tolerances between the different treatment groups (data not shown).

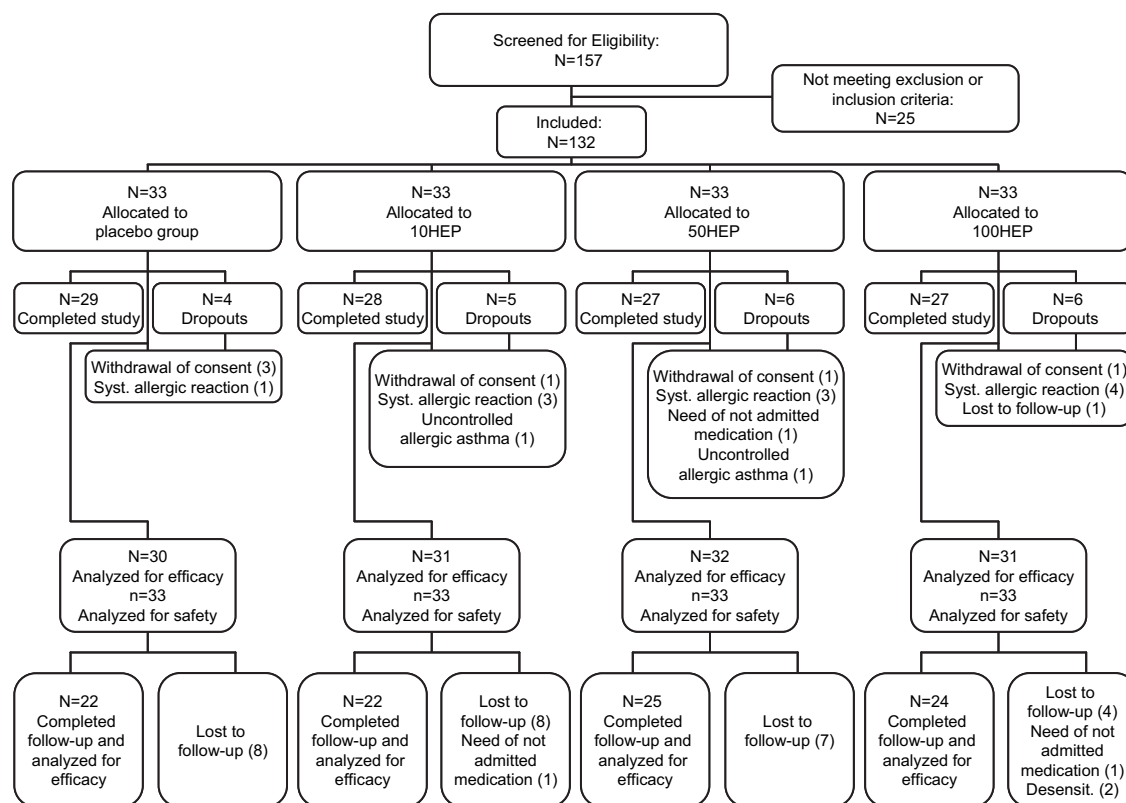


FIG 1. Participant flow. Flow diagram characterizing the study progress and participant flow through the different trial phases. Syst, Systemic.

TABLE II. Baseline characteristics

Characteristic	Treatment group			
	Placebo	10HEP	50HEP	100HEP
Age (y), mean $\pm$ SD	39 $\pm$ 10.8	35 $\pm$ 11.3	36 $\pm$ 10.2	36 $\pm$ 10.6
Female, n (%)	9 (27.3)	14 (42.4)	11 (33.3)	15 (45.5)
Duration of rhinitis (y), mean $\pm$ SD	23 $\pm$ 11.6	21 $\pm$ 11.2	21 $\pm$ 10.5	21 $\pm$ 10.6
Severity of rhinoconjunctivitis, mean total VAS $\pm$ SD	53 $\pm$ 14.9	47 $\pm$ 16.7	50 $\pm$ 17	51 $\pm$ 18.5
Severity of nasal symptoms, mean VAS $\pm$ SD	60 $\pm$ 19.8	56 $\pm$ 19.5	57 $\pm$ 24.0	56 $\pm$ 18.9
Severity of ocular symptoms, mean VAS $\pm$ SD	63 $\pm$ 20.1	52 $\pm$ 25.3	55 $\pm$ 19.0	52 $\pm$ 26.5
Severity of oropharyngeal symptoms, mean $\pm$ SD	28 $\pm$ 24.0	24 $\pm$ 24.2	31 $\pm$ 29.8	38 $\pm$ 26.7
Severity of lung symptoms, mean VAS $\pm$ SD	38 $\pm$ 33.7	24 $\pm$ 31.4	25 $\pm$ 28.9	24 $\pm$ 31.2
Seasonal asthma (%)	9 (27.3)	9 (27.3)	12 (36.4)	10 (30.3)
Monosensitized patients	7 (21.2)	5 (15.2)	11 (33.3)	7 (21.2)
Polysensitized patients	26 (78.8)	28 (84.8)	22 (66.7)	26 (78.8)
Baseline CPT threshold, mean $\pm$ SD				
5BU	2 $\pm$ 6.1	1 $\pm$ 3.0	1 $\pm$ 3.0	1 $\pm$ 3.0
50BU	5 $\pm$ 15.2	3 $\pm$ 9.1	8 $\pm$ 24.2	10 $\pm$ 30.3
500BU	21 $\pm$ 63.6	21 $\pm$ 63.6	19 $\pm$ 57.6	11 $\pm$ 33.3
5000BU	5 $\pm$ 15.2	8 $\pm$ 24.2	5 $\pm$ 15.2	11 $\pm$ 33.3
Baseline SPT threshold, mean $\pm$ SD				
0.05HEP	15 (45.5)	11 (33.3)	12 (36.4)	10 (30.3)
0.5HEP	6 (18.2)	11 (33.3)	11 (33.3)	7 (21.2)
5HEP	11 (33.3)	10 (30.3)	9 (27.3)	8 (24.2)
50HEP	1 (3.0)	1 (3.0)	1 (3.0)	8 (24.2)

BU, Biological units.

Safety

During the entire study, a total of 1566 AEs were reported (Table III), of which 825 (52.7%) were graded as likely or definitely drug related. The most frequent drug-related AEs were

pruritus (417 events, 26.6% of total AEs), erythema (90 events, 5.7%), wheals (149 events, 9.5%), or eczema (138 events, 8.8%). All local AEs occurred at the patch application site. The local reactions classified as eczema were short lived. Only 2

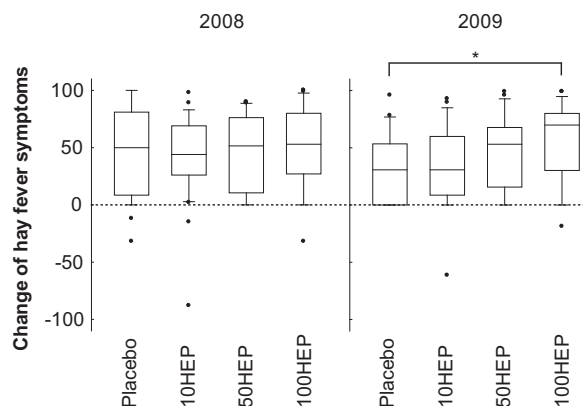


FIG 2. Patient-reported treatment outcome. Patients rated the general improvement/deterioration of hay fever symptoms on a scale from -100 (worst possible deterioration) to $+100$ (best possible improvement) during the seasons 2008 (treatment) and 2009 (follow-up). Box plots show the median, the 10th, 25th, 75th, and 90th percentiles, and outliers. Intergroup comparison by Mann-Whitney multiple testing with Hochberg procedure adjustment. $*P < .05$.

patients reported an eczema, which lasted for 3 and 4 days, respectively; eczema was treated with topical corticosteroids. All other reported eczemas lasted for 1 day only. Pruritus, erythema, wheals, and eczema were more frequent with higher allergen treatment doses (Fig 4, A). The occurrence of local reactions significantly decreased with each patch application in all the treatment groups ($P < .001$; Fig 4, B). The data did not suggest a correlation between the occurrence of local reactions and the amelioration of hay fever symptoms as analyzed by using the exact Mann-Whitney test for symptoms in patients with local reactions versus no local reactions ($P = .797$; data not shown).

Eleven patients (8.3%) stopped treatment because of a systemic allergic reaction. In these cases, patches were removed and the reaction was treated intravenously with corticosteroids (125 mg methylprednisolone) and antihistamines (4 mg clemastine). Of these 11 patients, 1 had received placebo, whereas 10 had been treated with allergen extract (Table IV). The reactions were rated as grade 1 or grade 2. All 11 patients reported cutaneous reactions such as sustained pruritus distant from the patch application site and sensation of heat, whereas 4 patients additionally suffered from rhinitis and cough originating in the upper airways, or vertigo. All systemic reactions responded to treatment with corticosteroids and antihistamines. Hospitalization or adrenaline treatment was never required.

DISCUSSION

The fact that allergic diseases constitute a global health problem with considerable impact on socioeconomic life¹ underlines the need to develop a novel and convenient route for SIT with high efficacy and broad acceptance. Since the burden of allergic diseases not only goes beyond symptom scores and medication use but also affects the quality of life, we chose the patient-reported outcome measure, the primary endpoint being changes in hay fever symptoms as described by VAS. A strong placebo effect was observed during the treatment season (2008), but as this effect declined in the second year, the results revealed that EPIT with grass pollen allergens caused a dose-dependent alleviation of allergy symptoms overall. Patients treated with 100HEP reported a 70%

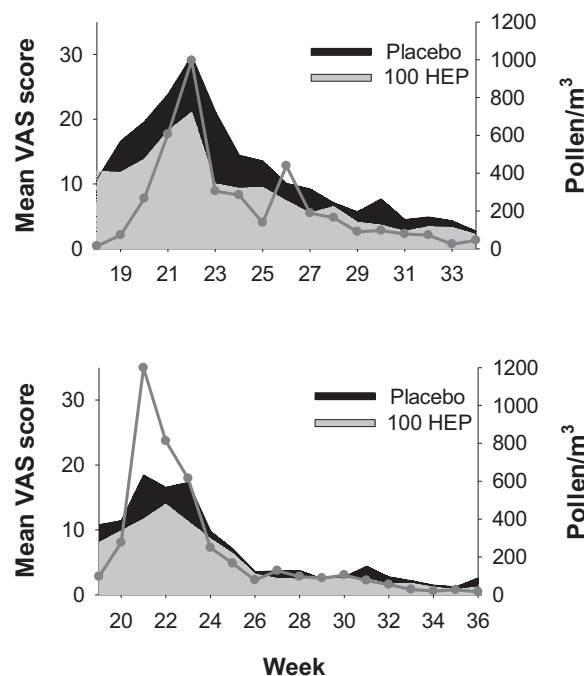


FIG 3. Mean VAS symptom score for pollen season 2008 (upper panel) and 2009 (lower panel). Mean weekly VAS symptom scores (except lung symptoms) of the 100HEP group compared with those of the placebo group (left axis). For each patient, the sum of all symptom scores was calculated and divided by the number of symptoms. The continuous line shows the weekly pollen counts in Zurich (right axis).

improvement overall in hay fever symptoms, and this result was significantly different from the that in the placebo-treated group. Interestingly, a weaker placebo effect was observed when assessing the specific seasonal rhinoconjunctivitis symptom scores, with allergen-EPIT-mediated symptom improvement recorded both in the first (32%) and second (24%) years.

Although statistical significance for self-reported symptom improvement was reached only for the high-dose treatment group, it is important to consider that statistical significance does not parallel treatment benefits under real-life conditions. Therefore, it has been proposed that every improvement of more than 30% is clinically relevant. Correspondingly, an improvement between 30% and 45% is judged as “little” treatment effect, whereas an improvement between 46% and 60% is assessed as “moderate” treatment effect and an improvement of more than 60% as a “strong” effect.²⁰ According to such a rating, treatment effect was strong with 100HEP, moderate with 50HEP group, and absent with 10HEP. Strikingly, such clinically relevant effect was achieved through the application of merely 6 patches over a time period of only approximately 8 weeks.

Overall, EPIT proved to be safe, but not without systemic allergic side effects, which required treatment and led to study exclusion in 8% of the treated patients. Yet, all these systemic allergic reactions were mild (grade 1 or 2) and occurred within 45 minutes after patch application. This is comparable to conventional subcutaneous allergen-specific immunotherapy where the number of systemic AEs using aeroallergens ranges from 1%²¹ to 29% of the patients.²² Local reactions such as pruritus, erythema, wheal, and eczema at the patch application site were frequent; although bothersome, the appearance of eczema suggests

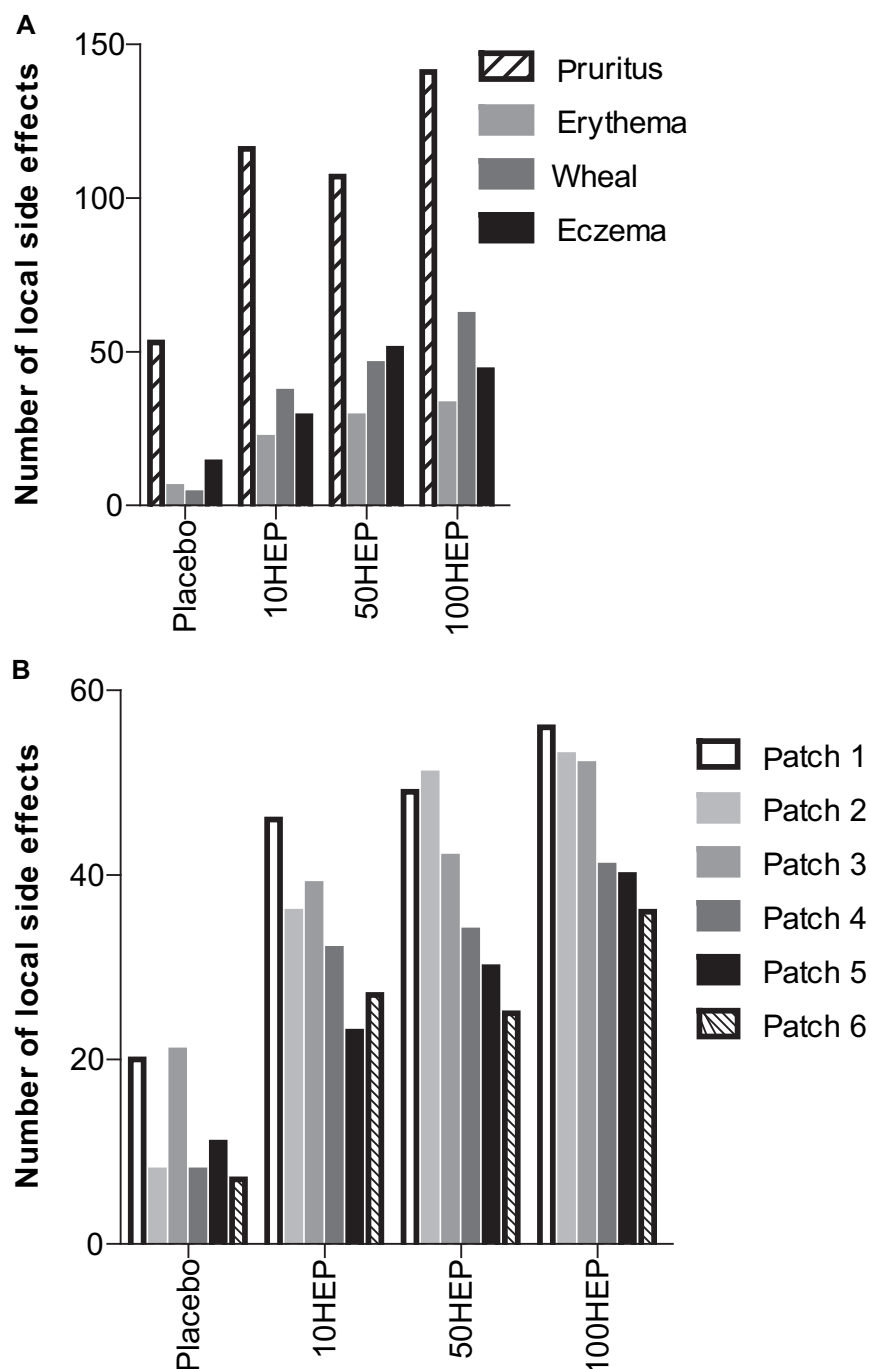


FIG 4. Local drug-related AEs. **A**, Numbers of the most frequent local drug-related AEs as reported in the different treatment groups. **B**, Frequency of local side effects per patch sequence. Distribution of the frequency of local AEs was tested by using the χ^2 test.

allergen presentation to T cells.^{23,24} T-cell involvement is a crucial mechanism in SIT and enables to redirect the pathogenic T_H2 response toward a more protective T_H1 or T-regulatory response.^{25,26} Interestingly and over time, local reactions decreased, suggesting a reduction in allergen-specific T-cell reactivity and the development of peripheral T-cell tolerance, which is a key feature of successful SIT.^{25,27-29} We are currently addressing these issues in a recently initiated clinical trial with epicutaneous immunotherapy.

The results of the present trial confirm the results and the conclusion of the previous and very first clinical trial on EPIT.¹³ In the latter pilot study, the patch preparation was guided by the patch test for the diagnosis of contact dermatitis, in which the allergen extracts are applied in soft paraffin for 48 hours to pulse skin resident antigen-presenting cells.^{24,30} One problem with the paraffin-based patch was that paraffin leaked from the patch. To overcome this problem, the patches used in the present study were prepared with aqueous allergen solutions.

TABLE III. Adverse events

	Treatment group			
	Placebo (n = 33)	10HEP (n = 33)	50HEP (n = 33)	100HEP (n = 33)
No. of patients with AEs, n (%)	33 (100)	33 (100)	33 (100)	33 (100)
No. of AE episodes	279	409	427	451
Local reactions				
Pruritus	53	116	107	141
Erythema	6	22	29	33
Eczema	14	29	51	44
Wheal	4	37	46	62
Causality assessment				
No relation, n (%)	14 (5.0)	14 (3.4)	18 (4.2)	11 (2.4)
Unlikely/possible relation, n (%)	182 (65.2)	191 (46.7)	162 (37.9)	148 (32.8)
Likely/definite, n (%)	82 (29.4)	204 (49.9)	247 (57.8)	292 (64.7)
Missing, n (%)	1 (0.4)	0	0	0
Severity assessment of likely/definite drug-related AEs, n (%)				
Mild	60 (76.9)	135 (66.2)	139 (56.3)	147 (50.3)
Moderate	14 (17.9)	58 (28.4)	82 (33.2)	115 (39.4)
Severe	4 (5.1)	11 (5.4)	26 (10.5)	30 (10.3)

TABLE IV. Systemic allergic reactions necessitating intervention*

	Treatment groups			
	Placebo (n = 33)	10HEP (n = 33)	50HEP (n = 33)	100HEP (n = 33)
Systemic allergic reaction				
Grade 1, n	1	1	3	2
Grade 2, n	0	2	0	2
Grade 3, n	0	0	0	0
Grade 4, n	0	0	0	0

*Treated with corticosteroids and antihistamines.

Another problem in the first trial¹³ was that 48 hours of allergen application caused incidents of strong local eczematous reactions. In the present study, we therefore shortened the treatment to 8 hours, assuming that a shorter application still pulsed skin resident antigen-presenting cells, but later, when T cells start infiltrating, the allergen would already be gone. In fact, while 160 eczema reactions were reported after 244 patch applications in 15 of the 21 allergen-treated patients in the original trial, only 44 and milder eczema reactions occurred after 198 patch applications in 20 of the 33 patients of the 100HEP-treated group in the current trial. In our first study, 10 of the 15 allergen-treated patients required treatment with topical corticosteroids, whereas only 2 of the 33 patients in the current trial used topical corticosteroids.

In comparison with the original EPIT trial,¹³ the number of patch applications in the current trial was reduced from 12 to 6. To compensate for this reduction, higher allergen doses were applied. In the first trial we used 300 Index of Reactivity Phleum pratense extract containing 21 µg of Phl p 5 equivalents. In the present trial, 100 HEP grass pollen extract containing 30 µg of Phl p 5 equivalents was used. Thus, the cumulative doses of Phl p 5 in the 2 trials were 252 and 180 µg of Phl p 5, respectively.

The changes described above had no notable impact on efficacy, as both trials produced comparable symptom amelioration. In our first trial,¹³ median symptom improvement in patients receiving allergen EPIT was 72% (18% with placebo), whereas in

the present study, median symptom improvement with allergen EPIT was 70% (31% with placebo).

The current trial demonstrates that higher allergen doses enhance the efficacy of EPIT. The maximal allergen doses applicable to the skin, however, are limited by safety issues. Local AEs, the most irritating being eczema, may be reduced by shortening the time period of carrying the patch. However, the application of high allergen doses to tape-stripped skin bears a significant risk of systemic allergic reactions due to the removal of the stratum corneum and, hence, disruption of the skin's natural barrier to xenobiotics. Alternative methods of skin preparation should therefore be evaluated in terms of safety for epicutaneous or transdermal allergen application. These methods include microneedle patches, liposomal allergen formulations, hydration of the stratum corneum, or a combination of these methods. In summary, the current study provides data that suggest a dose-response relationship in the treatment of hay fever with allergen EPIT, a method that extends the growing field of needle-free vaccination methods delivering the antigen via the skin. Transcutaneous vaccination against infectious diseases such as travellers' diarrhea³¹ and influenza³² are examples of this promising vaccination route. EPIT represents an easy and painless administration route that might also promote prescription of SIT in children, which is an important medical goal, since SIT has the potential to stop disease progression to asthma.^{33,34}

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Clinical implications: Epicutaneous allergen-specific immunotherapy is a convenient, safe, and efficacious treatment for IgE-mediated allergies, leading to symptom relief after a single treatment season.

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