

ORIGINAL ARTICLE

Bronchial allergen provocation: A useful method to assess the efficacy of specific immunotherapy in children

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Keywords

allergen specific immunotherapy (SIT); bronchial allergen provocation; house dust mites; asthma; allergoid; subcutaneous allergen-specific immunotherapy (SCIT)

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Abstract

Background: The clinical efficacy of subcutaneous allergen-specific immunotherapy (SCIT) varies between patients. New preparations are under development, and an objective tool with which to evaluate their efficacies in individual patients has become necessary. Our primary research question is whether bronchial allergen provocation (BAP) can be used to assess the efficacy of SCIT.

Methods: In 42 house dust mite (HDM) allergic children (average age: 8.6 yr) with asthma, we analysed the clinical and objective improvements of a standardised HDM allergoid. All patients underwent two BAPs, one before SCIT and another 1 yr after SCIT. Fourteen patients who were recommended but chose not to undergo SCIT represented the control group. The total and specific IgE were analysed before SCIT; in addition, after SCIT, specific IgG and IgG₄ were analysed.

Results: After SCIT, the patients' allergen-specific bronchial hyper-reactivity (BHR) was significantly improved; specifically, their PD₂₀FEV₁ was 34.4 AU before and 63.3 AU after SCIT ($p < 0.01$). The PD₂₀FEV₁ of the control group remained unchanged. Although BHR improved significantly in the treatment group, we were able to differentiate between the responders ($n = 17$, 60.7%) and non-responders ($n = 11$, no improvement in BAP). The patients in both groups stated that SCIT had led to a subjective improvement in their symptoms, in contrast to the untreated control group, but only the responders required less medication after SCIT ($p < 0.01$).

Conclusions: After 1 yr of SCIT against HDM, 60.7% of the patients observed in this study exhibited significant improvements, as defined by BAP. However, BAP was also able to identify the non-responders to treatment. Thus, BAP is a useful and objective method of estimating the effectiveness of SCIT and is not influenced by a placebo effect.

IgE-mediated allergic disorders such as asthma and allergic rhinitis have become an epidemic affecting up to 30% of the population.(1). Allergen-specific immunotherapy (SIT) is the only efficacious treatment with the potential to both ameliorate symptoms and alter the natural course of the disease. The most recent update of the Cochrane review confirmed that immunotherapy can reduce asthma symptoms, patients' need for medications and the risk of severe asthma attacks after future

exposure to the allergen (2). However, depending on the allergen, the success rates of SIT success rates range from 90% for insect venom allergies and 80% for pollen to approximately 70% for house dust mites (HDM). Thus, far the efficacy of subcutaneous allergen-specific immunotherapy (SCIT) has been assessed primarily according to symptom and medication scores, as well as quality-of-life questionnaires before and after treatment (3). Unfortunately, these scores fail to demonstrate significant improvement in small cohorts. To date, no consensus exists regarding the mechanism of successful SCIT, but it is thought to involve IgG antibodies, which inhibit IgE-facilitated allergen presentation and CD63 expression. Therefore, surrogate markers, including serum total and allergen-specific IgE, the formation of specific IgG and IgG₄ antibodies and

Abbreviations

AU, allergen units; BAP, bronchial allergen provocation; D.f., Dermatophagoides farina; D.pter., Dermatophagoides pteronyssinus; eNO, exhaled nitric oxide; HDM, house dust mite; SIT, allergen-specific immunotherapy; SCIT, allergen-specific immunotherapy.

allergen-induced Th1, Th2 and T-regulatory cytokines (3–5) are increasingly being used to evaluate the success of SCIT. However, these markers fail to reliably define the success of clinical treatment in individual patients. As the development of immunotherapeutic preparations progresses quickly, it is necessary to demonstrate the clinically meaningful improvement produced in individual patients by each preparation (6). In a recent Cochrane review by Abramson (2), the bronchial allergen challenge (BAP) is particularly recommended to determine the success of treatment by accurately evaluating the severity of bronchial sensitivity to a certain allergen (7, 8). However, the BAP has mainly been used to evaluate adults; paediatric data are rare due to safety and feasibility concerns. In our study, we evaluated the treatment success of 1 yr of SCIT with an allergoid by comparing established tools, such as symptom and medication scores and surrogate markers (e.g. IgE and IgG₄), with the results of a specific BAP.

Methods

Subjects

Our patient registry was retrospectively screened for patients who were sensitised to HDM and who underwent a specific BAP with mite allergens between November 2008 and February 2010. Of the 107 patients with a positive BAP who were advised to initiate a SCIT with an HDM allergoid, 86 (80%) were successfully contacted, 45 (42%) of whom were willing to take part in this study and underwent a second BAP. Three participants had to be excluded (one patient began treatment only 3 months before the second BAP, one patient was treated with a SCIT preparation containing no allergoid, and one patient had severe asthma). Of the remaining 42 patients (Table 1), only 28 began SCIT. The 14 patients who chose not to begin SCIT due to various personal reasons served as the control group.

The inclusion criteria were as follows:

- ≥ 5 and < 8 yr of age

Table 1 Characteristics of the patients

	SCIT – Group n = 28 Median (range)	Control Group n = 14 Median (range)
Age (years)	9 (5–16)	8 (5–10)
Gender (M/F)	15/13	7/7
FEV ₁ (%-pred.)	98 (77–132)	94 (73–129)
eNO (ppB)	36 (3–126)	20 (11–67)
Total IgE (IU/L)	453 (237–4566)	312 (15–2068)
Spec IgE D. pter. (IU/L)	44 (9–100)	46 (4–100)
Spec IgE D. f. (IU/L)	41 (12–100)	53 (2–100)
PD ₂₀ FEV ₁ D. f. (AU)	34 (6–358)	50 (2–332)

FEV₁: forced expiratory value in one-second; eNO: exhaled nitric oxide; spec IgE D. pter.: specific IgE for Dermatophagoides pteronyssinus; spec IgE D. f.: specific IgE for Dermatophagoides farinae; PD₂₀ FEV₁ D. f.: The individual allergen dose causing a 20% decrease in FEV₁ (early asthmatic response).

- Medical history of allergic asthma grade I–II according to the GINA guidelines (8) within the last 12 months
- HDM allergy
- FEV₁ decrease of more than 20% in the BAP with HDM (PD₂₀ FEV₁ mite)
- No significantly abnormal physical examination findings
- An exacerbation-free interval of at least 1 month prior to the beginning of this study
- Receipt by all patients actively undergoing treatment of an SCIT containing an allergoid
- An SCIT duration of ≥ 6 months

The exclusion criteria were as follows:

- FEV₁ of less than 75% of the predicted value
- Inability to perform lung function exercises correctly
- serious underlying conditions

Study design

The study was a non-randomised open trial. Before initiating a SCIT against HDM, we routinely determined the participants' lung function parameters, the exhaled nitric oxide (eNO) level, the total IgE and the specific IgE mite.

All patients willing to participate in a follow-up visit were asked to perform a second BAP with the HDM allergen between July and November 2010. In addition to a re-evaluation of the above-mentioned parameters, allergen-specific IgG and IgG₄ responses to HDM were analysed. The subjects were asked to record their symptoms, as described below (questionnaire).

Human guidelines of good clinical practice and the Helsinki (1964) and Edinburgh (2000) declarations were followed when conducting the trial. The study was approved by the local ethics committee (approval number 17/10). All the participants' parents and all the patients older than 16 yr of age provided written informed consent.

Allergen-specific immunotherapy

All patients received a SCIT with a HDM allergoid preparation (Acaroid® Allergopharma, Joachim Ganzer KG, Reinbek, Germany). The SCIT was performed according to the manufacturer's guidelines.

Lung function tests

Baseline spirometry (before the inhalation of 0.9% sterile saline and before the BAP) was performed using the MasterScreen® body plethysmograph (VIASYS Healthcare GmbH, Hoechst, Germany). Before and 0–8 h after the BAP, the subjects measured their FEV₁ hourly using the SpiroPro® spirometer (VIASYS Healthcare GmbH, Hoechst, Germany).

Bronchial allergen provocation

Solutions of HDM, Dermatophagoides farinae allergen extract (Allergopharma, Reinbek, Germany) were prepared according to the instruction manual resulting in aliquots of 5000 mg/ml.

All subjects were asked to inhale 0.9% sterile saline, followed 5 min later by increasing concentrations of HDM. The solutions were delivered via a medic aid nebuliser and the aerosol provocation system (APS[®]) powered by compressed air (VIASYS Healthcare GmbH, Hoechberg, Germany). The protocol resulted in the delivery of cumulative doses of 0, 10, 30, 70, 150, 310, 630 and 1270 AU. The challenge test was terminated if either the FEV₁ decreased $\geq 20\%$ from baseline (EAR) or a cumulative dose of 1270 mg was achieved. Clinical monitoring accompanied each provocation for at least 8 h, after which time the subjects were permitted to leave the hospital, remaining in touch with a physician involved in the study via telephone. To ensure safety, all patients received a peak flow meter to take peak expiratory flow measurements at home and were instructed to use 2 puffs of salbutamol (0.1 mg each) and seek medical advice in the case of a PEF loss of more than 20% of pre-test values, chest tightness, wheezing, coughing or other asthma-like symptoms. This procedure was utilised in earlier studies (7, 9), and was found to be extremely safe, avoiding the risk of overdose and enabling the detection of potential side effects as early as possible. The individual allergen dose causing a 20% decrease in FEV₁ in the EAR (PD₂₀ FEV₁ mite) was determined by the logarithmic interpolation of the measurements before and after the $\geq 20\%$ FEV₁ loss (7).

After each BAP, the patients received 0.2 mg of salbutamol until a FEV₁ of at least 80% of baseline was achieved.

Measurement of exhaled nitric oxide (eNO)

The exhaled NO measurements were performed using the NIOX[®] (Aerocrine, Solna, Sweden) that measures the NO in exhaled air according to ATS guidelines (10).

Blood sampling and laboratory allergy

The blood samples were taken after the first and second BAPs. The total IgE, specific IgE and specific IgG responses to HDM were analysed using a two-sided chemiluminescent assay (Immulate 2000, Siemens). The specific IgG₄ level resulting from HDM exposure was determined using ELISA (Diagnostic Systems & Technologies GmbH, Schwerin, Germany). The analysis was performed according to the manufacturer's instructions.

Blocking antibodies

The blocking antibodies were analysed according to their capacity to inhibit the CD63 expression of blood basophils after mite extract stimulation. The inhibitory properties of the serum antibodies were measured using the CAST stimulation system (Buehlmann Laboratories, Allschwil/Switzerland) in combination with HLA-DR/CD123/CD63 flow cytometry (Becton Dickinson, San Jose, CA, USA).

The leucocytes of a voluntary donor with a mite allergy (RAST 6) were isolated by dextran sedimentation from EDTA blood, and 1×10^5 cells/well were transferred to a 96-well plate. Each patient's serum was simultaneously incubated with

mite extract (Allergopharma, Joachim Ganzer KG, Reinbek, Germany) for 1 h at room temperature. This mixture of the patient's serum and mite extract was then used for cellular stimulation for 40 min. Ionomycin and anti-IgE-receptor antibody solutions were used as positive controls, and the patient's serum without mite extract was used as a negative control. The samples were analysed using a FACSArray Bioanalyzer (Becton Dickinson, San Jose, CA, USA), as described elsewhere(5).

Questionnaire

Patients were asked to rate any perceived improvement of asthma symptoms during treatment. Using a modified ISAAC questionnaire, the patients were further interviewed about their clinical complaints, nasal symptoms and medications during the 12 months prior to the second BAP. If the patients were unable to complete the questionnaire themselves (usually less than 10 yr of age), their parents were asked to complete it. The clinical and nasal symptoms were summed up using a clinical score with a range of 0–40. To obtain the medication score, the patients and controls were asked to record any specific medications they had taken during the previous 12 months. The reported use of each of five groups of anti-allergic/asthmatic medications (oral antihistamines, eye drops, nasal sprays, short-acting β_2 -agonists and inhaled steroids) was multiplied by 0 (no medication used), 1 (1–2 times), 2 (3–4 times), 3 (5–8 times), 4 (9–12 times), 5 (13–16 times) or 6 (16 or more times), respectively. Therefore, the calculated score for each patient ranged from 0 points to a maximum of 30 points.

End-points and statistical analysis

The primary end-point was the improvement of the PD₂₀FEV₁-mite. A probability (p) value of less than 0.05 was used to indicate statistical significance. Data from a previous study demonstrated that the early asthmatic response (EAR) has a standard deviation of approximately 11% (root mean square error from ANOVA with baseline correction). With $n = 14$ evaluable subjects in each treatment group, each of the two *t*-tests described below has an approximately 90% power of detecting a 14% difference in the EAR between the active treatment and placebo.

Secondary end-points were changes in the quality-of-life and use of allergen-specific medication. Data were statistically analysed and graphed with GraphPad Prism 5.01 (GraphPad Software Inc., La Jolla, CA, USA). The maximum percentage decrease in the FEV₁ after each BAP, the area under the curve (AUC) and the eNO after single-step challenges are expressed as the medians with their ranges (min-max). For the comparison of the before/after values, a paired *t*-test (Wilcoxon matched *t*-test) was used. Group comparisons were performed by ANOVA (Kruskal–Wallis test). A four-field table test (Fisher's exact test) was used in the analysis of the patients' current medications and clinical complaints. As results did not yield a Gaussian distribution, they are presented as median values with their ranges (min-max). The logarithmic graphs are presented using the log₁₀ scale.

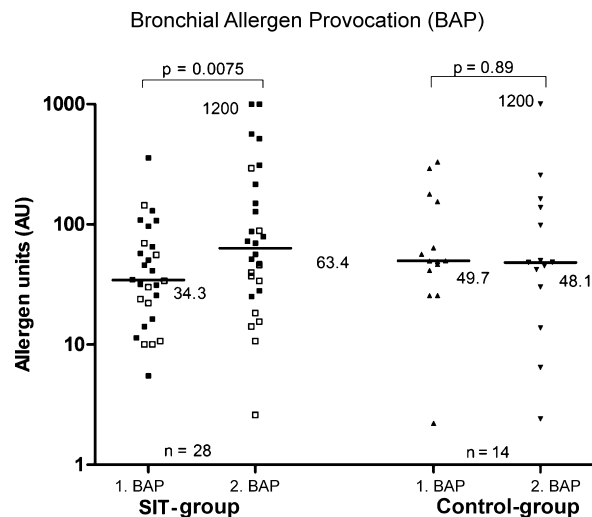


Figure 1 Results of the bronchial allergen provocation (BAP) before SCIT (1. BAP), and after 1 yr of SCIT (2. BAP) of the treatment group ($n = 28$) compared to the untreated control group ($n = 14$). Open squares define Non-Responder while closed squares define Responder. The values and lines represent the median.

Results

Bronchial allergen provocation

The overall specific bronchial reactivity of the treatment group improved significantly. The $PD_{20}FEV_1$ mite increased from a median of 34.3 AU (range 5.5–357.9) before treatment to a median of 63.3 AU (range 2.6–1200; $p = 0.0075$) after 1 yr of SCIT, and no change was detected in the control group median, which was 49.7 AU (range 2.22–331.7) before treatment and 48.1 AU (range 2.4–1200; $p = 0.89$) after treatment (Fig. 1).

While 17 of 28 of the treated patients (60.7%) improved significantly, 11 (39.3%) did not improve after one of treatment.

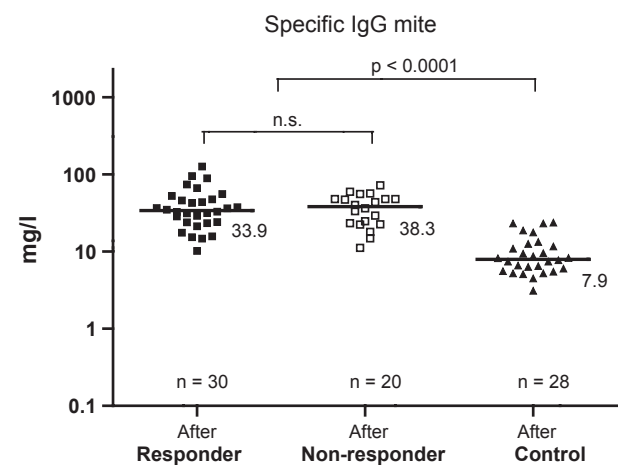


Figure 3 House dust mite (HDM) specific IgG after 1 yr of SCIT in the responder-, non-responder- and control-group respectively. The given values and lines represent the median.

Total and specific IgE levels in response to HDM

After 1 yr of treatment, the total IgE levels remained unchanged. In the SCIT group, the median total IgE level was 435 KU/l (range 78–4566) before and 410 KU/L (range 85–2260) after SCIT. In the control group, the median total IgE was 312 KU/l (range 15–2068) before and 466 KU/l (range 16–752; n.s.) after SCIT. The specific IgE decreased significantly after SCIT compared with the control group. However, neither the total nor the specific IgE (Fig. 2) was able to discriminate between responders and non-responders in the SCIT group.

HDM specific IgG and IgG₄ levels

IgG and IgG₄ were only analysed after 1 yr of treatment. Both levels were significantly higher in the treatment group (Fig. 3 and Fig. 4). After SCIT, the median IgG was 35.7 mg/l (range 10–127) compared with median of 7.9 mg/l (range 3.1–23.7;

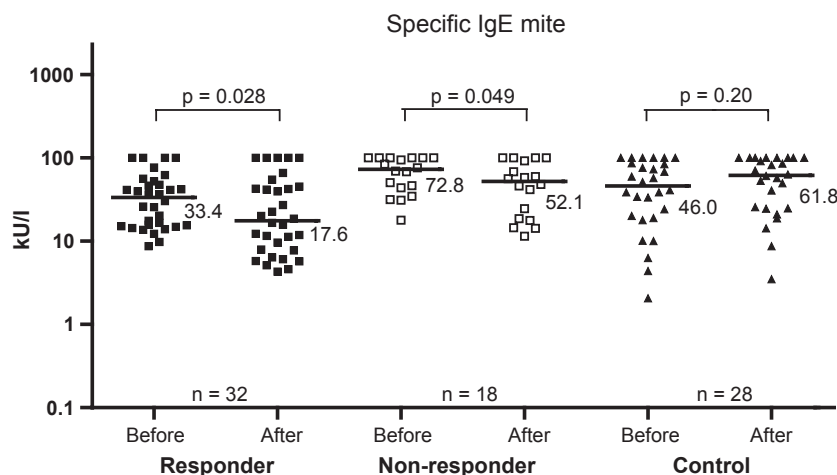


Figure 2 House dust mite (HDM) specific IgE before and after 1 yr of SCIT in the responder-, non-responder- and control-group respectively. The given values and lines represent the median.

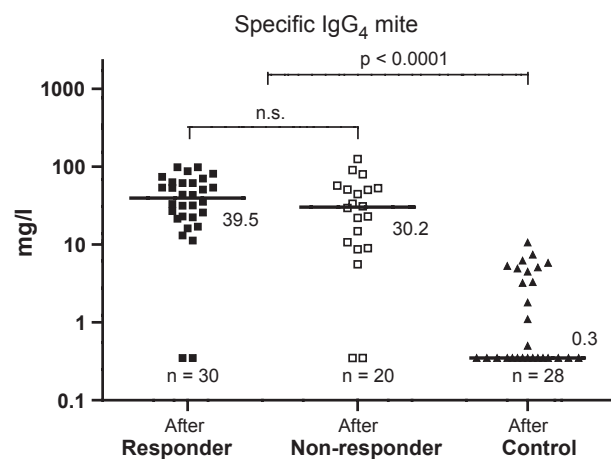


Figure 4 House dust mite (HDM) specific IgG₄ after 1 yr of SCIT in the responder-, non-responder- and control-group respectively. The given values and lines represent the median.

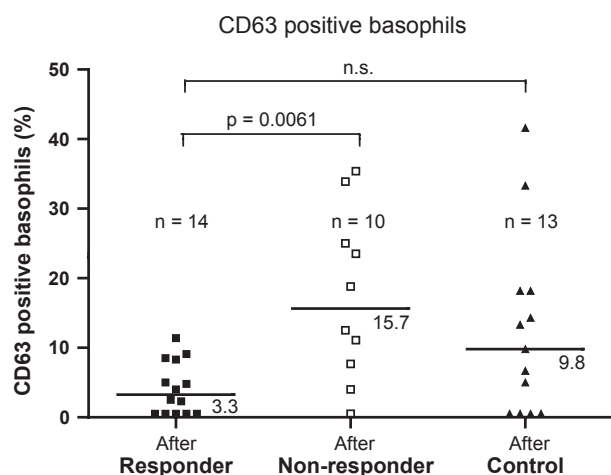


Figure 5 CD63 positive cells (in%-positive of all blood basophils) after 1 yr of SCIT in the responder-, non-responder- and control-group respectively. The given values and lines represent the median.

$p < 0.001$) and the median IgG₄ was 33.3 mg/l (range 0.35–126) compared with a median of 0.35 mg/l (range 0.35–10.6; $p < 0.0001$).

Neither specific IgG nor specific IgG₄ was able to discriminate between responder and non-responder states (median 0.35 mg/l, range 0.35–10.6 mg/l).

(median 0.35 mg/l, range 0.35–10.6).

Analysis of blocking antibodies analysed via CD63 staining

The blocking capability of the specific IgG and IgG₄ antibodies was analysed according to the changes in the CD63 expression of blood basophils after mite extract stimulation (Fig. 5). The responders exhibited a significant decrease in CD63 expression with a median of 3.25% CD63-positive cells

(range 0–11.4) compared with the median of 15.65% in the non-responder group (range 0–35.4) and a trend towards a lower level than the median of 9.8% in the control group (range 0–41). This result indicates that the activation of the basophils was decreased by the blocking antibodies in the serum of the responders.

Results of the questionnaire

Subjective (Patient-Reported) Outcome: The patients and their parents were asked whether they had observed a response to the SCIT. A total of 10 of the 17 patients in the responder group (70.5%) and 8 of the 11 patients (73%) in the non-responder group reported a meaningful clinical improvement in their symptoms. In the untreated control group, only 2 of the 14 patients (14.3%) described a mild improvement in their symptoms.

Furthermore, a modified ISAAC (9) questionnaire was used to document the patients' nasal and asthma symptoms and the use of allergy-directed therapeutics during the first year of therapy and before the second BAP. In contrast to the subjective ratings provided by the participants, the reported outcomes revealed significant differences between the responder and non-responder groups. The results were summarised as clinical and medication scores and are presented in Table 2.

Discussion

This study is the first to compare the established markers of treatment success such as serum markers and clinical scores of patients receiving SCIT with the results of a standardised BAP in children.

In our study, 60% of the patients treated with a HDM allergoid exhibited a significant improvement after only 1 yr of treatment. Comparable with the results of Blumberga et al. (11) in adults, our patients exhibited a 1.8-fold increase in bronchial tolerance compared with the untreated controls. To date, most of the paediatric SCIT studies have been conducted

Table 2 Subjective complaints and asthma- and medication scores

	SCIT – Group Responder n = 17	SCIT – Group Non-Responder n = 11	Control – Group n = 14
Patient-reported improvement	12 (70%)	8 (73%)	2 (14%)
Clinical score (maximum 16)	3 (1–12)	6 (0–9)	5 (2–14)
Medication score (maximum 30)	3 (0–21)	10 (2–16)	9 (0–21)
$p < 0.05$			

Patient-reported improvement: Number of parents answering "YES" to the question, if they felt an improvement of clinical symptoms after 1 yr; Clinical score: Frequencies of clinical complaints like asthma and nasal symptoms; Medication score: The use of each of the five groups of anti-allergic/-asthmatic medications multiplied with the frequency medications were used in the last year.

with unmodified allergen extracts, for example, Maestrelle et al. (12, 13). Recently, allergoids have been used more frequently due to the higher dose and longer dosing intervals of up to 8 weeks (14). The present study was one of the first to examine the immunological and clinical changes in children resulting from an SCIT containing an HDM allergoid. We were able to demonstrate a consistent immunomodulatory effect (a decrease in specific IgE and an increase in specific IgG/IgG₄) and a significant improvement in allergen-specific BHR in children with HDM asthma after 1 yr of treatment. However, SCIT did not work equally well in all our patients. With the use of BAP, we were able to identify non-responders ($n = 11$, 39.3% vs. responders $n = 17$, 60.7%) who exhibited no improvement.

In contrast to the serological markers and subjective ratings, only BAP was able to objectively discriminate between successful and non-successful SCIT therapy. Independently, the patients who received SCIT (responders and non-responders) described an improvement in their symptoms, while the control group exhibited no subjective improvement. Twelve of the 17 responders (70.5%) and 8 of the 11 non-responders (73%) described a good therapeutic effect. Only 2 of the 14 (14.3%) control group patients described a slight improvement. A total of 70% of the patients in the treatment group expressed feeling better; therefore, from the patients' perspective, it appears that the intervention worked. Although the subjects in the SCIT group subjectively described relief from their symptoms and exhibited an increase in allergen-specific IgG and IgG₄ levels, only the responder group exhibited significantly lower medication and asthma scores compared with the non-responder and control groups (Table 2). Thus, the subjective experiences of the patients in the non-responder group may potentially have been influenced by the placebo effect. Although the subjective perception was positive, the treatment success must be defined objectively because SCIT is an invasive and potentially harmful therapy.

The total IgE, specific IgE, IgG and IgG₄ measurements did not differ between the responder and non-responder groups and thus cannot be used to predict therapeutic success. After 1 yr of SCIT, 41% of the responders and 82% of the non-responders required medication. Therefore, the reduction in medication use was greater than was expected based on other trials (15, 16).

In principle, SCIT can be described as an immunisation procedure, and the prevailing immunological consensus is that T-regulatory (Treg) and blocking antibodies are induced during the treatment {Rosewich 2010 #500(17, 18). Non-responder states have been observed after immunisation for many years. For example, it is well known that 5–10% of individuals are non-responders to the hepatitis B vaccine, even after repeated immunisation.(19). The risk factors for non-responder status include an age of >40 years, smoking, vitamin D deficiency and genetic factors (20, 21). At least some of these risk factors, such as vitamin D deficiency, may be important determinants of whether successful immunotherapy can be achieved (22). Although they are clinically well known, non-responder states in individual patients after SCIT have not yet been well defined and characterised. BAP provides a reliable tool with which to objectively define responder and non-responder states. Therefore, BAP is an important tool for the development of new preparations in dose–response studies and may be the first step towards a new era of personalised medicine using SCIT tailored to individual patients' responses. In addition, we recently showed that BAP with the APS was a safe and reliable procedure in 350 children aged 5–18 yr. Although the majority of patients had an LAR, none of the patients exhibited severe asthmatic symptoms (23).

This study has several limitations. First, it was not a placebo-controlled study. In addition, it remains unclear whether our findings would extend to the second and third years of treatment. Interestingly, Blumberg et al. recently demonstrated that allergen-specific BHR did not improve further in the second and third years and may indicate a non-responder state, even after the end of treatment (10). Nevertheless, it is important that the findings of our study be replicated to assess their reliability and robustness.

Conclusion

Bronchial allergen provocation (BAP) is a useful tool with which to define asthmatic patient responders and non-responders to SCIT. It is the only test available to objectively define responders, and in contrast to the subjective rating, it is not influenced by the placebo effect. We were able to demonstrate significant therapeutic success in 60.7% of the patients who received an HDM allergoid preparation after only 1 yr of treatment.

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