

Efficacy and safety of birch pollen immunotherapy for local allergic rhinitis

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ABSTRACT

Background: Local allergic rhinitis (LAR) is a relatively new disease.

Objective: To ascertain the effects of allergen-specific immunotherapy in LAR.

Methods: A randomized, double-blind, placebo-controlled trial of birch subcutaneous allergen immunotherapy (AIT) for LAR was performed in 28 patients. The therapy was performed for 24 months in 15 patients with AIT and 13 patients given placebo. The primary end point was decrease in symptom medication score (SMS). In addition, we monitored serum-specific immunoglobulin E (IgE), serum-specific immunoglobulin G4, nasal-specific IgE to Bet v 1, and safety and quality-of-life parameters.

Results: After 24 months of treatment, there was a significant decrease in the median area under the curve for SMS of the active group vs the placebo group: 2.14 (range, 1.22–4.51) vs 6.21 (range, 5.12–7.89), at the $P < .05$ level. During AIT, the active group showed a significant decrease in SMS of up to 65% vs baseline. A significant increase in immunoglobulin G4 and decrease in nasal-specific IgE were observed in the active group during AIT compared with the placebo group. AIT was well-tolerated and without systemic reactions.

Conclusion: This study demonstrates that AIT for birch pollen in patients with LAR was clinically effective and exhibited good tolerance.

Trial Registration: [ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT03157505.

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Introduction

Local allergic rhinitis (LAR) is an underdiagnosed and under-treated disease characterized by the local production of immunoglobulin E (IgE) during natural exposure to aeroallergens. Patients with LAR have negative skin prick and serum-specific IgE test results but positive nasal provocation test (NPT) results for aeroallergens.^{1,2} More than 50% of patients with chronic nonallergic rhinitis may have problems due to the lack of an LAR diagnosis,^{2,3} because a misdiagnosis can lead to treatment inefficiency and errors.

In addition to the local IgE-mediated reaction, allergen immunotherapy (AIT) is a potential treatment method for these patients. However, sufficient data are only available to show that AIT is effective in allergic rhinoconjunctivitis and asthma in response to pollens, house dust mites, and some animals.^{4,5}

The aim of our study was to assess the safety and efficacy of AIT for birch pollen allergens in patients with LAR and a confirmed birch pollen allergy.

Methods

This study was a double-blind, placebo-controlled, randomized trial conducted at a single center. All patients participating signed an informed consent form. The study was approved by the local ethics committees of the Medical University of Silesia (34211/2014).

Patients

First, we screened 78 patients (43 women and 35 men; age range, 18–76 years) from a group of approximately 1,560 subjects with suspicion of inhalant allergies and suspicion of LAR. The number of screened participants received was based on a power calculation that took into account the expected effect size. The following formula was used to compare the 2 proportions: $N = 16p(1 - p) / (p_0 - p_1)^2$ and $p = (p_0 + p_1) / 2$, for $p = 0$ and $p_1 = 0.1$. All patients were recruited from January 2014 to February 2014 at one allergy outpatient center in southern Poland. Next, the screened patients were checked based on the following criteria:

1. Well-documented symptoms of rhinitis during birch pollen season.
2. A positive NPT to birch.
3. Negative skin prick test results for inhalant allergens, including *Dermatophagoides pteronyssinus*, *D farinae*, grass pollen, birch, hazel, alder, *Alternaria*, and cats.

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Table 1
Summary of Patients' Characteristics Before Randomization

	Active	Placebo	P value
Randomized patients (n)	16	13	.68
Age (y)	22.7 ± 3.1	24.9 ± 4.2	.87
Female (%)	8 (50)	6 (46)	.83
Duration of rhinitis (y)	5.3 ± 2.7	6.1 ± 3.9	.63
Mean weekly symptoms score in basement during birch pollen season	4.64 ± 1.54	4.32 ± 1.95	.49
Total serum IgE	62.81 ± 22.17	56.19 ± 17.65	.33
Specific nasal serum IgE to birch pollen in nasal lavage (kU/L) after NPT	1.89 ± 0.39	1.62 ± 0.84	.78
Mean symptom score after NPT to birch pollen	5.54 ± 2.11	5.38 ± 2.44	.72
Mean nasal flow decrease after NPT to birch pollen (%)	79.2	82.5	.33

Abbreviations: IgE, immunoglobulin E; NPT, nasal provocation test.

- Negative serum total and allergen-specific IgE results against the aforementioned allergens.
- Lack of diagnoses of bronchial asthma, nonallergic rhinitis (especially senile or vasomotor rhinitis), and severe, nonstable diseases.

Twenty-nine patients who fulfilled all criteria, ranging from 21 to 68 years of age, were included in the study. The subjects had moderate or severe intermittent allergic rhinitis during birch pollen

season (March–May) in Poland and fulfilled the Allergic Rhinitis and its Impact on Asthma (ARIA) criteria.⁶ Randomization Procedure and Blinding.

After the expected dropout period, 29 participants were randomized to 2 treatment groups at a 1:1 ratio. The randomization procedure with random selection relied on the use of computer-generated numbers via a flip-coin generator (Excel, version 14.2.0, 2011, Microsoft Corp, Redmond, WA). The patients were allocated to 2 groups: an AIT group for the administration of perennial AIT with Purethal Birch (HAL Allergy BV, Leiden, The Netherlands) and a placebo group for administration of subcutaneous placebo injections for 24 months.

Finally, 15 subjects in the AIT group and 13 subjects in the placebo group completed the 24-month observation period. The groups had comparable characteristics at baseline (Table 1). A diagram of the enrollment protocol is presented in Figure 1.

For study-blinding purposes, all patients received the same volume and same number of injections. The investigator, subjects, and personnel remained study-blind throughout the investigation until the database was locked.

The placebo was a sterile aluminum hydroxide suspension packed in bottle similar to that of the active drug and packed in the same type of unidentified white boxes with only the identification number of the patient and key number of the drug. All key codes to identify the active drug or placebo were locked by an independent coordinator who did not participate in the study until the study finished.

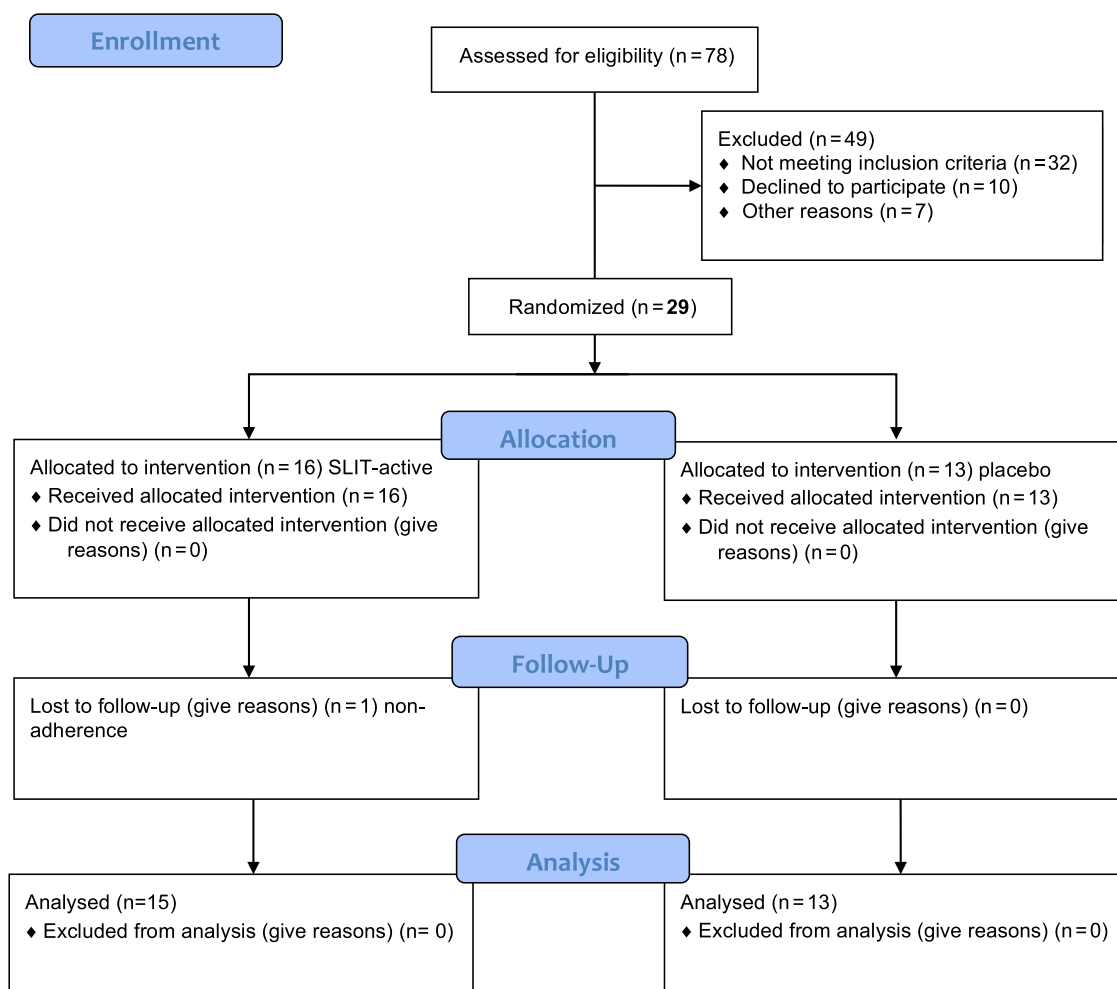


Figure 1. Number of participants assessed for eligibility who completed the study. SLIT, sublingual immunotherapy.

All subjects were required to abstain from antiallergy drugs and glucocorticoid nasal drops for at least 6 weeks before beginning the study.

Diagnostic Procedures

A careful examination of the eyes, ears, nose, and throat was performed for all patients. The severity of local seasonal allergic rhinitis was assessed using the ARIA guidelines.⁶ Patients with other nasal problems, such as chronic nasal obstruction, reduced olfaction, bacterial colonization confirmed by cultural examination, and chronic sinusitis, who were diagnosed based on computed tomography scan and nasal endoscopy, were excluded. Subjects with other chronic or acute clinical disorders or with a history of respiratory tract infection within 4 weeks of study initiation were also excluded.

Skin Prick Tests

The skin prick test (SPT) was performed using inhalant allergens (HAL Allergy BV) from a panel of *D pteronyssinus*, *D farinae*, 5 mixed grasses (*Phleum pratense*, *Dactylis glomerata*, *Anthoxanthum odoratum*, *Lolium perenne*, and *Poa pratensis*), birch, hazel, alder, mugwort, *Alternaria*, *Cladosporium*, and dog and cat allergens. Positive (10 mg/mL histamine) and negative (saline) controls were also included. A positive result was defined as a minimum wheal diameter of 3 mm greater than the negative control.⁷ Patients with positive tests for any allergens were excluded from subsequent analyses. The SPTs were performed during patients' initial qualification and at end of the study.

Serum-Specific IgE

The allergen-specific IgE (sIgE) reactivity to the aforementioned aeroallergens was determined using the ImmunoCAP 100 test (Thermo Fisher Scientific, Uppsala, Sweden). The results were considered positive when the sIgE concentration was >0.35 IU/mL (according to the manufacturer's instructions). Patients with a positive allergen test were excluded from the study. Measurement was performed at both the start and end of the study.

Nasal-Specific IgE Bet v 1 Detection

The nsIgE levels to Bet v 1 were examined as described for serum sIgE using an immunoassay (ImmunoCAP 100). These measurements were performed at baseline and at 30 minutes, 1 hour, and 6 hours after the NPT. In addition, nsIg Bet v 1 was measured during the birch pollen season before the start of and during the 2 years of immunotherapy and after 6, 12, 18, and 24 months of AIT.

A bilateral nasal lavage was performed according to the method described by Nacleiro et al.⁸ First, 6 mL of room temperature physiologic saline was added into each nostril dropwise. The mucus and saline sample was expelled after 10 seconds and stored on ice. The fluid was centrifuged at 4°C for 15 minutes at 1,500g, and the solid phase was separated. The samples were stored at –80°C before analysis. For analysis, 25-μL samples were incubated for 90 minutes at room temperature. Then, a biotin-labeled goat anti-human IgE antibody (1:1,000 dilution; Vector Laboratories, Burlingame, California) was added and incubated to detect specific IgE antibodies. After washing, the plates were incubated with streptavidin-peroxidase (1:1,000 dilution) and anti-goat alkaline phosphatase (1:10,000 dilution) for 1 hour at room temperature. After washing, O-phenylenediamine was added as a substrate solute, and the optical densities were measured using an enzyme-linked immunoassay reader (Metertech S-960, Metertech, Inc, Taipei, Taiwan). In addition, 3 mL of normal saline was added to each sample, and the concentrations of local antibodies were recalculated using the total

protein concentration for each measurement to correct for the dilution effect of the samples (Bradford protein analysis). All values were expressed as a calculated protein ratio.

Serum IgE and IgG4 to Bet v 1

Additional monitoring of sIgE and immunoglobulin G4 (IgG4) to Bet v 1 was performed during the NPT (30 minutes later) and during the birch pollen season before the AIT and at the start and after 6, 12, 18, and 24 months of treatment, during the birch pollen seasons in 2015 and 2106. The same method described in the assessment of serum sIgE was used.

The concentration of IgG4 against Bet v 1 was determined in the blood serum by enzyme-linked immunoassay according to the method described by Lai et al using the ImmunoCAP 100 test at the same timepoints used to measure IgE.⁹

Nasal Provocation Test

Nasal patency was assessed by acoustic rhinomanometry using a rhinometer (Model SRE 2000, Rhinometrics, Lynge, Denmark) and was performed according to the guidelines of the Standardisation Committee on Acoustic Rhinometry.¹⁰

The NPTs were performed from January to February 2014, due to the lack of concentration of birch pollen allergens during this time period in Poland. First, using a metered pump spray, the patients were intranasally challenged with saline to exclude nasal hyper-reactivity. If the NPT was negative, it was performed again 2 weeks later with extracts of the previously discussed aeroallergens (the concentration of each allergen was 5,000 standardized biological unit [SBE]/mL; Allergopharma, Reinbek, Germany). One hundred microliters of solution was applied to each nostril. The total volume of both nasal cavities was determined using acoustic rhinometry from 2 to 6 cm, and the results were compared with the baseline test. The immediate reaction was analyzed at 15 minutes, 1 hour, and 6 hours. Each subject was subjected to an NPT with saline first (to exclude nonspecific hyperreactivity).

Nasal symptoms (nasal obstruction, rhinorrhea, itching, and sneezing) were monitored using the 100-mm horizontal visual analog scale (VAS). Symptom severity was classified as severe (71–100), moderate (31–70), or mild (0–30). The response to an allergen was evaluated at baseline, immediately after allergen challenge, 15 minutes after saline application, and 15 minutes after aeroallergen application. The response was determined using a VAS and the total volume of the nasal cavity from 2 to 6 cm. An increase of $\geq 30\%$ in total VAS score and a $\geq 30\%$ decrease in 2- to 6-cm volume were considered to be positive responses.¹¹ As previously described, both nostrils underwent lavage.

Treatments

The patients were randomly selected to receive Purethol Birch 20,000 allergy unit milliequivalent (AUM)/mL (*Betula verrucosa*; HAL Allergy BV) or placebo (Fig 1). The recruitment period was limited to 2 months (January–February). The Purethol Birch was administered as a perennial therapy using a regimen of 1 dose of 0.1 mL, 2 doses of 0.2 mL, and 3 doses of 0.4 mL every week, followed by 0.5 mL every 4 weeks for 24 months (2015–2016).

One milliliter of Purethol contains 500 μg of modified birch pollen allergens. The Bet v 1 content was 52 μg. The other ingredients for injection were aluminum hydroxide (1.1 μg), sodium chloride (9.0 μg), phenol (5.0 μg), and water (1.0 mL).

Using this schedule, the average cumulative dose was approximately 650 μg of Bet v 1 administered to each patient undergoing active treatment for all 24 months of the study.

Efficacy Assessment

The primary outcome measurement was the area under the curve (AUC) for the combined symptom and medication scores (SMSs) over the birch pollen season after 2 years of AIT or placebo treatment (in 2016) compared with baseline before treatment (in 2014). The birch pollen season was defined as the period from March 20 to May 15 when birch spores are detected in the air in Poland. The patients recorded their symptom severities in a daily diary during the grass pollen season by scoring the following areas: nasal itching; sneezing; running; and blockage. Ocular itching was recorded on a separate VAS with a continuous scale from 0 cm (no symptoms) to 10 cm (very severe symptoms). Rescue medication was provided, and its use was recorded based on the following scoring: 1 point per spray for azelastine nasal spray, eye drops (levocabastine) or per 5-mg levocetirizine tablet; 2 points per puff per nostril for mometasone furoate nasal spray; and 3 points per 10-mg prednisolone tablet.¹¹ This combined symptom-medication score was calculated as the sum of the symptom score and medication score and was monitored daily with the use of the diary. The SMS was derived by adding the VAS for each of these 5 symptoms.

In addition, the symptom and medication scores were presented as the average adjusted symptom score (AAdSS). The AAdSS has been accepted for use as a primary end point in rhinoconjunctivitis allergen immunotherapy trials.¹² This score includes the nasal and ocular total symptom scores associated with birch pollen allergy as the rhinoconjunctivitis total symptom scores (RTSSs), which can be adjusted for the use of symptomatic treatment.¹²

The secondary outcome measures included: quality of life; reduction of the symptom score; and safety assessment and monitoring of allergen-specific IgE and IgG4 against Bet v 1 in the serum and specific IgE against Bet v 1 in the nasal lavage during the baseline birch pollen season (2014 to before AIT), at the start of AIT, after 6, 12, 18, and 24 months of therapy, and during the birch pollen season in the first and second years of AIT (2015 and 2016).

The local reactions were assessed 30 minutes after injection and were measured in centimeters. The systemic reactions were graded according to the criteria of the European Academy of Allergy and Clinical Immunology.¹³

Quality of Life

The quality of life of patients was evaluated using the Rhinoconjunctivitis Quality of Life Questionnaire score for adults who completed questionnaires for every birch pollen season during the study period.¹⁴

Pollen Counts

The local grass pollen counts from March to May were determined using a volumetric pollen trap (Burkard, Scientific, Ltd, Uxbridge, United Kingdom). The data are presented in Figure 2 for 2014, 2015, and 2016.

The statistical analysis was performed using Statistica, version 8.12 (SoftPol, Cracow, Poland). Nonparametric tests were used because the data were not normally distributed. The Wilcoxon test was used to analyze differences between groups. The χ^2 test was used to analyze differences between NPT flow and rate of adverse events in each study group. Analysis of variance testing was used to assess SMS data. The percentage reduction was calculated as the difference in AUC. Differences were considered significant at $P < .05$.

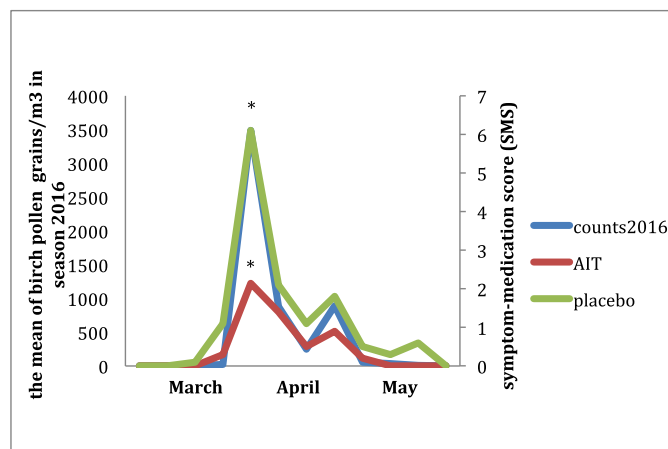


Figure 2. The birch spore count and the weekly average of the symptom and medication scores (SMSs) recorded by patients in the active and placebo groups during pollen season in southern Poland in 2016 (after 2 years of allergen immunotherapy [AIT]). * $P < .05$.

Results

Primary End Points

After 24 months of treatment, there was a significant decrease in median AUC for SMSs in the active group vs the placebo group: 2.14 (range, 1.22–4.51) vs 6.21 (range, 5.12–7.89), at $P < .05$. In the active group, the SMS score was reduced by 66%, the symptom score was reduced by 62%, and the medication score was reduced by 75% after 2 years of AIT.

Detailed data for SMSs, symptom scores, and medication scores before AIT and after 2 years of treatment are presented in Table 2.

After 24 months of AIT for birch allergy, a significant clinical effect was observed based on the AAdSS compared with baseline and compared with the placebo group (Fig 3).

Secondary End Points

Quality of life

The Rhinoconjunctivitis Quality of Life Questionnaire results showed a mean baseline level of 1.63 (95% confidence interval [CI], 1.19–1.95) in the randomized patients. The mean final result was decreased (1.23; 95% CI, 1.13–1.50) in the active group ($P < .05$), but it was not significantly decreased (1.59; 95% CI, 1.24–1.89) in the placebo group after 2 years of AIT ($P > .05$).

Allergen-specific IgE and IgG4 measurements

The serum allergen-specific IgE against Bet v 1 appeared in the active group between 4 and 6 months after the initiation of AIT (maximum mean level: 16.8 ± 8.14 IU/mL), decreased during the

Table 2

Combined SMS, Symptom Score, and Medication Score Comparisons Between Active and Placebo Group Patients at Baseline and After AIT

Type of scoring/year	Active (n = 15)	Placebo (n = 13)	P value
2014 baseline			
SMS	6.21 (4.11–9.01)	6.81 (5.72–10.01)	.121
Symptom score	5.16 (3.64–8.12)	6.11 (5.29–8.14)	.115
Medication score	4.03 (2.94–6.03)	4.10 (2.92–5.53)	.230
2016 until end of AIT			
SMS	2.14 (1.22–4.51)	6.21 (5.12–7.89)	.001
Total symptom score	1.98 (0.71–3.19)	6.43 (4.19–8.21)	.001
Medication score	4.10 (0.27–2.01)	3.84 (2.55–4.19)	.004

Abbreviations: AIT, allergen immunotherapy; SMS, symptom and medication score.

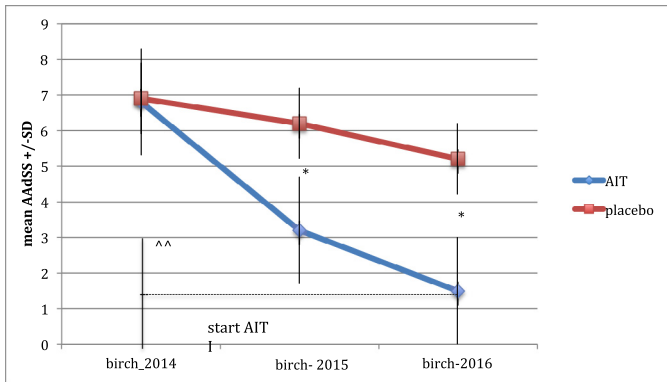


Figure 3. Clinical efficacy of allergen immunotherapy (AIT) after 2 years of AIT compared with placebo. AAdSS, average adjusted symptom score; birch_2014, birch pollen season of 2014 (baseline); birch_2015, birch pollen season of 2015 (first year of AIT); birch_2016, birch pollen season of 2016 (second year of AIT), * $P < .05$.

next 6 months, and was undetectable during the remainder of the treatment period (Fig 4A). In the placebo group, serum-specific IgE remained undetectable throughout the study. Serum IgG4 to Bet v 1 increased gradually to 0.89 ± 0.029 mgA/mL after 6 months of AIT in the active group (Fig 4B), and it was significantly higher compared with the placebo group (0.09 ± 0.011 mgA/mL).

The mean nasal allergen-specific IgE Bet v 1 level was 0.019 ± 0.006 as a protein ratio (in units per microgram) in the baseline birch pollen season and decreased to 0.011 ± 0.004 U/ μ g in the second birch pollen season during AIT in the active group. It was

significantly lower than the concentration in the placebo group (0.023 ± 0.007 U/ μ g) in the same second birch pollen season during therapy ($P < .05$). This trend is shown in Figure 4C.

Safety

During the study, no systemic anaphylactic reactions occurred in either group during AIT. There were comparable numbers of local adverse reactions between the active and placebo groups. In the active group, erythema or wheals <5 cm were observed after 19 of the 451 Purethal injections (0.042 per injection), and wheals >5 cm were observed after 10 of all injections (0.025 per injection). In the placebo group, the lower numbers of local reactions were noted as follows: erythema or wheals <5 cm after 15 of the 394 placebo injections (0.038 per injection), and wheals >5 cm after 8 of all injections (0.021 per injection).

One episode of headache 45 minutes after the injection was observed in the active group. In the placebo group, there was 1 episode of weakness 25 minutes after injection.

Discussion

Attempts to use AIT to treat LAR are not widespread. Our investigation is the first double-blind, placebo-controlled study in patients with LAR and allergy to birch pollen. Earlier work has shown that AIT is safe and successful for treatment of LARs and allergies to house dust mites and grass pollen, although the results are from single reports.^{4,5} The AIT conducted in patients with similar LARs but an allergy to birch pollen also gave encouraging results. The primary end point, a significant decrease in the SMS and AAdSS, was obtained in the first year of AIT. This finding provides evidence according to the objective criteria that AIT is effective for

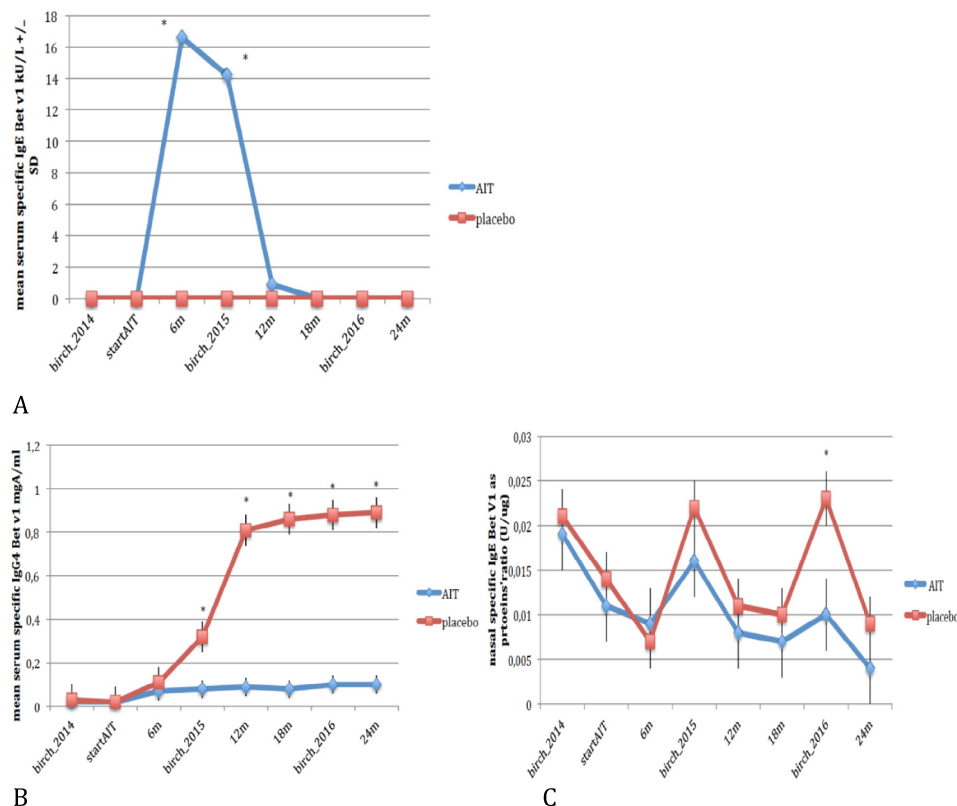


Figure 4. (A) Serum concentrations of allergen-specific immunoglobulin E (IgE) to Bet v 1. (B) Serum concentrations of allergen-specific IgG4 to Bet v 1 during the study period: 2014 birch pollen season (baseline); 2015 season (first year of allergen immunotherapy [AIT]); 2016 season (second year of AIT). (C) Nasal concentration comparison of the IgE to Bet v 1 ratio during the study period. * $P < .05$.

treatment of LARs.¹⁵ Despite the negative skin prick and serum IgE tests in patients with LARs, the presence of local IgE-mediated specific reactions to birch pollen is subject to AIT. Our study has confirmed that the short-term clinical effects after AIT in LAR patients are similar to those described in intermittent allergic rhinitis patients.^{16,17}

We also confirmed a lack of systemic adverse reactions along with only a small number of local reactions after therapy, as seen in other studies.^{4,5} Interestingly, the immunologic parameters were changed in the patients with typical seasonal allergic reactions who received AIT.^{18,19} During the first 6 months of AIT, the IgG4 to Bet v 1 ratio was detectable, and this concentration increased to a constant level after approximately 12 months of AIT. This is comparable to findings reported for AIT in allergic rhinitis.^{18,19} The serum IgE to Bet v 1 ratio was elevated after 6 months of AIT and decreased to an undetectable level in the active group. We found that serum IgE returned to pre-AIT levels in all patients in the active group, indicating that local immunologic status of the allergic disease was retained. We did not observe any transformation from LAR to typical seasonal allergic rhinitis in patients during and after AIT, but this scenario is possible in the future. Similar trends to those observed after AIT for seasonal allergic rhinitis were observed for IgG4.

The trends observed for nasal IgE to Bet v1 ratio are of interest. During the AIT, we observed a gradual decrease in nIgE Bet v 1n concentration in the next birch pollen season. This decrease was not observed in the placebo group. This result provides evidence of an inhibition of local IgE production and proof of effectiveness of AIT for patients with LARs. No other similar observations were made in this case.

The Purethal used in the study is an allergoid with high efficacy and safety, regardless of the dose regimen and type of allergen, which has been confirmed in several studies.^{20–22}

The main limitation of our study is the small number of patients, which resulted in slightly lower values. However, patients with birch sensitization are not common among patients with LARs. We also did not monitor IgG4 in the nasal lavage during AIT due to technical limitations. It would be of interest to obtain a precise assessment of the local nasal immune responses to AIT.

In a study similar to ours, the authors⁴ described a positive SPT and stable IgE concentration, indicating desensitization to the allergen. We did not share this observation, possibly because we assessed a more homogeneous group of patients.

This investigation has demonstrated significant effectiveness and safety of AIT in patients with LARs and an allergy to birch. Further studies are necessary to assess the clinical effect of AIT in patients with LARs. Proper patient qualification along with a precise diagnosis is crucial for this type of treatment.

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