

NLRP3 or neutralizing antibody against IL-1 β (Fig 2, K; see Fig E6, A-C, in this article's Online Repository at www.jacionline.org; $P < .001$). Moreover, we did the neutrophils' chemotaxis by culture supernatant from different treatments with macrophage or epithelial cells from patients with CRSwNP. Then, we found that LPS, poly I:C, and IL-4 also could chemotaxis neutrophils in the macrophage or epithelial cells (see Fig E7 in this article's Online Repository at www.jacionline.org), consistent with the results of mixed-cell experiments. Inhibition of NLRP3 expression in single-cell suspension of NPs abrogated the effect of LPS, poly I:C, or IL-4 exposure on IL-1 β secretion and neutrophil recruitment, indicating that NLRP3 is a central component in both tissue eosinophilia and neutrophilia. Meanwhile, the inhibitor of NLRP3 or abolished downstream IL-1 β maturation played a potential role in the control of chronic nasal mucosal inflammation.

Because glucocorticoid treatment is recommended as the primary treatment for patients with CRSwNP, we found that the treatment with glucocorticoids significantly downregulated NLRP3 mRNA and IL-1 β protein levels and alleviated the chemotactic neutrophils by IL-4 but not for LPS, poly I:C, and ATP (Fig 2, K; see Fig E6, D, and Fig E8 in this article's Online Repository at www.jacionline.org; $P < .001$). This finding indicates that glucocorticoid treatment might partially exert its anti-inflammatory effect by inhibiting the NLRP3 inflammasome and IL-1 β expressions and reducing local mucosal neutrophilic infiltration.

In summary, these studies suggest that the NLRP3 inflammasome and its associated downstream IL-1 β were significantly correlated and upregulated in high-neutrophil-infiltrated NPs, including both mixed eosinophil and neutrophil infiltration subjects and singular neutrophil infiltration subjects. The antigen-stimulated high expression of NLRP3 and secretory IL-1 β may be the key factor in neutrophilic inflammation of EOS⁻NEU⁺ and EOS⁺NEU⁺ CRSwNP subsets.

Meanwhile, IL-4-induced NLRP3 expression promotes mixed eosinophilic and neutrophilic infiltration in the EOS⁺NEU⁺ CRSwNP. Gurung et al⁸ reported that NLRP3 was the trigger of an adaptive T_H2-biased immune response upon parasite infection. In contrast, Niebuhr et al's published data⁹ from subjects with atopic dermatitis indicated that the function of the NLRP3 inflammasome was impaired in keratinocytes under a dominant T_H2 milieu. It is possible that NLRP3 inflammasome signaling exerts different roles in different types of cells, under certain disease settings. IL-4-NLRP3 signaling interactions may amplify IL-1 β production in single-cell suspension of NPs and modulate neutrophil chemotaxis, thus contributing to NP pathogenesis *in vivo*. These studies indicate that the NLRP3 pathway may be used as a therapeutic approach to target a certain CRSwNP subset.

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Intralymphatic immunotherapy in pollen-allergic young adults with rhinoconjunctivitis and mild asthma: A randomized trial



To the Editor:

Allergy immunotherapy (AIT) is the only disease-modifying therapy that induces long-term clinical benefits in allergic patients even several years after discontinuation of the treatment.¹ The most frequently used allergen administration is by subcutaneous injections (subcutaneous immunotherapy [SCIT]) and sublingual tablets (sublingual immunotherapy [SLIT]).¹ However, the long treatment period with multiple injections or daily sublingual tablets for several years is a major drawback that renders the therapy less attractive. In the past decade, AIT given as a few low-dose allergen injections in an inguinal lymph node (intralymphatic immunotherapy [ILIT]) has emerged as an efficient administration route with encouraging results.²⁻⁵ The short duration of ILIT is especially favorable for adolescents and young adults, a patient group where compliance to treatment is a major problem.

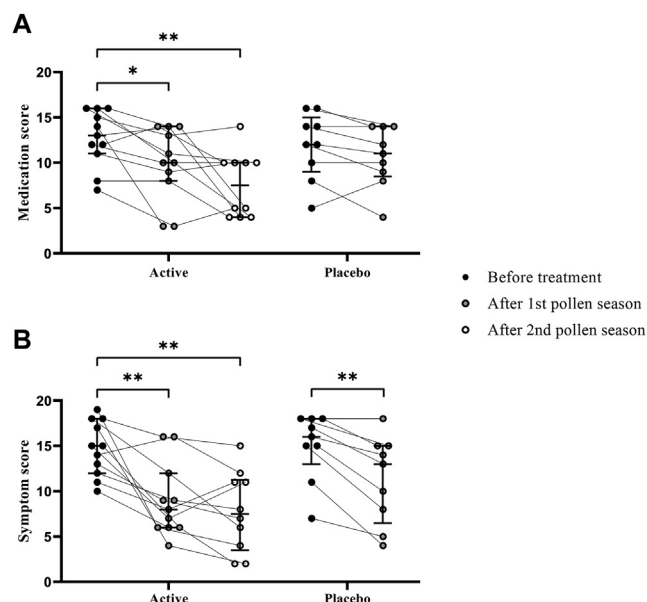


FIG 1. A, Medication score based on medications used was calculated. A maximum of 16 points could be obtained. **B,** Symptom score was calculated taking into account the frequency of symptoms. A maximum score of 20 points could be obtained. Differences were analyzed using Wilcoxon matched pairs signed rank test within the groups and with Mann-Whitney *U* test between the groups where $*P < .05$ and $**P < .01$. Lines connect the data points from single patients, and median $\pm$ interquartile range are indicated. Included patients were active $n = 11$ (before vs after first pollen season) and $n = 10$ (before vs after second pollen season), placebo $n = 9$.

This is the first randomized, double-blind, placebo-controlled ILIT study in pollen-allergic individuals investigating 3 pre-seasonal allergen injections and a booster dose 1 year later. The aims were to study the clinical efficacy and humoral effects of ILIT for the treatment of rhinoconjunctivitis and mild asthma in young adults. Thirty patients aged 16 to 42 years with rhinoconjunctivitis and mild asthma due to birch or timothy pollen were enrolled and randomized in parallel into active ($n = 17$) or placebo ($n = 13$) treatment. Four patients (active $n = 3$; placebo $n = 1$) were removed or left the study before its conclusion. See this article's Online Repository at www.jacionline.org for patient characteristics at inclusion (Table E1), data analysis, and flowchart of the study (Fig E1). The study was approved by the local ethics board in Lund and registered at ClinicalTrials.gov with study number NCT03394508, and performed according to the declaration of Helsinki. All participants gave their written informed consent.

Ultrasound-guided intralymphatic injections with 1000 SQ-U of allergen extract (Alutard birch [containing 0.15 μ g Bet v 1] or grass pollen [containing 0.2 μ g Phl p 5]; ALK-Abelló, Hørsholm, Denmark) or placebo (ALK diluent) were given in an inguinal lymph node at all injections. Patients were injected 3 times with 4 weeks in between before the start of the first pollen season. The study was unblinded at a follow-up visit in the fall, after the end of the first season. Active treated patients returned for a booster injection (1000 SQ-U) before the second pollen season. Blood was collected, questionnaires were answered, and physiological parameters were measured before treatment, after the first pollen season, and, for active treated patients only, after the second pollen season as well. For additional information about the study outline and the methods used, see this article's Online Repository and Fig E2 at www.jacionline.org.

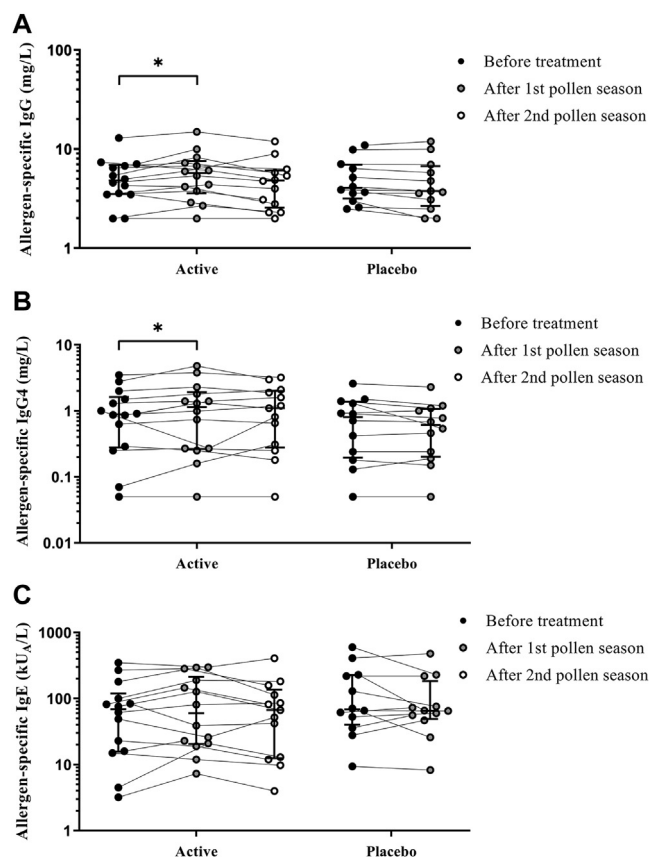


FIG 2. Allergen-specific immunoglobulin levels in serum measured by ImmunoCAP. **A,** IgG. **B,** IgG4. **C,** IgE. Differences were analyzed using Wilcoxon matched pairs signed rank test within the groups and with Mann-Whitney *U* test between the groups where $*P < .05$. Lines connect the data points from single patients, and median $\pm$ interquartile range are indicated. Active $n = 14$ ($n = 13$ after the second pollen season), black circles, and placebo $n = 12$, gray squares.

The primary outcome, change in the local nasal response to provocation (nasal provocation test [NPT]) with birch or timothy allergen, did not reach statistical significance. However, in the active treated group, the nasal symptom scores after the NPT decreased by 30% after the first pollen season compared with before treatment, whereas the decrease was only 12.5% in the placebo-treated group ($P = .1$, $n = 13$, and $P = .37$, $n = 11$, respectively; see Fig E3 in this article's Online Repository at www.jacionline.org). No statistically significant differences between the groups were detected.

A significant reduction in the medication scores (MSs) in the active treated group by 23.1% after the first pollen season ($P < .05$, Wilcoxon matched pairs signed rank test; Fig 1, A, $n = 11$) was detected. In the second year, after a pre-seasonal booster injection had been given, the reduction was 40% after the pollen season compared with before treatment ($P < .01$, Fig 1, A, $n = 10$). When we evaluated MSs in the placebo-treated group, no significant changes were noted before compared with after the first pollen season (reduction by 8.3%; Fig 1, A, $n = 9$). Thus, in contrast to placebo, the 4 intralymphatic allergen injections reduced the medication use during the pollen season.

There was a significant reduction in symptom scores (SSs) for both the active treated and the placebo-treated groups after the first pollen season compared with before treatment ($P < .01$; Fig 1, B, $n = 11$ and 9, respectively). However, the reduction was more

pronounced in the active treated group with 46.7% compared with 18.8% in the placebo-treated group. No statistically significant differences in MSs and SSs between the groups were detected. The results are in line with the pilot study by Patterson et al⁴ where 7 adolescents and young adults with grass pollen rhinoconjunctivitis were actively treated with 3 intralymphatic injections and 8 were given placebo. The authors found that the treatment was safe and reduced total combined SSs and MSs during the peak pollen season.

One cardinal feature of successful AIT, during which patients have been treated for several years, is the induction of allergen-specific IgG production with the capacity to inhibit allergen-specific IgE-mediated reactions.⁶ We found that the first course of 3 ILIT injections induced significantly increased allergen-specific IgG (18.8%) and IgG₄ (28.7%) levels after the first pollen season compared with before in the active treated group ($P < .05$; Fig 2, A and B, respectively, $n = 14$), whereas there was a reduction by 7.4% for IgG and by 24.2% for IgG₄ in the placebo-treated group ($n = 12$). The increases were modest compared with the levels observed after SCIT but in line with previous ILIT studies.^{5,7} After the second pollen season, after a preseasonal booster injection was given, the IgG and IgG₄ levels did not increase but remained at the same levels as after the first pollen season ($P > .05$, $n = 13$), suggesting that additional treatment is needed to further enhance the allergen-specific IgG and IgG₄ responses. We also evaluated the treatment effect for the allergen-specific IgE levels and found that ILIT did not induce any significant change at either time point (Fig 2, C). No statistically significant differences between the groups were detected.

All patients had a positive methacholine challenge and well-controlled asthma and most received treatment according to Global Initiative for Asthma step 1 or step 2, which probably explains why the secondary outcomes quality of life, PD₂₀ after methacholine challenge, treatment effect measured on a visual analogue scale, asthma control test, fraction of exhaled nitric oxide, FEV₁, and forced vital capacity did not reach statistical significance for neither within- nor between-group comparisons (see Table E2 in this article's Online Repository at www.jacionline.org).

With respect to treatment compliance, only 1 patient did not complete the therapy, emphasizing the favorable short treatment period of ILIT. The treatment was overall safe and mainly the active treated group reported mild side effects (pain, redness, itch or swelling at injection site, sneezing, and tiredness). One patient suffered from generalized urticaria in connection to the booster injection, and a second patient experienced increased asthma symptoms the day after the injection. However, no adrenaline was needed during the study. For detailed information on the adverse events, see this article's Online Repository at www.jacionline.org.

The small sample size is a limitation of the study as well as the fact that the patients had a mild and well-controlled asthma. Furthermore, recall bias cannot be excluded. Still, significance was reached with the important clinical parameters symptoms and medication use and the treatment was safe.

In conclusion, although the primary outcome was not reached, ILIT, given as 3 allergen injections and a booster dose before the second pollen season, reduced medication use and improved symptoms of birch- or grass pollen-induced allergy. The treatment had an immune-modulating effect by increasing allergen-specific IgG and IgG₄ levels without inducing allergen-specific IgE. However, the booster injection did not further enhance the IgG₄ and IgG levels, indicating that the therapy needs to be prolonged and the allergen dose optimized. Nevertheless,

ILIT would compose of fewer injections than regular SCIT and could potentially provide an attractive alternative for the treatment of allergic diseases in adolescents and young adults.

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METHODS

Study outline

All eligible allergic patients at the allergy clinics at the Karolinska University Hospital in Solna/Huddinge, Stockholm, Sweden, were screened and enrolled in the study during the inclusion period January 1, 2013, and December 31, 2015. Inclusion criteria were age 16 to 45 years, confirmed allergy to birch and/or timothy grass pollen together with rhinoconjunctivitis and mild asthma (controlled asthma [asthma control test score > 19] and a positive methacholine challenge), and otherwise healthy. Exclusion criteria were troublesome allergy to mites and animal dander, disease in the upper airways including sinusitis and nonallergic rhinitis, uncontrolled asthma, pregnancy, breast-feeding, other illnesses, and drug abuse. Unfortunately, the required number of patients (60 according to the power calculation; see the Statistical Analysis section below) was not reached because of narrow inclusion criteria. The study was randomized, double-blind, placebo-controlled, and performed between 2013 and 2017. Before each treatment period, an assistant prepared sealed opaque envelopes for both active and placebo treatments to achieve a final 1:1 ratio. After the first year of the study, it was revealed that 3 actively treated patients had to be excluded and therefore, patients were randomized 2:1, active:placebo, for the remainder of the study to achieve the 1:1 ratio. The envelopes were randomly mixed, and an independent nurse drew 1 envelope for each patient and prepared the medical product according to the envelope at all 3 injection visits. The active allergen extracts could not be distinguished from placebo. Using this method, all the participants, physicians who administered the interventions, and study staff who evaluated the outcomes remained blinded until the follow-up visit after the first pollen season was completed.

Assessment of clinical parameters

Clinical parameters were evaluated before the start of the treatment, in the fall after the first pollen season, and in the fall after the second pollen season (active group only). Treatment effect was evaluated by asking the patients to compare their allergic symptoms during the last pollen season with the pollen season before treatment on a visual analogue scale ranging from 0 (unchanged symptoms, no improvement) to 10 (total symptom relief, complete recovery). Asthma control 4 weeks before follow-up was estimated with the asthma control test, where a score of 19 or less suggests poorly controlled asthma.^{E1} Quality of life was assessed using the Juniper Asthma Quality of Life Questionnaire, giving a score ranging from 1 to 7, and a change in score of 0.5 points is considered clinically relevant.^{E2} Modified SSs and MSs^{E3} were calculated taking into account the frequency: daily (4 points); every second day (3 points); 1 to 3 days per week (2 points); occasionally (1 point); never (0 points), for the following symptoms: blocked nose, rhinorrhea, fatigue, sneezing, and asthma symptoms, and for the following medications used: local and systemic antihistamines, nasal steroids, asthma medication, and eye drops. A maximum score of 20 points for symptoms and 16 points for medication could be obtained.

Nasal provocation tests (NPTs) were performed with a commercially available birch or grass extract, Aquagen 100,000 SQ-E/mL (ALK-Abelló, Copenhagen, Denmark), according to a modified Lebel protocol.^{E4} One spray dose, 0.1 mL of the extract (10,000 SQ-E), was deposited in each nostril. Symptoms during NPTs were scored according to the Lebel scoring scale before and 5, 15, and 30 minutes after administration by a trained research nurse. The scoring system identifies nasal, eye, and ear symptoms: rhinorrhea, nasal pruritus, nasal congestion, ocular pruritus, watery eyes, and itchy ears, each graded on a scale from 0 to 3 points, and a total score was summarized after subtracting the starting score (maximum score is 54 + the number of sneezes).

Fraction of exhaled nitric oxide, FEV₁, and forced vital capacity were measured according to international guidelines.^{E5} Bronchial hyperresponsiveness to a challenge with methacholine was assessed, and the dose of methacholine causing a 20% reduction in FEV₁ (PD₂₀) was calculated.^{E6}

Assessment of serum antibodies

Blood was taken at each visit, and serum and plasma were collected for immunological analyses. Allergen-specific IgE level was measured by ImmunoCAP (Phadia, Thermo Scientific, Uppsala, Sweden) for birch (t3) and timothy grass (g6) pollen according to the manufacturer's instructions. A cutoff level ≥ 0.35 kU_A/L was considered positive. In addition, IgG to birch/timothy grass pollen and IgG₄ to birch/timothy grass pollen were measured by ImmunoCAP, with the cutoff 2 mg/L for IgG and 0.05 mg/L for IgG₄.

Outcomes

The primary outcome of the study was the change in symptoms score after NPT. The secondary outcomes were SSs and MSs, changes in response to a bronchial challenge with methacholine (PD₂₀), treatment effect measured on a visual analogue scale, changes in quality of life, changes in allergen-specific serum immunoglobulin levels, incidence of adverse events, changes in asthma symptom scores, changes in pulmonary function measurement (FEV₁ and forced vital capacity), and changes in airway inflammation assessed by fraction of exhaled nitric oxide.

Adverse events and patient analysis

Four patients were removed or left the study before analysis of results; 3 patients (active group) were excluded after the first treatment period because of deviations from the treatment protocol, and 1 patient (placebo) left the study after the first treatment period, before the follow-up (see Fig E1).

No adrenaline was needed during the study. In total 91 injections were given, 55 with active treatment and 36 with placebo. A total of 69 mild side effects (pain, redness, itch or swelling at injection site, sneezing, and tiredness) within 24 hours after injection were reported. Fifty-nine of these were reported from 13 patients receiving active treatment, and 10 from 5 placebo-treated patients. In the active group, 1 patient suffered from generalized urticaria 15 minutes after injection and 1 patient reported increased asthma symptoms the day after injection.

Eleven patients (6 receiving active and 5 placebo treatments) did not have full analysis data sets. In the active treated group, 1 patient reported side effects after the first treatment period (eczema) and declined the booster dose. This patient completed the follow-up after the first pollen season and is included in the analysis. One patient was diagnosed with sarcoidosis 3 months after the booster injection. After careful medical investigation by a physician in respiratory medicine, no causal relationship could be established. The patient participated in the final follow-up but did not take part in NPT and methacholine challenge and is not included in the analysis of spirometry and quality of life for that time point. In addition, 1 patient did not perform the NPTs and 3 did not answer the symptoms and medication questionnaires. In the placebo-treated group, 1 patient did not perform the NPT at the follow-up after the first pollen season, another did not perform the spirometry measurements at the follow-up after the first pollen season, and 3 patients did not answer the symptoms and medication questionnaires.

Statistical analysis

A power calculation was performed with a 2-sample *t* test for the primary outcome variable of NPT response. We expected 30% improvement in nasal symptoms in the active group at the NPT 6 to 9 months after treatment compared with the placebo group, based on a previous study,^{E7} and we assumed a mean of 18 in the placebo group and a mean of 14 ± 5.5 in the active group. Aiming at a power of 0.80 and using a type 1 error rate α level of .05, the calculated sample size was 60 in total. Data are presented as median $\pm$ interquartile range. Mann-Whitney *U* test was used for comparisons between placebo and active treatments. Wilcoxon matched pairs signed rank test was used within the active and placebo groups to compare before treatment to after the first pollen season and after the second pollen season. A *P* value $\leq .05$ was considered significant. Data were analyzed using Graphpad Prism v 8.0.2 (Graphpad Software, La Jolla, Calif; www.graphpad.com).

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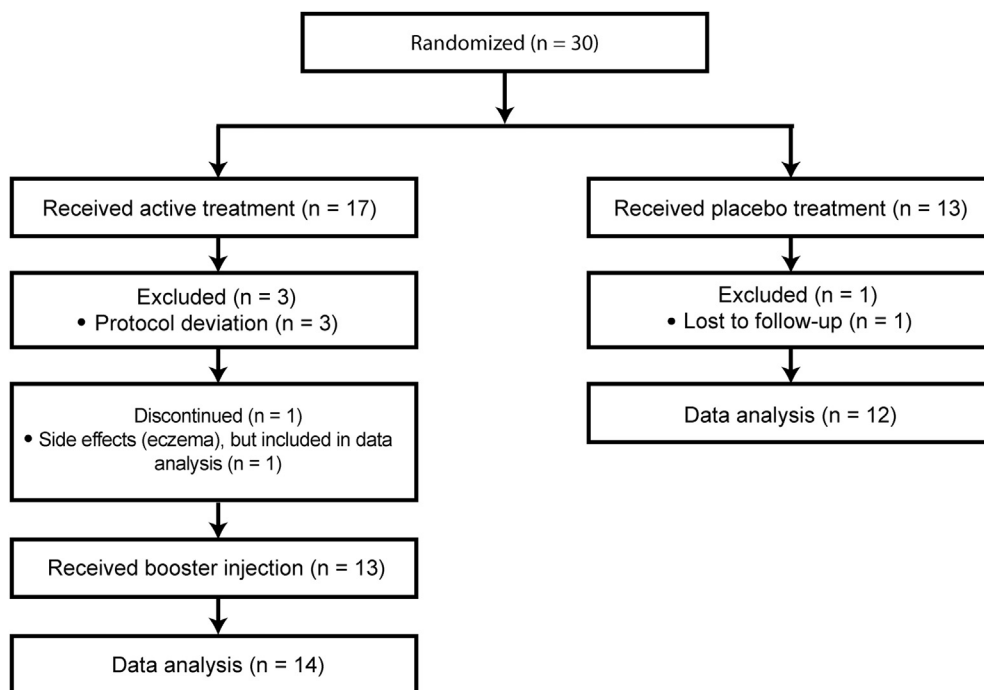


FIG E1. Flowchart of the study.

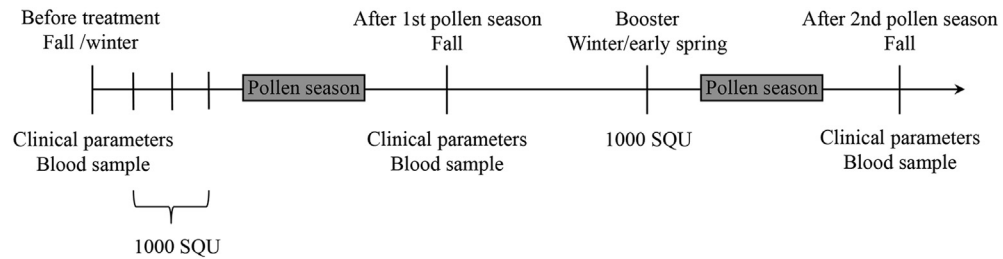


FIG E2. Study outline. Patients with confirmed allergy to birch and/or grass pollen together with rhinoconjunctivitis and mild asthma were enrolled and randomized in the study in the fall/winter before the first pollen season and received 3 injections with active or placebo treatment, 4 weeks apart, before the first pollen season. At a follow-up visit in the fall, treatment was revealed and active treated patients received a booster dose before the second pollen season.

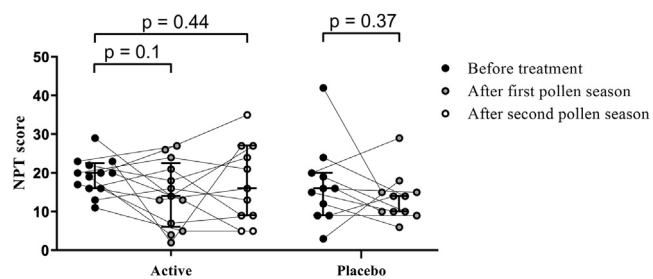


FIG E3. NPT score. Nasal symptoms were scored according to the modified Lebel protocol before and 5, 15, and 30 minutes after instillation of allergen in each nostril. A total score was calculated after subtracting the before score from each time point. Differences were analyzed using Wilcoxon matched pairs signed rank test within the groups and with Mann-Whitney *U* test between the groups. *Lines* connect the data points from single patients, and median $\pm$ interquartile range are indicated. Active: $n = 13$ before treatment vs after the first pollen season, and $n = 11$ before treatment vs after the second pollen season; Placebo: $n = 11$.

TABLE E1. Characteristics at inclusion of patients included in data analysis

Characteristic	Active treatment	Placebo treatment	P value
No. of patients	14	12	
Sex: M:F	6:8	10:2	
Age (y), median (range)	19.5 (16-42)	18 (16-36)	.85
Birch:Timothy	11:3	8:4	
Allergen-specific IgE* (kU _A /L), median (range)	69 (3.2-350)	66 (9.4-600)	.38
Asthma control, median (range)	21 (18-25)	23 (19-25)	.38
Quality of life (AQLQ), median (range)	6 (4.73-6.93)	6.3 (3.06-7)	.93
FEV ₁ (%), median (range)	97.5 (79-120)	101.5 (81-119)	.83
PD ₂₀ (μg), median (range)	180.5 (43-1027)	300.5 (23.13-1803)	.29
FENO (ppb), median (range)	20.5 (7-75)	20 (6-80)	.68
NPT score, median (range)	20 (11-29)	17.5 (6-43)	.16

AQLQ, Asthma Quality of Life Questionnaire; F, female; FENO, fraction of exhaled nitric oxide; M, male; ppb, parts per billion.

*Allergen-specific IgE to birch or timothy pollen extract corresponding to the treatment allergen (birch or timothy) for each patient.

TABLE E2. Clinical parameters measured at each time point

Parameter	Active treated patients, median (range), n		
	Baseline	First pollen season	Second pollen season
ACT score	21 (18-25), n = 14	22 (18-25), n = 14	23 (13-25), n = 11
AQLQ score	6 (4.73-6.93), n = 13	6.6 (5-6.93), n = 13	6.6 (4-7), n = 11
FEV ₁ (%)	97.5 (79-120), n = 14	99.5 (77-121), n = 14	92 (79-115), n = 11
PD ₂₀ (μg)	180.5 (43-1027), n = 13	205 (78-2000), n = 14	446 (37-2000), n = 11
FENO (ppb)	20.5 (7-75), n = 14	14 (5-88), n = 14	15 (5-103), n = 11
FVC (%)	98.5 (82-119), n = 14	101 (82-122), n = 14	93 (84-127), n = 11
Treatment effect (VAS score)	NA	5.5 (0-9.1), n = 14	6.85 (0-9.5), n = 12

Parameter	Placebo-treated patients, median (range), n	
	Baseline	First pollen season
ACT score	23 (19-25), n = 12	22.5 (16-25), n = 12
AQLQ score	6.3 (3.06-7), n = 12	6.53 (4.4-7), n = 12
FEV ₁ (%)	101.5 (81-119), n = 12	95 (78-111), n = 11
PD ₂₀ (μg)	300.5 (23.13-1803), n = 12	193 (60-2000), n = 11
FENO (ppb)	20 (6-80), n = 12	21 (5-50), n = 11
FVC (%)	101.5 (87-119), n = 12	99 (83-11), n = 11
Treatment effect (VAS score)	NA	3.5 (0-8.5), n = 12

ACT, Asthma control test; AQLQ, Asthma Quality of Life Questionnaire; FENO, fraction of exhaled nitric oxide; FVC, forced vital capacity; NA, not applicable; ppb, parts per billion; VAS, visual analogue scale.