

Comparative effect of pre-coseasonal and continuous grass sublingual immunotherapy in children

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Keywords

children; grass pollen; regimen of administration; sublingual immunotherapy.

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Abstract

Background: One of the most important aspects of sublingual immunotherapy (SLIT) is the regimen of administration. The aim of the study was to compare the efficacy and the safety of SLIT given pre-coseasonally (starting before the pollen season and continuing until the end of it) and continuously (all year round, independent of the pollen season) in children allergic to grass pollen.

Methods: Sixty children aged 6–18, sensitive only to grass pollen, with rhinitis (20 patients had concomitant asthma) participated in the 2-year prospective, randomized, double-blind, placebo-controlled trial.

Results: Both pre-coseasonal and continuous SLIT were associated with a substantial reduction in the combined symptoms/medication score when compared with placebo; there were no significant differences between the regimens (mean difference: 18.1 ± 12.4 , $P > 0.05$). Similar changes were observed in the total symptoms score. Pre-coseasonal therapy, compared with continuous, was more effective in the reduction of nasal symptoms (mean difference: -18.0 ± 2.5 , $P = 0.006$). We did not observe significant differences in medication, ocular, and asthma scores between the regimens. We did not observe changes in morning PEF, FEV₁, and PD20 in any of the three groups nor between the groups throughout the study. We showed a significant decrease in FeNO level comparable in both active groups. There were no differences between groups in the induction of CD4CD25Foxp3-positive cells in peripheral blood during the study.

Conclusion: Both protocols were effective compared with placebo and showed similar decreases for combined symptoms/medication score and all secondary endpoints, with the exception of nasal symptoms that were lower in the pre-coseasonal group.

Allergen-specific immunotherapy (SIT) is the only treatment of allergy modifying the immune response. Specific immunotherapy alleviates the symptoms, prevents the onset of new sensitizations, and reduces the risk of developing asthma. Moreover, its clinical efficacy lasts many years (1). One of the most important and debated aspects of sublingual immunotherapy (SLIT) is the regimen of administration. The results obtained in some trials suggest that the duration of the preseasonal treatment period might influence the

magnitude of the clinical effect (2–4); patients who received more than 8 weeks of therapy prior to the grass pollen season showed greater improvement than those who received <8 weeks of preseasonal treatment (5). On the other hand, there are studies with pollen SLIT given continuously, where considerable benefits in rhinitis and asthma were gained (6). However, there are no publications comparing how the pre-seasonal treatment and continuous treatment might influence the efficacy observed during continued coseasonal administration of SLIT. The induction of allergen-specific tolerance in the immune system of oral mucous membrane depends on the intensity of allergen exposure. In this study, the intensity of allergen exposure was modified in two ways: pre-coseasonal or continuous immunotherapy, and natural allergen exposure.

Abbreviations:

FENO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in one-second; ICS, inhaled glucocorticosteroids; MBPT, methacholine bronchial provocation test; SIT, specific immunotherapy; SLIT, sublingual immunotherapy.

The aim of our study was to compare the efficacy and the safety of SLIT given pre-coseasonally (starting 8 weeks before the pollen season) and continuously (all year round, independent of the pollen season) in children allergic to grass pollen.

Methods

Subjects

We studied 60 children, aged 6–18, living in the same geographic location, sensitive only to grass pollen as shown by positive skin prick tests and by the presence of specific IgE, with a clinical diagnosis of grass-related rhinoconjunctivitis with a duration of at least 2 years before the first study visit; 20 patients had concomitant asthma. All subjects had had seasonal symptoms of grass-related moderate-to-severe allergic rhinoconjunctivitis (according to Allergic Rhinitis and its Impact on Asthma 2008 recommendation) (7) with or without bronchial asthma (intermittent or mild-to-moderate persistent, according to Global Initiative for Asthma 2008 criteria) (8). The subjects with asthma and/or rhinitis, allergic to perennial allergens, or with severe or unstable asthma were excluded from the study. Exclusion criteria also included active upper respiratory tract infection within 1 month before the first visit and between the first and the second visit as well as the known contraindications of SIT according to the EAACI (9). Patients with any previous immunotherapy or suffering from clinically significant pulmonary, hematologic, hepatic, gastrointestinal, renal, endocrine, neuronal, cardiovascular, and/or psychiatric diseases or malignancies, which could either put them at risk during their participation in the study or might have influenced the results of the study (as judged by the investigator), were also excluded. Finally, excluded medications were systemic corticosteroids or immune suppressive drugs, used within 4 weeks before the study.

Study design

We performed a prospective, randomized, double-blind, placebo-controlled study to compare the efficacy of pre-coseasonal and continuous regimens of SLIT in children allergic to grass pollen. There were 2 years (between September 2008 and March 2011) of a follow-up period, without a baseline assessment in a run-in pollen season. All suitable subjects were randomized according to a computer-generated allocation schedule into the three treatment arms: the pre-coseasonal SLIT group ($n = 20$), the continuous SLIT group ($n = 20$), and the placebo group ($n = 20$). All subjects were recruited from our specialty clinic setting. Flowchart of the study is presented in Fig. 1. The primary endpoint was the efficacy assessed on the basis of the reduction in combined symptoms/medication score. Secondary endpoints were assessed: total symptoms score, medication score, and individual organ symptoms scores (nasal, ocular, asthma) as well as lung function, exhaled nitric oxide (NO) concentration, methacholine bronchial provocation test (MBPT), and the percentage of T regulatory Foxp3-positive cells in peripheral blood. Combined symptoms/medication score, total symptoms score, medication score, and individual organ symptoms scores were adjusted to 1000 pollen grains/m³. Safety was estimated on the basis of adverse symptoms.

The spore count of grass pollen was monitored by a professional center (Professor Marek L. Kowalski, Department of Immunology, Rheumatology and Allergy, Medical University of Lodz) during the entire study period. Counts were expressed as number of grass pollen per cubic meter.

Treatment

Both SLIT groups were treated with Staloral 300 IR as a standardized extract of five grass pollen (*Dactylis glomerata*, *Anthoxanthum odoratum*, *Lolium perenne*, *Poa pratensis*, and

	Inclusion 2008	Start of first year of intervention	Middle of the pollen season 2009	End of the pollen season 2009	Start of second year of intervention	Middle of the pollen season 2010	End of the pollen season 2010	Final visit 2011
Visit	1	2	3	4	5	6	7	8
Month	0	6	9	12	18	21	24	28
Participants I/II/III*	20/20/20	20/20/20	19/20/20	18/20/20	18/18/19	18/17/19	18/17/19	18/17/19
Adherence of the therapy^	—	—	3	3	7	3	3	—

Visit 1

1. Subjects were informed of the purpose of the study
2. Subjects received diary card
3. Randomization into the treatment arms
4. MBPT**

Visit 2 and 5

1. SLIT induction
2. Diary card
3. Blood sampling

Visit 3 and 6

1. Diary card evaluation
2. Lung function
3. FeNO measurement

Visit 4 and 7

1. Diary card evaluation
2. Lung function
3. MBPT

Visit 8

1. Blood sampling

*Participants in three arms: I placebo, II pre-coseasonal SLIT, III continuous SLIT

^ number of empty vials brought to visit

Figure 1 Flowchart of study.

Phleum pratense); the placebo group received an identically looking verum dispensed in the same glycerosaline diluents and supplied by a pharmaceutical company (Stallergenes SA, Antony, France). The mean concentration of major allergens (Dact g5, Antx o5, Lol p5, Poa p5, Phl p5) in the extract was 25 µg/ml. Immunotherapy was administered blindly by a treatment team that was also responsible for the assessment and treatment of any adverse reactions. On the average of 8 weeks, before the pollen season (2009 and 2010), all children received immunotherapy/placebo following a recommended induction scheme: 1 drop on first day followed by 3, 5, 7, and 12 drops (four puffs) on each consecutive day. In the maintenance phase every morning before breakfast, all patients received four puffs from a dispenser: for the continuous SLIT group – active drug (10 µg of major allergens) for 12 months, for the pre-coseasonal SLIT group – active drug (10 µg of major allergens) for 6 months followed by 6 months of placebo, and for the placebo group – placebo for 12 months. It was then followed by a second induction phase (in March 2010) with an active drug in pre-coseasonal group and with placebo in the continuous and placebo groups. Therefore, cumulative doses of major allergens for continuous SLIT group and pre-coseasonal SLIT group were 7.3 and 3.6 mg, respectively. To assess their adherence to the treatment, all subjects were asked to return empty vials to each visit (see Fig. 1). The definition of the pollen season was based on the analysis of three previous pollen seasons in the specified region. The onset of the season was defined as the first of three consecutive days with grass pollen counts of at least 10 grains/m³, and its ending as the last day before three consecutive days with a grass pollen count of < 10 grains/m³. Pollen count was calculated throughout the study for the region of interest (<http://www.umed.lodz.pl/KatedraImmunologii/>). During the pollen season, all children received at baseline the standard treatments of the allergic rhinoconjunctivitis – systemic antihistamines. Subjects were allowed to use rescue medications for symptoms of hay fever (nasal steroids, local antihistamines, or cromones for eyes) and asthma (inhaled corticosteroids, short-acting β₂-agonist). Also, the standard treatments of the infections were allowed (antibiotics, mucolytics, and pyrolytics if necessary).

Efficacy assessments

Combined symptoms/medication score was calculated as a sum of total symptoms score and the medication score recorded by the patients on a daily basis during the season from March till August (10, 11). Total symptoms score was recorded with the use of a point score system for the most common symptoms of rhinitis (rhinorrhea, sneezing, itching, and nasal congestion) – nasal score; conjunctivitis (ocular pruritus, watery eyes, and itching) – ocular score; and respiratory disorders (cough, wheeze, and dyspnea) – asthma score. A score ranging from 0 to 3 was used for each symptom: 0, no symptoms; 1, mild symptoms (symptoms clearly present but easily tolerated); 2, moderate symptoms (definite awareness of bothersome but tolerable symptoms); 3, severe symptoms (symptoms hard to tolerate and causing

interference with daily activities and/or sleeping). The minimum score for each day was 0 (no symptoms during the day, no symptoms at night) and the maximum score was 30 (severe symptoms during the day and at night). Medication score was recorded as follows: 1 point for each rescue medication multiplied by the number of treatment days; inhaled corticosteroids treatment was scored 0.5 point for 100 µg of budesonide or equivalent multiplied by the number of treatment days during the total season.

All symptoms and medication scores were adjusted to pollen count. Adjustment was calculated according to the following rule: total season score/cumulative concentration of grass pollen during the season × 1000.

Lung function test

Pulmonary function testing was performed with a Master Screen unit (Erich Jaeger GmbH, Hochberg, Germany). Predicted values for all lung function variables were based on a previous study of healthy controls, provided by the manufacturer of the lung function test equipment (12). Flow–volume curves were drawn according to American Thoracic Society standards (12). The highest of three successful measurements was taken. The results were expressed as the percentage of a predicted value. Reversibility test was performed after administration of salbutamol (200 µg). Percent of change from baseline in FEV₁, prebronchodilatory FEV₁, and PEF was included in the analysis.

Nitric oxide (NO) measurement

The NO measurements were taken according to ERS/ATS recommendations (13) with a chemiluminescence analyzer (model 280i NO analyzer; Sievers, Boulder, CO, USA) and defined in parts per billion. The analyzer provides an online continuous measurement of NO in a single exhalation with a detection range of 0.1–500 ppb. Environmental NO was measured before and after each test and never exceeded 5 ppb. All subjects were studied in the sitting position, without wearing a nose clip. The subjects exhaled at a constant flow rate (50 ml/s) from total lung capacity to residual volume without breath holding. They maintained a constant mouth pressure (17 cm H₂O) by monitoring a visual display to eliminate contamination from nasal NO. Dead space and nasal NO (which are reflected by the NO concentration peak during exhalation) and NO from the lower respiratory tract (determined by the plateau value after the peak) were recorded automatically by using the manufacturer's software. Three FeNO measurements of the plateau phase were obtained, with less than 10% variation. The mean value of three successive, reproducible recordings was retained for statistical analysis.

Methacholine bronchial provocation test (MBPT)

Methacholine bronchial test of the provocation was performed at the beginning of the study – before immunotherapy (September 2008), after the first (September 2009) and

after the second (September 2010) year of SLIT, using the dosimeter technique APS Pro (Erich Jaeger GmbH) with controlled tidal breathing. After the pulverization physiological diluent, methacholine was delivered in four cumulative doses: 0.015, 0.045, 0.18, and 0.72 mg. Methacholine bronchial provocation test was continued with 2-min intervals between the inhalations until a fall in forced expiratory volume in 1 s (FEV1) of $\geq 20\%$ was obtained. PD20 was calculated by a linear interpolation on a log-dose-response curve (12).

T regulatory cell induction

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation on Histopaque[®]-1077. CD4, CD25, and Foxp3 expression on PBMCs was estimated by flow cytometry (LSRII instrument; BD Biosciences, Franklin Lakes, NJ, USA) using appropriate fluorophore-conjugated monoclonal antibodies (14). Peripheral blood mononuclear cells were surface-stained with anti-human CD4 FITC and anti-human CD25 APC, then fixed and permeabilized using the Foxp3 Staining Buffers and subsequently stained with Rat IgG2a κ -isotype control PE or anti-Human Foxp3 PE. The cells in the lymphocyte gate were used for analysis.

Safety

Before inclusion in the study and during each consecutive visit (see Fig. 1), an interview and a physical examination were carried out. The doctor assessed changes in asthma scores, drug use, and the lung function test in comparison with the baseline. All adverse events were recorded.

Sample size and statistics

The sample size was determined on the basis of previously published immunotherapy studies (5, 11) and available recommendations (15). Primary endpoint – combined symptoms/medication score – was used to calculate the sample size. Taking into account previous findings showing that the magnitude of the reductions in symptoms and medication scores increased with longer duration of pre-coseasonal treatment (5), we expected consistent and spectacular effect of continuous over pre-coseasonal SLIT. Therefore, we used less rigorous assumptions during sample size estimation: standardized difference between groups of 0.5, a significance level of $\alpha = 0.05$ (one-tailed) and a power of $1 - \beta = 0.8$, at least 18 subjects per treatment arm should complete the second grass pollen season. This estimation includes 10% dropout rate. Before the analysis, scores for each individual patient – combined symptoms/medication, total symptoms, medication and individual organ symptoms – were adjusted to pollen count in 2009 and 2010. Next, the study endpoints were compared within and among the treatment groups by the analysis of variance (ANOVA) for the repeated-measurement design. The χ^2 test was used for categorical data. P -value < 0.05 (two-tailed) was regarded as statistically significant.

Results

Subjects

Fifty-four children completed the 2-year study; there was a total dropout rate of 10%: three subjects (15%) in the pre-coseasonal group, 1(5%) in the continuous group, and 2 (10%) in the placebo group. In all cases, the reason for dropping out was a lost follow-up (two patients in the first season and four patients during the second year). Therefore, the number of 18 subjects per treatment arm, as statistically planned, was not reached. The results of the intention-to-treat and per-protocol analysis were consistent (dropouts from per-protocol population were close to the median values, see Fig. 2). As in most of the dropout cases we had concerns regarding compliance, here we showed the results of per-protocol analysis only. Baseline characteristics of the subjects who completed the study are shown in Table 1.

There were no differences between SLIT and placebo group during the first course of treatment (Table 2).

Combined symptoms/medication score

Diaries were available for evaluations for the seasons of 2009 and 2010. Figure 2 presents the pollen count in the two seasons. The mean total season score for combined symptoms/medication, total symptoms, medication, and individual organ symptoms, adjusted to 1000 pollen grains/m³ recorded during two consecutive grass pollen seasons, is shown in Table 2 and Fig. 2. The pre-coseasonal and the continuous groups demonstrated significant decreases in the combined symptoms/medication score within the groups and in comparison with the placebo. There were no differences between both active groups in the combined symptoms/medication scores (mean difference: -18.0 ± 12.4) (Table 2, Fig. 2).

Total symptoms score

The pre-coseasonal and the continuous groups demonstrated significant decreases in the total symptoms score within the groups and in comparison with the placebo. There were no differences between both active groups in the total symptoms score (Table 2, Fig. 2).

Medication score

There were no differences between both active groups in the medication score (Table 2, Fig. 2). The pre-coseasonal group demonstrated significant decreases in the medication score within the group and in comparison with the placebo; decrease in the medication score in the continuous group was shown within the group only.

Individual organ symptoms score

Over the period of 2 years, treatment with pre-coseasonal and continuous SLIT significantly reduced all symptoms (nasal, asthma, and ocular) within the groups (Table 2).

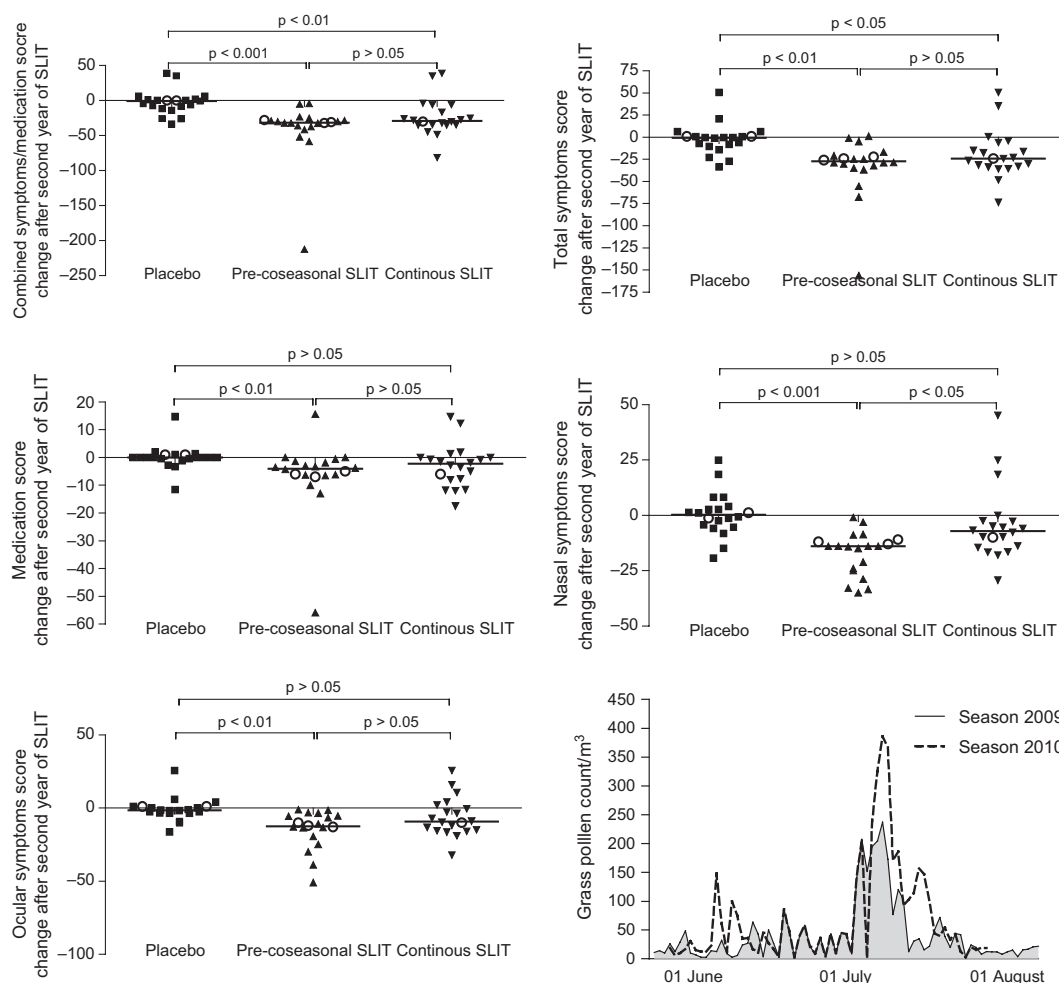


Figure 2 Changes in combined symptoms/medication, total symptoms, medication, nasal and ocular scores in the second compared with the first year of sublingual immunotherapy (SLIT) among study groups. The scores were adjusted to 1000 per m^3 according to the following rule: total season score/cumulative concentration of grass

pollen during the season multiplied by 1000. Data are presented as individual patients' value and median for the group. Open circles represent dropouts from per-protocol population. Grass pollen count (grains/ m^3) during season 2009 and season 2010 (two consecutive years of SLIT).

Table 1 Baseline characteristics of study groups before starting SLIT

	Placebo group <i>N</i> = 18	Pre-coseasonal SLIT group <i>N</i> = 17	Continuous SLIT group <i>N</i> = 19	<i>P</i>
Age (years) mean (min–max)	8.1 (4–15)	8.3 (5–17)	10.1 (3–16)	0.68
Male gender <i>n</i> (%)	11 (61)	11 (65)	14 (74)	0.45
Asthma <i>n</i> (%)	5 (28)	6 (35)	5 (26)	0.78
Asthma duration (years) median (quartile range)	2 (1–3)	2 (0–3)	2 (1–4)	0.87
FEV1 mean (SEM)	99.7 (2.4)	98.3 (2.8)	101.9 (2.4)	0.69
Daily inhaled budesonide (or equivalent) dosage during the season before entering study (μg) median (quartile range)	200 (100–400)	200 (200–400)	200 (100–400)	0.78

SLIT, sublingual immunotherapy.

When compared with the placebo, only in the pre-coseasonal SLIT group there was reduction in nasal and ocular symptoms (Fig. 2); there was no difference in asthma score in both

active groups compared with placebo. When compared with the continuous, the pre-coseasonal therapy was more effective in the reduction of nasal symptoms (mean difference:

Table 2 Study endpoints by study groups: season 2009 and season 2010 of SLIT

	Placebo group N = 18			Pre-coseasonal SLIT group N = 17			Continuous SLIT group N = 19			Plevel (ANOVA) season 2009		Plevel (ANOVA) season 2010 vs 2009	
	Season 2009	Season 2010	Season 2009	Season 2009	Season 2010	Season 2009	Season 2009	Season 2010	Season 2010	Pre-coseasonal SLIT vs placebo	Continuous SLIT vs placebo	Pre-coseasonal SLIT vs continuous SLIT	Continuous SLIT vs placebo
Clinical parameters (points)													
Combined symptoms/medication score	77.4 (25.6)	76.1 (26.7)	80.5 (16.9)	41.7 (7.6)	73.8 (10.3)	59.2 (10.1)	0.375	0.271	<0.001	<0.01	<0.01	>0.05	>0.05
Medication score	11.8 (4.1)	10.8 (4.8)	9.1 (3.8)	3.8 (1.6)	12.5 (3.7)	11.9 (4.3)	0.760	0.363	<0.01	>0.05	>0.05	>0.05	>0.05
Total symptoms score	62.0 (22.0)	65.3 (23.2)	71.4 (13.7)	36.9 (7.2)	63.1 (9.8)	45.2 (8.4)	0.454	0.421	<0.01	<0.05	<0.05	>0.05	>0.05
Nasal symptoms	31.5 (9.0)	34.2 (9.7)	35.8 (5.4)	20.6 (4.6)	32.6 (5.0)	28.0 (6.1)	0.118	0.298	<0.001	>0.05	>0.05	<0.05	<0.05
Ocular symptoms	16.5 (6.1)	17.2 (6.8)	28.5 (10.9)	12.0 (3.7)	16.1 (4.0)	10.8 (3.1)	0.111	0.284	<0.01	>0.05	>0.05	>0.05	>0.05
Asthma symptoms	14.1 (8.4)	13.8 (8.5)	7.1 (2.1)	4.3 (1.7)	12.6 (4.8)	5.9 (2.0)	0.911	0.480	>0.05	>0.05	>0.05	>0.05	>0.05
Additional parameters													
FEV1 (% pred.)	100.7 (3.4)	102.3 (1.9)	99.3 (3.0)	100.2 (2.9)	102.9 (2.7)	102.8 (2.7)	0.351	0.991	>0.05	>0.05	>0.05	>0.05	>0.05
PEF (% pred.)	88.8 (4.6)	90.1 (4.9)	95.7 (4.2)	99.5 (3.1)	98.9 (3.6)	98 (3.9)	0.851	0.391	>0.05	>0.05	>0.05	>0.05	>0.05
PD20 (mg)	0.73 (0.09)	0.25 (0.02)	0.80 (0.09)	0.25 (0.02)	0.75 (0.09)	0.19 (0.03)	0.757	0.858	>0.05	>0.05	>0.05	>0.05	>0.05
FeNO (ppb)	29.2 (5.2)	43.6 (11.7)	32.8 (4.5)	19.1 (1.9)	47.7 (7.3)	36.6 (7.9)	0.423	0.590	<0.01	<0.01	<0.01	>0.05	>0.05
Foxp3 (%CD4CD25)	3.9 (1.1)	10.5 (1.0)	2.5 (0.4)	10.1 (1.1)	3.8 (0.9)	8.9 (0.8)	0.290	0.480	>0.05	>0.05	>0.05	>0.05	>0.05

SLIT, sublingual immunotherapy.

Data are presented by mean with standard error mean.

-18.0 ± 2.5 ; $P = 0.006$) (Fig. 2); there were no difference in asthma and ocular scores between both regimens.

Lung function and bronchial provocation test

We did not observe any significant changes in morning PEF, FEV1, and PD20 in all groups throughout the study; there were no significant changes among the groups (Table 2, Fig. 3).

Nitric oxide (NO) measurement

We observed a significant decrease in the in-season FeNO level in the pre-coseasonal and the continuous group both within the groups and in comparison with the placebo. There were no significant changes in FeNO level between the pre-coseasonal and the continuous groups nor within the placebo group (Table 2, Fig. 3).

T regulatory cell induction

After the second year of SLIT, significant increases in the percentage of Foxp3 expression in CD4CD25-positive cell in peripheral blood were observed in all study groups; however, there were no differences among the groups (Table 2, Fig. 3).

Safety

The number of patients with allergic reactions possibly related to the trial medication is shown in Table 3. Predominantly local reactions – such as sublingual itching (45% vs 15.3%) – were reported. Other adverse events possibly associated with the treatment were the following: headaches in the first year of immunotherapy, 4.1% vs 4%, and 0 vs 5.2% in the second year of immunotherapy; and stomachaches in the first year of immunotherapy, 3.5% vs 5.5%, and 6% vs 5.6% in the second year of immunotherapy. There were no cases of termination of the study owing to adverse events in none of the groups. The global assessment of tolerability performed

Table 3 Number of patients with allergic reactions possibly related to the trial medication in the first and second year of study

	Pre-coseasonal SLIT group	Continuous SLIT group	Placebo group
First year of the study <i>N</i> (%)	5 (29.4)	4 (21.0)	4 (22.2)
Second year of the study <i>N</i> (%)	3 (17.6)	2 (10.5)	4 (22.2)

SLIT, sublingual immunotherapy.

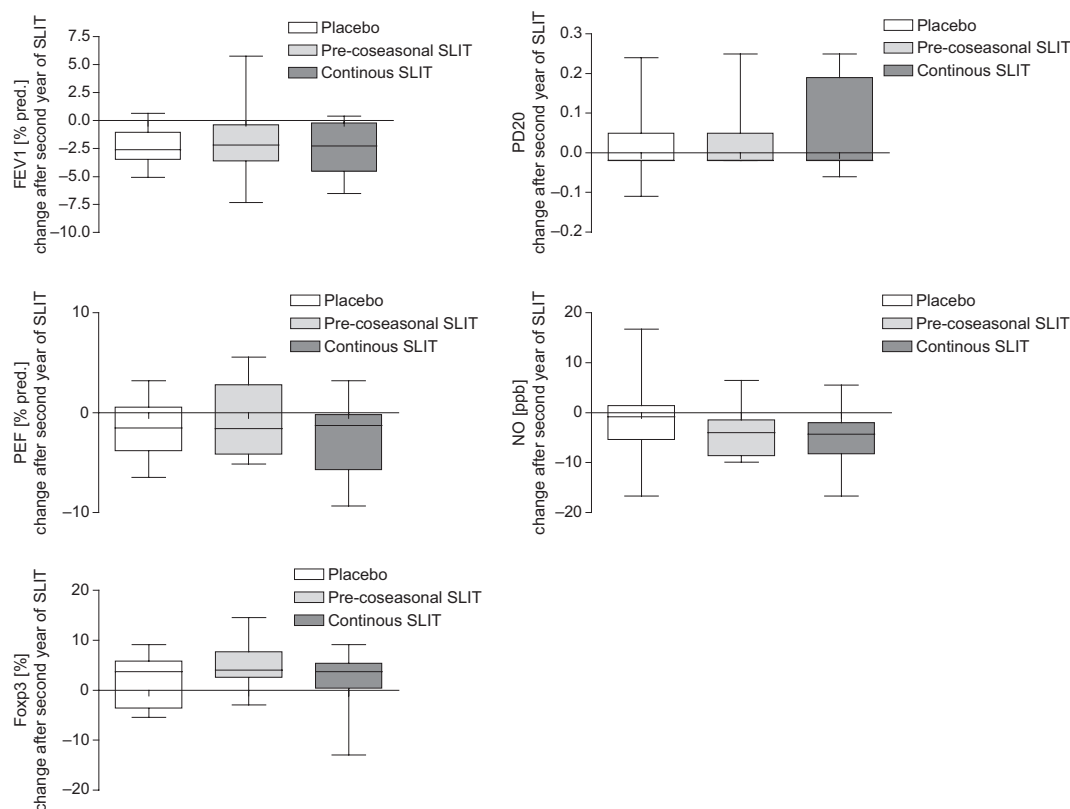


Figure 3 Changes in FEV1, PD20, PEF, FeNO, and Foxp3 in the second compared with the first year of sublingual immunotherapy among study groups. Data are presented as median (inner-box line) with interquartile range (box) and range of extreme values (whisker).

by the subjects and doctors was better in the second year of treatment (data not shown). All subjects returned empty vials of drugs to visits according to the study design (on average three empty vials every 3 months; details are shown in Fig. 1); the remaining volume was measured. Adherence was the same in all protocols.

Discussion

To our knowledge, as far as pollen allergens are concerned, there is no study directly comparing the various treatment alternatives – preseasonal, pre-coseasonal, coseasonal and continuous. The present study is the first one specifically designed to compare the clinical and immunologic effects of pre-coseasonal and continuous SLIT in children allergic to grass pollen.

The pre-coseasonal and continuous groups showed significant decreases in the combined symptoms/medication score within the groups and compared with the placebo; there were no significant changes in the combined symptoms/medication score between the pre-coseasonal and the continuous group. Similar changes were observed in the total symptoms score. There were no differences between both protocols also in the medication score. Surprisingly, our data indicate that, when compared with continuous, pre-coseasonal therapy was more effective in the reduction of nasal symptoms. However, there was no significant difference in asthma and ocular scores. We did not observe any significant changes in morning PEF, FEV1, and PD20 in any of the three groups nor among the groups throughout the study. We showed a significant decrease in FeNO level comparable in both active groups. Interestingly, although we observed significant increases in the percentage of Foxp3 expression in CD4CD25-positive cells in peripheral blood in all study groups after second year of SLIT, there were no differences among the groups. There were no cases of the termination of the study owing to adverse events in none of the groups. The global assessment of tolerability made by the subjects and doctors was better in the second year of treatment.

Recent studies have documented the efficacy and safety of SLIT in pediatric patients with rhinitis (11, 16). Unfortunately, so far, the choice of SLIT regimen has been based mainly on economic implications and compliance rather than its effectiveness in head-to-head comparison of different regimens. A recently published literature analysis, including 41 randomized controlled trials on pollen SLIT in derived experimentally supported suggestions about the preferable regimens of administration, did not include the comparison of the effectiveness of different regimens (17). In our study, both active treatments – the pre-coseasonal and the continuous – resulted in similar clinical improvement (30% reduction in combined symptoms/medication score); pre-coseasonal therapy was even more effective in the reduction of nasal symptoms. Pajno et al. (18) in a randomized open study showed that in grass pollen allergy, the continuous regimen performs better than the coseasonal in the first season, whereas in the subsequent years, the two regimens are nearly equivalent. However, the absence of a baseline evaluation in our study does not allow us to assess the effect of SLIT in the first season and compare

our results to Pajno et al. Keeping in mind a general rule that the effectiveness of immunotherapy depends on the cumulative dose of administered allergen, our findings raise a question whether it is the seasonal rather than total cumulative SLIT allergens that decide about the clinical efficacy of SLIT in the treatment of seasonal allergies.

The derivation of the power calculation needs to be explained. Data, coming from three randomized, double-blind, placebo-controlled, multi-center trials with sublingual grass allergen and varying preseasonal treatment periods, suggest that patients who received more than 8 weeks therapy prior to the grass pollen season improved more (up to 30%) than those who received <8 weeks preseasonal treatment (5). Therefore, we expected consistent and spectacular effect of continuous over pre-coseasonal SLIT and used less rigorous assumptions during sample size estimation. Both regimens were equally effective, and because present study was not designed so as to assess the noninferiority effect, the conclusion on lack of difference between protocols is underpower.

In SLIT, the mechanisms of action are not fully understood but seem to be similar to those of SCIT, with the particular involvement of mucosal dendritic cells (19). It has been previously shown that Treg Foxp3-positive cells are induced locally in mucosa during effective SLIT, and this effect is at least partially involved in the mechanism of SLIT (20–22). It is also possible that SLIT induces a significant increase in mRNA expression of Foxp3 in peripheral blood PBMC; however, this effect was shown in a single trial and was marginal compared with placebo (23). In our study, the induction of peripheral Foxp3 cells in both active groups was comparable with the placebo group. Additionally, those immunologic changes did not correlate with the clinical effect of SLIT. These findings have two implications: firstly, the immune tolerance during SLIT is induced mainly locally and, secondly, monitoring Foxp3 cells in peripheral blood during SLIT does not seem justified.

The results of the secondary endpoints, such as morning PEF, FEV1, PD20, and FeNO, revealed no differences between the active treatment groups. Moreover, lung function parameters did not change significantly within the active treatment groups, which could be explained by the lack of lung function limitation at baseline, and small numbers of asthmatic patients distributed across all groups. The decrease in in-season FeNO contradicts above general conclusion on secondary endpoints, and it shows positive impact on lower airway inflammation of the active treatments.

Owing to ethical reasons, methacholine challenge was performed outside the pollen season. Nevertheless, we recognized the assessment of bronchial hyperresponsiveness just after the pollen season as a valuable study endpoint. The other limitation of the study was the fact that there were only 2 years of a follow-up period, without a baseline assessment in a run-in pollen season, which was the result of ethical concerns regarding a long-lasting period without immunotherapy. Our study does not allow addressing SIT-modifying effects because it was only a 2-year follow-up study.

In conclusion, the present study indicates that both pre-coseasonal and continuous protocols, compared with placebo, were safe and had a similar positive effect on the reduction in

combined symptoms/medication score, with the exception of nasal symptoms that were lower in the pre-coseasonal group. However, to justify which regimen is a more suitable option for children allergic to grass pollen, more studies are warranted.

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Author contributions

The concept of the study was designed by Iwona Stelmach, Joanna Jerzynska, Włodzimierz Stelmach, and Pawe Majak.

Izabela Kaluzińska-Parzyszek and Piotr Stelmach helped in acquisition of data. Iwona Stelmach, Izabela Kaluzińska-Parzyszek, Joanna Jerzynska, Piotr Stelmach, Włodzimierz Stelmach, and Pawe Majak contributed to analysis and interpretation of data and manuscript preparation.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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