

Comparison of the effect of 5-grass pollen sublingual immunotherapy tablets and drops in children with rhinoconjunctivitis

Joanna Jerzynska, M.D., Ph.D.,¹ Włodzimierz Stelmach, M.D., Ph.D.,² Paweł Majak, M.D., Ph.D.,³ Rafał Stelmach, D.M.D.,⁴ Anna Janas, D.M.D., Ph.D.,⁴ and Iwona Stelmach, M.D., Ph.D.¹

ABSTRACT

Background: One of the most important aspects of sublingual immunotherapy (SLIT) is the regimen of administration.

Aim: To find any differences in symptom-medication scores between the two groups of SLIT tablets and drops, given pre-coseasonally (starting 8 weeks before the pollen season) in children with rhinoconjunctivitis allergy to grass pollen. The secondary outcome were the differences in lung function and induction of T-regulatory forkhead box P3 (FOXP3) positive cells.

Methods: This was a retrospective, secondary analysis of pooled data obtained from our two prospective randomized placebo controlled trials that involved children who underwent SLIT. Forty-one children, ages 6–18 years, with allergic rhinitis (AR), sensitive to grass pollen, participated in the study.

Results: Treatment with both tablets and drops significantly reduced all symptoms (nasal, asthma, and ocular) within the groups; there was no significant difference between both groups. When compared with the tablet therapy, there was a trend for drops therapy to be more effective in the reduction of combined symptom-medication score, but the difference was not statistically significant ($p = 0.1036$); there was no significant difference in asthma and nasal scores. We showed a significant decrease in the fractional exhaled nitric oxide level comparable in both immunotherapy groups. There were no differences between the groups in the induction of CD4⁺ CD25⁺ FOXP3⁺-positive cells in peripheral blood.

Conclusions: Both protocols showed similar decreases in symptom-medication scores; however, when compared with tablet therapy, there was a trend for drops therapy to be more effective in the reduction of combined symptom-medication score.

(Allergy Asthma Proc 39:66–73, 2018; doi: 10.2500/aap.2018.39.4099)

As of today, allergen-specific immunotherapy (SIT) remains the only disease-modifying treatment offered to patients with allergic rhinoconjunctivitis and allergic asthma. SIT alleviates the symptoms and prevents the development of allergic asthma in children with allergic rhinoconjunctivitis and the onset of new sensitizations. After many years of subcutaneous applications, the sublingual route has been of growing importance in the past few years.¹ Positive outcomes on the symptom and medication scores in pivotal trials convinced authorities to approve sublingual allergen immunotherapy (SLIT).^{2–4} Moreover, patients treated with subcutaneous immunotherapy (SCIT) are at risk of both local and systemic adverse reactions.

The safety profile of SLIT is good; therefore, SLIT can be self-administered by patients in their homes and has been included in international guidelines for the treatment of allergic rhinitis (AR) with or without conjunctivitis. Dosing of the allergen in SLIT has been a major issue that has still not been completely resolved. This could be because, with SLIT, not only the quality and quantity of the administered extract determine its efficacy but also the vehicle and other factors that are involved in the local allergen uptake.⁵ The two widely used forms of administration, such as drops and tablet, are available on the market. They both contain the same natural allergens of pollens from several cross-reacting grasses. The primary outcome of our study was to find any differences in symptom-medication score between the two groups, SLIT tablets and drops, given pre-coseasonally (starting 8 weeks before the pollen season) in children with rhinoconjunctivitis allergy to grass pollen. The secondary outcomes were differences in lung function and induction of T-regulatory forkhead box P3 (FOXP3) positive cells.

METHODS

This was a *post hoc* secondary analysis of pooled data obtained from our two previously published prospective, randomized, placebo controlled trials that in-

From the ¹Department of Pediatrics and Allergy, Medical University of Łódź, N. Copernicus Memorial Hospital, Łódź, Poland, ²Department of Social and Preventive Medicine, Medical University of Łódź, Łódź, Poland, ³Department of Internal Medicine, Asthma and Allergy, Medical University of Łódź, Łódź, Poland, and ⁴Department of Oral Surgery, Medical University of Łódź, Łódź, Poland
This study was funded by grant 503/2–056-01/503–01 from the Medical University of Łódź, Łódź, Poland

The authors have no conflicts of interest to declare pertaining to this article
Address correspondence to Iwona Stelmach, M.D., Department of Pediatrics and Allergy, N. Copernicus Memorial Hospital, 71 Al. Pilsudskiego, 90–329, Łódź, Poland
E-mail address: alergol@kopernik.lodz.pl
Copyright © 2018, OceanSide Publications, Inc., U.S.A.

volved children who underwent SLIT.^{6,7} Both trials, which assessed the clinical and immunologic effect of different ways of SLIT delivery, were conducted during different grass pollen seasons, in the same allergy center, and according to the similar study protocol. From these trials, children treated with immunotherapy only were compared in the present analysis.^{6,7} In the “drops” trial,⁷ there was a true placebo, whereas, in the “tablet” trial,⁶ there was a lack of immunotherapy (tablet) placebo.⁶ In one study (tablet),⁶ immunotherapy was maintained for 5 months and, in the other study (drops), for 6 months in all the subjects.⁷

The combined study population consisted of 41 boys and girls ages 5–18 years (24 participants from the tablet trial and 17 from the drops trial).^{6,7} Nine children (three participants from tablet trial and six from drops trial)^{6,7} had moderate immunoglobulin E (IgE) dependent asthma with a duration of at least 2 years, treated with inhaled corticosteroids (intermittent or mild-to-moderate persistent, according to Global Initiative for Asthma 2008 and 2014 criteria).⁸ The patients were sensitive only to grass pollen, with a clinical diagnosis of grass-related moderate-to-severe rhinoconjunctivitis (according to Allergic Rhinitis and its Impact on Asthma 2008 and 2014 recommendation)⁹ and with a duration of at least 1 year before the first study visit. The patients were classified as atopic based on a history and positive skin-prick testing result (12 tested allergens in total) and/or by the presence of serum-specific IgE (list of specific IgEs detected: *Dactylis glomerata*, *Anthoxanthum odoratum*, *Lolium perenne*, *Poa pratensis*, *Phleum pratense*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, *Alternaria tenuis*, ryegrass, birch, hazel).

Asthma was stable in all the patients; there had been no exacerbations of the disease and no change in maintenance medications or symptoms for 6 months. The subjects with asthma and/or rhinitis, allergy 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 European Academy of Allergy and Clinical Immunology.¹⁰ The patients with any previous immunotherapy or with 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 [J.J.]), were also excluded. Also, excluded medications were systemic corticosteroids or immune suppressive drugs, used within 4 weeks before the study.

Study Design of Included Trials

During the prestudy visit, all the participants were informed about the purpose of the study, were told how to score the asthma symptoms, and underwent skin testing and/or blood sampling. During the first visit, all the patients received the first sublingual dose of commercial drops (Staloral 300 IR, Stallergenes Greer, Antony, France) or tablets (Oralair, Stallergenes Greer) as a standardized extract of 5-grass pollen (Stallergenes Greer). The next visit took place 5 months after the first visit in patients who received tablets and 6 months in patients who received drops at the end of the first year of SIT. Blood samples were obtained from all the patients before and after SLIT, clinical symptoms were assessed, lung function was measured, and the patients' diaries were evaluated. The visits were scheduled in such a way that blood sampling and lung function testing in individual patients always took place at the same time and on the same day of each week. All the children remained in the same environment during the study. The primary end points were the following: the efficacy assessed based on the reduction in symptom-medication score and in symptoms (nasal, ocular, asthma) and medication use separately. Secondary end points included lung function, exhaled nitric oxide (NO) concentration, methacholine bronchial provocation test (MBPT), and the percentage of T-regulatory FOXP3+ cells in peripheral blood. The safety was estimated based on adverse symptoms.

Treatment

The SLIT drops group was treated with Staloral 300 IR as a standardized extract of 5-grass pollen (*Dactylis glomerata*, *Anthoxanthum odoratum*, *Lolium perenne*, *Poa pratensis*, *Phleum pratense*) (Stallergenes SA, Antony, France). The mean concentration of group 5 allergens (Dact g5, Antx o5, Lol p5, Poa p5, Phl p5) in the extract was 25 µg/mL. On an average of 8 weeks before the pollen season (2009), all the children received immunotherapy by following a recommended induction scheme: 1 drop on the first day, followed by 3, 5, 7, and 12 drops (4 puffs) on each consecutive day. In the maintenance phase every morning before breakfast, all the patients received 4 puffs from a dispenser (10 µg of the major allergens) for 6 months. It was then followed by a second induction phase (in March 2010) with SLIT drops.

The SLIT tablet group was treated once daily with Oralair 300 IR tablet as a standardized extract of the 5-grass pollen (*D. glomerata*, *A. odoratum*, *L. perenne*, *P. pratensis*, *P. pratense*) (Stallergenes); the mean concentration of group 5 allergens in the tablet was 25 µg/mL daily. The first SLIT dose (100 IR) was administered at the physician's office by following the manufacturer's recommendations and the overall favorable safety pro-

file shown in previous studies.^{11,12} All subsequent doses of Oralair were self-administered by the children or their guardians at home as follows: second dose 200 IR and the rest as consecutive daily doses of 300 IR. Every morning before breakfast, all the patients received Oralair. Each tablet was placed in an empty mouth, under the tongue, held until complete dissolution (at least 1 minute), and then swallowed. The children were treated at least 8 weeks before the grass pollen season and throughout the entire season for a total of at least 20 weeks.

In both studies, to assess adherence to the treatment, all the subjects were asked to bring all empty medication packages to the last visit. The onset of the pollen season in both studies was defined as the first of 3 consecutive days with grass pollen counts of ≥ 10 grains/m³ and its ending as the last day before 3 consecutive days with a grass pollen count of < 10 grains/m³. Pollen count was performed throughout the study for the region of interest.¹³ During the pollen season, all the children received, at baseline, the standard treatments of the allergic rhinoconjunctivitis: systemic antihistamines. Other permissible treatments included nasal steroids, local antihistamines, or cromones for eyes, inhaled corticosteroids in minimal doses necessary to control the symptoms, short-acting β_2 -agonist as a quick reliever, and the standard treatments of the infections and exacerbations of asthma.

Symptom-Medication Score

A combined symptom-medication score was calculated as a sum of the symptom score and the medication score recorded by the patients on a daily basis during the season from March until August. The symptom score was recorded with the use of a point score system for the most common symptoms of rhinitis (rhinorrhea, sneezing, itching, and nasal congestion), conjunctivitis (ocular pruritus, watery eyes, and itching), and respiratory disorders (cough, wheeze, and dyspnea). In addition, the amount of as-needed β_2 -agonist (salbutamol) was recorded daily as the number of puffs. A score that ranged 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); and 3, severe symptoms (symptoms hard to tolerate and that cause 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). In addition, every use of medication was registered (maximum 1 point daily).¹⁴ The two scores (symptom and medication) were added together to get the combined score.

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.¹⁵ Flow-volume curves were performed according to American Thoracic Society standards.¹⁵ The highest of three successful measurements was taken. The results were expressed as the percentage of a predicted value. A reversibility test was performed after administration of salbutamol (200 μ g). The percentage of change from baseline in forced expiratory volume in 1 second (FEV₁), prebronchodilatory FEV₁, and peak expiratory flow were included in the analysis.

NO Measurement

The NO measurements were performed according to European Respiratory Society/American Thoracic Society recommendations,¹⁶ with a chemiluminescence analyzer (model 280i NO analyzer; Sievers Biotech, Boulder, CO) and defined in parts per billion. The analyzer provides an on-line continuous measurement of NO in a single exhalation, with a detection range of 0.1–500 ppb. The environmental NO level was measured before and after each test, and never exceeded 5 ppb. All the subjects were studied while they were in the sitting position and without wearing a nose clip. The subjects exhaled at a constant flow rate (50 mL/s) from the 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 concentrations from the lower respiratory tract (determined by the plateau value after the peak) were recorded automatically by using the manufacturer's software. Three fractional exhaled NO (FeNO) measurements of the plateau phase were obtained, with $< 10\%$ variation. The mean value of three successive, reproducible recordings was retained for statistical analysis.

MBPT

MBPT was performed at the beginning of the study, before immunotherapy, and after the first year of SLIT by using the dosimeter technique APS Pro (Erich Jaeger) with controlled tidal breathing. After the pulverization physiologic diluent, methacholine was delivered in four cumulative doses: 0.015, 0.045, 0.18, and 0.72 mg. MBPT was continued with 2-minute intervals between the inhalations until a fall in FEV₁ of $\geq 20\%$ was obtained. Provocation dose causing a 20% decline

Table 1 Clinical characteristics of study groups

	SLIT Tablet Group (n = 24)			SLIT Drops Group (n = 17)			p Value*
	Median	Lower Quartile	Upper Quartile	Median	Lower Quartile	Upper Quartile	
Conjunctivitis score	13.3	9.7	26.9	9.0	4.5	18.0	0.1349
Rhinitis score	16.6	9.1	31.8	16.3	14.2	23.7	0.9472
Drug consumption score	3.8	2.1	8.2	3.5	1.3	4.2	0.2607
Combined symptoms-medication score	52.8	36.4	79.4	33.8	28.3	46.0	0.1036
FeNO value, ppb	23.2	17.5	30.7	26.7#	16.3#	46.8#	0.2878
FoxP3 cells, %	7.2	6.2	11.0	9.3§	6.5§	13.5§	0.3967

SLIT = Sublingual immunotherapy; FeNO = fractional exhaled nitric oxide; FOXP3 = forkhead box P3.

*Mann-Whitney test.

#Based on n = 16.

§Based on n = 14.

in FEV₁ was calculated by linear interpolation on a log dose-response curve.¹⁷

T-Regulatory Cell Induction

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by density gradient centrifugation by using an Histopaque-1077 (Sigma-Aldrich, St. Louis, MO). CD4, CD25, and FOXP3 expression on PBMCs was estimated by flow cytometry (LSRII instrument; BD Biosciences, San Jose, CA) by using appropriate fluorophore-conjugated monoclonal antibodies. PBMCs were surface stained with antihuman CD4 fluorescein isothiocyanate and antihuman CD25 Antibody Conjugation Kit, then fixed and permeabilized by using the FOXP3 staining buffers and, subsequently, stained with rat immunoglobulin G2a κ -isotype control phycoerythrin or antihuman FOXP3 phycoerythrin. The cells in the lymphocyte gate were used for analysis.

Safety

Before the inclusion in the study and during each consecutive visit, an interview and a physical examination were performed. The physician assessed the changes in asthma scores, drug use, and the lung function test in comparison with the baseline. All adverse events were recorded at each visit.

Sample Size and Statistics

The sample sizes in both trials were determined based on previously published results for symptom-medication scores.¹⁸ Both analyses of the effect of SLIT were performed on an intention-to-treat basis according to the randomized treatment allocation. By using an expected standardized difference 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 grass pollen season. This estimation included a 20% dropout rate. In the first step, the symptom and medication scores were adjusted to the pollen count in the season. Next, the study end points were compared within and among the treatment groups by the analysis of variance for the repeated measurement design. The χ^2 test was used for categorical data. A p value of <0.05 (two-tailed) was regarded as statistically significant.

RESULTS

Subjects

Forty-one children were included in the analyses. One child from the drops group did not perform FeNO measurements and, in three children, blood collection was not possible. The mean specific IgE for the tablet group was 21.2 kU/L and for drops group was 28.6 kU/L. The mean skin prick test (SPT) diameter for patients in “tablet” group was 5 mm and for the drops group was 6 mm. Baseline characteristics of the subjects who completed the study are shown in Table 1.

Symptom Scores and Combined Symptom-Medication Score

Diaries were available for evaluations for the whole grass pollen season. The pollen count was performed throughout both studies for the region of interest and was comparable in both seasons (Fig. 1). Receiver operating characteristic curve analysis showed a lack of difference between both seasons in the area under the curve of natural allergen exposition ($p = 0.2297$). Then, the mean symptom-medication score was then adjusted to 1000 pollen grains/m³ and recorded during both grass pollen seasons.^{6,7} The adjustment was cal-

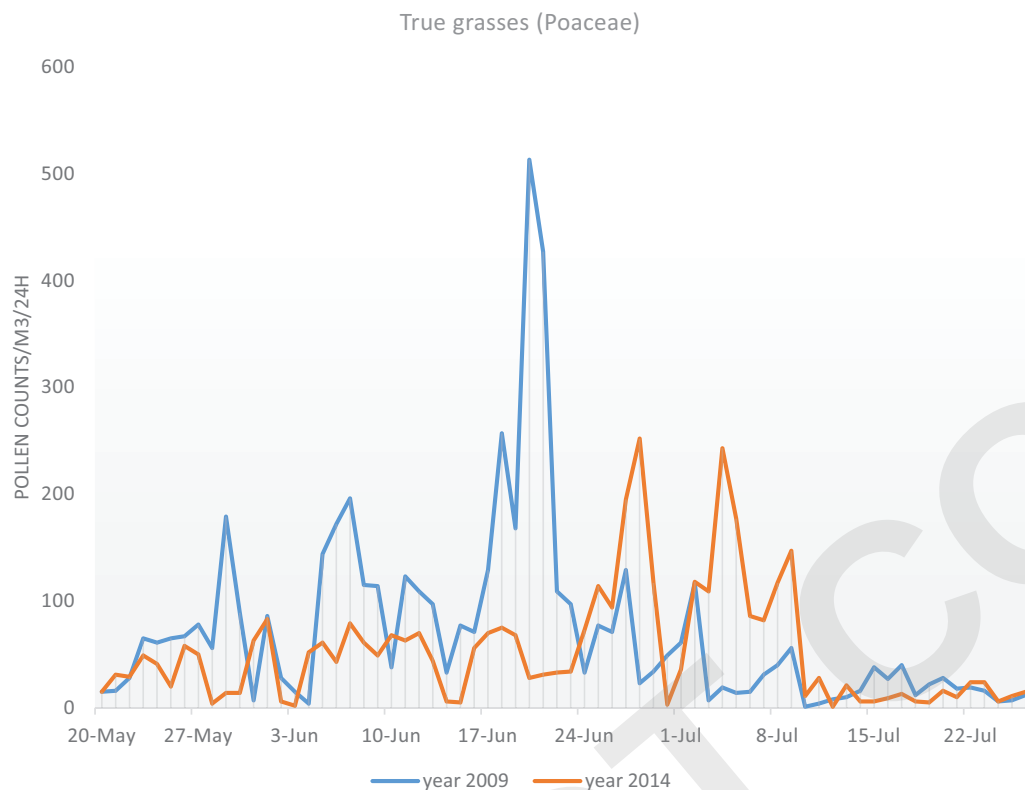


Figure 1. True grass (Family: Poaceae) pollen counts per m³ per 24 hours in both seasons. Receiver operating characteristic (ROC) curve analysis showed a lack of difference between both seasons in the area under the curve of natural allergen exposition ($p = 0.2297$).

culated according to the same following rule: the total of ([seasonal score]/[cumulative concentration of grass pollen during the season]) $\times 1000$. Therapy treatment with tablets and drops significantly reduced all symptoms (nasal, asthma, and ocular) within the groups; there was no significant difference between both groups (Fig. 2). When compared with the tablet therapy, the drops therapy was more effective in the reduction of the combined symptom-medication score, but the difference was not statistically significant ($p = 0.1036$).

Lung Function and Bronchial Provocation Test

There were no statistical differences in methacholine bronchial test and FEV₁ between the tablet and the drops groups at the beginning of each study. We did not observe any significant changes in FEV₁ and provocation dose causing a 20% decline in FEV₁ in either of the groups after SLIT (when compared with the values from the beginning of the studies).

NO Measurement

There was a significant decrease of the in-season FeNO level in both groups in comparison with the placebo. There were no significant changes in the FeNO level between both groups (Fig. 2).

T-Regulatory Cell Induction

After the first season of SLIT, significant increases in the percentage of FOXP3 expression in CD4 CD25–

positive cells in peripheral blood were observed in both study groups (Fig. 2). We observed more significant increases in the percentage of FOXP3 expression in CD4 CD25–positive cells in peripheral blood in the drops group; however, there were no statistical differences among the groups.

Safety

In both trials, there were predominantly local reactions, *e.g.*, sublingual itching. Other adverse events possibly associated with the treatment were headaches and stomachaches. There was no difference between both groups in the number of adverse reactions. There were no cases of termination of the study due to adverse events in any of the groups. Adherence was the same for all protocols.

DISCUSSION

The primary outcome of our study was to find any differences in the symptom-medication score between two forms of SLIT, tablets and drops, given pre-seasonally in children with rhinoconjunctivitis allergy to grass pollen. The secondary outcomes were the differences in lung function and induction of T-regulatory FOXP3+ cells. Analysis of our data indicated that, when compared with drops, the tablets were no more effective in the reduction of separate symptoms; however, when compared with the tablet therapy, the drops therapy was more effective in the reduction of the combined symptom-medication score, but the dif-

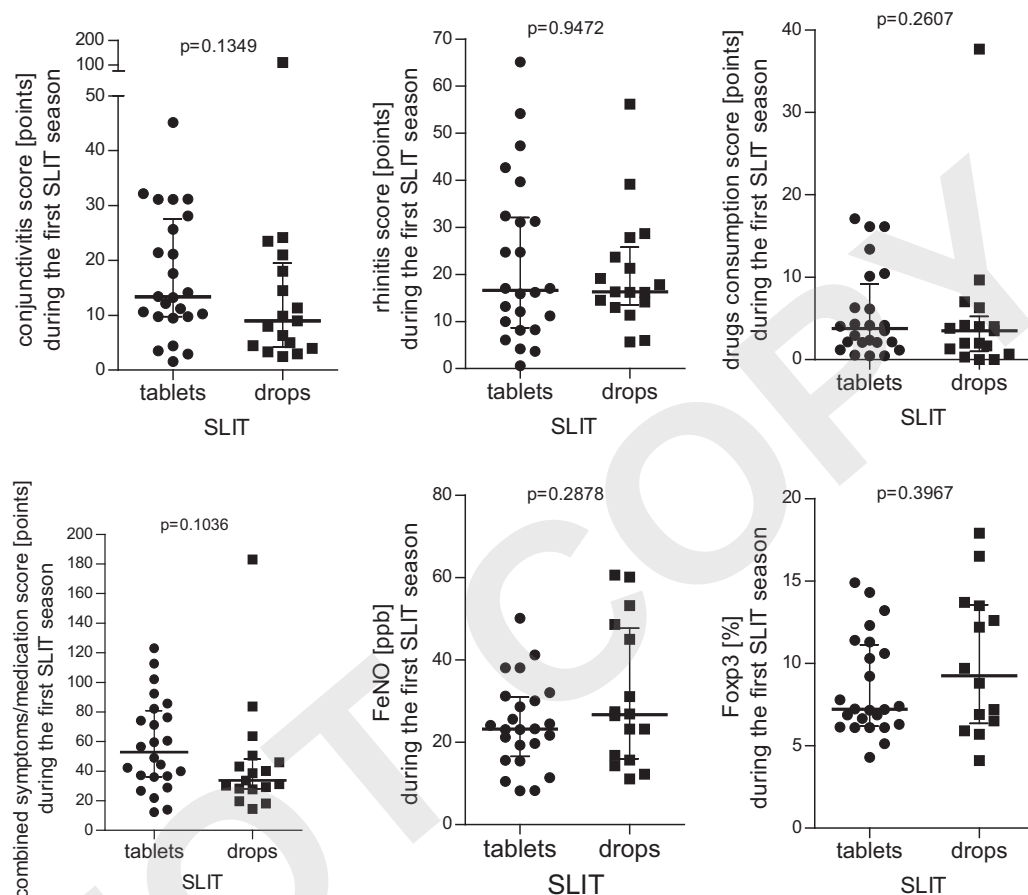


Figure 2. Changes in nasal, ocular, medication, and combined symptom-medication scores in fractional exhaled nitric oxide (FeNO) and forkhead box P3 (FOXP3) between the study groups. The scores were adjusted to 1000 per m^3 according to the following rule: $[(\text{total season score})/(\text{cumulative concentration of grass pollen during the season})] \times 1000$. Data are presented as median for the group.

ference was not statistically significant. We did not observe any significant changes in FEV₁, FeNO, and MBPT values among the groups throughout the study. Interestingly, although we observed more significant increases in the percentage of FOXP3 expression in CD4 CD25-positive cells in peripheral blood in the drops group, there were no differences between the groups.

Recent studies documented the efficacy and safety of SLIT in pediatric patients with rhinitis.^{19,20} 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 comparisons 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.²¹ In our study, both immunotherapy treatments, the drops and the tablet, resulted in similar clinical and immunologic improvement. In our comparison, drops therapy was more effective in the reduction of the combined symptom-medication score, but the difference was not statistically significant.

Because our study was not designed to assess the noninferiority of the drops when compared with the tablet immunotherapy, the conclusion on the lack of differences could have been underpowered. However, we did find that the tablet regimen was more effective than the drops treatment in the reduction of one of the major end points. Although some studies confirmed that the tablet is more potent, in our study, the differences were not statistically significant.²² Therefore, the clinical implication of our study that indicated that both regimens should be equally recommended for treatment of allergic rhinitis in children was justified.

Because both symptoms and medication use are two linked variables, a combined symptom-medication score is the preferred primary efficacy variable. However, some trials use only the symptom score as their main outcome. Another variable that was similar in both trials was the definition of the pollen season; we defined the start of the pollen season in both trials as the presence of 3 consecutive days with a pollen count of 10 grains/ m^3 . This was important for the outcome of the trials because it directly affected the symptom score difference between both treatments. Although both forms were effective in reducing total symptoms and medication scores, the drops dose was more so. Treat-

ment was started 8 weeks before the season and continued throughout the season, for an average of 38 weeks in both trials. As for safety, in the tablet trial, similar results were found to those in the drops trial.

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.²³ It was previously shown that T-regulatory FOXP3+ cells are induced locally in mucosa during effective SLIT and that this effect is at least partially involved in the mechanism of SLIT.^{24–26} It is also possible that SLIT induces a significant increase in messenger RNA expression of FOXP3 in PBMC; however, this effect was shown in a single trial and was marginal compared with placebo.²⁷ In our study, the induction of peripheral FOXP3 cells in both immunotherapy groups was comparable, slightly higher in the drops group. The results of the secondary end points, *e.g.*, lung function, revealed no differences between the treatment groups. The decrease of in-season FeNO value contradicted the abovementioned general conclusion on secondary end points and showed a positive impact on lower airways inflammation of the immunotherapy treatments.

For ethical reasons, methacholine challenge was performed outside the pollen season in both trials. Nevertheless, we recognized the assessment of bronchial hyperresponsiveness just after the pollen season as a valuable study end point. The main limitation of the study was the failure to achieve the calculated sample size due to a high dropout rate, with fewer patients than expected to detect differences in symptoms and medication scores. Therefore, the limitation in both trials was the small number of subjects. However, the sample size was determined based on the recommendations to reach the necessary power and limit the administration of placebo to children with symptoms, which is always a matter of ethical debate.²⁸

The other limitation of the study was that there was only 1 year of follow-up. The other limitation was that both studies were in two different pollen seasons, which could be an additional problem. However, the pollen count was performed throughout both studies for the same region and was comparable in both seasons. Fig. 1 compares the pollen count in two seasons. The receiver operating characteristic curve analysis showed a lack of difference between both seasons in the area under the curve of natural allergen exposition ($p = 0.2297$). Due to differences between the allergen immunotherapy trials in patient populations, data analysis, comparisons between products with existing data (different daily dose of allergens), a lack of placebo in one of the trials was not possible nor valid. However, the evidence available about the efficacy and safety in our trials could be compared because both trials had the same design, the same quality, details in the administration schedules, and safety issues.

CONCLUSION

SLIT, especially in childhood, offered practical advantages over SCIT because injections could be avoided, the treatment was generally well tolerated, and the risk of severe allergic reactions seemed to be negligible. Further details on the comparative efficacy of tablets and drops would only be possible if both were evaluated in the same, adequately powered trial.

ACKNOWLEDGMENTS

The pollen count was provided by M.L. Kowalski, Ph.D., and B. Wojciechowska, Ph.D., Department of Immunology, Rheumatology and Allergy, Medical University of Lodz, Lodz, Poland.

REFERENCES

1. Canonica GW, Cox L, Pawankar R, et al. Sublingual immunotherapy: World Allergy Organization position paper 2013 update. *World Allergy Organ J*. 2014; 7:6.
2. Durham SR. Sublingual immunotherapy: What have we learnt from the 'big trials'? *Curr Opin Allergy Clin Immunol*. 2008; 8:577–584.
3. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: Systematic review and meta-analysis. *Allergy*. 2005; 60:4–12.
4. Radulovic S, Calderon MA, Wilson D, Durham S. Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst Rev*. 2010; CD002893.
5. Serra HM, Alignani DO, Passalacqua G, Canonica GW, Correa SG. What do we need to learn to optimize the SLIT alternative. *Eur Ann Allergy Clin Immunol*. 2006; 38:158–165.
6. Jerzynska J, Stelmach W, Balcerak J, et al. Effect of *Lactobacillus rhamnosus* GG and vitamin D supplementation on the immunologic effectiveness of grass-specific sublingual immunotherapy in children with allergy. *Allergy Asthma Proc*. 2016; 37:324–334.
7. Stelmach I, Kaluzińska-Parzyszek I, Jerzynska J, Stelmach P, Stelmach W, Majak P. Comparative effect of pre-coseasonal and continuous grass sublingual immunotherapy in children. *Allergy*. 2012; 67:312–320.
8. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. Available at www.ginasthma.com/download.asp?intId=345; accessed 2011.
9. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008. *Allergy*. 2008; 63(suppl. 86):8–160.
10. Alvarez-Cuesta E, Bousquet J, Canonica GW, et al. Standards for practical allergen-specific immunotherapy. *Allergy*. 2006; 61(suppl. 82):1–20.
11. Nelson HS, Nolte H, Creticos P, Maloney J, Wu J, Bernstein DI. Efficacy and safety of timothy grass allergy immunotherapy tablet treatment in North American adults. *J Allergy Clin Immunol*. 2011; 127:72–80, 80.e1–e2.
12. Blaiss M, Maloney J, Nolte H, Gawchik S, Yao R, Skoner DP. Efficacy and safety of timothy grass allergy immunotherapy tablets in North American children and adolescents. *J Allergy Clin Immunol*. 2011; 127:64–71, 71.e1–e4. Erratum in *J Allergy Clin Immunol*. 2011; 128:436.
13. Klinika Immunologii, Reumatologii i Alergii. Available at www.umed.lodz.pl/KatedraImmunologii/; accessed September 30, 2008 and October 1, 2014
14. Wasserfallen JB, Gold K, Schulman KA, Baraniuk JN. Development and validation of a rhinoconjunctivitis and asthma symptom score for use as an outcome measure in clinical trials. *J Allergy Clin Immunol*. 1997; 100:16–22.

15. Miller MR, Hankinson J, Brusasco V, et al. ATS/ERS TaskForce. Standardisation of spirometry. *Eur Respir J*. 2005; 26:319–338.
16. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med*. 1999; 160:2104–2117.
17. Valovirta E, Jacobsen L, Ljorring C, Koivikko A, Savolainen J. Clinical efficacy and safety of sublingual immunotherapy with tree pollen extract in children. *Allergy*. 2006; 61:1177–1183.
18. Penagos M, Passalacqua G, Compalati E, et al. Metaanalysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. *Chest*. 2008; 133:599–609.
19. Penagos M, Compalati E, Tarantini F, et al. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3 to 18 years of age: A meta-analysis of randomized, placebo-controlled, double-blind trials. *Ann Allergy Asthma Immunol*. 2006; 97:141–148.
20. Stelmach I, Kaczmarek-Wozniak J, Majak P, Olszowiec-Chlebna M, Jerzynska. Efficacy and safety of high-doses sublingual immunotherapy in ultra-rush scheme in children allergic to grass pollen. *Clin Exp Allergy*. 2009; 39:401–408.
21. Lombardi C, Incorvaia C, Braga M, Senna G, Canonica GW, Passalacqua G. Administration regimens for sublingual immunotherapy to pollen allergens: What do we know? *Allergy*. 2009; 64:849–854.
22. Didier A, Malling HJ, Worm M, Horak F, Jäger S, Montagut A, et al. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. *J Allergy Clin Immunol*. 2007; 120:1338–1345.
23. Xystrakis E, Urry Z, Hawrylowicz CM. Regulatory T cell therapy as individualized medicine for asthma and allergy. *Curr Opin Allergy Clin Immunol*. 2007; 7:535–541.
24. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. *J Allergy Clin Immunol*. 2008; 121:1467–1472, 1472.e1.
25. Scadding G, Durham S. Mechanisms of sublingual immunotherapy. *J Asthma*. 2009; 46:322–334.
26. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2011; 127:18–27; quiz 28–29.
27. Nieminen K, Valovirta E, Savolainen J. Clinical outcome and IL-17, IL-23, IL-27 and FOXP3 expression in peripheral blood mononuclear cells of pollen-allergic children during sublingual immunotherapy. *Pediatr Allergy Immunol*. 2010; 21(pt. 2): e174–e184.
28. Bousquet J, Schünemann HJ, Bousquet PJ, Bachert C, Canonica GW, Casale TB, et al. How to design and evaluate randomized controlled trials in immunotherapy for allergic rhinitis: An ARIA-GA(2) LEN statement. *Allergy*. 2011; 66:765–774. □

Copyright of Allergy & Asthma Proceedings is the property of OceanSide Publications Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.