Perennial is more effective than preseasonal subcutaneous immunotherapy in the treatment of seasonal allergic rhinoconjunctivitis

Damian Tworek, M.D., Ph.D.,[1] Malgorzata Bochenska-Marciniak, M.D., Ph.D.,[2] Izabela Kuprys-Lipinska, M.D., Ph.D.,[3] Maciej Kupczyk, M.D., Ph.D.,[3] and Piotr Kuna, M.D., Ph.D.[3]

ABSTRACT

Background: *Two different regimens of subcutaneous immunotherapy (IT), perennial or preseasonal, may be used in the treatment of seasonal allergy. The aim of this study was to compare the efficacy and safety of perennial IT (PIT) and preseasonal IT (PSIT) in patients suffering from seasonal rhinoconjunctivitis.*

Methods: *The study was planned as a randomized, double-blind, comparative study on the efficacy and safety of PIT and PSIT. The study group comprised 120 patients allergic to grass and rye pollen. After the observational season they were randomized to receive PIT or PSIT for 3 years. The effect of IT was assessed based on symptom severity and medication use recorded in diaries.*

Results: *Ninety-nine patients completed the study. No difference was seen between the groups regarding combined symptom medication score (SMS) in the first season of IT. During the second season, the difference between PIT and PSIT regarding combined SMS was 27.9% ($p = 0.063$) and reached 42.7% ($p = 0.012$) in favor of PIT in the third season. Both treatments had a similar safety profile.*

Conclusion: *PIT was more effective than PSIT in the treatment of rhinoconjunctivitis in patients allergic to grass and rye pollens. Clinicaltrials.gov registration number NCT01555736.*

(Am J Rhinol Allergy 27, 304–308, 2013; doi: 10.2500/ajra.2013.27.3935)

Allergen-specific immunotherapy (IT) was introduced in 1911 by Noon who was the first to report a successful application of grass pollen extract in patients with seasonal allergy.[1] Today, IT is considered to be the only disease-modifying treatment for allergic diseases such as allergic rhinoconjunctivitis and allergic asthma. It is highly effective in controlling allergy symptoms and reveals long-term clinical effects even years after cessation of IT.[2] Long-term clinical benefit of IT can be observed 6 and even 12 years after discontinuation of treatment.[3,4] Furthermore, IT can modify the course of the allergy—allergen vaccination, decreases the risk of new sensitizations and the risk of asthma development in patients suffering from allergic rhinitis.[2,5]

IT with seasonal allergens may be performed with one of two regimens: preseasonal IT (PSIT) or perennial IT (PIT). PSIT is a periodical administration of a vaccine only before the pollen season. PIT consists of an induction phase with increasing dose, followed by a maintenance phase through the rest of the course of IT. As a result, in the first method, the patient undergoes an induction phase once every treatment year before the pollen season, and in the second method, only once in the 1st year of IT, continuing the maintenance phase up to the end of treatment. So far, it is unknown which of these two methods is more effective and safer because there has been no study directly comparing subcutaneous PIT and PSIT.

Therefore, this study was intended to evaluate the efficacy and safety of grass and rye pollen allergoid preparation in subcutaneous PSIT versus PIT in patients suffering from seasonal allergic rhinoconjunctivitis triggered by grass and rye pollens.

From the [1]Divisiont of Internal Diseases, Asthma and Allergy, Barlicki University Hospital, Lodz, Poland, and Departments of [2]Pneumonolgy and Allergy and [3]Internal Diseases, Asthma and Allergy, Medical University of Lodz, Poland

P Kuna received fees for speaking and reimbursement for attending symposia from Allergopharma and Stallergens. I Kuprys-Lipinska received fees from Allergopharma for speaking. The remaining authors have no conflicts of interest to declare pertaining to this article

Address correspondence to Damian Tworek, M.D., Ph.D., Division of Internal Diseases, Asthma and Allergy, Kopcinskiego 22, 90-153 Lodz, Poland
E-mail address: damian.tworek@gmail.com

Published online April 29, 2013

Copyright © 2013, OceanSide Publications, Inc., U.S.A.

METHODS

Study Design

The study was planned as prospective, randomized, and double blind with two active arms: one arm being PSIT before the pollen season with subsequent placebo injections until next preseasonal phase, and the second arm being PIT during the 1st, 2nd and 3rd years of IT. The random assignment of subjects to groups at a ratio of 1:1 was performed externally by Allergopharma (the manufacturer of the study drug; Reinbeck, Germany). The personnel at the study site were blinded until all data-related queries had been resolved at the end of the study.

The study was performed from January 2003 to August 2006 and consisted of three phases: screening (January to April 2003), the baseline pollen season between May and July 2003, and 3 years of active treatment with subcutaneous IT (from 2004 to 2006 with efficacy data collected from May to July). The study flowchart is presented in Fig. 1.

The study protocol was approved by the Medical University of Lodz Ethics Committee (RNN/124/02/KE) and the trial was conducted in accordance with Good Clinical Practice. The study was registered with Clinicaltrials.gov (registration number NCT01555736). The study was planned and self-funded by the investigator. The contribution of the pharmaceutical company (Allergopharma) and drug distributor (Nexter, Katowice, Poland) was limited to the supply of free study drugs and matched placebo, blinding procedures, providing pollen count data, and determination of specific immunoglobulin G4 (sIgG4) levels.

Study Group

The study group comprised 120 outpatients who had given their informed written consent. All were men and women aged 18–60 years suffering from allergic rhinoconjunctivitis triggered by grass and rye pollen. Inclusion criteria were

1. IgE-mediated seasonal allergic rhinitis with symptoms during the grass and rye pollen seasons (May, June, and July)
2. Symptoms of allergic rhinoconjunctivitis requiring medication during the last season
3. Positive skin-prick test to grass and rye pollen
4. For female patients effective contraception and negative pregnancy test results were necessary.

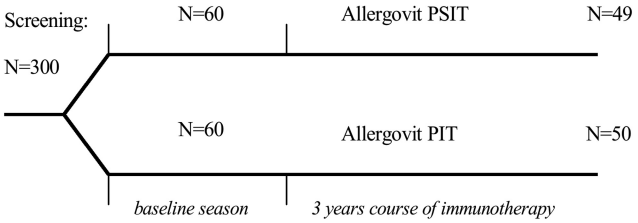

Figure 1. *Study flowchart. PSIT, preseasonal immunotherapy; PIT, perennial immunotherapy.*

Exclusion criteria were

1. Previous course of IT with grass and rye pollen extracts or allergens that were unknown during the previous 5 years
2. Forced expiratory volume in 1 second of <80% of predicted
3. Uncontrolled bronchial asthma according to the Global Initiative for Asthma
4. Nonallergic rhinoconjunctivitis
5. Severe acute or chronic diseases and/or severe inflammatory diseases
6. Autoimmune diseases, immunosuppression, and/or neoplastic diseases
7. Severe psychiatric and psychological disorders including alcohol or drug abuse
8. Contraindication for application of adrenaline
9. Treatment with β-blockers
10. Pregnancy or lactation period
11. Female patients seeking to become pregnant
12. Low compliance.

Skin-Prick Tests

Skin-prick tests were performed with 12 common aeroallergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, grass, rye, birch, hazel, alder, mugwort, cat, dog, *Alternaria tenuis*, and *Cladosporium herbarum* (Allergopharma). Histamine at a concentration of 1.7 mg/mL (Allergopharma) and standard glycerosaline solution (Allergopharma) were used, respectively, as positive and negative controls. A wheal diameter of ≥3 mm was considered as a positive result of the test.

Study Drugs. IT was performed with the allergoid preparation Allergovit (Allergopharma), consisting of 80% grass pollen extract and 20% rye pollen extract. The vials contained 1000 therapeutic units (TU)/mL (vial A) and 10.000 TU/mL (vial B).

The placebo was blinded by using histamine dihydrochloride (0.125 mg/mL). The placebo and active drug were visually indistinguishable.

Patients used intranasal antihistamines, α-mimetics nasal spray, antihistamines and nedocromil eye drops, and oral antihistamines as the rescue medications during the pollen seasons. Antileukotrienes and nasal glucocorticosteroids were prohibited during the diary phase.

Immunotherapy

IT was started in February 2004. During the induction phase, the patients received four injections from vial A (0.1, 0.2, 0.4, and 0.8 mL) and three injections from vial B (0.15, 0.3, and 0.6 mL) at intervals from 1 to 2 weeks. After reaching the maintenance dose, patients in the PSIT arm received 0.6 mL of placebo at 4- to 6-week intervals, whereas patients in the PIT arm received 0.6 mL of allergoid vaccine at 4- to 6-week intervals. The induction phase was repeated in both groups in 2005 and 2006 starting in February. If necessary, because of clinical reasons and compliance, dosing and intervals between injections were modified by the physicians according to the manufacturer's recommendations. IT was performed in the outpatient clinic. All injections were subcutaneous in the upper side of the arm and were performed by nurses under the supervision of a physician after previous medical examination. Patients stayed in the clinic 30 minutes after the injections for safety reasons. The information about the adverse events was collected by physicians and nurses through the whole study and recorded in the patient documentation. Adverse events were classified according to the European Academy of Allergology and Clinical Immunology (EAACI) guidelines as local or systemic reactions (grades I, II, III, and IV).[6]

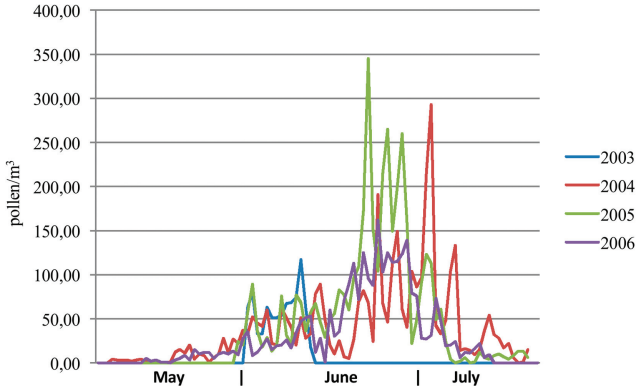

Figure 2. *Grass pollen count (pollen/m^3) during subsequent years of the study.*

Evaluation of the Effectiveness of the Therapies

The primary end point was a difference between PIT and PSIT with regard to the area under curve (AUC) values of combined symptom medication score (SMS), calculated as a sum of rhinoconjunctivitis symptoms and use of rescue medications during subsequent seasons of IT.[7] Secondary end points were the frequency and type of adverse reactions during IT, as well as the differences from the baseline and between study groups in mean daily eye, nose, total rhinoconjunctivitis symptoms score and SMS, and in serum level of sIgG4. The data concerning symptoms of rhinoconjunctivitis and antiallergic medication consumption were collected using diaries during the pollen seasons.

The severity of symptoms in the diaries was scored as follows: 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, and 3 = severe symptoms separately for eyes (itching, tear flow, and redness) and nose (sneezing, running nose, and blockage). The drug intake was recorded using generic name and number of tablets, drops, or puffs. Medication usage was scored as follows: intranasal antihistamines or α-mimetics nasal spray, 0.5/puff; antihistamines or nedocromil eye drops, 0.5/drop; and oral antihistamines, 6 per 10 mg.

The level of grass pollen was monitored through the whole period of the study from the beginning of May to the end of July. The pollen concentration was assessed by a "Burkard Pollentrap" and the pollen species were identified microscopically. The data were received thanks to Allergopharma (Fig. 2).

Specific IgG4

Blood samples for serum specimens were collected at the peak of each pollen season. The serum was stored at −20°C for further sIgG4 levels determination by Allergopharma.

Statistical Analysis

Data are analyzed using Statistica 9.1 (StatSoft, Tulusa, OK) with the Student's *t*-test for paired data and Wilcoxon test (comparisons within groups) and Student's *t*-test and Mann-Whitney *U* test (comparisons between groups) when appropriate. Normality of distribution was analyzed with the Shapiro-Wilk test. Frequencies were compared using the χ^2 or exact Fisher test, where appropriate. The SMS values were analyzed using one-way ANOVA. The percentage of difference in SMS values between PIT and PSIT was calculated as a difference in AUC: (PIT-PSIT)/PSIT × 100. AUCs were calculated using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA).

Table 1 **The demographic and clinical characteristic of the study groups for the PP and ITT population**

	PSIT	PIT
PP		
All subjects	49	50
Male subjects, no. (%)	25 (51%)	23 (46%)
Age (yr)	23.59 ± 5.87	25.07 ± 7.69
Duration of rhinoconjunctivitis (yr)	7.4 ± 5.22	8.37 ± 3.35
ITT		
All subjects	60	60
Male subjects, no. (%)	28 (47%)	27 (45%)
Age (yr)	24.72 ± 6.21	25.87 ± 4.74
Duration of rhinoconjunctivitis (yr)	7.64 ± 6.01	8.02 ± 2.65

PP = per protocol; ITT = intention-to-treat; PSIT = preseasonal immunotherapy; PIT = perennial immunotherapy.

A value of $p < 0.05$ was considered statistically significant. A 20% difference between study groups regarding the AUC of SMS was accepted as clinically significant.

RESULTS

Characteristic of the Study Population

The study groups did not differ significantly when compared in analysis per protocol or intention to treat. The demographic and clinical characteristic of the study groups is presented in Table 1. None of the participants had previously undergone any IT course.

Eleven patients in the PSIT group and 10 patients in the PIT group were withdrawn from the study after randomization. There were no discontinuations due to adverse reactions or lack of efficacy of IT. The reasons for dropouts were lost to follow-up (18 patients) and withdrawal of the consent (3 patients).

Combined SMS

The SMS did not differ significantly between PIT and PSIT groups at baseline (307.36 ± 132.61 versus 316.76 ± 117.27; $p = 0.58$). Both treatments reduced SMS values compared with baseline beginning with the 1st year of IT ($p < 0.0001$). The reduction from the baseline SMS for PIT and PSIT was 39.77 and 40.84% in the 1st year, 59.37 and 45.49% in the 2nd year, and 72.56 and 55.76% in the 3rd year, respectively.

The differences in AUC of combined SMS during baseline and first season of IT were not clinically or statistically significant. During the 2nd season the difference was 27.9%, in favor of PIT ($p = 0.063$). In the third season of IT the difference between PIT and PSIT reached 42.7% and became statistically significant ($p = 0.012$; Fig. 3).

Cumulative Dose and Correlation with Improvement in SMS

The cumulative dose in the PIT group was significantly higher compared with the PSIT group ($p < 0.001$), and after the 3rd year of IT reached 157,656.5 ± 26,028.74 TU (range, 112,300–214,800 TU). The cumulative dose in the PSIT group after 3rd year of IT averaged 66,704.00 ± 13,858.00 TU (range, 37,500–84,000 TU).

The strong significant correlation between cumulative dose and improvement in SMS was detected in the 3rd year of IT ($R = 0.53$; $p < 0.001$).

Mean Daily Nasal Symptoms Score

IT effectively reduced nasal symptoms in both study groups from the 1st year of IT: 40.3 and 41.5% reductions from the baseline in the PIT and the PSIT groups ($p < 0.0001$ for both analyses). The values reached a 73.7 and 53.4% decrease in the PIT and the PSIT group, respectively, in the in the 3rd year ($p < 0.0001$ for both analyses).

Mean daily nasal symptoms score did not differ between PIT an PSIT groups during baseline and first grass pollen season (1.32 ± 0.69 versus 1.47 ± 0.6; $p = 0.41$, and 0.74 ± 0.46 versus 0.78 ± 0.36, $p = 0.78$, respectively). However, during the second and third seasons, PIT was significantly more effective in ameliorating nasal symptoms (0.45 ± 0.33 versus 0.68 ± 0.32, $p = 0.01$, and 0.27 ± 0.15 versus 0.57 ± 0.3, $p < 0.001$, respectively).

Eye Symptoms Score

IT effectively diminished eye symptoms in both study groups starting from the 1st year of treatment: 42.1 and 45.9% reduction from the baseline in the PIT and the PSIT group ($p < 0.05$ in both analyses). The value reached 76.7 and 62.5% of reduction in PIT and PSIT groups, respectively, in the 3rd year ($p < 0.05$ in both analyses).

The mean daily eye symptom score did not differ between PIT and PSIT groups at baseline (1.04 ± 0.75 versus 1.06 ± 0.71, $p = 0.836$, respectively) during subsequent pollen seasons (first season, 0.63 ± 0.56 versus 0.53 ± 0.56, $p = 0.476$; second season, 0.39 ± 0.36 versus 0.53 ± 0.49, $p = 0.543$; third season, 0.19 ± 0.17 versus 0.37 ± 0.43, $p = 0.201$).

Total Rhinoconjunctivitis Score

The total rhinoconjunctivitis score did not differ significantly between PIT and PSIT groups at baseline (2.37 ± 1.36 versus 2.60 ± 1.17, $p = 0.49$). When the efficacy of IT was analyzed, a clinical improvement was seen in both study groups from the 1st year of IT and 42.05 and 44.91% of reduction in total rhinoconjunctivitis score from the baseline in PIT and PSIT, respectively ($p < 0.0001$ for both analyses). The efficacy reached a decrease in total rhinoconjunctivitis score of 76.42 and 57.11% in PIT and PSIT groups, respectively, in the 3rd year ($p < 0.0001$ for both analyses). PIT had a greater efficacy compared with PSIT in the 2nd ($p = 0.024$) and 3rd ($p = 0.001$) year of IT.

Medication Consumption

The mean daily medication use did not differ significantly between PIT and PSIT groups at baseline (0.98 ± 0.39 versus 0.90 ± 0.21; $p = 0.91$). Both treatments reduced the need for antiallergic medications compared with baseline starting from the 1st year of IT ($p < 0.05$). The reductions for PIT and PSIT were 30.12 and 25.82% in the 1st year, 51.79 and 38.13% in the 2nd year, and 61.71 and 49.52% in the 3rd year from the baseline usage, respectively. The mean daily medication use did not differ significantly between PIT and PSIT groups during the first (0.65 ± 0.37 versus 0.64 ± 0.29, $p = 0.628$, respectively) and second seasons (0.47 ± 0.32 versus 0.55 ± 0.32, $p = 0.381$, respectively). However, a significant difference between the PIT and PSIT groups was observed in the third pollen season (0.24 ± 0.13 versus 0.46 ± 0.24, $p = 0.026$, respectively).

Safety

During the whole period of the study, 5354 injections were performed, 2977 of them during induction phase.

All noted systemic adverse reactions were mild to moderate, grades I–II. The frequency and characteristics of adverse reactions are presented in Tables 2 and 3.

Specific IgG4

The difference in the level of sIgG4 between study groups was not statistically significant at baseline. A significant increase in the levels of sIgG4 compared with baseline was observed in both groups from the 1st year of IT ($p < 0.001$ for subsequent seasons in both groups). The 2nd year of IT there was a trend toward more pronounced production of IgG4 in the PIT group; however, the differences did not reach statistical significance (Fig. 4).

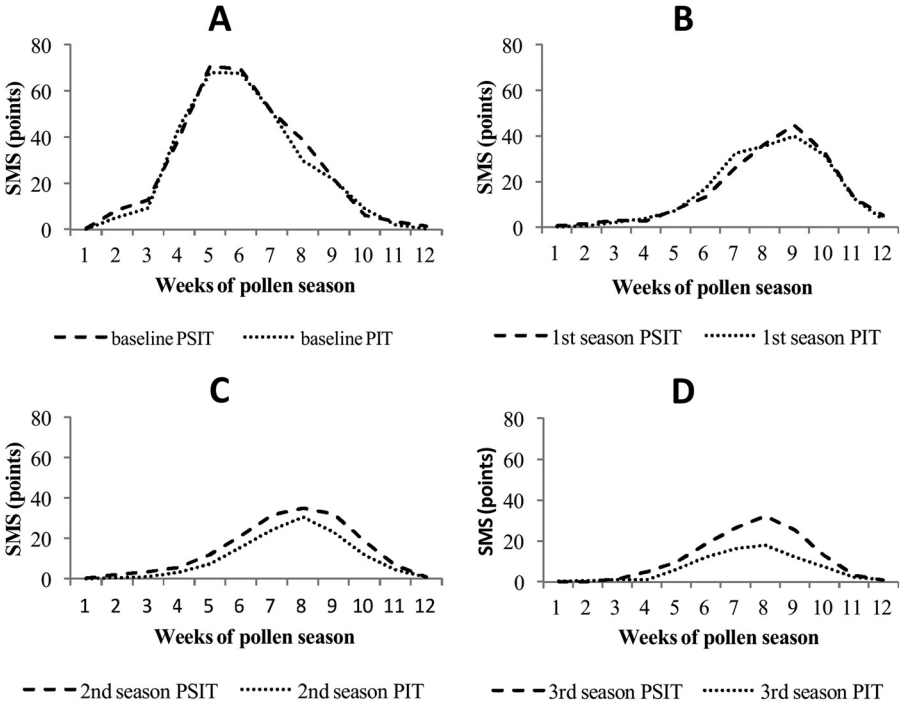

Figure 3. Combined symptom medication score (SMS) at (A) baseline and during the (B) first, (C) second, and (D) third pollen season. The difference in SMS in perennial immunotherapy (PIT) group compared with preseasonal immunotherapy (PSIT) were 27.9 and 42.7% during the second and third season of treatment, respectively.

Table 2 **Large local reactions and systemic reactions during subsequent years of IT presented in percent as the number of reaction per injection in the group**

	Large Local Reactions			Systemic Reactions		
	PSIT	PIT	*p* Value	PSIT	PIT	*p* Value
Induction phase						
1st yr	3.25%	3.68%	0.70	1.21%	0.97%	0.7
2nd yr	2.83%	1.63%	0.20	0.6%	0%	0.084
3rd yr	1.83%	0.39%	0.03	0%	0%	—
Maintenance phase (PSIT, placebo; PIT, active treatment)						
1st yr	0.74%	1.17%	0.52	0%	0.47%	0.17
2nd yr	0.47%	0.72%	0.65	0%	0.72%	0.15
3rd yr	0.55%	0.28%	0.58	0%	0%	—

PSIT = preseasonal immunotherapy; PIT=- perennial immunotherapy; IT = immunotherapy.

Table 3 **Charactersistics of systemic adverse reactions in PSIT and PIT groups**

	PSIT	PIT
Up-titration phase		
1st yr	Rhinoconjunctivitis (4) Mild asthma (1) Generalized acute urticaria (1)	Rhinoconjunctivitis (4) Mild asthma (1) Generalized acute urticaria (1) Localized urticaria (1)
2nd yr	Rhinoconjunctivitis (4) Mild asthma (1)	None
3rd yr	None	None
Maintenance phase (PSIT, placebo; PIT, active treatment)		
1st year	None	Mild rhinitis (1)
2nd year	None	Mild rhinitis (1) Generalized acute urticaria (1)
3rd year	None	None

The number of particular reactions is given in parentheses.
PSIT = preseasonal immunotherapy; PIT=- perennial immunotherapy.

DISCUSSION

The results of the study show that the PIT with grass and rye pollen has significantly better clinical effect than PSIT, as measured by SMS, and that this effect is related to the total dose of vaccine during the whole period of IT. The EAACI underlines the requirement of such studies in a task force report on dose–response relationship in allergen-specific IT.[8]

The main problem in the comparison of PIT and PSIT regimens was the procedure of blinding. The schema of the study is unique. In the preseasonal arm, the building-up phase alternates with the placebo phase, whereas in the perennial phase, the building-up and the maintenance phases alternate without a placebo phase. The repeating building-up phase in the further arm is somewhat artificial but it greatly simplifies the blinding procedure, without having a significant influence on the total dose of vaccine received by patients in the study. The adverse events were evaluated separately for building-up and maintenance phases, and it was concluded that the schema of the injections did not influence the safety results.

Both regimens of IT were clinically effective as shown by the reduction of allergic symptoms and usage of antiallergic medications beginning from the 1st year of the treatment. The reduction from baseline was statistically significant and >20% for severity of rhinoconjunctivitis symptoms as well as medication usage and SMS. The difference in clinical efficacy between regimens measured by SMS was seen in the 3rd year of IT and amounted to a 42.7% reduction of SMS in favor of the PIT group. The difference in improving symptoms appeared earlier, *i.e.*, in the 2nd year of IT. Lower consumption of medications was seen in the 3rd year of IT. The reason for this result is probably the higher cumulative dose of allergen administered to patients from the PIT group. This is confirmed by the strong correlation between the decrease of SMS and cumulative dose of allergen in the 3rd year of IT.

Because there are no objective parameters of IT efficacy, the evaluation was based on the clinical assessment. The active treatment was preceded by baseline period to received datum point for further evaluation. The

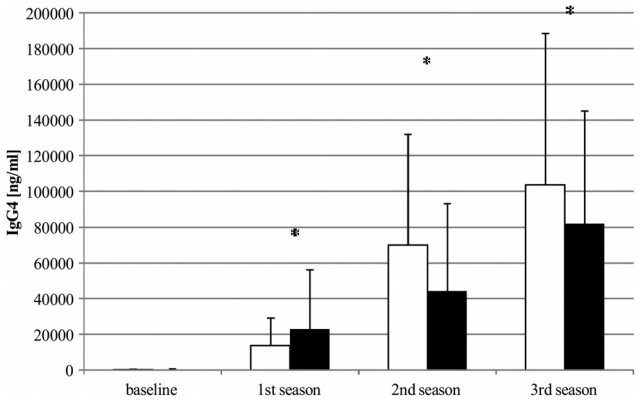

Figure 4. *Serum-specific immunoglobulin G4 (IgG_4) levels in perennial immunotherapy (PIT; white bars) and preseasonal immunotherapy (PSIT; black bars) groups. *Baseline versus subsequent seasons for both groups ($p < 0.001$).*

evaluation was performed according to everyday medical practice and based on clinical symptoms and medication usage. Patients completed the diaries and recorded the severity of symptoms and rescue medication usage *au courant* to maximize the accuracy and minimize the subjectivity of the evaluation. The criteria for clinical improvement were adopted form the Future of Allergists and Specific Immunotherapy-Workshop experts report.[9]

Frew *et al.* have published a study comparing the effectiveness of two maintenance doses of allergen vaccine (Alutard; ALK, Horsholm, Denmark) containing 10.000 and 100.000 s.q. in the treatment of allergic rhinoconjunctivitis in patients allergic to grass pollen.[10] The results of this study show that a high maintenance dose is more effective in the reduction of both allergy symptoms and medication consumption, as well as in the improvement of quality of life. Furin *et al.* showed that antigen-induced eosinophil migration into the nasal cavity induced by nasal allergen challenge, as well as during pollen season, is significantly lower when the maintenance dose of allergen vaccine is 24 μg compared with 2 μg.[11] A similar phenomenon was shown in a study with venom IT: an increase in the dose from 100 to 200 μg resulted in better efficacy.[12] Patients receiving higher maintenance doses of vaccine were found to have a lower level of serum sIgE for wasp venom and lower skin reactivity in intracutaneous test with this allergen.

The sIgG4 is a marker of allergen exposition and its level gradually increased in both groups over the course of IT. Despite the trend toward higher levels of IgG4 in the PIT group, the difference did not reach statistical significance in any year of IT. It is known from other studies that the levels of sIgG4 increase 10–100 times during IT but do not correlate with clinical effect.[13,14]

The safety of the medications is at least as important as their efficacy, especially in long-term therapy. There is always a risk of anaphylactic events over the course of IT because of administration of the allergen to sensitized individuals.[15] A greater number of injections and the administration of the allergen vaccines during the time of natural exposure during PIT may arouse concern regarding the increased frequency of adverse events. A number of studies showed that an increased dose leads to better efficacy but also more frequent adverse events. In the article mentioned previously by Frew *et al.*[10] the higher maintenance dose was shown to have better efficacy but also a higher incidence of both local and systemic adverse events. Similar to other reports, the adverse events occurred mainly during the induction phase.[16] In our study, the incidence of adverse events was approximately the same in both arms. Generally, the course of IT with grass and rye allergoid performed in both regimens was safe and well tolerated and there was no systemic grades III and IV adverse effects, according to the EAACI guidelines.

In conclusion, we found that PIT with allergoid preparation may be more effective than PSIT in ameliorating rhinoconjunctivitis symptoms, while maintaining a similar safety profile.

ACKNOWLEDGMENTS

The authors thank Dr. Joanna Nizio-Masior and Ireneusz Heczko from Allergopharma-Nexter Sp. z o. o., Poland, and Allergopharma Joachim Ganzer KG, Reinbek, Germany, for their support in supplying the study medication, blinding the IT preparations, performing sIgG4 immunoassays and for providing pollen counts. They thank Dr. Ewa Bogacka for help in the recruitment of patients. They thank all nurses, and, in particular, Katarzyna Czerwinska, M.Sc., Aneta Sawicka, M.Sc., and Elzbieta Karkowska from our Allergy Department, for their devotion and help. They also thank Dr. Monika Antczak-Marczak and Dr. Ewa Zielinska-Wyderkiewicz for their support during the trial.

REFERENCES

1. Larenas Linnemann DE. One hundred years of immunotherapy: Review of the first landmark studies. Allergy Asthma Proc. 33:122–128, 2012.
2. Möller C, Dreborg S, Ferdousi HA, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). J Allergy Clin Immunol 109:251–256, 2002.
3. Eng PA, Reinhold M, and Gnehm HP. Long-term efficacy of preseasonal grass pollen immunotherapy in children. Allergy 57:306–312, 2002.
4. Eng PA, Borer-Reinhold M, Heijnen IA, and Gnehm HP. Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. Allergy 61:198–201, 2006.
5. Pajno GB, Barberio G, De Luca F, et al. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. Clin Exp Allergy 31:1392–1397, 2001.
6. Alvarez-Cuesta E, Bousquet J, Canonica GW, et al.; EAACI, Immunotherapy Task Force. Standards for practical allergen-specific immunotherapy. Allergy 61(suppl 82):1–20, 2006.
7. Canonica GW, Baena-Cagnani CE, Bousquet J, ET AL. Recommendations for standardization of clinical trials with allergen specific immunotherapy for respiratory allergy: A statement of a World Allergy Organization (WAO) taskforce. Allergy 62:317–324, 2007.
8. Calderón MA, Larenas D, Kleine-Tebbe J, et al. European Academy of Allergy and Clinical Immunology task force report on 'dose-response relationship in allergen-specific immunotherapy.' Allergy 66:1345–1359, 2011.
9. Future of Allergists and Specific Immunotherapy—FASIT-Workshop 2008 Report. Matricardi P (Ed).
10. Frew AJ, Powell RJ, Corrigan CJ, and Durham SR; UK Immunotherapy Study Group. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 117:319–325, 2006.
11. Furin MJ, Norman PS, Creticos PS, et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. J Allergy Clin Immunol 88:27–32, 1991.
12. Glérant JC, Martinez P, Guillaume C, and Jounieaux V. Comparison of 2 maintenance doses (100 microg vs 200 microg) in Hymenoptera venom immunotherapy: Influence of the maintenance dose on the immunologic response. Ann Allergy Asthma Immunol 94:451–456, 2005.
13. Jutel M, and Akdis CA. Immunological mechanisms of allergen-specific immunotherapy. Allergy 66:725–732, 2011.
14. Jutel M, Jaeger L, Suck R, et al. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 116:608–613, 2005.
15. Lieberman P. The risk and management of anaphylaxis in the setting of immunotherapy. Am J Rhinol Allergy 26:469–474, 2012.
16. Walker SM, Varney VA, Gaga M, et al. Grass pollen immunotherapy: Efficacy and safety during a 4-year follow-up study. Allergy 50:405–413, 1995. □