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Efficacy of Sublingual Immunotherapy with *Dermatophagoides Pteronyssinus*: A Real-life Study

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ABSTRACT

Sublingual allergen immunotherapy (SLIT) is considered to be safer and more convenient than subcutaneous immunotherapy. SLIT trials with house dust mites involving patients with allergic rhinitis (AR) and asthma reported discordant results. The aim of the study was to investigate the clinical efficacy and safety of SLIT with *Dermatophagoides pteronyssinus* (*D.pt*) extract produced in Serbia and patient's satisfaction through open-label trial.

Adult patients with allergic rhinitis were randomized into two groups: one received drugs and SLIT, while other received only drugs. Symptom score (SS), medication score (MS) and cumulative score (CS), skin prick tests (SPT) and serum level of *D. pt* specific IgE were assessed. One year after, the patients were re-evaluated.

In total, 61 patients were enrolled in the study, but 52 of them were analyzed at the end of the year. CS (29.3%, $p < 0.001$) and MS (54.3%, $p < 0.05$) reduced significantly in the SLIT group. There was a significant improvement of MS and CS in the SLIT compared to control group ($p < 0.001$ and $p < 0.05$ respectively). There was no significant improvement of SS as well as specific sIgE. Patients in the SLIT group were more satisfied with treatment ($p < 0.001$). The incidence of mild adverse reaction was 38.4%. Specific IgG was not done.

One year SLIT with *D.pt* extract was clinically efficient treatment in AR patients.

Keywords: Allergic rhinitis; Asthma; *Dermatophagoides pteronyssinus*; Efficacy; Sublingual immunotherapy

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INTRODUCTION

Allergen immunotherapy (AIT) has been used for

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more than one century as a desensitizing therapy for immunoglobulin E (IgE) antibody (Ab) mediated allergic diseases and represents the only curative and preventive method of treatment. AIT is the practice of administering gradually increasing amounts of allergen(s), with the aim to achieve hyposensitization, which means, reducing the symptoms occurring during natural exposure to the allergen(s).¹

While pharmacotherapy reduces symptoms of allergic rhinitis (AR) and allergic asthma (AA), AIT is the only treatment offering potential long-term immune modification, reduces development of new sensitizations as well as progression from AR to AA.² Due to conventional subcutaneous immunotherapy (SCIT) safety concerns, multiple non-injection routes have been investigated. For the last 25 years sublingual immunotherapy (SLIT) has been the most promising treatment and has been accepted as a viable alternative to SCIT.³

Meta-analyses have confirmed that SLIT is an effective and safe therapy for AR^{4,5} and/or AA.^{6,7} Although SLIT appeared globally effective, the sub-analyses for seasonal and perennial allergens⁵ such as house dust mites (HDM) has been insufficiently documented^{1,3} and reported effects have varied greatly between studies.⁸ In Serbia, SLIT with allergenic extracts produced by the national manufacturer of allergenic extracts was introduced in 1995. To our knowledge no reported study evaluated efficacy and safety of *Dermatophagoides pteronyssinus* (*D. pt*) SLIT extract available in Serbia.

The aim of the present study was to evaluate efficacy and safety of SLIT with Serbian extract of *D. pt*, in adult AR patients with or without AA, as well as patient's subjective satisfaction of clinical improvement after one year of therapy.

MATERIAL AND METHODS

Study Design

Prospective, randomized, open-label, controlled, clinical trial took place at the Clinic of Allergy and Immunology, Clinical Center of Serbia (CCS) in Belgrade, Serbia, from November 2009 to December 2010. The adult patients with AR with or without AA, sensitized to *D. pt*, eligible to SLIT, were randomized by computer generated list, in two groups to receive: (i) SLIT (anti-allergic drugs plus SLIT) and (ii) control group (anti-allergic drugs only). All patients included in

the study gave their written informed consent. The study was approved by the CCS's Ethics Committee (No. 4687/10).

Patients and Diagnosis

Outpatients, having AR due to *D. pt* were enrolled in the study, according to ARIA⁹ and WAO¹ position paper guidelines for SLIT. The diagnosis of allergic respiratory diseases due to *D. pt* was made on the basis of clinical and immunological criteria, according to published Allergic Rhinitis and its Impact on Asthma (ARIA)⁹ and Global Initiative for Asthma (GINA) guidelines.¹⁰

Inclusion criteria were:

1. Clinical history of moderate to severe persistent AR with or without mild AA due to *D. pt*, in the past two years according to GINA criteria (10) with FEV1 being within normal limits (>80% of predicted value)
2. Aged between 16 and 65 years
3. Positive skin prick test (SPT) response and specific IgE (sIgE) Ab to *D. pt* (class ≥ 2).

Clinical history of all study patients sensitized to *D. pt* alone, or with concomitant sensitization to other inhalant allergens (except animal dander and molds), clearly indicated the relevance of HDM for the patient's symptoms.

The exclusion criteria were a history of previous courses of AIT, systemic or autoimmune diseases, psychiatric disorders, malignancies and pregnancy.

SPT was performed according to the published guideline⁹ with standard glycerinated extracts: HDM (*Dermatophagoides* spp.), cockroach, mold, pollens (tree, grass and weed) and animal dander (Institute of Virology, Vaccines and Sera TORLAK, Belgrade, Serbia). *D. pt* extracts used in the study were from the same batch. Histamine and saline solution were used as positive and negative controls, respectively. A drop of each allergen was placed on the forearm volar surface and penetrated with a separate lancet. After 15 minutes, the wheal reaction was measured as the mean value of the longest diameter and the diameter perpendicular to it. Reactions with diameter ≥ 3 mm were considered as positive.

Serum sIgE Ab to *D. pt* (d1, Phadia, Uppsala, Sweden) was assayed with an automated immunofluorimetric method (ImmunoCAP 100; Phadia, Uppsala, Sweden). Results were expressed as CAP scores from class 0 to 6, according to the

manufacturer's instruction (\geq class 2 was accepted as relevant).

SLIT and Concomitant Treatments

The prescribed SLIT was prepared as sublingual-swallow drops (SLITOR, Institute of Virology, Vaccines and Sera TORLAK, Belgrade, Serbia). The patients were carefully instructed by the allergist about the self-administration technique. Detailed written instructions were provided.

The *D. pt* protein extract (mixture of major and minor allergens, molecular weight 14-116 kD) was used for the preparation of sublingual-swallow "vaccines" in phosphate-buffered saline with 50% glycerol. Quality of allergen extract was tested with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot technique (Figure 1). The potency of the solution was expressed as protein nitrogen unit (PNU)/ml and prepared in three strengths: 16, 125 and 1000 PNU/ml. According to manufacturer's recommendations, in the build-up phase (45 days), patients received progressively increasing concentration of the extract, starting with one drop of 16 PNU/ml and increasing to 15 drops in 15 days. Daily dose was taken sublingually applied on a sugar cube in the morning, half an hour before breakfast. This process was repeated also for the 125 and 1000 PNU/ml. Finally, patient was switched to maintenance phase regimen, using 15 drops of the 1000 PNU/ml twice a week for the following 12 months. Allergen proteins concentration in maintenance therapy was equivalent to 19.9 $\mu\text{g}/\text{ml}$ i.e. 0.995 μg of allergen proteins in one drop of extract. Calculated mean cumulative monthly dose of allergen proteins was 119.4 μg , while the mean cumulative dose per year was about 1.4 mg.

Patients from both groups (irrespective to SLIT) received an appropriate pharmacological treatment according to ARIA and GINA guidelines depending on symptoms oral antihistamines (loratadine 10 mg once daily), intranasal corticosteroid (fluticason propionate 200 $\mu\text{g}/\text{daily}$), inhaled corticosteroid (fluticason propionate 100-250 $\mu\text{g}/\text{daily}$) and inhaled bronchodilator (salbutamol one to four puffs, 100 $\mu\text{g}/\text{puff}$).

Clinical Evaluation

All patients were followed up with: (i) 3 regular clinical visits on every 15 days intervals and then once monthly, (ii) additional clinical visits, telephone calls

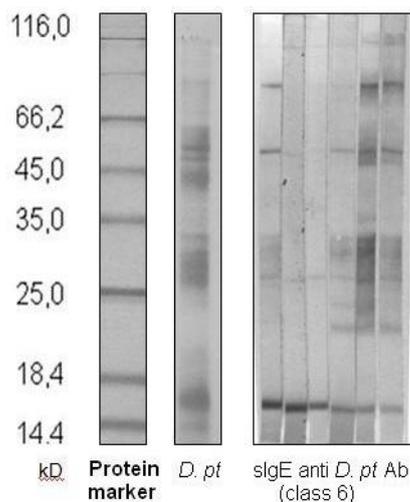


Figure 1. Pre-treatment SDS-PAGE analysis of *Dermatophagoides pteronyssinus* extract and IgE-binding profiles class 6 from six sIgE positive patients from SLIT group

Abbreviations: SDS-PAGE, sodium dodecyl sulfate - polyacrylamide gel electrophoresis; *D. pt*, *Dermatophagoides pteronyssinus*; Ab, antibody; sIgE, specific Immunoglobulin E; SLIT, sublingual immunotherapy.

and (iii) diary of symptoms, drug intake and adverse reactions. Before the introduction of SLIT as well as one year later, during the same period of the year (winter season, the highest level of exposure to HDM), the patients were asked to fill in the symptom and medication score diary on a daily basis during one month period. Adverse reactions have been recorded separately by questionnaire and by clinic visits, during SLIT. At the end of the study, patients were asked to complete a self-assessment symptoms and medication intake diary card. The study timeline is summarized in Figure 2.

The following symptoms of AR were scored: rhinorrhea, sneezing, nasal itching and blocked nose. In addition, for the AR with AA patients, next symptoms were scored: chest tightness, shortness of breath, cough and wheezing. Each symptom was scored as 0 (absent), 1 (mild), 2 (moderate), 3 (severe) and was calculated as the mean monthly symptom score (SS). The use of symptomatic medications was also recorded daily, during the same period. Each dose of each drug was scored: one point was attributed to each dose of oral antihistamines and bronchodilator and two points for

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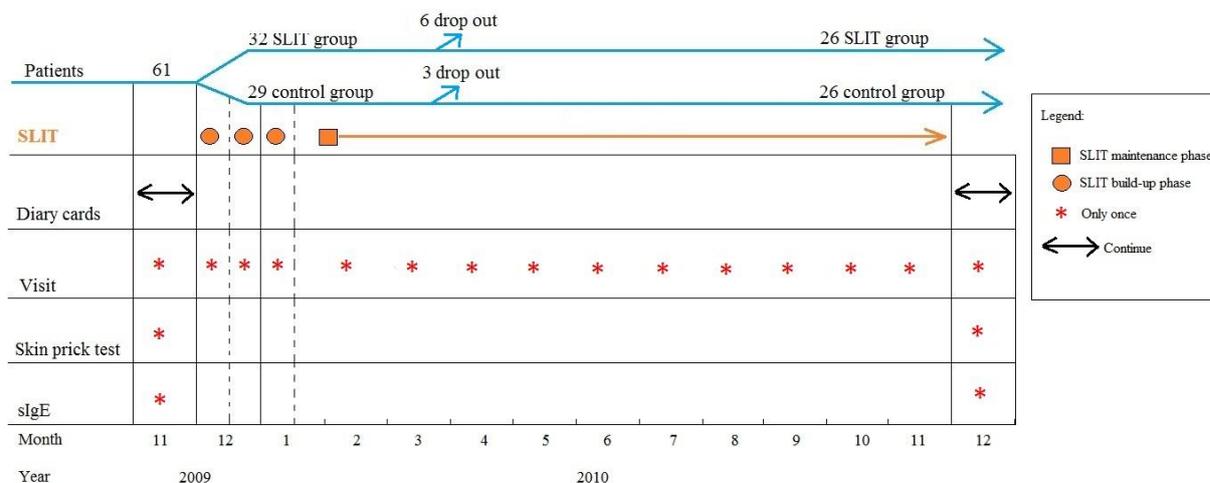


Figure 2. Timeline of the study

Abbreviations: sIgE, specific Immunoglobulin E; SLIT, sublingual immunotherapy;

nasal or inhaled corticosteroids. Anti-allergic medication requirement was evaluated as the mean monthly medication score (MS). Total monthly score for clinical symptoms and symptomatic medication consumption was calculated for each patient. Sum of SS plus MS was used in statistical analysis as cumulative score (CS).

Patient satisfaction, measured by subjective assessment of symptoms and effectiveness of treatment after one year of therapy was evaluated by 4 categories: “very satisfied”, “satisfied”, “unchanged” and “worse”. The need for anti-allergic drugs, measured by subjective assessment of drug intake was assessed as “increased”, “similar”, “decreased”, “more decreased” and “no use of drugs”.

The patients receiving SLIT were required to record and give their report on a specific diary card, in the case of side effects: local (oral itching/burning, swelling, oedema of the uvula or tongue) or systemic adverse reactions (asthma, rhinitis, urticaria, angioedema, generalized itching, gastrointestinal symptoms – abdominal pain, nausea, vomiting, shock).

At the beginning and at the end of the study, SPT was performed and *D. pt* sIgE Ab level was determined.

Adherence to SLIT was not assessed, but patients were asked to show empty vials of extracts consumed during period of treatment.

All the patients were asked to continue their normal house cleaning activities. No additional environmental care was recommended during the time of enrollment.

Avoidance measures needed to remain unchanged throughout the study, in order to maintain the same level of exposure to HDM.

Statistical Analysis

The differences between frequencies were tested by Pearson's chi-square test, while Student's t-test was performed to verify differences between mean values. The Wilcoxon rank sum test was used for comparisons within the groups and the Mann-Whitney U test for intergroup comparisons and $p < 0.05$ was considered as significant. All statistical analyses were performed using SPSS 15.0 version software (SPSS Inc., Illinois, USA).

RESULTS

Seventy five patients were assessed for eligibility, 14 were excluded (10 did not meet inclusion criteria while 4 refused to participate). Sixty one patients (mean age 29.5 ± 10.7 , range 16 to 45 years; 44.3% males; 4.9% with nasal polyposis) were randomized: (i) 32 (52.4%) to receive SLIT as an add-on to drug therapy and (ii) 29 (47.5%) to receive anti-allergic drug therapy alone. Six (18.75%) patients dropped out from the SLIT and three (10.4%) from the control group. Total of 52 patients were analyzed at the end of one year of follow-up (26 patients in SLIT and 26 in control group) (Figure 3).

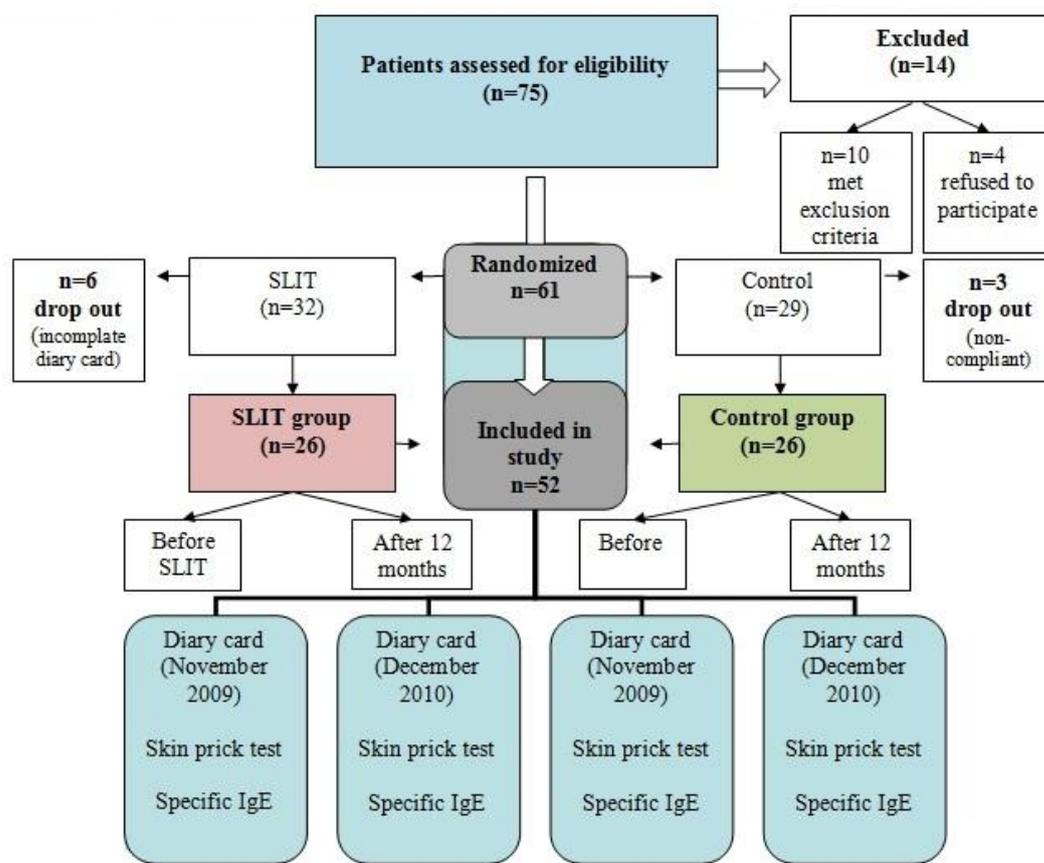


Figure 3. Algorithm of study design

Abbreviations: IgE, Immunoglobulin E; SLIT, sublingual immunotherapy.

Table 1. Demographic and clinical characteristics of patients from SLIT and control group

Variable	SLIT group (n=32)	Control group (n=29)	p
M:F ratio	15/17	12/17	>0.05
Age (in years) (mean ± SD)	28.3 ± 11.1	30.8 ± 10.2	>0.05
min	16	45	
max	16	43	
Rhinitis, No (%)	15 (46.9)	19 (65.5)	
Rhinitis + asthma, No (%)	17 (53.1)	10 (34.5)	>0.05
Nasal polyposis (%)	2 (3.3)	1 (1.6)	
Single sensitization to <i>D. pt.</i> , No (%)	0 (0.0)	3 (4.9%)	
Poly sensitization (<i>D. pt.</i> + pollens) (%)	32 (100)	26 (89.7)	>0.05
Duration of disease, mean ± SD (in years)	5.69 ± 4.39	6.07 ± 2.55	>0.05

Abbreviations: M, male; F, female; SLIT, Sublingual immunotherapy; *D. pt.*, *Dermatophagoides pteronyssinus*

Patients from SLIT and control group were homogenous for all demographic and clinical characteristics (Table 1). An average duration of the

disease was 5.7 in the SLIT and 6.1 years in the control group.

We found clinical improvement in the SLIT group,

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demonstrated by statistically significant decrease in SS ($Z=-4.05$, $p<0.001$), MS ($Z=-2.17$, $p<0.05$) and CS ($Z=-4.11$, $p<0.001$), after one year of SLIT vs. baseline. We also found a statistically significant inter-group difference in MS ($p<0.001$) and in CS ($p<0.05$). Patients from the SLIT group halved (54.3%) the use of anti-allergic drugs after one year (Table 2).

Level of *D. pt* sIgE Ab after one-year of SLIT treatment was slightly increased in SLIT group, but the difference was not statistically significant ($p>0.05$).

Unlike the control group, the majority of patients from SLIT group (83.4%) were "satisfied" or "very satisfied", which gave statistically significant difference in overall subjective treatment satisfaction between

groups ($p<0.001$) (Table 3).

Half of the patients from the SLIT group (13/26) „decreased” anti-allergic drug intake, while almost 60% of patients from the control group answered that use of drugs was "similar", giving statistically significant difference between groups, according to subjective assessment of drug usage after one year of treatment ($p<0.001$) (Table 4).

The incidence of adverse effects during SLIT treatment was 38.4% in the up-dosing phase. Only 2.2% of applied doses (78/3458) resulted in adverse reactions. Ten patients reported mild local reaction (itching and slight swelling), while no systemic adverse effects were reported.

Table 2. Changes in clinical outcome measures between SLIT and control group from baseline to the end of the study

Variable	SLIT group (n=26)			Control group (n=26)			p
	Baseline, mean±SD	After 1 year, mean±SD	YRe -YRb (%)	Baseline, mean±SD	After 1 year, mean±SD	YRe -YRb (%)	
SS	314.32±188.13	237.75±178.91	24.4	330.92±236.05	336.96±243.49	1.8	>0.05
MS	60.84±64.6	27.81±31.69	54.3	58.52±19.28	43.48±24.18	25.7	<0.001*
CS	375.18±209.43	265.22±206.20	29.3	389.21±245.28	380.14±256.60	2.3	<0.05*
<i>D. pt</i> SPT (mm)	7.53±3.03	7.34±3.81	2.5	6.07±2.55	6.65±2.26	9.5	>0.05
<i>D. pt</i> sIgE class	3.75±1.19	3.83±1.20	2.1	3.28±0.94	3.29±0.95	0.3	>0.05

Abbreviations: *D. pt*, *Dermatophagoides pteronyssinus*; sIgE, specific IgE; YRe-YRb, Pre-treatment – post treatment × 100; SS, symptom score; MS, medication score; CS, cumulative score; SPT, skin prick test.

* Statistically significant

Table 3. Patients satisfaction with outcome and effectiveness after one year of treatment between SLIT and control group

Subjective assessment	SLIT group (n=26)	Control group (n=26)	p
Very satisfied, n (%)	7 (29.6)	5 (19.2)	<0.001*
Satisfied, n (%)	14 (53.8)	4 (15.4)	
Unchanged, n (%)	5 (19.2)	17 (65.4)	
Worsen, n (%)	0 (0.0)	0 (0.0)	

* Statistically significant intergroup difference of patient's treatment satisfaction

Table 4. Subjective assessment of drug intake after one year of treatment between SLIT and control group

Subjective assessment of drug intake	SLIT group (n=26)	Control group (n=26)	p
Similar, n (%)	8 (30.8)	15 (57.7)	<0.001*
Decreased, n (%)	9 (34.6)	7 (26.9)	
More decreased, n (%)	4 (15.4)	0 (0.0)	
Increased, n (%)	0 (0.0)	0 (0.0)	
No use of drugs, n (%)	5 (19.2)	4 (15.4)	

* Statistically significant intergroup difference of subjective assessment of drug intake after treatment

DISCUSSION

This is the first study evaluating efficacy, safety as well as patients' satisfaction with *D. pt* SLIT in Serbia. In the current study we found: a) statistically significant improvement in MS and CS after one year of treatment in SLIT group (54.3%, 29.3%; respectively) compared to control group (25.7%, 2.3%; respectively), b) only 38.4% of (10/26) patients reported mild local adverse reactions, c) 80.8% (21/26) patients from the SLIT group were "satisfied" or "very satisfied" with the subjective assessment of the SLIT and d) 50% (13/26) patients had decreased subjective assessment of anti-allergic medication usage after SLIT treatment.

Since SLIT was first introduced in 1986 and later accepted as a viable alternative to SCIT, need for an assessment of its efficacy and safety in respiratory allergy has emerged. Consequently, many randomized double blind placebo controlled and open controlled trials,^{1,3,12,13} as well as a number of systematic reviews and meta-analyses have been carried out to determine the efficacy and safety of SLIT.^{1,3,4,9,14,15} Data from literature suggest overall clinical effectiveness of SLIT in patients with AR and AA, although the conclusions were limited by heterogeneity of the studies.^{3,5} Subgroup analyses for perennial allergens, such as HDM, provided controversial and inconclusive results regarding several important issues: standardization of allergen extracts, heterogeneity of allergen dose, duration and schedules of treatment, selection of patients, etc.^{5,14,16}

We chose perennial allergy and polysensitization as the most common in patients with respiratory allergy in Serbia¹⁷ as well as in Europe.¹⁸ HDM is the dominant inhalant allergen in our region followed by grass and weed pollens.¹⁷ Most AR patients are polysensitized, however, polysensitized patients are not necessarily clinically polyallergic. The efficacy of AIT, especially single-AIT in these patients is still a matter of debate and it is often suggested that polysensitized patients have no such benefit from AIT as monosensitized patients.^{5,19} Some authors concluded that SLIT with clinically relevant allergen could be effective in polysensitized patients.^{20,21} In our study almost all patients were polysensitized, only 3 patients in the control group were monosensitized to *D. pt* and the causal role of the *D. pt* allergen used for immunotherapy was clearly ascertained. Patients were included in the study between November and February,

which is the optimal time for "pick" of *D. pt* allergen concentration. Clinical outcomes were assessed one year later in the same season aimed to avoid bias in efficacy assessment.

The comparison between immunotherapy and therapy with anti-allergic drugs is still a matter of debate. Clinical effects of SLIT can be appreciated only in the long term period (months), whereas traditional drugs act immediately. The only available head-to-head trials comparing SLIT to inhaled budesonide²² and montelukast²³ in patients with AA and AR, respectively, showed a favorable outcome of SLIT. According to our results, SLIT used complementing pharmacotherapy seems to be beneficial therapeutic strategy.

AIT trials often assess SS and MS independently, although the treatment reduces both. Pragmatic view assumes equivalent importance of SS and MS indicating that each of these accounts for half of the clinical burden of the disease. Therefore, it is recommended that combined SS and MS should be utilized as the primary outcome measure²⁴ and that magnitude of SLIT clinical efficacy should be estimated as the percentage reduction of combined SS/MS score (CS) in the actively treated group vs. control group.²⁵ In our study, before the introduction of SLIT, patients from both groups had similar mean values of all clinical scores and they did not differ much in respect to symptom severity and drug intake. After 12 months, reduction in all clinical scores was observed in the SLIT group. It is important to notice that SLIT decreased drug usage (54.3%) more than relieving symptoms (24.4%), although both reductions were statistically significant. Reduction of drug intake indicates that medication treatment does not enhance SLIT efficiency. Comparison between groups showed statistically significant reduction of MS and CS in SLIT group. In our study, patients were not divided in groups regarding comorbidity with asthma, what could have an impact on SLIT effectiveness and results of clinical scores. Statistically significant reduction of CS by almost 30% after 12 months of treatment, favors efficacy of SLIT with *D. pt* extract used in our study, but according to parameters of SLIT efficacy,²⁵ our results would be considered as borderline clinical efficacy.

In the present study, we have considered several possible reasons why SLIT did not demonstrate higher clinical efficacy. At the same time these were the main limitations of the study. Namely, the small number of

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patients included in both groups increased the risk of underestimating the treatment efficacy, as well as the lack of a placebo group (considered to be mandatory for immunotherapy trials).²⁴ Additionally, the difference in exposure to causal allergen as well as fluctuations in indoor allergen levels, were not monitored in the study. Also, the quality of SLIT extract is questionable, given that it consists of minor and major allergens. We presume that observed clinical efficacy could be higher with usage of SLIT extract containing only major allergens (Der p1, Der p2). Data from literature showed that efficacy of SLIT is dose-dependent and sufficient duration of treatment is essential to elicit the immunologic changes underlying its clinical effectiveness.¹ One more thing to note is that manufacturer's variability of allergen content in commercial extracts makes the comparison amongst extracts and regimens difficult.

The only available *D. pt* SLIT extract in Serbia, used in our study, is not standardized and it was difficult to determine the exact dose in micrograms of major Der p1 allergen. According to calculated total concentration of allergen proteins mixture (19.9 µg/ml), the mean cumulative dose per year taken by our patients was 1.4 mg. Some immunotherapeutic protocols^{26,27} used low-dosed SLIT with mean cumulative dose per year of 0.57 mg Der p1 (only 5 x higher than SCIT dose) and found no significant clinical effect. Also, taking in account that given allergen dose in our study corresponds to about 34 times the dose recommended for SCIT from the same manufacturer, our protocol could be considered as intermediate-dosed SLIT (6-50 x SCIT).²⁷ Although we found satisfactory and statistically significant clinical improvement using recommended protocol by national manufacturer, we plan to conduct study using higher dose SLIT regimen (doubled maintenance dose) in order to compare results.

It was demonstrated that AIT results in early induction of peripheral IL-10 response and elevated serum concentration of IgG4 and IgA Ab, as early as 3 months after its commencement. That in turn results in suppression of cutaneous allergen responses.^{28,29} In our study, no significant inter-group differences in skin reactivity were found after 12 months of SLIT. Some data from literature showed no SPT changes after 12 months, but significant suppressive response after 18 to 24 months of SLIT treatment.³⁰

Besides cutaneous reactivity, the most frequently assessed immunologic responses to SLIT are changes in

allergen-sIgE and IgG4 Ab production. In this regard, increase of *D. pt* sIgE Ab from baseline to the end of SLIT was observed in several studies.^{31,32} In low-dosed SLIT studies, no significant changes of sIgE Ab were observed, all the more, in some studies decrease of sIgE Ab was observed, but such response was not dose-dependent.²⁷ Other studies found transitional increase in the level of sIgE Ab followed by its decrease, what was confirmed as a parameter of successful treatment.⁵ Although not statistically significant, a trend of slight increase in the level of *D. pt* sIgE Ab recorded in our study could indicate positive response to SLIT.

In our study, after one year of SLIT, none of the patients assessed their symptoms as "worse". Subjectively, more than 80% of these patients felt better in comparison to the control group. Better subjective assessment of symptoms and effectiveness of treatment with high compliance results in better quality of life and better therapy adherence.

One of the advantages of SLIT is its improved safety.^{1,27} In general, the majority of side effects of SLIT are represented by local adverse reactions. Cox et al. reported that such reactions occurred in 40-75% of SLIT during the build up phase which did not lead to dose reduction or discontinuation of treatment.³³ Systemic side effects are reported as very rare and occur in less than 1% of patients.³ Only six cases of anaphylaxis have been described so far, with some of them not using standardized extracts. Finally, no fatal event has ever been reported.³ Our results are in accordance with the previously mentioned findings. About 38% of patients receiving SLIT reported mild local adverse reaction, most of them (70%) in the up-dosing phase, with a tendency to subside without specific treatment during continuation of SLIT.

Although the positive impact of SLIT for most allergens is well known, we gave new insight into the efficiency and safety of *D. pt* SLIT, as well as patient's subjective satisfaction with clinical improvement. As this is the first study of SLIT with *D. pt* extract made in Serbia conducted in AR patients with or without AA, evaluated in a real-life setting with non-standardized allergenic extract, we can conclude that this kind of AIT is safe and clinically efficient add-on treatment to standard pharmacotherapy.

Further observations are needed to evaluate effects of SLIT in our clinical practice: follow-up of other functional and immunological parameters, sub-analysis of asthma patients, longer duration of SLIT and

standardization of extract in respect to concentration of major allergens expressed in micrograms would allow better comparison between various trials and products.

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