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Clinical trial to assess tolerability and subrogate efficacy effects of an abbreviated schedule with house dust mites mixture subcutaneous immunotherapy

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Key words

House dust mites; DPT/DF mixture; subcutaneous immunotherapy; rhinoconjunctivitis; abbreviated schedule.

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Doi
10.23822/EurAnnACI.1764-1489.140

Summary

Objective. *To evaluate the tolerability and efficacy of Dermatophagoides pteronyssinus/Dermatophagoides farinae mixture subcutaneous immunotherapy (SCIT).* ***Methods.*** *Patients received an abbreviated build-up schedule. The aims were: number, percentage, and severity of adverse reactions. Secondary outcomes included: changes in immunoglobulin titers and changes in dose-response skin prick tests.* ***Results.*** *Out of 289 administrations, 17% elicited any clinically relevant adverse reaction. Most of them were local reactions (LR) (9.4%) and the rest (7.6%) were systemic. Significant increases in sIgG and sIgG4 were detected in serum samples. Cutaneous reactivity decreased significantly.* ***Conclusions.*** *SCIT with house dust mites mixture of ROXALL Medicina España S.A. seems to have an acceptable tolerability profile, induces blocking IgG and decreases skin reactivity.*

Abbreviations

AEs: Adverse events
AIT: Allergen immunotherapy
AR: Allergic rhinitis
ARIA: Allergic Rhinitis and its Impact on Asthma
ARs: Adverse reactions
DBU: Diagnostic biological unit
DF: *Dermatophagoides farinae*
PPT: *Dermatophagoides pteronyssinus*
EAACI: European Academy of Allergy and Clinical Immunology
ELISA: Enzyme-Linked Immune Sorbent Assay
EMA: European Medicines Agency
HDM: House dust mites
ITT: Intention to treat
LR: Local reaction
MedDRA: Medical Dictionary for Regulatory Activities
PP: Per Protocol
SAS: Statistical Analysis Software
SMs: Storage Mites
SPT: Skin prick test
SP: Safety population
SR: Systemic reaction
TSU: Treatment Standardized Unit

Introduction

Respiratory allergy brings together a set of conditions with a highly health burden in the world (1, 2). Mites cause allergy disease in more than 10% of globe population and 90% of the people diagnosed from allergic asthma presents sensitization to domestic mites (3). House dust mites (HDM) are the most abundant aeroallergen in indoor environments, especially in warm and moist areas like Iberian countries (4).

Usually, mites are classified in two vast groups: HDMs belonging to the *Pyroglyphidae* family and storage mites (SMs) belonging to *Glycyphagidae* and *Acaridae* families (5). *Dermatophagoides pteronyssinus* (DPT) and *Dermatophagoides farinae* (DF) for HDM, followed by *Lepidoglyphus destructor* for SMs in a specific area of the north-west of Spain, were the most common species found in an epidemiologic study describing the prevalence of mites' sensitization in four different areas of the country (6). This geographical mites' distribution can imply different sensitization profile in patients of diverse areas and different allergen immunotherapy composition needs (3, 7).

It is well ascertained that allergy immunotherapy (AIT) is the unique available therapeutic option to target the disease and not only symptoms (8-11). Thanks to their disease-modifying effect, specific AIT gets immune system tolerance to clinically relevant allergens through triggering specific blocking antibodies, activating mediators and achieving the decrease of the inflammatory response in tissues. Probably, the AIT prescription is considerably lower than 10% of patients with AR or asthma (12, 13).

According to EMA guidelines, mixture of different allergenic sources is only recommended when they are taxonomically related (14). In the case of DPT and DF mixture, the similarity and cross-reactivity is so high, between 80-90 % (15, 16) that it could be enough to receive unique vaccine with one of these two allergen extracts. As a consequence of the enormous homology, any of the two allergenic sources could be proposed as the representative homologous specie (17).

However, a considerable number of clinicians remains prescribing HDM mixture (50% DPT and 50% DF) mainly based on patient's sensitization results (18,19).

Therefore, an open multicentre clinical trial in adult patients with allergic rhinitis (AR), (with or without asthma) using a standardized native depot HDM mixture extract for subcutaneous immunotherapy was conducted. The principal objective was to establish the tolerability and safety of an abbreviated treatment schedule in patients sensitized to HDM.

Materials and methods

Study design and ethical considerations

Five hospitals in Spain collaborated in this open, multicentre and phase I clinical trial. Likewise, the study was conducted in accordance with the principles of the Declaration of Helsinki and the ICH guideline on Good Clinical Practice. It was approved by relevant ethics committees and by the Spanish regulatory authorities, (EudraCT 2015-004712-38). Prior to their participation, written informed consent was given by every patient.

Study population

Patients had to meet the following criteria: age 18-60 years, clinical history of perennial AR due to HDM for at least 2 years prior to the study inclusion, a positive skin prick test to DPT or DF (wheal diameter ≥ 3 mm) and specific immunoglobulin E (sIgE) against DPT or DF levels ≥ 0.7 kUa/L determined by ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden). Results of SPT performed within 12 months prior to the inclusion were accepted. Only patients with concurrent mild asthma were allowed to participate.

The following were defined as exclusion criteria: to have received immunotherapy against HDM or a cross-reactive allergen in the 5 years prior the study inclusion, current administration of immunotherapy for any other allergen, moderate to severe asthma, forced expiratory volume in 1st second (FEV1) < 70%, clinically relevant perennial sensitization different of HDM. The following conditions were additional exclusion criteria: history of anaphylaxis, chronic urticaria, moderate to severe atopic dermatitis; immunological, cardiac, renal or hepatic diseases; current treatment with immunosuppressants, anti-IgE, tricyclic antidepressants, psychotropic drugs, beta-blockers, or angiotensin-converting enzyme inhibitors and pregnant or breast-feeding women.

Study interventions

Patients were treated with a native depot mixture of DPT and DF subcutaneous treatment, (Allergovac® Depot, ROXALL Medicina España S.A., Zamudio, Spain) consisting of two different strengths. The abbreviated build-up schedule comprised 6 doses at weekly intervals (± 2 days): 3 doses (0.2, 0.5 and 1 mL) from vial 2 (100 Treatment Standardized Units (TSU)/mL), and 3 subsequent administrations (0.2, 0.5 and 1 mL) from vial 3. The last dose of the increasing period, 1 mL of vial 3, 1000 TSU/mL, was the target maintenance dose and was administered at monthly intervals, during one trimester, being the whole treatment duration of 17 weeks. The concentration of the major HDM allergens for group 1 were: Der p1 0.44 µg/mL and 0.34 µg/mL Der f1 and for group 2 were Der p2 0.69 µg/mL and 0.45 µg/mL Der f2. Some dose schedule variations were allowed in the event of adverse reactions according to the standards for practical allergen-specific immunotherapy recommendations (20).

Outcome measures

In this study all adverse events (AEs) were registered for tolerability assessment. The primary outcome was the incidence of adverse reactions (ARs), recorded at participating sites during the

30 minutes after each vaccine administration. In addition, ARs were also collected by reviewing the patients' diaries designed to register any unpleasant experience outside immunotherapy units and by telephone calls. ARs were defined as all noxious and unintended responses to any dose of the investigational allergen vaccine administered. These reactions were classified as immediate (within 30 minutes after the vaccine administration) or delayed (> 30 minutes after vaccine administration).
In the same way, adverse reactions were classified as local (LR, reactions taking place at the arm where vaccine was administered), or systemic reactions (SRs, generalised symptoms taking place far away from the administration site). According to LRs extension, we consider clinically significant the immediate LR ≥ 5 cm and the delayed LR ≥ 10 cm or those implying a dose modification in the next administration. Additionally, LRs were described as diffuse inflammation, redness, erythema, local painfulness, pruritus or reaction in injection site (when two or more local symptoms took place simultaneously). SRs were classified by the investigators according to the European Academy of Allergy and Clinical Immunology EAACI guidelines (20) and also by the Medical Dictionary for Regulatory Activities (MedDRA).
Dose-response skin prick test (SPT) was performed using four increasing concentrations of HDM mixture extract (100, 1.000, 10.000 and 100.000 DBU/mL, Diagnostic Biologic Units) as well as positive (histamine 10 mg/mL) and negative (saline) controls. Titrated skin prick test for basal and final visits, were provided by ROXALL Medicina España S.A. to the study participants. The batch used for the whole study population was the same. The change in cutaneous reactivity (wheal area in mm^2) from baseline to the final visit was measured.
Regarding the immunological effects' assessment, serum samples were obtained at baseline and final visits to determinate immunoglobulin levels (sIgE, sIgG and sIgG4) against DPT and DF whole extract by ELISA (Enzyme-Linked Immune Sorbent Assay) as previously described in Sola J. *et al.* (2015) (21). Samples were frozen and sent to ROXALL's central laboratory for bioanalysis in accordance with Good Laboratory Practices.
Moreover, specific immunoglobulin titters IgE against Der p1, Der p2 and Der p10 were analysed at baseline and final visit by ImmunoCAP® (Thermo Scientific, Uppsala, Sweden).

Statistical methods

We described three populations: safety population (SP), patients who received at least one dose of treatment, intention-to-treat (ITT) population, patients who met all inclusion/exclusion criteria, received at least one dose of treatment, and had available data on subrogate efficacy variables, and the per-protocol (PP) population, patients who met previous criterial and moreover achieved their target maintenance dose and completed the study without any major protocol deviation.
Tolerability and safety were analysed using descriptive statistics. Categorical variables were described by absolute and relative frequencies and in continuous variables the mean and the standard deviation were applied.
Changes in immunoglobulin levels and SPT values from baseline to final visit, were analysed by means of the Wilcoxon non-parametric test for paired samples. A bilateral statistical significance level of 0.05 was displayed to all statistical tests. Statistical analyses were performed using the Statistical Analysis software (SAS) version 9.4.
Sample size was calculated considering a percentage of ARs of 22,9% (22). Establishing a confidence interval of 95% with a precision of ± 4 percentage unit and assuming a 5% of drop outs, the number of patients to provide adequate data on the primary endpoint was 42.

Results

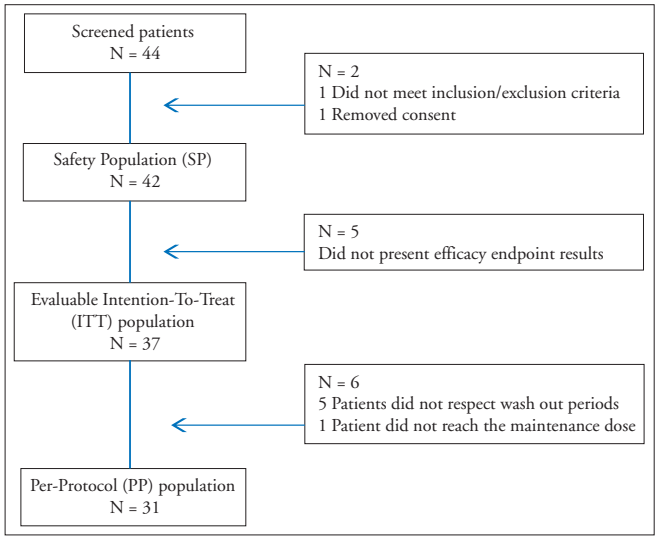
Descriptive data

A total of 44 patients were recruited from August 2016 to April 2017. Out of them, one was a screening failure and other one removed the consent prior to start treatment so, 42 patients were assigned to receive HDM mixture AIT and were analysed in SP. Additionally, in this study there were 6 early discontinuations: 1 due to AE, defined as hearing loss, 1 for surgery intervention, 3 for loss of follow up and 1 for change in the residence address. Rhinoconjunctivitis secondary to sensitization to *Dermatophagoides* was confirmed in each participant by allergy diagnostic tests and a rigorous clinical history. Regarding other sensitizations, the percentage of patients sensitized to grass pollen was 7.14 %, to weed pollen 4.76%, and to tree pollen 2.38%. However, these aeroallergen sensitizations were not clinically relevant or did not interfere with the collecting data period. ITT population included 37 patients since 5 were excluded due to the absence of data on immunoglobulins or dose-response SPT at final visit. Finally, 31 patients remained in the PP population, mainly as a result of major protocol deviations. Patient's distribution is shown in **figure 1**. Most patients showed sIgE class ≥ 4 against whole DPT and DF extract: 38.1% and 50% respectively. Subjects' baseline clinical characteristics and sIgE profile is presented in **table I**.

Tolerability and safety

All patients presented at least 1 AE in the study, being classified the majority of them as mild to moderate intensity. The most frequent reported AEs were, injection site reaction, headache, upper respiratory tract infections and digestive system disorders. All AE were non-serious and the vast majority were resolved with symptomatic medication.
ARs were summarized in **table II** and **III**. Out of 289 dose administrations, 6 (2.1 %), were considered as clinically relevant immediate LR, and 21 (7.3%) clinically relevant delayed LRs. All of them described as injection site reaction.

***Figure 1** - Study flow chart.*



Regarding systemic reactions, 22 SRs (7.6% of dose administrations) were recorded; six grade 0 (2.1%), fifteen (5.2%) grade I and one grade II (0.3%). There were no systemic reactions grades III or IV. All of them are described in **table III**.

SRs were resolved with symptomatic treatment or a change in the next administration dose. All patients recovered of the ARs at the end of the study. One patient in spite of the dose modifications performed in the schedule, did not reach the maintenance dose established in the study protocol due to adverse reactions.

This patient with the worst systemic reaction, described as delayed rhinitis with dyspnoea responded to the symptomatic treatment with beta 2 blockers, inhaled corticosteroids and antihistamines. At basal period, she/he presented class 4 levels of sIgE against DPT and DF. However, papule areas before immunotherapy treatment showed a size similar to the mean of the sample population.

Clinically relevant changes in blood count and biochemistry parameters were not observed in any patient after receiving immunotherapy treatment.

Immunoglobulin levels

Statistically significant increases in serum sIgG and sIgG4 titters against DPT and DF whole extract at final visit were observed compared with basal visit (both $p < 0.0001$; Wilcoxon test). Serum sIgE levels to DPT and DF slightly decreased at final visit, achieving statistical significance ($p < 0.0002$; Wilcoxon test) (**figure 2**). On contrary, a statistically significant increases in serum sIgE against Der p1 and Der p2 at final visit were observed in comparison with basal visit ($p < 0.0001$ and $p < 0.02$, respectively; Wilcoxon test). As it was expected, these results were maintained in PP population.

***Table I** - Patients' baseline clinical characteristics.*

Baseline characteristics	
Number of patients *(SP)*	*42*
Age *(years), mean ± (SD)*	*33.6 ± 9.1*
Women *n (%)*	*21 (50.0)*
Race *n (%)*	
Caucasians	*31 (73.8)*
Hispanics	*7 (16.7)*
Arabs	*2 (4.8)*
Asians	*2 (4.8)*
Rhinitis ARIA classification (32)	
Intermittent mild n (%)	*1 (2.4)*
Persistent mild n (%)	*9 (21.4)*
Intermittent moderate-severe n (%)	*2 (4.8)*
Persistent moderate-severe n (%)	*30 (71.4)*
Main concomitant allergic condition	
Asthma n (%)	*7 (16.7)*
Time from diagnostic *(years), mean ± (SD)*	*4.7 ± (8.4)*
(BMI), Kg/m² *mean ± (SD)*	*24.7 (4.85)*
Vital signs *mean ± (SD)*	
Systolic blood pressure, mmHg	*114.7 (14.1)*
Diastolic blood pressure, mmHg	*71.7 (10.6)*
Heart rate, bpm	*71.6 (9.2)*
sIgE DPT CAP class *n (%)*	
2	*1 (2.4)*
3	*9 (21.4)*
4	*16 (38.1)*
5	*16 (38.1)*
sIgE DF CAP class *n (%)*	
2	*1 (2.4)*
3	*11 (26.2)*
4	*21 (50.0)*
5	*9 (21.4)*

(SP) safety population, (SD) standard deviation, (BMI) Body Mass Index, (mmHg) millimetres of mercury and (bpm) beats per minute.

Cutaneous reactivity

Mean values of wheal area in mm^2 were significantly reduced at final visit compared with baseline in each one of the four tested vials against HDM mixture (**figure 3**). Moreover, a statistical significance was achieved with all tested vials ($p < 0.04$; Wilcoxon test from vial 1 to vial 4). These cutaneous results were also reproduced in the PP population.

Discussion

Despite the fact of great homology between DPT and the rest of the mites belonging to the family *Pyroglyphidae,* (15, 16, 23)

Table II *- Summary of adverse reactions in SP.*

	Schedule Phase	**Patients number n (%)** *42 (100%)*	**Administered doses n (%)** *289 (100%)*
Local reactions		*37 (88.1%)*	*187 (64.7%)*
Clinically relevant immediate LRs	*Initiation Phase*	*5 (11.9%)*	*6 (2.1%)*
Clinically relevant delayed LRs	*Initiation Phase*	*12 (28.6%)*	*21 (7.3%)*
Systemic reactions		*14 (33.3%)*	*22 (7.6%)*
Grade 0	*Initiation Phase*	*6 (14.3%)*	*6 (2.1%)*
Grade I	*Initiation Phase*	*9 (21.4%)*	*15 (5.2%)*
Grade II	*Initiation Phase*	*1 (2.4%)*	*1 (0.3%)*

n (%) number and percentage of adverse reactions, LR (local reaction) and SP (safety population).

Table III *- Description of systemic adverse reactions by administration doses.*

(N = 289 doses administered)

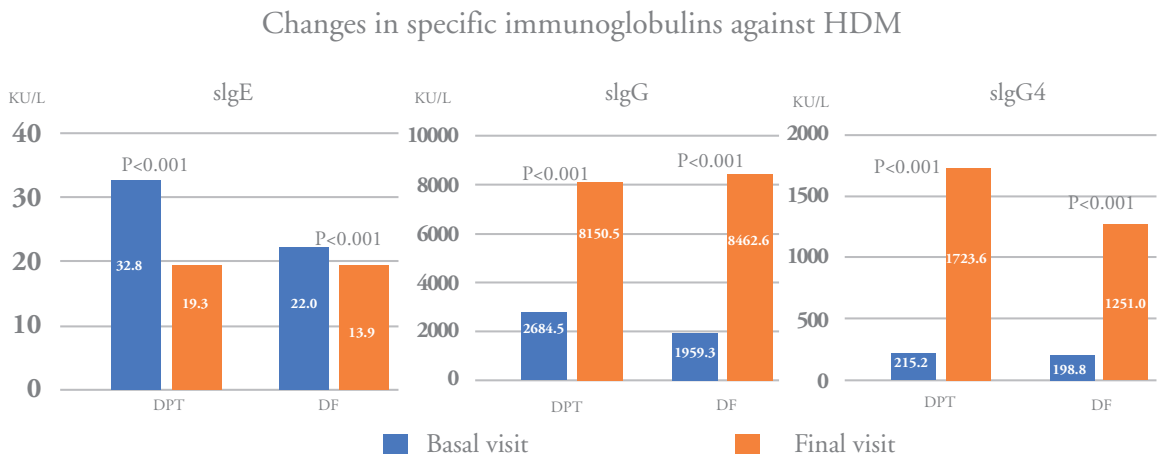
	n (ARs) (%)	**Number**	**Description**
Grade 0	*6 (2.1%)*	*2*	*Headache*
		3	*General desconfort + nausea + dizziness*
		1	*Non-specific cough*
Grade I	*15 (5.2%)*	*1*	*Conjunctivitis*
		1	*Dermatitis*
		4	*Allergic rhinitis*
		2	*Urticaria*
		1	*Generalized pruritus*
		1	*Pruritus out of the injection site*
		2	*Erythema out of the injection site*
		1	*Throat irritation*
		1	*Pharyngitis*
		1	*Allergic cough*
Grade II	*1 (0.3%)*	*1*	*Rhinitis + dyspnea*

n (%) number and percentage of adverse reactions. (ARs) Adverse Reactions.

allergy clinicians commonly prescribe mixed vaccines to treat patients polysensitized to mites. As it was mentioned, the most common mites with positive results in diagnostic prick tests performed in patients with respiratory allergy in Spain, were DPT, DF and *Lepidoglyphus destructor* (6), excluding the islands. As a consequence, immunotherapy containing a mixture of DPT and DF is frequently prescribed, although a recent publication did not find differences in efficacy between two commercial mites' extracts: one with DPT as single source and other with a mixture of DPT and DF (50/50) (24).

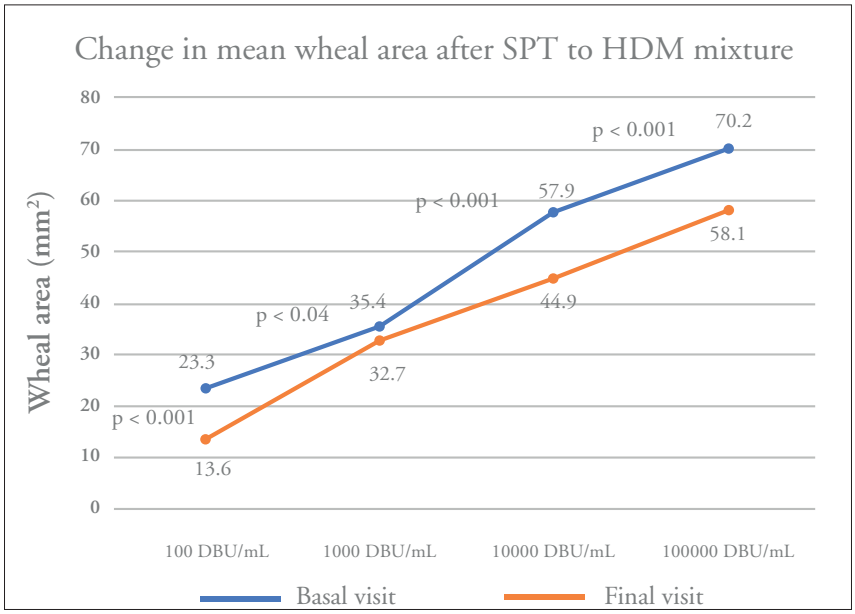
ROXALL Medicina España S.A. (formerly Bial) conducted two clinical trials with Allergovac® Depot native DPT 100%. A placebo-controlled Phase I study to evaluate three different build-up schedules (22) and a dose finding randomized controlled trial to compare the efficacy of five different doses (25). In both studies, the tolerability profile of the abbreviated schedule could be ascertained as good. However, this evidenced data must be interpreted with caution when they are extrapolated to another marketed product with HDM mixture. A new safety and tolerability trial, with the same schedule and a treatment containing

Figure 2 *- Changes in specific immunoglobulins against HDM.*



Changes in specific immunoglobulins against HDM. Corresponding p values according to Wilcoxon test are indicated.

Figure 3 *- Change in mean wheal area after SPT with HDM mixture.*



Change in mean wheal area after SPT with HDM mixture at final visit versus baseline. p values according to Wilcoxon test are indicated.

a DPT/DF mixture (50/50), was designed in order to avoid this extrapolation.

The percentage of systemic ARs with the vaccine under study was slightly higher than with the DPT 100% vaccine used in previous ROXALL clinical trials. In current study, a 7.6% of systemic ARs was described versus 4.8 % in the phase I study (22) and 3.8% with the group containing the commercial dose, in the dose finding trial (25). In comparison with other similar marketed products, the encountered results are in a similar range, thus a study conducted by Tabar *et al.* (26) showed a systemic ARs percentage of 8.8% by patient. In another clinical trial with a DPT 100% formulation, a 9.8% of ARs was report-

ed (27). In a comparative study of two schedules with HDM depot native immunotherapy (28), the percentage of ARs with conventional schedule reached 13.8% and 10.7% with cluster scheme. Additionally, in similar designed studies, but with different extracts composition apart from HDM, the percentage of ARs was even higher reaching in some cases 21% (29).

This depot formulation induced an early immunological response, confirmed by statistically significant increments of sIgG and sIgG4 levels against DPT and DF, after approximately 3 months of therapy. Similar results could be observed in other studies, where a fast increase in sIgG and sIgG4 can be associated with the effect of blocking IgE-binding to allergens and immune response modification (26, 30, 31). These results are in line with the immunologic and skin prick test outcomes observed in previous studies with DPT 100% (Allergovac® Depot, ROXALL Medicina España S.A., Zamudio, Spain) (22, 25). Regarding sIgE determination against Der p1 and Der p2, surprisingly a statistically significant increased at final visit was observed. These increases could be attributed to the effect of other allergens different to Der p1 and Der p, with relevance in the study patients' immune response.

Considering the cutaneous reactivity to the causal allergens, a statistically significant reduction in immediate skin response to the different concentrations of DPT and DF combination was observed, showing a decrease in the mean papule size produced by each concentration tested. This result is in the line of another clinical trial with an extract of HDM mixture after a short administration of specific immunotherapy (26).

Conclusions

Given the heterogeneity in participants, allergens, schedules, dosing treatment and adverse reactions reporting methodology, it is difficult to compare tolerability results between different available studies. However, this clinical trial shows that the assayed abbreviated schedule with native depot HDM mixture, (Allergovac® Depot ROXALL Medicina España), has an acceptable tolerability profile. Moreover, preliminary positive efficacy response can be observed due to a significant immunological and cutaneous reactivity changes in subjects suffering from allergic rhinoconjunctivitis. These promising results should be worth to be confirmed in a larger controlled clinical trial.

Acknowledgements

The authors would like to thank participating hospitals and the inestimable help of all collaborator investigators like: Ignacio Jáuregui, Nathalie Depreux, Clara Padró, Blanca Andrés, Marta Velasco, Marta Frías, Verónica Salinas, Mª Cruz Santín, Mª Jesus Ortiz and Eva González-Morchón.

Fundings

This study was supported by ROXALL Medicina España S.A.

Conflict of interests

Ignacio Antépara has received research fees from, Novartis, GSK, Astra-Zeneca, Sanofiand Bial. Albert Roger has received research fees from Roxall, Allergy Therapeutics, Stallergenes, Leti, Hal, Merck, Diater. Consultant fees: Allergy Therapeutics, Stallergenes, Merck. Speaker fees: Roxall, Allergy Therapeutics, Leti, Merck. Nagore Bernedo has received consultant fees from ALK and Allergy Therapeutics, speaker fees from Roxall and research fees from Merck. Fernando Rodríguez has received research fees from GSK, ALK and Roxall. Speaker fees: GSK, Novartis, Astra-Zeneca and Chiesi. Begoña Madariaga, Juan A Asturias, Leire Begoña, Alberto Martínez, Araitz Landeta and María C Gómez are fulltime employees of ROXALL Medicina España S.A. Ramón Lleonart has no conflicts of interest to declare. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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