



Efficacy and safety of subcutaneous immunotherapy with a mixture of glutaraldehyde-modified extracts of *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blomia tropicalis*

Ricardo Cardona-Villa, MD, MSc^a, Susana Uribe-Garcia, MD^a,
Víctor Daniel Calvo-Betancur, BSc, MSc^a, Jose Fernando Cantillo, PhD^b and
Enrique Fernández-Caldas, PhD^{c*}

ABSTRACT

Background: Allergen Immunotherapy (AIT) is an effective treatment of allergic respiratory diseases induced by the inhalation of house dust mite allergens.

Objectives: To evaluate the efficacy and safety of glutaraldehyde polymerized allergen extracts using a mixture of *Dermatophagoides pteronyssinus*, *D. farinae* and *Blomia tropicalis* in mite allergic individuals residing in Colombia.

Methods: Two hundred and fifty (250) patients with allergic rhinoconjunctivitis with, or without asthma and sensitized to *D. pteronyssinus*, *D. farinae* and *B. tropicalis* were included. A glutaraldehyde-modified extract containing a mixture of *D. pteronyssinus*, *D. farinae* and *B. tropicalis* was employed, using a cluster up-dosing schedule followed by a monthly maintenance dose. The primary endpoints to evaluate the clinical impact were the Combined Symptom and Medication Scores (CSMS) for allergic rhinitis, the Asthma Control Test (ACT) and the reduction in medication consumption.

Results: Significant improvement was found after 3 months of treatment regarding CSMS ($p < 0.0001$) and ACT ($p < 0.0001$). Additionally, a significant decrease in medication consumption was found after 3 months of treatment ($p < 0.0001$). Adverse reactions, either local or systemic were mild and no severe reactions related to the vaccines were observed.

Conclusion: After 12 months of allergen immunotherapy, glutaraldehyde-modified mixture of *D. pteronyssinus*, *D. farinae* and *B. tropicalis* proved to be safe and effective in the treatment of patients with rhinoconjunctivitis with or without asthma due to allergy to mites.

^aGrupo de Alergología Clínica y Experimental (GACE), University of Antioquia, Medellín, Antioquia, Colombia
^{*}Corresponding author. Immunotek, SL, Calle Punto Mobi, 5, 28805 Alcalá de Henares, Spain. E-mail: efcaldas@immunotek.com
Full list of author information is available at the end of the article
<http://doi.org/10.1016/j.waojou.2022.100692>

Received 23 February 2022; Received in revised form 24 June 2022; Accepted 5 August 2022
Online publication date xxx
1939-4551/© 2022 The Author(s). Published by Elsevier Inc. on behalf of World Allergy Organization. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords: Immunotherapy, Allergic rhino-conjunctivitis, House dust mites, Glutaraldehyde-polymerized extracts

INTRODUCTION

Allergic diseases are one of most prevalent disorders worldwide, regardless of gender, age, or race and represent a major health burden.¹ Up to one-fifth of the global population is affected by allergic diseases, causing considerable direct and indirect costs related to its high morbidity.¹⁻⁷

Allergen immunotherapy (AIT) consists of the administration of increasing doses of extracts prepared from the allergenic material to which the patient is sensitized.^{8,9} This treatment induces changes in the immune response to the allergens, including regulatory responses by allergen specific regulatory T cells (Treg) and the production of IgG antibodies.¹⁰⁻¹⁷ AIT is the only treatment available to improve or cure an allergic process.¹⁸ Most of the placebo-controlled immunotherapy studies have shown the advantages of this type of treatment. From a clinical point of view, this treatment provides great medical benefit and a reduction in health related costs, since it reduces the consumption of medication and health resources.^{19,20} International guidelines recommend AIT in patients with allergic rhinitis, with or without concomitant asthma.^{6,21-23} To treat allergic respiratory diseases, AIT is mainly administered through the subcutaneous (SCIT)²⁴ or sublingual routes (SLIT).^{6,16,17} SLIT is effective and has a favorable safety profile. However, it requires large doses of allergen and the duration of the treatment is still particularly long, which could be translated into low patient adherence to AIT. SCIT must be administered at the doctor's office, which allows for a better control of the outcomes of treatment and a better monitoring of the progress of the treatment.

D. pteronyssinus (Dpt), *D. farinae* (Df) and *B. tropicalis* (Bt) are the most common house dust mites and are among the most prevalent sources of indoor allergens worldwide, especially in subtropical and tropical regions.²⁵⁻²⁷ These species are very abundant in humid and warm regions,

where most allergic individuals are sensitized to house dust mites. In Latin American countries, about 30% of the total population is sensitized to mites²⁸ and most patients are allergic to Dpt, Df and Bt.²⁹⁻³⁴ In Colombia, *B. tropicalis* is among the most common mite species.³⁵ In the city of El Salvador, Brazil, *B. tropicalis* was the most frequent mite in residences.³⁶ This mite species is the source of several allergens and 14 have been included in the WHO/IUIS Allergen Nomenclature Sub-Committee (<http://www.allergen.org>). Several studies have shown a lack of cross reactivity between *Dermatophagoides* spp. and Bt.³⁷⁻³⁹ These observations indicate that Bt should be included in vaccine preparations for AIT, especially in individuals residing in the tropics and sensitized to these species.⁴⁰ Specific immunotherapy with mite extracts has demonstrated clinical benefits in several double-blind, placebo-controlled trials that are included in recent reviews of subcutaneous immunotherapy, including pediatric and adult patients with rhino-conjunctivitis and, or, asthma.⁴¹⁻⁴⁴

Successful studies of mite immunotherapy have used native allergen extracts adsorbed onto aluminum hydroxide or chemically modified mite-allergen extracts. Several studies have also shown efficacy using SLIT in pediatric and adult patients with asthma and/or rhinitis.⁴³ Because the delivery of high doses of allergen carries with it the risk for immunoglobulin E (IgE)-mediated events, several methods have been developed to reduce specific IgE binding to mite-allergen extracts. Studies of AIT with standardized *B. tropicalis* extracts mixed with other mite species are scarce, but successful and well tolerated.^{41,45} Attempts to design and obtain novel recombinant therapeutic molecules derived from Bt and/or Dpt for AIT have also been proposed.⁴⁶ A hybrid molecule constructed with portions from the allergens Blo t 5 and Blo t 21 showed promising results in *in vitro* and *in vivo* analysis.⁴⁷ However, to the best of our knowledge there are no clinical studies of allergoids including this mite species. Since low to non-cross-reactivity between

Dermatophagoides spp. and *Bt* has been demonstrated, we included the 3 mite species in 1 vaccine to obtain a better hyposensitization to all the allergenic molecules present in these mite species.

Although AIT is commonly used, long-term treatment periods and safety issues still raise some major concerns. Therefore, it is necessary to develop short-term AIT approaches that induce rapid improvement with few administrations. An alternative, to reduce such effects, consists in the use of allergoids: chemically modified extracts obtained by treating the allergen extracts with glutaraldehyde or formaldehyde, which react with free amino groups and produce covalent bonds. As a result, high-molecular-weight aggregates with low IgE-binding capacity are produced. Efficacy⁴⁸ and safety⁴⁹ of AIT with allergoids have been demonstrated in several clinical trials excellent safety profile remains in both adults and children.

In the present study, we evaluated the efficacy and safety of an allergoid prepared with the mixture of allergenic extracts from *Dpt*, *Df*, and *Bt*, in allergic patients from Medellin, Colombia.

MATERIALS AND METHODS

Trial design

This was an open label, prospective study on the efficacy and safety with alum-adsorbed polymerized allergenic extracts (Alxoid), prepared

from the house dust mite species *Dermatophagoides pteronyssinus* (*Dpt*), *Dermatophagoides farinae* (*Df*) and *Blomia tropicalis* (*Bt*).

Trial population

Two hundred and fifty patients (250) were included in this study (Table 1). A Consort flow diagram of the selection process is included in Fig. 1. They were recruited from the Research About Tropical Trends in Asthma (RATTA) project.⁵⁰ Informed consent was obtained from the patients. The protocol was approved by the Ethics Committee of the Institución Prestadora de Salud Universitaria (IPS) of the University of Antioquia (Project Code: 21612948). All patients were diagnosed with allergic rhino-conjunctivitis, with or without asthma (intermittent, mild or moderate) induced by house dust mites (*Dpt*, *Df* and *Bt*), and were treated during 12 months with allergen specific subcutaneous immunotherapy with alum-adsorbed glutaraldehyde-polymerized extracts prepared from the 3 mite species (Alxoid, Immunotek, S.L. Madrid, Spain). The mean age was 15.7 years (± 11 ; 3-72 years of age). From the 250 patients, 133 (45%) were males (mean age = 18.6 years old; SD ± 13.5) and 137 (55%) were females (mean age = 13.4; SD ± 8.5). 217 patients (87%) lived in urban areas and 33 (13%) in rural areas.

All were diagnosed with rhinoconjunctivitis due to allergy to house dust mites, presenting positive

	All	Male	Females	Asthma	No asthma	Urban	Rural
Mean age	15.7	18.6	13.4	14.2	16.8	16.0	13.9
C.I. < 95%	14.3	16.0	11.9	12.0	15.0	14.4	10.9
C.I. > 95%	17.1	21.1	14.8	16.3	18.7	17.6	16.9
S.D.	11.3	13.5	8.5	11.2	11.3	11.7	8.5
Median	12.0	14.0	12.0	11.0	13.0	12.0	12.0
Quartile 1	9.0	10.0	9.0	8.0	11.0	10.0	8.0
Quartile 3	17.0	19.0	14.0	15.5	18.0	17.0	15.0
Maximum	72.0	72.0	72.0	72.0	72.0	72.0	39.0
Minimum	3.0	3.0	3.0	3.0	3.0	3.0	5.0
n	250	113	137	107	143	217	33

Table 1. Demographic data from all patients entered in the study. C.I., Confidence Interval.

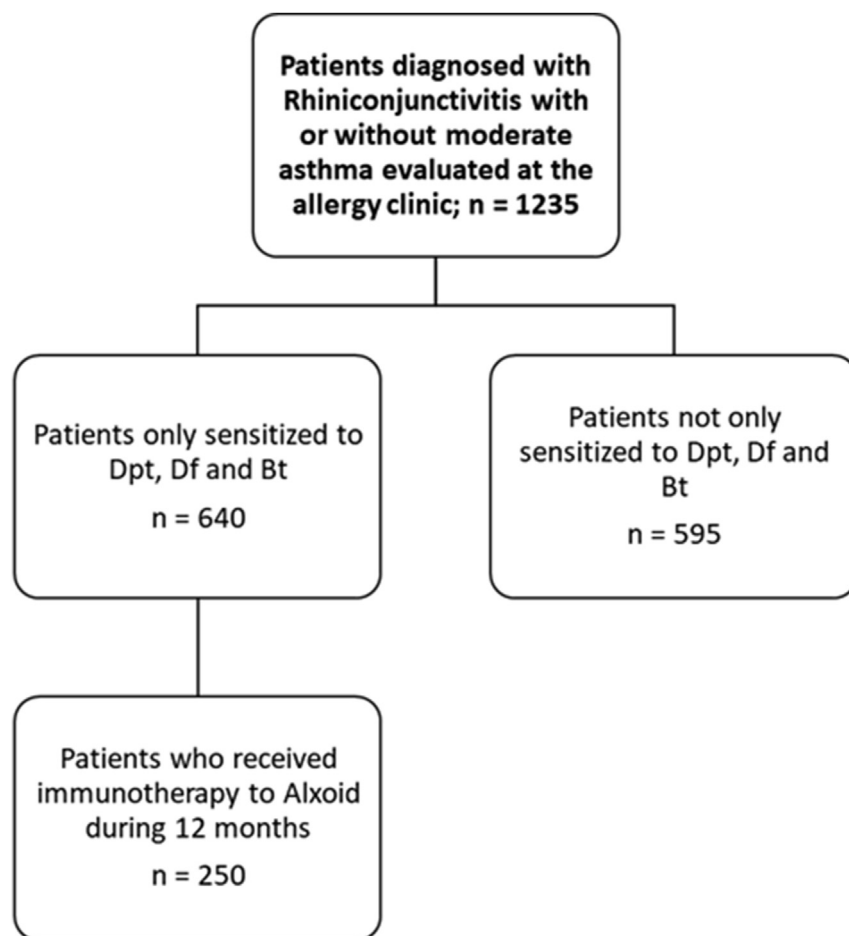


Fig. 1 Consort flow diagram of the study. DpT, *Dermatophagoides pteronyssinus*; Df, *Dermatophagoides farinae*; Bt, *Blomia tropicalis*.

skin prick tests to the 3 mite species and negative to other common aeroallergens, including grasses, weeds and tree pollens, animal dander, and molds. One hundred and seven patients (43%) had intermittent mild to moderate asthma.

Allergen vaccines

Alxoid is an alum adsorbed glutaraldehyde-polymerized allergenic extracts prepared from *Dpt* (33%), *Df* (33%) and *Bt* (34%). The final concentration of the preparation was 10.000 TU/mL. The concentration for each individual extract is: *Dpt*: 3.300 TU/mL, *Df*: 3.300 TU/mL and *Bt*: 3.400 TU/mL. Two injections, containing 0.2 mL (2000 TU) and 0.3 (3000 TU) mL, respectively, 30 min apart were applied on the first day of treatment. One injection containing 0.5 mL (5000 TU) was subsequently applied monthly during the length of the study.

Each polymerized extract originates from its corresponding native extract that has been standardized according to biological units. By

definition, 1 TU contains the same amount of protein as 1 Biological Unit (BU) of its corresponding native extract. These units have been defined in the regulations of the Nordic Countries: an extract contains 10.000 Biological Units (BU) when it induces, in the skin of an allergic subject, a wheal of the same size induced by Histamine Dihydrochloride at 10 mg/mL.⁵¹

Outcome measures

Combination of rhino-conjunctivitis Medication and Symptom Score (CSMS)

Clinical data collected from the patients were evaluated before the start of immunotherapy (T0), and at 3 (T1), 6 (T2) and 12 (T3) months. The European Academy of Allergy and Clinical Immunology (EAACI) published a consensus for CSMS related to allergen immunotherapy trials for allergic rhinoconjunctivitis,⁵² based on a weight equal to the total daily symptom score (dSS) and the total daily medication score (dMS). In this report, the authors considered the use of this

score as advantageous since it has a well-defined terminology of the ocular and nasal symptoms, together with the 0-3 symptom score accepted by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA). The dSS is the sum of all individual symptoms divided by the number of symptoms, the dSS range being 0 to 3 (the maximum score is 3).

The following variables were evaluated:

- Oral and/or topical antihistamines (eyes and/or nose) non-sedating (H1A): 1
- Intranasal corticosteroids (INS) with/without H1A: 2
- Oral corticosteroids with/without INS, with/without H1A: 3

The dMS ranges from 0 to 3. The CSMS is the sum of dSS (range 0-3) and sMS (range 0-3). CSMS values are in the range of 0-6.

Asthma Control Test™-ACT questionnaire

In patients who, in addition to rhinoconjunctivitis, had allergic asthma, a test that consisted of 5 questions in relationship to the frequency of asthma symptoms and use of rescue medication that the patient has needed in the previous 4 weeks was conducted. The scores could range from 5 (worst control) to 25 (total control).⁵³

Global Initiative for Asthma (GINA) score

The Global Initiative for Asthma (GINA) score represents the medication needed to control asthma. This was scored according to the therapeutic steps described in GINA.⁴⁵ It comprises 5 steps, assigning to each step a score of 1-5. Steps 1 and 2 are those that include medication to control mild-intermittent asthma, steps 3 and 4 for moderate asthma, and step 5 for severe asthma.⁵⁴

Statistical analysis

For descriptive statistics, the mean with standard deviation (SD) and the median with the respective first (IQ1) and third quartiles (IQ3) were used. For the efficacy variables, the Shapiro-Wilk test was used to check whether the data obtained followed a normal distribution. In all cases it was found that they did not follow a normal distribution.

For the comparative statistics used in the evaluation of the evolution of CSMS and ACT, the Friedman test for repeated measures was used. Nemeny's procedure was used for the peer-to-peer analysis of each of these measurements.

To evaluate the number of patients who experienced improvement in the GINA Score at the different evaluation points a contingency table analysis (Chi-square) was used.

Ethical conduct of the study

The clinical trial was conducted within the ethical and legal framework established by the local Health Authorities, in accordance with the Declaration of Helsinki,⁵⁵ the Good Clinical Practice (ICH E6: Good Clinical Practice: Consolidated Guideline, CPMP/ICH/135/9).⁵⁶ Patients provided written informed consent.

RESULTS

Efficacy

Combination of rhinoconjunctivitis Medication and Symptom Score

Sustained and significant reductions of the need for medication and allergic symptoms were observed during the immunotherapy treatment, as evaluated by the CSMS. At the beginning of the study, the median for CSMS was 5. After 3 months the value was 4 (20% reduction), at 6 months it remained at 4 and at 12 months the value was 3 (reduction 40%) (Table 2). Patients showed a clear statistical significance ($P < 0,0001$) to improve the scores over time as a consequence of the allergen specific immunotherapy, according to the Friedman test and Nemeny's procedure (Fig. 2A).

Medication use according to the GINA scale

This score was performed only in patients with asthma. The median value at baseline was 3 (Table 3). After 12 months the GINA value was 2 (33% reduction). The analysis was performed considering steps 1 through 5. Steps 1 and 2 corresponded to mild asthma control, while steps 3, 4, and 5 corresponded to moderate-severe asthma control. The evolution in the treatment scale can be observed in Table 4. At T0, the number of patients who were medicated with

	T0	T1	T2	T3
Mean	5.1	4.3	3.7	3.1
C.I. < 95%	5.0	4.2	3.6	3.0
C.I. > 95%	5.3	4.4	3.8	3.2
S.D.	0.9	1.0	1.1	1.1
Median	5.0	4.0	4.0	3.0
Quartile 1	5.0	4.0	3.0	2.0
Quartile 3	6.0	5.0	4.0	4.0
Maximum	6.0	6.0	6.0	6.0
Minimum	1.0	1.0	0.0	0.0
n	250	250	250	250

Table 2. Descriptive statistics from the CSMS at the beginning of the study (T0), T1, T2 and T3. C.I.: Confidence Interval.

steps 1 and 2 was 29, going from 36 in T1 (P Chi-Square = 0.2982), to 43 in T2 (P Chi-Square = 0.0428), and at 60 in T3 (P Chi-Square <0.0001), which indicates a statistically significant improvement in the consumption of medication (Fig. 1B and Table 4). There is a clear trend towards

less consumption of medication for asthma control. Considering steps 1 and 2 to treat mild asthma and steps 3, 4 and 5 to treat moderate asthma,⁴⁵ a clear trend is observed to decrease the medication of steps 3, 4 and 5 from the beginning T0 to T3. Patients with moderate-

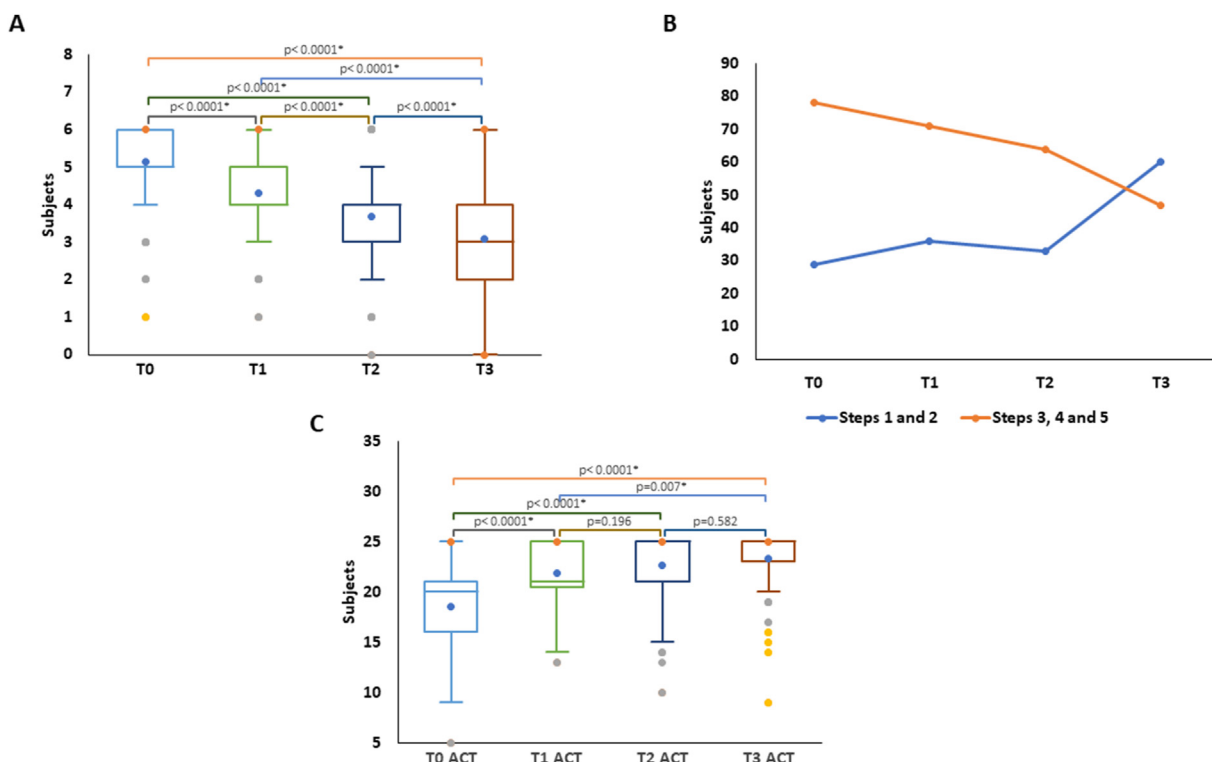


Fig. 2 A. Box plot comparison between CSMS pairs. *: $p < 0,0001$. **B.** Evolution of patients with medication in steps 1 and 2, and 3, 4 and 5. **C.** Box plot comparison between ACT pairs. *: Statistically significant.

	TO GINA	T1 GINA	T2 GINA	T3 GINA
Mean	3.4	3.2	3.0	2.6
C.I. < 95%	3.3	3.1	2.8	2.3
C.I. > 95%	3.5	3.4	3.2	2.8
S.D.	0.5	0.7	0.9	1.1
Median	3.0	3.0	3.0	3.0
Quartile 1	3.0	3.0	3.0	2.0
Quartile 3	4.0	4.0	4.0	3.0
Maximum	4.0	5.0	5.0	5.0
Minimum	3.0	1.0	1.0	1.0
n	78	78	78	72

Table 3. Descriptive statistics of the medication score according to GINA at the beginning (T0) and, T1, T2 and T3

			Patients per GINA scale			
	GINA step		T0	T1	T2	T3
	1	n	4	11	14	28
		%	3.7%	10.3%	13.3%	28.3%
	2	n	25	25	29	32
		%	23.4%	23.4%	27.6%	32.3%
	3	n	46	44	39	30
		%	43.0%	41.1%	37.1%	30.3%
	4	n	32	25	23	16
		%	29.9%	23.4%	21.9%	16.2%
	5	n	0	2	2	1
		%	0.0%	1.9%	1.9%	1.0%
Total patients			107	107	107	107
Steps 1 and 2 (Mild asthma)			29	36	43	60
			27.1%	33.6%	40.2%	56.1%
Steps 3 to 5 (Moderate/severe asthma)			78	71	64	47
			72.9%	66.4%	59.8%	43.9%
Chi Square <i>p</i> value (Compared to T0)				0.2982	0.0428	<0.0001

Table 4. Patients that received during each medical visit according to GINA and statistical analysis (Chi square).

		Patients that improved from T0 to		
		T1	T2	T3
Yes	n	21	32	57
	%	19.6%	30.5%	57.6%
No	n	86	75	50
	%	80.4%	69.5%	42.4%

Table 5. Improvement of clinical criteria according to GINA

severe asthma go from 78 in T0 to 47 in T3 (12 months), which means a decrease of 40%.

Regarding the GINA step of T0, the number of patients who experienced improvement in medication intake in T1 was 21 (20%), in T2 it was 32 (30%) and in T3 it was 57 (58%) (Table 5).

Asthma Control Test TM-ACT questionnaire

For patients with asthma, the median value at the beginning was 20, which increased to 25 after 12 months (Fig. 1C). Comparative statistics (Friedman test) indicated that the differences between the groups are highly significant ($P < 0.0001$) (Fig. 1C) even after only the first 3 months of treatment with Alxoid.

Safety

No adverse reactions, either local (>5 cm for immediate reactions and >10 cm for late reactions) or systemic (immediate or delayed, greater than grade II) were reported.

DISCUSSION

In Latin America allergic diseases are common and house dust mites are the most important sources of indoor allergens.^{57,58} These pathologies negatively affect sleeping schedules, work productivity, and quality of life. Unlike food and drug allergies, it is seldom possible to completely avoid contact with the responsible allergens that cause this disease.⁵⁹ In patients with moderate-severe symptoms, demonstrable sensitization by skin test, or by determination of specific IgE to the relevant allergen, and that do not respond adequately to antiallergic drugs, immunotherapy with aeroallergens is recommended.^{59,60} Subcutaneous and intradermal

allergen immunotherapy is effective for both seasonal allergic rhinitis caused by pollen⁶¹ and perennial allergic rhinitis with or without asthma caused by house dust mites.^{41,62} Furthermore, this kind of treatment is indicated in patients with mild to moderate asthma, which is supported by data showing that standardized allergenic extracts of Dpt y Df induce protection when used in immunotherapy approaches.⁶³⁻⁶⁵

In this report we present, to the best of our knowledge, the first prospective study of immunotherapy using alum-adsorbed glutaraldehyde-polymerized mixture of allergenic extracts, containing the allergenic material from three house dust mite species (Dpt, Df, and Bt) in patients from Medellín, Colombia. In this clinical trial, we demonstrate clinical efficacy and good tolerance to the preparations. The study lasted 12 months, and in this period all parameters tested indicated that the use of such preparation was successful and safe.

The allergic patients recruited for the study (250 individuals) had allergic rhino-conjunctivitis, with or without asthma (intermittent, mild or moderate) induced by house dust mites, and showed a statistically significant clinical improvement with reductions in the allergic symptoms and the need of anti-allergic medication. These results resemble what have been observed in other studies of immunotherapy with extracts from *Dermatophagoides* spp., where allergic symptoms and the need of medication is reduced because of the treatment, as evidenced in improvement of symptom, medication and cumulative scores.^{66,67}

The CRMSS is a reliable way to measure the success of the immunotherapy for allergic rhino-

conjunctivitis since such treatment decreases the incidence and severity of allergic symptoms, and the use of concomitant rescue medication. The CRMSS evaluates both parameters in a single unbiased data set and is a good tool to test the results of the immunotherapy. At T0, the number of patients who were medicated with steps 1 and 2 was 29, and it gradually increased with time until reaching 60 in T3. Similarly, patients who were medicated with steps 3, 4, and 5 showed improvement as they went from 78 in T0 to 47 in T3 (12 months), which means a decrease of 40%, indicating that both severity of allergic symptoms and the use of concomitant rescue medication, improved.

For a successful immunotherapy, it is important that the allergenic preparation contains the allergens to which the patient is sensitized. Dpt, Df, and Bt are the most common mite species in Colombia and neighboring countries, especially in the tropical area.²⁵⁻²⁷ About 95% of the allergic population is sensitized to *Bt*.⁶⁸ Several data indicate that specific immunotherapy with Dpt or Df is successful but there is not too much information for immunotherapy with Bt extracts. Therefore, in the event that the allergic patient presents a skin sensitization to *B. tropicalis* and has signs and symptoms of allergic rhinitis with or without asthma, the inclusion of this species in the extract for immunotherapy is indicated. Previous studies have shown that sensitization to *B. tropicalis* is specific, and therefore should be treated. Co-sensitization to *Dermatophagoides* spp. and *Bt* is common in Colombia, mainly due to exposure to both mite species and not due to cross-reactivity.

In the present study, none of the subjects presented immunotherapy-related adverse reactions [local (>5 cm for immediate reactions and >10 cm for late reactions), or systemic (immediate or delayed, greater than grade II)] at any time during the study, demonstrating that this preparation is effective and safe to treat mite allergic patients. In conclusion, we have conducted an immunotherapy study using a mixture of polymerized extracts of Dpt, Df and Bt and the results clearly demonstrate that this treatment is safe and effective to treat patients with rhinoconjunctivitis with, or without asthma.

Abbreviations

ACT, Asthma Control Test; AIT, Allergen immunotherapy; Bt, *Blomia tropicalis*; BU, Biological units; CSMS, Combined Symptom and Medication Scores; Df, *Dermatophagoides farinae*; Dpt, *Dermatophagoides pteronyssinus*; dMS, daily medication score; dSS, daily symptom score; EAACI, European Academy of Allergy and Clinical Immunology; EMA, European Medicines Agency; FDA, Food and Drug Administration; GINA, Global Initiative for Asthma; IgE, Immunoglobulin E; IgG, Immunoglobulin G; INS, Intranasal corticosteroids; mL, milliliter; RATA, Research About Tropical Trends in Asthma; SCIT, Subcutaneous immunotherapy; SLIT, Sublingual immunotherapy; Treg, Regulatory T cells; TU, Therapeutic units; US, United States; WHO/IUIS, World Health Organization/International Union of Immunological Societies.

Acknowledgments

We thank all the participants, coordinators, and investigators for their participation.

Funding

Not applicable.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available.

Author contributions

RC conceived and designed the clinical work. SUG, VD, RC conducted and completed the clinical aspects of the study. All the analysis of the data was performed by JFC, SUG and VD. The manuscript was written by JFC, and critically revised EFC and RC. All authors approved the final version of the manuscript.

Ethical considerations

The Ethic Committee of the University of Antioquia from Medellín-Colombia approved this study. Because some of the participants were minors, the parents gave written informed consent. According to the request of the ethics committee, children also gave their assent, which was supervised by a child psychologist in children under 10 years of age.

Author's consent for publication

After individually checking the final version of the manuscript, all authors manifest consent for its publication, and are willing to confirm such statement at any given time.

Declaration of competing interest

- Uribe-Garcia, Susana: Grupo de Alergología Clínica y Experimental (GACE). University of Antioquia. Medellín, Antioquia. Colombia and reports no conflict interest

- Calvo-Betancur Víctor Daniel: Grupo de Alergología Clínica y Experimental (GACE). University of Antioquia. Medellín, Antioquia. Colombia and reports no conflict of interest
- Cantillo, Jose Fernando: Is currently employed by the Spanish Pharmaceutical company Inmunotek, S.L., which provided the medication.
- Fernández-Caldas, Enrique: Is currently employed by the Spanish Pharmaceutical company Inmunotek, S.L., which provided the medication.
- Cardona-Villa, Ricardo: Is currently employed by Grupo de Alergología Clínica y Experimental (GACE). University of Antioquia. Medellín, Antioquia. Colombia, and reports no conflict of interests

Submission declaration

We declare that all the authors confirm that they have read and agreed to the following submission statements:

- the submission is original and has not been previously published. This manuscript is not currently under evaluation by another journal.
- all permissions (when applicable) have been obtained
- the manuscript includes all the relevant statements and acknowledgements

Author details

^aGrupo de Alergología Clínica y Experimental (GACE), University of Antioquia, Medellín, Antioquia, Colombia.

^bInmunotek, S.L. Alcalá de Henares, Madrid, Spain.

^cInmunotek, S.L. Madrid and University of South Florida College of Medicine, Tampa, FL, USA.

REFERENCES

1. Burney P, Jarvis D, Perez-Padilla R. The global burden of chronic respiratory disease in adults. *Int J Tuberc Lung Dis*. 2015;19(1):10-20.
2. Lai CK, Beasley R, Crane J, et al. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax*. 2009;64(6):476-483.
3. Chinn S, Jarvis D, Burney P, et al. Increase in diagnosed asthma but not in symptoms in the European community respiratory health survey. *Thorax*. 2004;59(8):646-651.
4. Sullivan PW, Ghushchyan VH, Campbell JD, Globe G, Bender B, Magid DJ. Measuring the cost of poor asthma control and exacerbations. *J Asthma*. 2017;54(1):24-31.
5. Tavakoli H, FitzGerald JM, Chen W, et al. Ten-year trends in direct costs of asthma: a population-based study. *Allergy*. 2017;72(2):291-299.
6. Agache I, Lau S, Akdis CA, et al. EAACI Guidelines on Allergen Immunotherapy: house dust mite-driven allergic asthma. *Allergy*. 2019;74(5):855-873.
7. Khan DA. Allergic rhinitis and asthma: epidemiology and common pathophysiology. *Allergy Asthma Proc*. 2014;35(5):357-361.
8. Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am J Respir Crit Care Med*. 1995;151(4):969-974.
9. Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev*. 2000;(2):CD001186.
10. Larsen JN, Broge L, Jacobi H. Allergy immunotherapy: the future of allergy treatment. *Drug Discov Today*. 2016;21(1):26-37.
11. Schmidt-Weber CB, Blaser K. New insights into the mechanisms of allergen-specific immunotherapy. *Curr Opin Allergy Clin Immunol*. 2005;5(6):525-530.
12. Larché M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol*. 2006;6(10):761-771.
13. Walker SM, Durham SR, Till SJ, et al. Immunotherapy for allergic rhinitis. *Clin Exp Allergy*. 2011;41(9):1177-1200.
14. Bousquet J, Schunemann HJ, Samolinski B, et al. Allergic rhinitis and its impact on asthma (ARIA): achievements in 10 years and future needs. *J Allergy Clin Immunol*. 2012;130(5):1049-1062.
15. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol*. 2001;108(5 Suppl):S147-S334.
16. Jutel M, Agache I, Bonini S, et al. International consensus on allergy immunotherapy. *J Allergy Clin Immunol*. 2015;136(3):556-568.
17. Jutel M, Agache I, Bonini S, et al. International consensus on allergen immunotherapy II: mechanisms, standardization, and pharmacoeconomics. *J Allergy Clin Immunol*. 2016;137(2):358-368.
18. Malling HJ. The position of immunotherapy in the European Academy of allergology and clinical immunology. *J Investig Allergol Clin Immunol*. 1997;7(5):356-357.
19. Ariano R, Berto P, Tracci D, Incorvaia C, Frati F. Pharmacoeconomics of allergen immunotherapy compared with symptomatic drug treatment in patients with allergic rhinitis and asthma. *Allergy Asthma Proc*. 2006;27(2):159-163.
20. Nieto A, Alvarez-Cuesta E, Boquete M, Mazon A, de la Torre F. The cost of asthma treatment in Spain and rationalizing the expense. *J Investig Allergol Clin Immunol*. 2001;11(3):139-148.
21. Bousquet J, Dahl R, Khaltaev N. Global alliance against chronic respiratory diseases. *Allergy*. 2007;62(3):216-223.
22. Bousquet J, Clark TJ, Hurd S, et al. GINA guidelines on asthma and beyond. *Allergy*. 2007;62(2):102-112.
23. Fokkens WJ, Lund VJ, Mullol J, et al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology*. 2012;50(1):1-12.
24. Wahn U, Klimek L, Ploszczuk A, et al. High-dose sublingual immunotherapy with single-dose aqueous grass pollen extract in children is effective and safe: a double-blind, placebo-controlled study. *J Allergy Clin Immunol*. 2012;130(4):886-893 e5.
25. Caraballo L, Zakzuk J, Lee BW, et al. Particularities of allergy in the tropics. *World Allergy Organ J*. 2016;9:20.

26. Manolio TA, Barnes KC, Naidu RP, Levett PN, Beatty TH, Wilson AF. Correlates of sensitization to *Blomia tropicalis* and *Dermatophagoides pteronyssinus* in asthma in Barbados. *Int Arch Allergy Immunol.* 2003;131(2):119-126.
27. Oliveira-Santos S, Motta-Franco J, Barreto I, Sole D, Gurgel R. Asthma in adolescents-Prevalence trends and associated factors in northeast Brazil. *Allergol Immunopathol.* 2015;43(5):429-435.
28. Arlian LG, Platts-Mills TA. The biology of dust mites and the remediation of mite allergens in allergic disease. *J Allergy Clin Immunol.* 2001;107(3 Suppl):S406-S413.
29. Caraballo L, Puerta L, Fernandez-Caldas E, Lockey RF, Martinez B. Sensitization to mite allergens and acute asthma in a tropical environment. *J Investig Allergol Clin Immunol.* 1998;8(5):281-284.
30. Cardona ROJ, Montoya F, Orduz C, Betancourt M, Dennis R, Rojas MX. Sensibilidad cutánea a aeroalergenos en pacientes asmáticos de la ciudad de Medellín, Colombia. *Revista de la Asociación Colombiana de Alergias e Inmunología.* 2003;11(2):7.
31. Dennis R, Caraballo L, Garcia E, et al. Asthma and other allergic conditions in Colombia: a study in 6 cities. *Ann Allergy Asthma Immunol.* 2004;93(6):568-574.
32. Fernandez-Caldas E, Baena-Cagnani CE, Lopez M, et al. Cutaneous sensitivity to six mite species in asthmatic patients from five Latin American countries. *J Investig Allergol Clin Immunol.* 1993;3(5):245-249.
33. Sánchez Medina M. *Ácaros en Colombia y su Relación con las Alergias Respiratorias: Enrique Fernández-Caldas (Editores).* Bogotá, Colombia: Editora Guadalupe Ltda; 1994.
34. Sánchez-Caraballo JD-ZS, Cardona-Villa R. Sensibilización a aeroalérgenos en pacientes alérgicos de Medellín, Colombia. *Rev Alerg Mex.* 2012;59(3):139-147.
35. Fernandez-Caldas E, Puerta L, Mercado D, Lockey RF, Caraballo LR. Mite fauna, Der p I, Der f I and *Blomia tropicalis* allergen levels in a tropical environment. *Clin Exp Allergy.* 1993;23(4):292-297.
36. Baqueiro T, Carvalho FM, Rios CF, dos Santos NM, Alcantara-Neves NM, Medical Student G. Dust mite species and allergen concentrations in beds of individuals belonging to different urban socioeconomic groups in Brazil. *J Asthma.* 2006;43(2):101-105.
37. Stanaland BE, Fernandez-Caldas E, Jacinto CM, Trudeau WL, Lockey RF. Positive nasal challenge responses to *Blomia tropicalis*. *J Allergy Clin Immunol.* 1996;97(5):1045-1049.
38. Stanaland BE, Fernandez-Caldas E, Jacinto CM, Trudeau WL, Lockey RF. Sensitization to *Blomia tropicalis*: skin test and cross-reactivity studies. *J Allergy Clin Immunol.* 1994;94(3 Pt 1):452-457.
39. Santos da Silva E, Asam C, Lackner P, et al. Allergens of *Blomia tropicalis*: an overview of recombinant molecules. *Int Arch Allergy Immunol.* 2017;172(4):203-214.
40. González-Pérez R, El-Qutob D, Letrán Antonio MV. Precision medicine in mite allergic rhinitis. *Front Allergy.* 2021;2:6.
41. Rondon C, Sanchez-Borges M, Cupello ER, Fabiano F, Capriles-Hulett A. Aqueous intradermal low-dose house dust mite immunotherapy in tropical settings: a valid cost-effective approach for developing nations? *Allergol Immunopathol.* 2021;49(2):31-39.
42. Fernandez-Caldas E, Iraola V, Boquete M, Nieto A, Casanovas M. Mite immunotherapy. *Curr Allergy Asthma Rep.* 2006;6(5):413-419.
43. Tang RB. House dust mite-specific immunotherapy alters the natural course of atopic march. *J Chin Med Assoc.* 2020;83(2):109-112.
44. Yang L, Zhu R. Immunotherapy of house dust mite allergy. *Hum Vaccines Immunother.* 2017;13(10):2390-2396.
45. Vieira-Hernandez A, Capriles-Hulett A, Sanchez-Borges M, Fabiano F, Albarran-Barrios C. [Intradermal immunotherapy with low-dose house dust mite allergens in patients with allergic rhinitis: a proof-of-concept study]. *Rev Alerg Mex.* 2018;65(1):41-51.
46. Martinez D, Cantillo JF, Herazo H, et al. Characterization of a hybrid protein designed with segments of allergens from *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Immunol Lett.* 2018;196:103-112.
47. da Silva ES, Aglas L, Pinheiro CS, et al. A hybrid of two major *Blomia tropicalis* allergens as an allergy vaccine candidate. *Clin Exp Allergy.* 2020;50(7):835-847.
48. Jutel M, Bruggenjurgen B, Richter H, Vogelberg C. Real-world evidence of subcutaneous allergoid immunotherapy in house dust mite-induced allergic rhinitis and asthma. *Allergy.* 2020;75(8):2050-2058.
49. Guzman-Fulgencio M, Caballero R, Lara B, et al. Safety of immunotherapy with glutaraldehyde modified allergen extracts in children and adults. *Allergol Immunopathol.* 2017;45(2):198-207.
50. Gaviria R, Ocampo J, Londoño J, Calvo V, Cardona R, Sánchez J. Sensibilización IgE y las condiciones sociodemográficas como determinantes en la gravedad del asma. *Rev Alerg Mex.* 2017;64:439.
51. Nordic-Council-on-Medicines. *Registration of allergen preparations. Nordic Guidelines. Prepared by the Nordic Council on Medicines in Cooperation with the Drug Regulatory Authorities in Denmark, Finland, Iceland, Norway and Sweden.* Uppsala (Sweden): Nordiska Läkemiddelsnämnden; 1989.
52. Pfaar O, Demoly P, Gerth van Wijk R, et al. Recommendations for the standardization of clinical outcomes used in allergen immunotherapy trials for allergic rhinoconjunctivitis: an EAACI Position Paper. *Allergy.* 2014;69(7):854-867.
53. Cloutier MM, Schatz M, Castro M, et al. Asthma outcomes: composite scores of asthma control. *J Allergy Clin Immunol.* 2012;129(3 Suppl):S24-S33.
54. The Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. Accessed on 2019 <http://www.ginasthma.com/>.
55. Rosenau H. Legal prerequisites for clinical trials under the revised declaration of Helsinki and the European convention on human rights and biomedicine. *Eur J Health Law.* 2000;7(2):105-121.
56. ICH. *ICH topic E 6 (R1) guideline for good clinical Practice.* 1996. Note for Guidance on Good Clinical Practice.
57. Calderon MA, Linneberg A, Kleine-Tebbe J, et al. Respiratory allergy caused by house dust mites: what do we really know? *J Allergy Clin Immunol.* 2015;136(1):38-48.
58. Guillemainault L, Viala-Gastan C. [*Blomia tropicalis*: a house dust mite in the tropics]. *Rev Mal Respir.* 2017;34(8):791-801.

59. Scadding GK, Kariyawasam HH, Scadding G, et al. BSACI guideline for the diagnosis and management of allergic and non-allergic rhinitis (Revised Edition 2017; First edition 2007). *Clin Exp Allergy*. 2017;47(7):856-889.
60. Roberts G, Pfaar O, Akdis CA, et al. EAACI guidelines on allergen immunotherapy: allergic rhinoconjunctivitis. *Allergy*. 2018;73(4):765-798.
61. Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev*. 2007;2007(1). CD001936-CD.
62. Durham SR, Penagos M. Sublingual or subcutaneous immunotherapy for allergic rhinitis? *J Allergy Clin Immunol*. 2016;137(2):339-349. e10.
63. Abramson MJ, Puy RM, Weiner JM. Injection allergen immunotherapy for asthma. *Cochrane Database Syst Rev*. 2010;8:CD001186.
64. Bousquet J, Lockett R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol*. 1998;102(4 Pt 1):558-562.
65. Boonpiyathad T, Pradubpongsa P, Mitthamsiri W, Satitsuksanoa P, Jacquet A, Sangasapaviliya A. Allergen-specific immunotherapy boosts allergen-specific IgD production in house dust mite-sensitized asthmatic patients. *Allergy*. 2020;75(6):1457-1460.
66. Vesna TS, Denisa D, Slavenka J, et al. Efficacy of sublingual immunotherapy with *Dermatophagoides pteronyssinus*: a real-life study. *Iran J Allergy, Asthma Immunol*. 2016;15(2):112-121.
67. Xu CX, Zhang ML, Li BZ, et al. Efficacy of sublingual immunotherapy with *Dermatophagoides farinae* extract in monosensitized and polysensitized patients with allergic rhinitis: clinical observation and analysis. *BioMed Res Int*. 2015;2015, 187620.
68. Puerta Llerena L, Fernandez-Caldas E, Caraballo Gracia LR, Lockett RF. Sensitization to *Blomia tropicalis* and *Lepidoglyphus destructor* in *Dermatophagoides* spp-allergic individuals. *J Allergy Clin Immunol*. 1991;88(6):943-950.