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Subcutaneous allergen immunotherapy for asthma: A randomized, double-blind, placebo-controlled study with a standardized *Blomia tropicalis* vaccine

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ABSTRACT

Background: Sensitization to *Blomia tropicalis* (*Bt*) is very frequent in the tropics, and particularly in Cuba, being a significant cause of allergic asthma. Allergen immunotherapy (AIT) with *Bt* can be a therapeutic option, however, placebo-controlled clinical trials have not been reported.

Objective: To assess the therapeutic effect and safety of AIT for asthma using a standardized allergen vaccine of *B. tropicalis* by subcutaneous route, in allergic asthmatic patients exposed and sensitized to this mite species.

Methods: A double-blind, placebo-controlled Phase II trial was conducted in 35 adults (18 with treatment and 17 with placebo), with mild to moderate asthma, predominantly sensitized to *Bt*. AIT was administered subcutaneously in increasing doses from 4 to 6000 Biological Units using a locally manufactured standardized extract (BIOCEN, Cuba). Patient assessment was performed using symptom-medication score (SMS), peak expiratory flow and skin reactivity relative to Histamine as measured by skin prick test (SPT).

Results: The 12-month treatment achieved a significant ($p < 0.001$) decrease of SMS. Symptom score showed only 41% (CI: 26–61) of placebo values, whereas medication was 34.5% (22.4%–63.3%). Treatment was regarded clinically effective in 67% of patients (OR 32; 95%CI: 17 to 102). The effect size on symptoms and medication was higher than has been reported with equivalent allergen dosages of *D. pteronyssinus* and *D. siboney* in Cuban asthmatic patients. Skin reactivity to *Bt* was also significantly reduced ($p = 0.0001$), increasing 148-fold the allergen threshold to elicit a positive skin test. This desensitization effect was specific to *Bt* and did not modify the reactivity to *Dermatophagoides*. The change of specific skin reactivity was significantly ($p < 0.05$) correlated to clinical improvement. All adverse events were local with a frequency of 2.4% of injections.

Conclusions: Subcutaneous AIT with *Blomia tropicalis* was effective and safe in asthmatic adults exposed and sensitized to this mite species in a tropical environment.

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Keywords: Subcutaneous immunotherapy, *Blomia tropicalis*, Standardized allergen extract, Clinical trial

INTRODUCTION

World Health Organization (WHO) reports that approximately 235 million people worldwide have asthma.[1] In Latin American, several studies confirm an increasing trend in asthma prevalence.[2] Prevalence in Cuba is also high, ranging from 8.5% to 10% of adults and up to 17.8% of children.[3,4] In addition, the direct and indirect costs of asthma represent a major economic burden to the Cuban health care system and society.[5]

House Dust Mite (HDM) allergens are strongly involved in the pathogenesis of allergic asthma. In addition to well-known Dermatophagoides species, sensitization to *Blomia tropicalis* is highly prevalent in several "Latin American" countries, e.g. Brazil, Colombia, Cuba, and the Caribbean, which are regions with humid tropical climates.[6–10] Particularly, in Cuba the sensitization frequency to *B. tropicalis* among asthmatics has been reported to be similar to *Dermatophagoides pteronyssinus* and *D. siboney*.[11–13] Unlike the species belonging to the *Pyroglyphidae* family (e.g. *Dermatophagoides*), the allergens of *Blomia tropicalis* share limited sequence homology and cross-reactivity with them.[6,11,13] These species have unique allergenic molecules and a distinctive profile of major allergens. Thus, sensitization to *Blomia* results from relevant exposure to this mite, frequently found in the house environment including house dust and not as a consequence of cross-reactive IgE antibodies.[6,11–17]

Allergen immunotherapy (AIT) is still considered a cornerstone of the management of allergic diseases (allergic rhinitis and asthma) as the only disease-modifying treatment for allergic patients and it is effective in reduction of symptoms and medication usage.[18–21] HDM SCIT is indicated for treatment of mild to moderate asthma in sensitized patients, supported by extensive clinical data using standardized allergen extracts of *D. pteronyssinus* and *D. farinae*.[20] In contrast, very little is known about AIT with *Blomia tropicalis*, and no reports of rigorous randomized controlled clinical trials have been published. Therefore, establishing the efficacy and safety of AIT to *B. tropicalis* is important for countries with high prevalence of sensitization to this mite because of the economic costs/burden of allergic asthma to health care system. Thus, the aim of this work was to assess the efficacy and safety of a standardized allergen vaccine of *Blomia tropicalis* by subcutaneous route in allergic asthmatic adults sensitized to this mite species.

METHODS

Study design

A Phase II (therapeutic effect), double-blind, placebo-controlled, randomized clinical trial was performed in 35 adult allergic patients, with mild to moderate persistent asthma. Eighteen patients received active treatment with injections of the *Blomia tropicalis* allergen extract and seventeen received placebo. The trial was conducted at the Allergy and Immunology Service of the "Calixto García" University Hospital in Havana (Code in the Cuban Public Registry of Clinical Trials: RPCEC00000026).[22] The study was designed and conducted according to international guidelines for Good Clinical Practice and was approved by the Research Ethics Committee of the clinical site and the Cuban medicine regulatory authority (CECMED).

Treatment

Allergen injections were administered by subcutaneous route in a dose escalation scheme, first,

an induction phase with weekly injections during the first 13 weeks, followed by a maintenance phase of monthly injections up to 12 months (Table 1). The standardized allergen extract VALERGEN-BT (*Blomia tropicalis*) developed and manufactured by BIOCEN (Bejucal, Cuba) was used. This allergen product is licensed in Cuba as medicine. Standardization is expressed in Biological Units (BU) according to the definition of Nordic Guidelines for the Registration of Allergen Products, which takes Histamine HCl 10 mg/mL as a reference for defining the biological activity at 10 000 BU/mL.[23] The maintenance dose was 6000 BU, which is equivalent roughly to 150 µg of total protein and 60 µg of the fraction between 12 and 21 Kda encompassing the allergens Blo t 5, Blo t 21, Blo t 13 and Blo t 2.[24] The extract is available as a freeze-dried product, which is reconstituted in an aqueous phosphate buffered saline (PBS) albumin diluent. Both the active and control group received concomitant symptomatic treatment consisting of antihistamines, bronchodilators, β2-agonists, and oral steroids, as prescribed by "blinded" physicians.

Participants

The study involved men and women aged 16–45 years with clinical diagnosis of mild to moderate persistent asthma diagnosed according to GINA guidelines,[25] possibly including concomitant manifestations of rhinitis, atopic dermatitis, and/or conjunctivitis. Diagnosis of allergic asthma was based on clinical history and confirmed by PEF measurement and the airway response reversibility test with bronchodilators. Patient's allergic status was supported by a positive skin prick test (SPT) to *Blomia tropicalis* allergen extract (BIOCEN, Cuba, 20 000 BU/mL) and clinical assessment consistent with allergic respiratory symptoms (cough, wheezing, chest tightness and dyspnea) upon exposure to house dust. The skin test was considered positive if the wheal diameter was greater or equal to 3 mm. In addition to *Blomia tropicalis*, reactivity to *D. siboney* and *D. pteronyssinus* was also tested. Since strict monosensitization in this case is very difficult, patients with predominant sensitization to *Blomia tropicalis* (i.e. with a reaction size larger

Week	Concentration (BU/mL)	Volume (mL)	Dose (BU)
1	20	0,2	4
2	20	0,5	10
3	20	1	20
4	200	0,2	40
5	200	0,5	100
6	200	1	200
7	2000	0,2	400
8	2000	0,4	800
9	2000	0,6	1200
10	2000	0,8	1600
11	20000	0,1	2000
12	20000	0,2	2000
13	20000	0,3	6000
27 to 52	20000	0,3	6000

Table 1. Dosing schedule

than 2 mm, with respect to the other mite species) were selected. Patients were excluded from the trial if they had been treated with AIT in the last 2 years, or if they had had severe intermittent or persistent asthma or received immunostimulant or immunosuppressive treatment one year prior to the study.

Outcomes

The therapeutic effect was measured through symptom and medication scores, and the combination of both as the main endpoint. In addition, lung function and skin reactivity relative to histamine (Ch_{10}) were used also as secondary outcomes.

Symptom and Medication (SM) scores were collected using a form completed by the patient with a 4 level scale with the following values: 0 = absent, 1 = mild, 2 = moderate, 3 = severe, for each symptom manifestation (cough, wheezing, chest tightness, and dyspnea). In addition, Medication Score measured the frequency of drug intake. Each time a drug was consumed the score was incremented by 1 point, except oral steroids that were worth 2 points. At the end of the month, all points were summed up.[26,27] For the semiannual partial evaluations (at months 6 and 12) the values of the last 2 months were averaged: 5–6, and 11–12 months, respectively. Baseline pre-treatment value was established measuring SM scores during 1 month. For comparing with published meta-analysis the Standard Mean Difference (SMD) was calculated (i.e. difference between active and placebo, divided by the standard error) according to Cochrane methodology.[28]

Allergen specific reactivity was assessed by (SPT) to *Bt*, *Dp,* and *Ds* allergen extracts at 20,000 BU/mL. Stainless steel lancets with tips of 1 mm (ALK, Denmark) were used according to Dreborg.[29] Histamine HCl 10 mg/mL was used as a positive control, and PBS as negative control. In order to record the test results, a line was drawn around the wheal and transferred to a transparent adhesive tape, which was finally taped on to the data recording book. The largest and the orthogonal diameters were measured on the wheal drawing, and the mean diameter was calculated. Furthermore, the mean diameter (d) between the 2 arms was calculated. The test was considered valid if the difference between both arms wheals was less than 2 mm for wheals between 3 and 6 mm, or less than 3 mm for larger wheals; besides being positive for Histamine (positive control) and negative for the diluent solution (negative control). The test was considered positive for $d \geq 3$ mm. Wheal area was calculated from the mean diameter (d) according to the expression: $A = \pi (d^2/4)$. Allergen specific reactivity was calculated relative to the Histamine HC 10 mg/mL, using this expression:[29,30]

$$Ch_{10} = (A_a/A_h)^{2.5}$$

(where A_a: allergen wheal area; A_h: histamine wheal area).

Pulmonary function was measured through peak expiratory flow (PEF) with the use of a portable PEF meter (Ferraris, UK). This measurement was performed daily by the patient at home, whereas daily PEF variability was calculated by the physician.[31,32]

A secondary dichotomical variable was used to assess the overall clinical improvement (OCI) of each patient, with two levels: "better" and "not better". The "better" value was assigned when the symptom/medication score was reduced below 60%,[26] with respect to the baseline value and the skin reactivity (Ch_{10}) and lung function category did not worsen with respect to the beginning of the treatment.

Safety assessment

Adverse reactions were recorded and classified as local or systemic, or immediate or delayed.[31] Local reactions were assessed by erythema diameter. Local immediate reactions with a diameter of less than 5 cm and delayed reactions of less than 10 cm were considered mild. Systemic reactions were graded in accordance with the World Allergy Organization (WAO).[33,34]

Statistical methods

The statistical package GlaxoWellcome C4-SDP was used for the calculation of the sample size and randomization to intervention groups. Thus, the sample size needed to obtain a relevant preset minimum difference, between two proportions

from two groups P1 (placebo) and P2 (active), was calculated. The following input data were used: P2–P1 = 53%; $\alpha = 0.05$; $\beta = 0.10$. Output: required subjects n = 14. The study used 20 patients taking into account possible dropouts. For data processing and hypothesis testing the statistical package STATISTICA v.5.0 (Statsoft, USA) was used. Outcome data was analyzed in a blinded manner at the 2 planned evaluative points: 6 and 12 months. Cumulative symptom and medication scores were calculated at 12 months, as the area under the curve (AUC), starting from month 1–12, with linear interpolation in the intervals where no data was recorded. Testing to normality of variables was assessed by Kolmogorov-Smirnov and Shapiro-Wilks tests. Non-parametric methods were applied to each variable (except skin reactivity, Ch_{10}). Thus, the central values were expressed by the median and its 95% Confidence Interval and comparisons between the active and placebo groups were performed using the Mann-Whitney *U* test. Comparisons within each group with respect to pre-treatment values were performed using the Wilconxon test for paired samples. For Ch_{10}, a log transformation was performed and, after verification of its adjustment to normality, parametric methods were applied: the Student test for comparison between groups, and the Student test with paired data for comparison within the same group with respect to the baseline values. Finally, a correlation analysis between variables

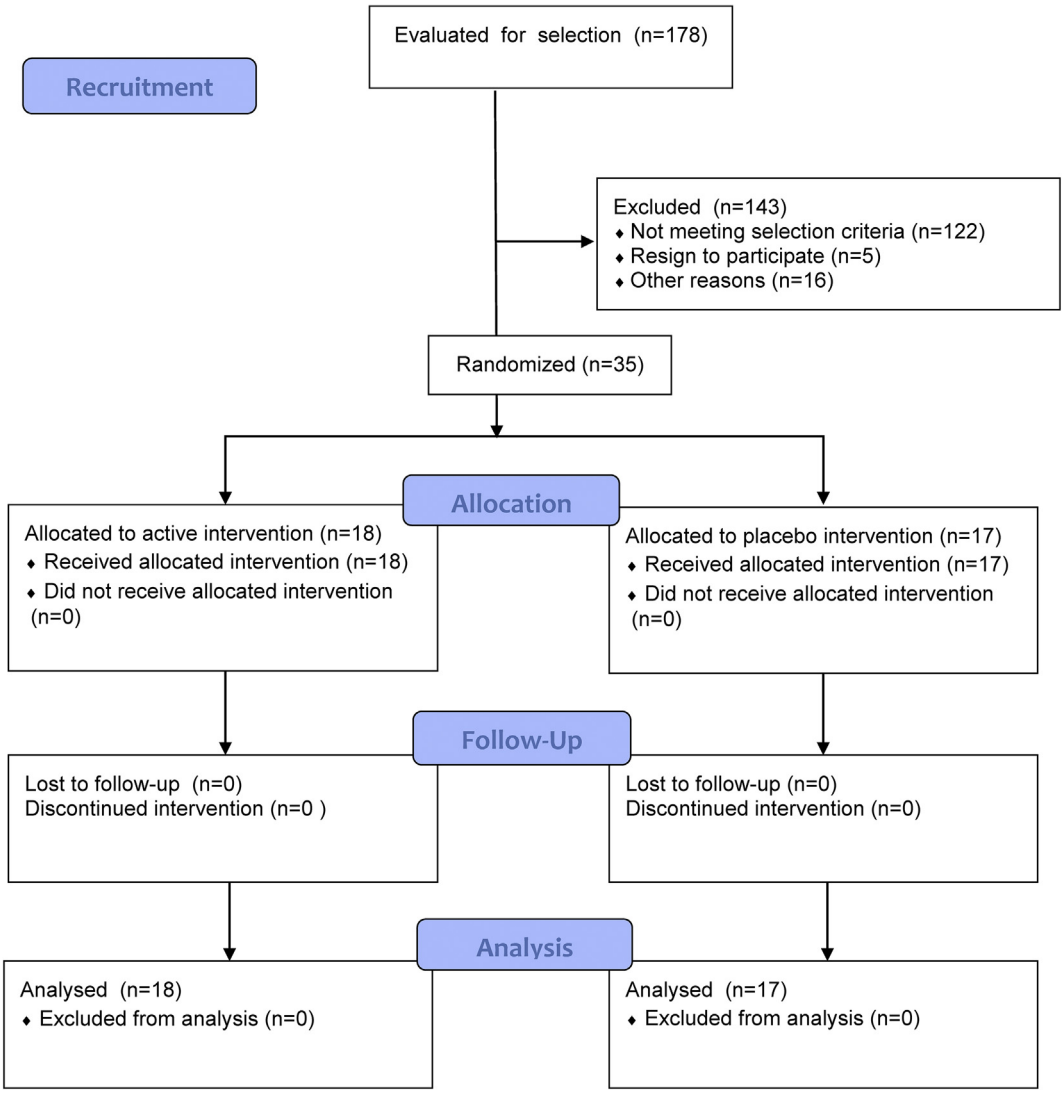

Fig. 1 Flow diagram of the progress through the clinical trial stages

was performed using from the non-parametric Spearman correlation coefficient.

RESULTS

Of 178 patients evaluated with asthma symptoms, presumably related to exposure to house dust, and who expressed their consent to take part in the trial, only 35 patients (19.7%) were selected (Fig. 1). In patients that failed to meet the inclusion criteria (122), the most frequent factor (74.6%) was lack of predominant sensitization to *B. tropicalis*, showing either sensitization to *D. pteronyssinus* or *D. siboney,* or polysensitization to the 3 mite species in a similar degree. Only 31 patients (25.4%) failed to show a positive response to the three tested mite species.

As expected by design, the SCIT and Placebo groups were comparable in terms of demographic and clinical variables with no statistical differences ($p > 0.05$) (Table 2). All 35 included patients completed the trial. There were no dropouts or patients who were withdrawn from the study. Total cumulative dose was 63 035 BU, in an average of 20.6 injections.

Regarding outcomes, at 12 months of treatment, the clinical symptom score significantly ($p < 0.001$) decreased, showing only 20% (95%CI: 16–39%) of the median value of the placebo group. Medication score also was significantly ($p < 0.001$) reduced, achieving 13% (11–36%), as compared to median value in the placebo group. Thus, the combined symptom/medication score (the main outcome) significantly decreased to 17% (14–38%) of the placebo value. The difference between the active and placebo groups was significant ($p < 0.01$) even at 6 months and deepened at 12 months ($p < 0.001$). The effect was also significant as compared to the beginning of the treatment within the active group ($p < 0.001$) (Fig. 2).

The 12-month cumulative median value for symptom score was 40.7% (25.7%–61.0%), whereas the cumulative median value for medication score was 34.5% (22.4%–63.3%). Then, cumulative SM score rendered a median value of 38.2% (24.2%–61.5%). The difference between the active and placebo groups, for the 3 cumulative scores, was highly significant $p < 0.001$.

Following the shift in the main clinical outcome, a significant although modest improvement of the lung function was observed in the active group, expressed both in a PEF increase ($p < 0.05$) and decrease of PEF daily variability ($p < 0.05$), both as compared to placebo group and baseline values within the active group. (Table 3).

According to the Overall Clinical Improvement (OCI) variable, 12 patients (67%) from the active group were reported as "better" relative to initial data. Moreover, the patient-reported clinical improvement was also significant (OR 32: 95%CI: 17 to 102).

In agreement with the clinical improvement, the skin reactivity specific to *B. tropicalis* showed a

	Active Group (n = 18)	Placebo Group (n = 17)	P (Mann Whitney *U* Test)
Sex (M/F)	8/10	9/8	0,29
Age (years) Median Range	 32 19–44	 33 18–43	 0,88
Height (cm) Median Range	 166,5 154,2 - 177	 164,6 151,5 - 180	 0,98
Weight (kg) Median Range	 72,5 61,2–79.9	 78,5 63.5–90.6	 0,81
Asthma Mild/Moderate	10/8	8/9	0,67

Table 2. Demographic and clinical characteristics of patients

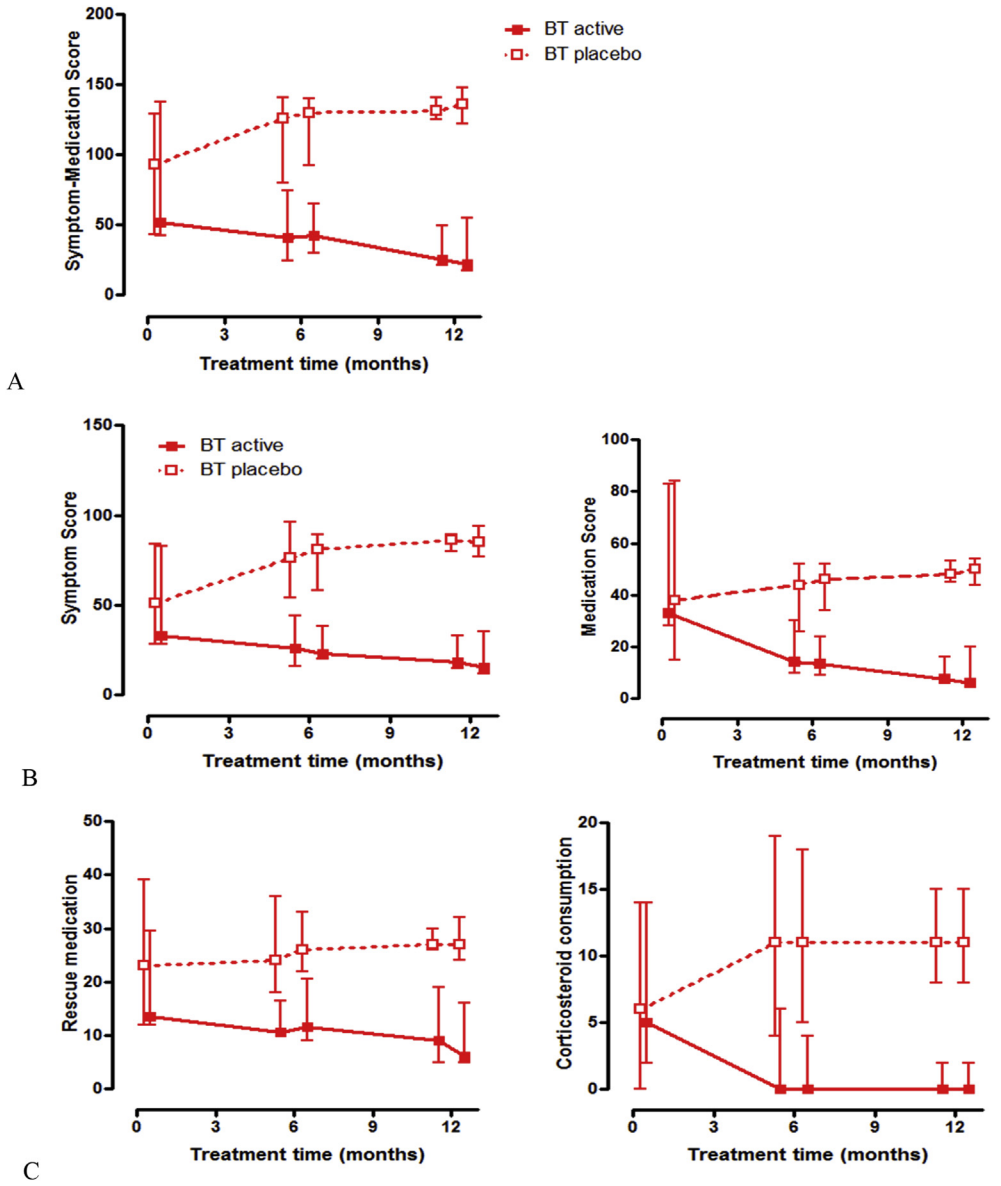

Fig. 2 AIT clinical effect as measured by the Symptom/Medication score in the active and placebo groups at time intervals (Median values and 95% CI). A: Combined SM score. B: Separate Symptom and Medication scores. C: Medication score corresponding to rescue medication (Salbutamol or Teophiline) and oral corticosteroid use

remarked decrease (Fig. 3). The Ch_{10} value decreased significantly ($p < 0.05$), both in comparison to the baseline and placebo values, at 6 months, and even more significant at 12 months. Noteworthy, the effect was quite specific since no influence was observed on to skin reactivity towards both *Dermatophagoides* species. Overall, 15 patients (83%) decreased Ch_{10} in more than 1 logarithm (i.e. 10-fold increase in the allergen threshold) and 9 of them (50%) completely abolished the skin reactivity to *Bt*, showing no wheals, or small reactions under 3 mm diameter. In support of the clinical significance of allergen-specific skin reactivity, the reduction of the same was correlated significantly with the change in primary clinical outcome: the SM score ($r = 0.666$; $p < 0.01$). Furthermore, there was also a significant correlation between the decrease in daily variability of PEF and change of skin reactivity ($r = 0.869$; $p < 0.001$).

Frequency of adverse reactions and maximum tolerated allergen dose are safety indicators. During this study 720 injections were given, 373 in the

Respiratory Function variables	Treatment Time (months)		
	0	6	12
PEF Active (95% CI)	82.4% (78.2%–86.7%)	82.7% (78.5%–87.6%)	83.7% *§ (78.7%–88.6%)
PEF Placebo (95% CI)	79.1% (76.8%–82.8%)	77.2% (79.4%–83%)	76.8% (78.4%–82.2%)
PEF-V Active (95% CI)	29.7% (21.9%–36.8%)	29.7% (21.4%–35.6%)	27.8% *§ (20.3%–32.6%)
PEF-V Placebo (95% CI)	31.3% (24.7%–37.5%)	31.3% (24.2%–37.5%)	33.8% (29.1%–37.5%)

Table 3. Peak Expiratory Flow and PEF daily variability (median values in active and placebo groups). *PEF: Peak Expiratory Flow; PEF-V: Peak Expiratory Flow Variability; (*) Statistically significant difference ($p < 0.05$) with respect to $t = 0$ (Wilconxon Matched Pairs Test); (§) Statistically significant difference ($p < 0.05$) between active and placebo group (Mann Whitney U Test)*

active group. Each patient reached the expected maximum dose of 6000 BU with equal treatment duration of 12 months. There were 9 adverse events during the treatment; all of them were classified as immediate local reactions occurring in the up-dosing phase (0.8 mL of vial number 3). Importantly, no systemic reactions were reported. The observed local reactions consisted of pruritus with wheals at the injection site with diameters below 5 cm, symptoms featuring an immediate allergic reaction. The frequency of patients with treatment-related adverse events in the active group was 33.3% (6 patients). The frequency of adverse reactions with respect to the number of injections administered was only 2.4% in the active group by 0.6% in placebo group.

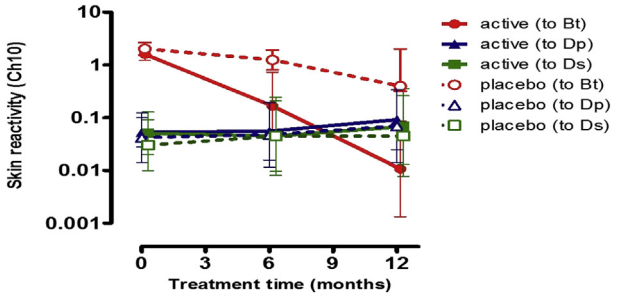

Fig. 3 AIT effect on skin reactivity, as measured by Ch_{10} value to the allergen extracts of *Blomia tropicalis* (*Bt*), *D. pteronyssinus* (*Dp*) and *D. siboney* (*Ds*). AIT with *Bt* induced only an allergen-specific decrease of skin reactivity towards the *Bt* extract, whereas no cross-effect towards *Dp* or *Ds* was noted

DISCUSSION

This work describes a successful AIT clinical trial using a standardized allergen extract of the tropical mite, *Blomia tropicalis*. In spite of the growing relevance of this mite species regarding allergen characterization and epidemiological data,[12,13,15,35–37] no placebo-controlled double blind clinical trials of *B. tropicalis* AIT have been published before in PUBMED-indexed journals.

The role of *Blomia tropicalis* as strong sensitizer in the tropics and its association with allergic asthma has been evidenced by several authors.[35–42] Prevalence of IgE sensitization to *Blomia tropicalis* in humid tropical climates is usually close to the values displayed by *Dermatophagoides pteronyssinus*; particularly in Cuba, it achieves values ranging from 56.2 to 68.1% among asthmatics.[12,39] Even though co-sensitization is common in asthmatics usually exposed both to *Blomia* and *Dermatophagoides*, sensitization to *Blomia* has own features and distinctive markers, including the immunodominant allergens Blo t 5 and Blo t 21 and the species-specific Blo t 12,[14,16,38–44] overall, with very limited or no cross-reactivity towards the *Dermatophagoides* counterparts. For instance, Kidon et al.,[14] in a major study in Singapore revealed that Blo t 21 was the third more prevalent allergen molecule in atopic children in this country (56%) preceded only by Der p 2 and Der p 1. Moreover, polysensitization to *Blomia* and *Dermatophagoides* has been associated to

increased asthma severity and systematization of the allergic disease.[14,37]

Thus, AIT using *Dermatophagoides* allergen vaccines is thought to be ineffective against *Blomia* sensitization and *Blomia* triggered allergic symptoms. In fact, a previous clinical trial performed in Cuba has shown the lack of cross-desensitization effect towards *Blomia tropicalis* using *D. pteronyssinus* or *D. siboney* allergen subcutaneous immunotherapy.[45] Therefore, an optimal AIT approach for allergic asthma in the tropics should include *Blomia tropicalis*. The findings of the present work are fully in line with this statement. The study results were highly satisfactory in terms of clinical improvement and decrease of allergen-specific skin reactivity.

Both, the effect sizes on cumulative symptom and medication were higher in this study than the reported values in a well-known Abramson meta-analysis of subcutaneous AIT using HDM allergens of *D. pteronyssinus* and *D. farinae* species.[20] The Standard Mean Difference (SMD) for symptoms between active and placebo was here: −2.03 (−1.89 to −2.86) versus −1.13 in the Abramson meta-analysis; whereas the SMD on medication was: −2.74 (−1.79 to −3.69) versus −0.51 in the Abramson review.[20] Comparing with the above mentioned similar study, performed in the same hospital with allergen extracts of *D. pteronyssinus* and *D. siboney*, using the same standardized dosage schedule, current results with *B. tropicalis* seem to be also better, both in terms of SMD or relative values of symptom/medication score decrease as compared to placebo group.[45] Concerning the respiratory function, the results on decreased PEF variability, although modest in size, are in agreement with primary clinical outcomes and with previous results with *D. pteronyssinus* and *D. siboney*.[45] The treatment effect on respiratory function could be masked by the fact that placebo patients took more medication than patients under AIT.

The effect on allergen-specific skin reactivity was also large, although similar to what has been noted with *Dp* and *Ds* in the previous study.[45] The magnitude of this effect, which by the Ch_{10} definition can be translated into a 148-fold increase of the allergen threshold able to elicit a positive skin reaction, was higher than the reported in most publications.[19–21] This effect size as well as its correlation to the main clinical outcomes become factors in support of the use of carefully measured skin reactivity (adjusted to histamine reactivity),[30] as a surrogate marker of clinical efficacy during AIT.[46] In agreement with previous results that showed that AIT with *Dermatophagoides* species was unable to induce a cross-desensitization effect towards *Blomia tropicalis*, present results have shown that *Blomia* AIT has no effect on skin reactivity towards *D. pteronyssinus* or *D. siboney* in patients that had polysensitization, which is in line with the scarce cross-reactivity reported between *Blomia* and *Dermatophagoides* extracts as well as with the marked difference in the allergen profile.[6,15–17,40,41,45,47]

Despite the beneficial aspects of AIT, safety of injection route has been a major concern, since severe systemic reactions can pose a life-threatening risk. The reported frequency of systemic reactions is approximately 0.1–0.2% of injections in 2%–5% of patients.[18,19,21,31,32,48,49] In this study, the frequency of adverse reactions related to the product did not differ substantially to what has been reported internationally, both with respect to the total number of injections and number of patients. However, here no systemic allergic reactions were noticed, i.e., all reactions were only local. A previous study using *Dp* and *Ds* allergen extracts sharing the same standardized dosage schedule,[45] the observed frequency of adverse reactions was quite similar (25% of patients/2.5% of injections). Standardization of the allergen vaccine is an important factor in improving AIT safety, because it assures more consistent composition of the allergen extract. Other factors could involve proper patient selection and careful clinical examination prior to the injection administration as well as surveillance during the injection procedure.[18,19,21]

A novel aspect on this work resides on the use of a *Blomia tropicalis* allergen extract for treating asthma in patients sensitized to this mite species. This novelty is backed by the use of a standardized product. Standardization of allergen extracts is still a technically challenging theme and a regulatory issue lacking proper harmonization at the

international level.[50] Especially for *Blomia tropicalis*, since it is a tropical species not addressed by the leading manufacturers focused on developed countries in temperate regions, no clear technical guidance and reagents are available. Unlike *Dermatophagoides*, there are no clear molecular markers of total allergenic potency such as Der p 1 or Der p 2, since the hierarchy of the major/immunodominant allergens for *Blomia* is different, with Blo t 5 and Blo t 21 heading the list of candidates.[41,47] However, the combination of both plus Blo t 7 did not account for 65% IgE binding frequency against the whole allergen extract and its content in the extracts is usually very low.[14]The standardization approach used here (Biological Units) is based on median allergen-specific skin reactivity in a given population, and it is complemented by allergen profiling by SDS-PAGE Western Blotting, using an IgE serum pool of representative patients, implementing indicators of binding intensity and relative content in the region of 12–21kD.[24] This fraction comprises Blo t 5 and Blo t 21 as well as other potentially important allergens as Blo t 2, Blo t 12 and Blo t 13.[40,51] The standardization in Biological Units has the advantage to be clinically meaningful (particularly, for safety) and equivalent among different allergen products. The fact that the results obtained here, both in terms of efficacy and safety are similar to those obtained by equivalent allergen products and dosages of *Dp* and *Ds* in a comparable population sample,[43] is an argument in support of the usefulness of this standardization approach.

The current study was performed in adults with an age range of 16–45 years in a population sample of Cuban adult allergic asthmatic patients. Taking into account the high prevalence of sensitization to *Blomia tropicalis* in this population, more studies should be conducted to expand and confirm these results, particularly in children.

In summary, subcutaneous AIT with a *Blomia tropicalis* standardized allergen vaccine was effective and safe for the control and amelioration of allergic asthma in a population commonly exposed not only to this mite species but also *Dermatophagoides species*, showing, however, a species-specific desensitization effect. Thus, AIT with *Blomia tropicalis* should be considered as an important element in AIT strategies in the tropics taking into account internationally recommended safety standards.

Abbreviations

Bt: Blomia tropicalis or B. tropicalis; AIT: Allergen immunotherapy; BIOCEN: National Center of Bioproducts; HDM: House Dust Mite; HDM SCIT: Immunotherpy Subcutaneous with allergens of House Dust Mite; Dp or D: pteronyssinus: Dermatophagoides pteronyssinus; Ds or D: siboney: Dermatophagoides siboney; CECMED: Center for State Control of Drugs, Equipment and Medical Devices; VALERGEN-BT: Standardized allergen extract of Blomia tropicalis; BU: Biological Units; BU/mL: Biological units per milliliter; Blo t: Mayor allegen of Blomia tropicalis; SM: Symptom and Medication; SMD: Standard Mean Difference; ALK: Denmark-based pharmaceutical company; PEF: Peak Expiratory Flow; OCI: Overall clinical improvement; Ch10: Allergen specific reactivity calculated relative to the Histamine HC 10 mg/mL

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Consent for publication

All authors gave its written consent for publication.

Ethics approval

The study was conducted following the ethical principles contained in the Declaration of Helsinki, 64th WMA General Assembly, Fortaleza, Brazil, October 2013. Subjects were asked for their written informed consent to participate in the study. The study was approved by the Ethics Committee of the "Calixto García" University Hospital and was approved by the Cuban medicine regulatory authority (CECMED).

Availability of data and materials

Please contact author for primary data requests.

Authors' contributions

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CI: Have been involved in drafting the manuscript or revising it critically for important intellectual content.
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The authors have no conflicts of interest.

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