Molecular allergen profiling of dual mite sensitization in severe allergic rhinitis

Running title: A Molecular Mite Allergen Profiling in Severe Rhinitis

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0439
Abstract

**Background:** Mites are the most prevalent source of indoor allergens. The aim of the present study is based on a component resolved diagnosis (CRD) model approach to investigate the mite specific IgE sensitization profile to *Dermatophagoides pteronyssinus* and *Blomia tropicalis* and the performance outcomes of commercially available CRD in subjects with severe allergic rhinitis (SAR).

**Methods:** We selected 63 consecutive patients showing a dual sensitization to *D. pteronyssinus* and *B. tropicalis* with persistent severe rhinitis according to the ARIA Guidelines. Skin prick test with standardized extracts and specific serum IgE to both mites along with serum specific IgE to Der p 1, Der p 2, Der p 23, Der p 10 and Blo t 5 was measured.

**Results:** Fifty-eight and 59 patients showed a positive sIgE to the whole extracts of *D. pteronyssinus* and *B. tropicalis*, respectively. While 91.67% of patients were sensitized to specific IgE Der p 1, Der p 2 and/or Der p 23, specific IgE to Blo t 5 (≥0.3 ISU-E) was not detected in the majority (55%) of the serum samples.

**Conclusions:** Although the combination panel of the commercially available major allergens Der p 1, Der p 2 and Der p 23, identified more than 90% of the *D. pteronyssinus* allergic patients, Blo t 5 showed a very limited performance in those sensitized to *B. tropicalis*. Improvements in CRD and further research concerning the prevalence and clinical relevance of serodominant allergens are needed to achieve a genuine molecular diagnosis along with a patient-centred mite-allergy specific immunotherapy.

**Key words:** Allergen, Severe Allergic Rhinitis, Component Resolved Diagnosis, Skin Prick Test, House Dust Mites, *Dermatophagoides pteronyssinus, Blomia tropicalis*, Precision Medicine.
RESUMEN

Introducción: Los ácaros son los alérgenos de interior más prevalentes. El presente estudio investiga el perfil de sensibilización a *Dermatophagoides pteronyssinus* y *Blomia tropicalis*, así como el rendimiento del diagnóstico por componentes (CRD) disponible comercialmente en pacientes con rinitis alérgica grave persistente.

Material y métodos: Seleccionamos 63 pacientes con rinitis grave persistente (Guía ARIA) con sensibilización dual a *pteronyssinus* and *B. tropicalis*. Se realizaron pruebas cutáneas en prick con extractos estandarizados, IgE sérica específica a ambos ácaros además de IgE específica a alérgenos individuales Der p 1, Der p 2, Der p 23, Der p 10 y Blo t 5.

Resultados: Cincuenta y ocho y 59 pacientes presentaron IgE específica positiva a extractos crudos de *D. pteronyssinus* and *B. tropicalis*, respectivamente. Aunque el 91.67% mostraron sensibilización a Der p 1, Der p 2 y/o Der p 23, Blo t 5 (≥0.3 ISU-E) no fue detectado en la mayoría (55%) de las muestras estudiadas.

Conclusiones: Aunque la combinación de alérgenos principales Der p 1, Der p 2, Der p 23, pudo identificar más del 90% de los pacientes sensibilizados a *D. pteronyssinus*, Blo t 5 presentó un rendimiento diagnóstico muy limitado para aquellos sensibilizados a *B. tropicalis*. Conocer la prevalencia y relevancia clínica de los alérgenos acarianos serodominantes en cada territorio contribuiría a una mejor identificación de sensibilizaciones genuinas en la era de la medicina de precisión.

Palabras clave: Alérgeno, rinitis alérgica grave, diagnóstico por componentes, Skin Prick Test, ácaros, *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, medicina de precisión.
Background
Inhalant allergens play a main role in the development of allergic disease in sensitized and genetically predisposed individuals, with the house dust mite (HDM) as the most important allergen source worldwide and a global health problem [1, 2]. The prevalence of sensitization to mites is also very high (50–90%) in the respiratory allergic population [3, 4]. It is increasingly recognized that the dominant causative allergen in a population might differ regionally and could also vary among patients [5]. The mite species *Blomia tropicalis* (Acari: Astigmata: Echimyopodidae) was originally identified in the 1970s and characterized as a storage mite [6] but today it is widely agreed that constitutes a crucial house dust mite species not just limited to the tropical and subtropical areas of the world [7]. *Blomia tropicalis* (*B. tropicalis*) coexist with *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) mites and dual sensitization by allergens from both species are common among atopic individuals in these regions [8]. The relative prevalence of both mites’ families in areas of cohabitation is very dynamic, depending on both geographic and micro-environmental factors [9]. In the subtropical Canary Islands, despite both pyroglyphid and echimyopodidae mites are found in the domestic environment, respiratory allergies are most frequently triggered by exposure to *Dermatophagoides spp* [10].

The World Health Organization and the International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee currently includes up to 39 *Dermatophagoides spp.* allergens in the systematic nomenclature [11], with wide variations in prevalence rates for the major allergens Der p1 and Der p2 in different countries [12]. The commercially available allergen component Der p10 also showed varying prevalence rates being partly responsible for the cross-reactions to arthropods and mollusks [13]. The Der p23 is a new described and relevant HDM allergen which might explain perennial allergic symptoms when the known major HDM allergen components Der p1 and Der p2 as well as Der p10 are not detected [14]. Concerning the Echimyopodidae family, 14 *B. tropicalis* allergens have been currently acknowledged by the WHO/IUIS allergen nomenclature subcommittee [15]. Unlike *D. pteronyssinus*, the group 5 allergen of the *B. tropicalis*, Blo t5, is described as the dominant major allergen [16] showing up to 70% of specific IgE reactivity in asthmatic patients. Despite the sequence homology between the group 5 allergens, the IgE cross-reactivity of the major allergen Blo t5 and the minor allergen Der p5 is unexpectedly low [17].

Interestingly, different clinical expressions have been attributed to each mite family. In
fact, *B. tropicalis* seems to be preferentially involved as a cause of respiratory allergy and *Dermatophagoides* mites is a key feature in children with atopic dermatitis while dual sensitization increased the risk of asthma and allergic rhinitis [18]. Although different repertoires of specific HDM allergen sensitization have been specially described in asthma [19, 20] there is yet little evidence regarding persistent rhinitis. Allergic rhinitis (AR) is a chronic and highly prevalent condition affecting children and adults that may lead to considerable morbidity and impairment of quality of life in terms of ability to concentrate, professional performance, interference with daily activities and sleep [21, 22] serving as a risk factor for the development of asthma [23]. HDM induced AR typically evolves perennial with seasonal exacerbations in the spring and fall, which corresponds to an increase of proliferation of mites. The persistent nature of AR is more pronounced in temperate regions while the bi-annual rhythmicity (spring and late summer/spring) or intermittent character is more marked in the Mediterranean regions [24].

The concept of component-resolved diagnosis (CRD) for allergy diagnosis was introduced in 1999 [25]. This microarray technique uses a multi-allergen analyses enabling a comprehensive analysis of the patient’s IgE binding pattern to a large number of individual allergens [26]. The *Dermatophagoides* spp. group 1, group 2 and group 23 allergens, are immunodominant based on the prevalence of IgE responses [27, 28]. The association of IgE responses to these dominant group of allergens along with the clinical phenotypes of allergies has not been yet established.

The aim of the present study lays on a CRD-model approach to investigate the IgE dual sensitization profile to *D. pteronyssinus* and *B. tropicalis* and the usefulness of CRD in European patients with severe AR in a subtropical region with a high perennial HDM exposure [29]. Thus, a specific commercial molecular panel for *Dermatophagoides pteronyssinus* and *Blomia tropicalis* was depicted including the classical major allergens Der p1, Der p2 and Blo t5, the recently described peritrophin-like protein Der p23 and Der p10 as a marker of minor mite allergen and arthropod food-related allergy.

**Methods**

**Subjects**

We recruited patients consecutively with a clinical diagnosis of severe persistent AR according to the ARIA Guidelines [22] from the Hospital Universitario de Canarias
Allergy Outpatient Office in Tenerife, Spain. All patients had to fulfil the following clinical criteria:
- Persistent symptoms with seasonal exacerbations (Spring, Fall)
- Symptoms improve in altitude (>1500 m) and
- Aggravation of symptoms by contact with household dust and domestic/indoors activities.

AR severity was evaluated through patient-reported assessments of the intensity of 6 individual symptoms (sneezing, rhinorrhoea, nasal pruritus, nasal congestion, ocular pruritus and watery eyes) on a scale of 0–3. Symptoms were rated from “0: no symptoms to 3: severe symptoms”. The sum of the 6 symptom points yielded the rhinoconjunctivitis total symptom score (RTSS) on the day of the visit and could range from 0 to 18 [30].

Only patients with an immediate positive skin prick test (SPT) to both *D. pteronyssinus* and *B. tropicalis* extract were included in the study. Serum blood samples were obtained from all participating subjects identified with a code label, stored at -40°C and thawed immediately before the in vitro analysis. Pregnant and breast-feeding women were excluded. The study was approved by the local Ethical Committee of our Institution and informed consent was signed by all subjects and parents/guardians for those participants <18 y.o.

**Skin Prick Test**
Skin Prick tests (SPT) were performed according to European standards [31] with standardized extracts of *D. pteronyssinus* and *Blomia tropicalis* (Diater, Madrid, Spain). Histamine (10 mg/ml) and saline were used as positive and negative controls. Following everyday practice, antihistamines were withdrawn a week prior to the SPT. The wheal diameter were measured immediately after 20 minutes and those diameters greater than 3 mm were regarded as positive.

**Mite allergenic extracts**
Proteins from mites bodies of *D. pteronyssinus* and *B. tropicalis* were extracted in phosphate-buffered saline buffer (PBS), 0.01 M, pH 7.4, for 2 hours at 5±3°C. Both protein solutions were clarified by filtration and centrifugation (1 hour at 16,000 g).
Afterwards, the isolated supernatants were ultra filtrated against highly purified water (Ph. Eur. specification), sterile filtered, frozen and lyophilized.

SDS PAGE/IgE Western blot

Proteins from *D. pteronyssinus* extracts and *B. tropicalis* were analysed by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), according to Laemmli [32] in 15% polyacrylamide gels under reducing conditions. Proteins were visualised by Coomassie Brilliant Blue R-250 staining and transferred to polyvinylidene difluoride (PVDF, Trans-blot turbo TM. BIORAD, Hercules, CA, USA). The binding of IgE antibody to allergens was analysed by western blot using individual patients’ sera and anti-human IgE peroxidase conjugate (Southern Biotech, Birmingham, USA). Chemiluminescence detection reagents (Western lightning® Plus-ECL. Perkin Elmer. Waltham, MA, USA) were added following the manufacturer’s instructions. IgE binding bands were identified using the BioRad Diversity database program.

Serological analysis

Total IgE levels, specific IgE to *Dermatophagoides pteronyssinus* and *Blomia tropicalis*, Der p1, Der p2, Der p10, Der p23 and Blo t5 were measured by ImmunoCAP and ISAC multiplexing (ThermoFisher Scientific, Uppsala, Sweden) according to the manufacturer’s instructions. Total IgE levels were expressed in international units per unit volume (kU/L), specific IgE levels were expressed in kU\_A/L and ISU (ISAC Standardized Units). Values ≥0.35 kU\_A/L and ≥ 0.3 ISU were considered positive.

Statistical analysis

Baseline and demographic characteristics will be summarized by standard descriptive summaries (medians and standard deviations for continuous variables and percentages for categorical variables). To compare differences analysis of variance, Kruskal-Wallis, and chi-square tests are required for parametric continuous, nonparametric continuous, and categorical variables respectively.

Results

**Demographic Characteristics of Patients**

Sixty-three (39 > 18 y.o.) patients (Table I) were recruited from the outpatient allergy
clinic to assess their eligibility for the study. All 63 subjects (36 females and 27 males, median age 26.1 y.o.) who fulfilled the ARIA criteria for severe persistent AR had a positive skin prick test (SPT) to both D. pteronyssinus and B. tropicalis. The median values of individual symptom scores for the RTSS recorded at the same day of the visit was 15 with no differences between the younger (≤18 y.o.) and older individuals. Most of the subjects (77.77%, 49 out of 63 individuals) referred AR for a long period (>5 years) and none of them had been previously in treatment with specific immunotherapy (SIT). Regarding comorbidities, 6 subjects had mild atopic dermatitis, 2 had confirmed food allergy (egg and milk) while 5 had controlled mild intermittent allergic asthma. Fifty-four patients had a known family history of atopy.

**Total IgE**
A quantitative analysis of total IgE was performed in order to evaluate the basal atopic status in the study population. The total IgE ranged from 10.28 UI/ml to 998.9 UI/mL, with a median value of 523.65 UI/ml (Table II). The younger patients showed a median total IgE (728 UI/ml) value higher than adults (396 UI/ml).

**Prevalence and IgE reactivity of D. pteronyssinus, B. tropicalis, Der p 1, Der p 2, Der p10, Der p 23 and Blot 5 serum IgE in HDM-Allergic Patients**
Fifty-eight and fifty-nine patients were sIgE positive (≥ 0.35 kU/l) to the allergenic whole extracts of D. pteronyssinus (1.99 to >100 kU/L) and B. tropicalis (0.5 to >100 kU/L), respectively. Four subjects were exclusively sensitized to the crude extract of B. tropicalis (0.5 to >100 kU/L). Concerning individual allergens, Der p1, Der p2 and Der p23 were the most prevalent allergens and 91.67% of patients were sensitized to group 1 and/or group 2 and/or Der p23. Fifty-three of the patients (83.33%) were positive for sIgE against Der p1 and/or Der p2, 45 (75%) subjects were positive for Der p1, 48 (80%) for Der p2, 48 (80%) for Der p23 and 5 (8.33%) for Der p10. Two patients (3.33%) were exclusively sensitized to Der p2 or Der p23 sIgE, 1 patient showed a single sIgE to Der p1, while no subjects were solely sensitized to Der p10. Interestingly, 2 subjects (#42 and 45) had a positive sIgE response to the crude extract of D. pteronyssinus showing no detection to any of the 4 individual available allergens (Table II).

The median values of sIgE (kU/L) against Der p1, Der p2 Der p10 and Der p23 were significantly different: 3.72, 10.16, 0.02 and 2.42 respectively. The median ratio of sIgE
(kU/L) against Der p 2 relative to sIgE (kU/L) to the crude extract was significantly higher (55.50) than those observed for Der p 1(28.31), Der p10(0.05) and Der p23 (16.54). Also, the median ratio of sIgE (kU/L) against Der p 2 relative to total IgE (UI/ml) was higher (3.67) than those observed for Der p1(1.23), Der p10(0.0) and Der p23 (0.76). Relationship between Serum IgE Levels of Der p1, Der p2, Der p10 and Der p23 and correlations between the sIgE levels of HDM extract and single-component Der p1, Der p2, Der p10 and Der p23 are shown in Figure 1.

Concerning *B. tropicalis*’ major allergen, the measurements of sIgE to Blo t5 in 20 subjects, detected values ranging from 0.1 to 96 ISU-E (mean value: 10.5 ISU-E). Specific IgE to Blo t5 (≥0.3 ISU-E) was not detected in the majority (55%) of the serum samples.

**Relation of sensitization profile to sIgE with age**

Specific IgE (sIgE) to crude extracts of *D. pteronyssinus* and *B. tropicalis* showed median values (kU/L) of 39.57 and 15.33 respectively finding a higher median level of sIgE to both mites in the younger subject’s group (*D. pteronyssinus*: 49.01 to 33.16 and *B. tropicalis*: 21.64 to 11.91). Again, younger individuals showed a higher median level of sIgE (kU/L) to single allergens compared to elderly patients (Figure 2): Der p1 (4.54 to 2.07), Der p2 (12.65 to 7.34), and Der p23 (8.65 to 1.17).

**SDS PAGE/IgE Western blot**

Western blot of selected patients with a dual sensitization to *D. pteronyssinus* and *B. tropicalis* showed different patterns of sensitization (Figure 3). A marked stain intensity was found at 14-15 kDa for *D. pteronyssinus* in almost all subjects (>95%), followed by protein staining at 24 kDa. Concerning *B. tropicalis*, the majority of patients (>90%) displayed a notable protein staining about 55 kDa stain followed by a protein band at 14-15 kDa.

**Discussion**

A key feature of mite sensitization in the tropics is the larger repertoire of specific mite allergens that the atopic individuals are sensitized to, possibly due to the presence of a more diverse group of mites being co-dominantly present in the environment (e.g., the concurrent presence of both *B. tropicalis* and *Dermatophagoides spp.*) [33] as well as host genetic factors, with family history being the strongest predictor of allergic
diseases [34]. This is in contrast to the predominant group 1 and/or 2 house dust mite specific immuno responses in more temperate regions (with more than 70 and 80% of HDM allergic patients having specific IgE to these allergens, respectively) [35]. The se variations in terms of geographical and poblational mite allergen serodominance may have direct implications for the design, production and standardization of dust mite allergen immunotherapy extracts [5, 28, 36].

In the current investigation we confirmed a dominant role for sIgE sensitization to Der p2 (80%), Der p23 (80%) and Der p1 (75%) amongst a selected population with severe AR in this subtropical part of Spain. This is in line with previous reports from different parts of the world [37], showing a higher prevalence of IgE responses to Der p1 and Der p2 than those reported for Australia [33] (up to 77%) and Singapore [38] (63%) probably explained by a high perennial exposure to HDM favoured by the specific local weather conditions. Batard et al. reported that in temperate regions between 20 and 47% of 1302 HDM allergic patients also showed sIgE to allergens from groups 4, 5, 7, 13, 15, 21, and 23 [5].

The close correlation between serum IgE titters against Der p1, Der p2 and Der p23, on the one hand, and against *D. pteronyssinus* on the other, further supports the dominant role of these allergens in the human IgE response against HDM in our area. We also measured IgE against Der p 10 in 63 patients, and 5 (8.33%) of them had detectable levels of IgE against this allergen, showing a higher prevalence compared to earlier works from China [39, 40]. The IgE response against Der p2 and Der p23 represented a larger fraction compared to Der p1 as shown by former reports in Spain [41]. Mean specific IgE responses to Der p2 and Der p1 allergens were higher to those elicited to Der p23 in terms of quantity.

Several studies support that the proteolytic activity of Der p1 seems more important at an early age and that Der p2-*a lipopolysaccharide coreceptor for the Toll-like receptor 4*-takes the lead later in life, reflecting that exposure to Der p 1 in house dust is generally higher than to Der p2 [42, 43]. Interestingly, although younger individuals showed a higher mean level of sIgE (kU/L) to the crude extract of *D. pteronyssinus* and *B. tropicalis* and single allergens compared to elderly patients, we found that the sIgE response to Der p2 was more prevalent and quantitatively higher than sIgE to Der p1 and Der p23 in both children and adults.

Previous investigations by Kidon [33], Zheng [39] and co-workers have shown that sIgE
responses to an increased number of distinct mite allergens correlates with the complexity of the allergic phenotype, and that both Der p1 and Derp2 are risk factors for multisystemic phenotypes of allergic diseases in Asia. Our study found different IgE profiles to Der p1, Derp2, Der p23 and Derp10, within the same organ and degree of the underlying disease (i.e. severe AR) excluding another atopic condition such as asthma or atopic dermatitis. In this regard, more specific disease centred studies are warranted to display a more accurate allergenic profile of each condition related to different populations in the age of precision medicine.

In contrast to Becker and coworkers [14] findings, with 5 out of 16 patients showing positive results for Der p 23 solely, Weghofer et al. [44] found that 6 of the 158 HDM allergic patients showed exclusive IgE reactivity to Der p23 but not to any of the other tested HDM allergens (i.e., Der p1, Der p2, Der p5, Der p7, Der p10, and Der p21). Despite the high prevalence of sIgE response to Der p23, only 2 subjects (3.33%) where exclusively sensitised to Der p23 in our sample. The high prevalence (80%) of Der p 23 sIgE confirmed the importance of this allergen component as a major HDM allergen supported by former research from Austria [45] and Thailand [46]. Although, Der p 1 and Der p 2 dominate the IgE response, patient variability has been shown in the individual responses, suggesting that molecular diagnostic may be useful in formulating personalised immunotherapy [28].

Tropomyosin (sIgE to Der p10) prevalence was scarce (8.33%) and only present in the younger patients. In contrast to previous studies our findings do not support the proposal that tropomyosin sensitization indicates a true food allergy independent of mite respiratory disease as no clinical correlation with shrimp allergy was found in any of those individuals [47]. None of the subjects were found to be exclusively sensitized to tropomyosin. In line with earlier reports, the Der p 10-positive group exhibited significantly higher total IgE levels (1,046.9 kU/L) than those patients without IgE towards Der p10 (480.0 kU/L) [48]. This observation was also described in grass pollen allergic patients indicating a close connection between wider sensitization profiles and higher IgE levels [23]. As observed by Posa and co-workers, a sensitization to Der p10 may be taken as a marker for broad sensitization, thus the immune response is more susceptible to be modified in the first, asymptomatic, mono-oligomolecular stages, early monitoring of the IgE response at a molecular level may be helpful to start an allergen-specific (immunotherapy)treatment at once [49, 50].

Twenty sera with positive specific IgE to the crude extract of B. tropicalis (ranging
from 0.35 to >100 kU/L) were also selected and although measurements of specific IgE to Blo t5 detected values ranged from 0.1 to 96 ISU-E (median value: 10.5 ISU-E), specific IgE to Blo t5 (≥0.3 ISU-E) was only detected in 45% of the studied serum samples. In this regard, Barber and co-workers observed that in the Canary Islands, *B. tropicalis*-determined by its major allergen Blo t 5- was only relevant locally in the Gran Canaria island but not in Tenerife, where the present work is originally based [47]. In the same study some of those patients with Lep d 2 -the major allergen for the storage mite *Lepidoglyphus destructor*- sensitivity were found to react to *B. tropicalis* by SPT, thus suggesting the existence of a Lep d 2-like allergens not yet characterized. Despite different patterns of sensitization to *B. tropicalis* were obtained and the quantification of relevant allergens for *B. tropicalis* (i.e. Blo t 4 and Blo t 21) was not commercially available, stain intensities (figure 3) designate mite amylase Blo t4 -a minor allergen from *B. tropicalis*- as the most prevalent profile (>90%) in our sample compared to the major allergens Blo t5 and Blo t21. Concerning this issue, Blo t4 (28%) has also been described as a local serodominant allergen showing an unusually higher frequency than to the major allergen Blo t5 (22%) in allergic subjects from China [51] and subtropical Spain [52]. In a recent paper by Huang et al. [53] the underrepresentation of certain allergens and/or competition by nonallergenic proteins, are responsible for IgE levels measured with HDM allergen extract–based ImmunoCAP being lower than those measured with molecular ImmunoCAP suggesting the replacement of allergen extract–based tests for the detection of HDM sensitization with more specific molecular tests. The current investigation has some limitations as almost 10% and 55% of the subjects respectively sensitized to the crude extract of *D. pteronyssinus* and *B. tropicalis* could not be identified through the proposed CRD panel. Our findings showed that a combination panel of the major allergens Der p1, Der p2 and Der p23, could identify more than 90% of the patients with severe AR amongst those *D. pteronyssinus* allergic patients in our area. In contrast, specific IgE to Blo t5 was only detected in 45% of the serum samples of those individuals sensitised to the crude whole extract of *B. tropicalis*. While this CRD panel approach may be sufficient to obtain a precise *D. pteronyssinus* mite severe AR diagnosis, it showed a very limited performance in those patients specifically sensitized to *B. tropicalis*. The measurement of *Blomia tropicalis*-specific IgE levels could be enhanced either by adding recombinant allergen molecules to crude allergen extracts or displaying molecular panels containing the most important allergens, as
previously described for HDM [54]. Improvements in CRD and further research concerning the prevalence and clinical relevance of serodominant allergens are needed to achieve a genuine diagnosis leading to a tailored mite-allergy specific immunotherapy in the near future.
Declarations

Ethics approval and consent to participate
The study was approved by the local Ethical Committee CEIC Hospital Universitario Nuestra Señora de Candelaria on 2017 March, 28 with the reference number P.I.-14/17.

Consent for publication
Institutional consent form was obtained by all subjects and parents or legal guardians for those <18 years old taking part of the study.

Availability of data and material
The data that support the findings of this study are available from Servicio Canario de Salud but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Servicio Canario de Salud.

Competing Interests:
All of the authors declare no competing interest in this section.

Funding:
The present study has been entirely funded by the Spanish Society for Allergy and Clinical Immunology (Fundación SEAIC).

Authors’ Contributions
RG-P, FP and PP-G designed the study and wrote the manuscript. VM, IS-M, PP-G and RG-P contributed to data collection. FP and MC performed the in vitro immunoassays and the statistical analysis. All authors contributed to the critical interpretation of the results. All authors approved the final manuscript.
References


Table Legends

**Figure 1.** Serum IgE Levels of Der p1, Der p2, Der p10 and Der p23 and correlations between the sIgE levels of HDM extract, total IgE and single-component Der p1, Der p2, Der p10 and Der p23.

![Figure 1](image1)

**Figure 2.** Serum IgE Levels of Der p1, Der p2, Der p10 and Der p23 of younger compared to elderly patients.

![Figure 2](image2)

**Figure 3.** Western blot of selected patients with a dual sensitization to *Dermatophagoides pteronyssinus* and *Blomia tropicalis*.

![Figure 3](image3)
Table 1. Descriptive statistics.

<table>
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<th>Total (n)</th>
<th>&lt;18 y.o.</th>
<th>&gt;18 y.o. (%)</th>
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<td>26.1</td>
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</tr>
<tr>
<td>Sex (F/M)</td>
<td>36/27</td>
<td>(16/8)</td>
<td>(25/14)</td>
</tr>
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<td>SAR</td>
<td>63</td>
<td>24 (100%)</td>
<td>39 (100%)</td>
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<td>Atopic Dermatitis</td>
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<td>2 (100%)</td>
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<td>SPT+Dpt. and Bt.</td>
<td>63</td>
<td>24 (100%)</td>
<td>39 (100%)</td>
</tr>
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<td>Total IgE (UI/ml)</td>
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<td>728</td>
<td>396</td>
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<td>Family Hx of Atopy</td>
<td>54</td>
<td>20 (83.33%)</td>
<td>34 (87.1%)</td>
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SAR: Severe Allergic Rhinitis. SPT: Skin Prick Test

Table 2. Detailed data of the 63 selected patients with a co-sensitization to *Dermatophagoides pteronyssinus* and *Blomia tropicalis*.