ORIGINAL ARTICLE

Molecular Allergen Profiling of Dual Mite Sensitization in Severe Allergic Rhinitis

González-Pérez R[1], Pineda F[2], Poza-Guedes P[1], Castillo M[2], Matheu V[1], Sánchez-Machín I[1]

[1]Allergy Department, Hospital Universitario de Canarias, Tenerife, Spain
[2]Diater Laboratory, Madrid, Spain

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Abstract

Background: Mites are the most prevalent source of indoor allergens. The present study used a component-resolved diagnosis (CRD) approach to investigate the mite-specific IgE sensitization profile for *Dermatophagoides pteronyssinus* and *Blomia tropicalis*. We also assessed the performance of a commercially available CRD approach in patients with severe allergic rhinitis.
Methods: We selected 63 consecutive patients with dual sensitization to *D pteronyssinus* and *B tropicalis* and persistent severe rhinitis according to the ARIA guidelines. We performed skin prick tests with standardized extracts and determined 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.
Results: Fifty-eight and 59 patients had positive sIgE to the whole extracts of *D pteronyssinus* and *B tropicalis*, respectively. While 91.67% of patients were sensitized to specific IgE to 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 most of the serum samples (55%).
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 performed somewhat poorly 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, as well as patient-centered 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 *D. pteronyssinus* y *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* y *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 components. Skin prick test. Ácaros. *Dermatophagoides pteronyssinus*. *Blomia tropicalis*. Medicina de precisión.

Introduction

Inhalant allergens play a key role in the development of allergic disease in sensitized and genetically predisposed individuals, with house dust mite (HDM) being the most important allergen source worldwide and the cause of a global health problem [1,2]. The prevalence of sensitization to mites is also very high in persons with respiratory allergy (50%-90%) [3,4]. It is increasingly recognized that the dominant causative allergen in a population may vary by region and by patient [5]. The mite species *Blomia tropicalis* (Acari, Astigmata, Echimyopodidae) was originally identified in the 1970s and characterized as a storage mite [6]. However, today it is widely agreed that *B tropicalis* is a crucial house dust mite species that is not limited to tropical and subtropical areas [7]. *B tropicalis* coexists with *Dermatophagoides pteronyssinus* mites, and dual sensitization by allergens from both species are common among atopic individuals in these regions [8]. The relative prevalence of cohabitation of both mite families is very dynamic, depending on both geographic and microenvironmental factors [9]. In the subtropical Canary Islands, respiratory allergies are most frequently triggered by exposure to *Dermatophagoides* species, even though both pyroglyphid and Echimyopodidae are found in the domestic environment [10].

The World Health Organization and the International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-Committee currently includes up to 39 *Dermatophagoides* species allergens in the systematic nomenclature [11], with wide variations in prevalence rates for the major allergens Der p 1 and Der p 2 in different countries [12]. The prevalence of the commercially available allergen component Der p 10, which is partly responsible for cross-reactions to arthropods and mollusks, also varies [13]. The newly described and relevant HDM allergen Der p 23 might explain perennial allergic symptoms when the known major HDM allergen components Der p 1, Der p 2, and Der p 10 are not detected [14]. Concerning the Echimyopodidae family, 14 *B tropicalis* allergens are currently acknowledged by the WHO/IUIS Allergen Nomenclature Sub-Committee [15]. Unlike *D pteronyssinus*, the group 5 allergen of *B tropicalis*, Blo t 5, is the dominant major allergen [16], with up to 70% specific IgE reactivity in asthmatic patients. Despite the sequence homology between the group 5 allergens, IgE cross-reactivity between the major allergen Blo t 5 and the minor allergen Der p 5 is unexpectedly low [17].

Interestingly, the clinical expression attributed to each mite family differs. *B tropicalis* seems to be preferentially involved as a cause of respiratory allergy, and *Dermatophagoides* species is a key feature in children with atopic dermatitis; dual sensitization increases the risk of asthma and allergic rhinitis (AR) [18]. Although different repertoires of specific HDM allergen sensitization have been described in asthma [19,20], there is little evidence regarding persistent rhinitis. AR is a chronic and highly prevalent condition affecting children and adults that can lead to considerable morbidity and impaired quality of life in terms of ability to concentrate, professional performance, and interference with daily activities and sleep [21,22]. AR is also a risk factor for the development of asthma [23]. HDM-induced AR typically progresses as a perennial disease with seasonal exacerbations in the spring and fall corresponding to an increase in the proliferation of mites. The persistent nature of AR is more pronounced in temperate regions, whereas the twice-yearly rhythm (spring and late summer/spring) or intermittent character is more marked in the Mediterranean region [24].

Component-resolved diagnosis (CRD) of allergy was introduced in 1999 [25]. This microarray technique uses a multiallergen analysis to provide a comprehensive picture of the patient's IgE binding pattern [26]. *Dermatophagoides* species group 1, group 2, and group 23 allergens are immunodominant based on the prevalence of IgE responses [27,28]. The association between IgE responses to these dominant groups of allergens and the clinical phenotypes of allergies has not yet been established.

In the present study, we used a CRD-based model 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 high perennial exposure to HDM [29]. Thus, we describe a specific commercial molecular panel for *D pteronyssinus* and *B tropicalis* including the classic major allergens (Der p 1, Der p 2, and Blo t 5), the recently described peritrophin-like protein Der p 23, and Der p 10 as a marker of minor mite allergen and arthropod-induced food-related allergy.

Methods

Patients

We consecutively recruited patients 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)
- Improvement in symptoms at altitude (>1500 m)
- Aggravation of symptoms by contact with household dust and domestic/indoor activities.

Severity of AR was evaluated through patient-reported assessments of the intensity of 6 specific symptoms (sneezing, rhinorrhea, 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) result to both *D pteronyssinus* and *B tropicalis* extract were included in the study. Serum blood samples were obtained from all participants, identified with a code label, stored at –40°C, and thawed immediately before the in vitro analysis. Pregnant and breastfeeding women were excluded.

The study was approved by the local ethics committee (CEIC Hospital Universitario Nuestra Señora de Candelaria) on March 28, 2017 (reference number P.I.-14/17).

Informed consent was signed by all participants and parents/guardians for those participants aged <18 years.

Skin Prick Test

SPTs were performed according to European standards [31] with standardized extracts of *D pteronyssinus* and *B tropicalis* (Diater). Histamine (10 mg/mL) and saline were used as positive and negative controls. Following daily clinical practice, antihistamines were withdrawn a week prior to the SPT. The wheal diameters were measured after 20 minutes, and diameters >3 mm were considered positive.

Mite Allergen Extracts

Proteins from mite bodies (*D pteronyssinus* and *B tropicalis*) were extracted in phosphate-buffered saline, 0.01 M, pH 7.4, for 2 hours at 5±3ºC. Both protein solutions were clarified by filtration and centrifugation (1 hour at 16 000*g*). The isolated supernatants were then ultrafiltered against highly purified water (European Pharmacopoiea specification), sterile filtered, frozen, and lyophilized.

SDS PAGE/IgE Western Blot

Proteins from *D pteronyssinus* extracts and *B tropicalis* were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [32] in 15% polyacrylamide gels under reducing conditions. Proteins were visualized using Coomassie Brilliant Blue R-250 staining and transferred to polyvinylidene difluoride membranes (Trans-Blot Turbo, Bio-Rad). The binding of IgE antibody to allergens was analyzed using Western blot with individual patients' sera and antihuman IgE peroxidase conjugate (Southern Biotech). Chemiluminescence detection reagents (Western Lightning Plus-ECL, Perkin Elmer) were added following the manufacturer's instructions. IgE binding bands were identified using the BioRad Diversity database program.

Serological Analysis

Total IgE levels and specific IgE to *D pteronyssinus* and *B tropicalis*, Der p 1, Der p 2, Der p 10, Der p 23, and Blo t 5 were measured using ImmunoCAP and ISAC multiplexing (Thermo Fisher Scientific) according to the manufacturer's instructions. Total IgE levels were expressed in international units per unit volume (kU/L), and specific IgE levels were expressed in kU_A/L and ISAC standardized units (ISU). Values ≥0.35 kU_A/L and ≥0.3 ISU were considered positive

Table 1. Descriptive Statistics

	Total, No.	<18 y	>18 y (%)
No.	63	24 (38.1%)	39 (61.90%)
Age, y	26.1	13.5	39.4
Sex, F/M	36/27	(16/8)	(25/14)
SAR	63	24(100%)	39 (100%)
Atopic dermatitis	6	4 (66.6%)	2(33.3%)
Asthma	5	3 (60%)	2 (40%)
Food allergy	2	2 (100%)	0
SPT+DP and BT	63	24 (100%)	39 (100%)
Total IgE, IU/mL	523.65	728	396
Family history of atopy	54	20 (83.33%)	34 (87.1%)

Abbreviations: BT, *Blomia tropicalis*; DP, *Dermatophagoides pterynossinus*; SAR, severe allergic rhinitis; SPT, skin prick test.

Statistical Analysis

Baseline and demographic characteristics are summarized using standard descriptive measures (medians and standard deviations for continuous variables and percentages for categorical variables). Differences were compared using analysis of variance, the Kruskal-Wallis test, and the χ^2 test for parametric continuous, nonparametric continuous, and categorical variables, respectively.

Results

Demographic Characteristics

We recruited 63 patients (39 aged >18 years) (Table 1) from the outpatient allergy clinic to assess their eligibility for the study. All 63 patients (36 females and 27 males; median age, 26.1 years) who fulfilled the ARIA criteria for severe persistent AR had a positive SPT result to both *D pteronyssinus* and *B tropicalis*. The median RTSS value recorded on the day of the visit was 15, with no differences between younger patients (≤18 years) and older patients. Most patients (77.77%, 49/63) reported having AR for a long period (>5 years), and none had previously received specific immunotherapy. Regarding comorbidities, 6 patients had mild atopic dermatitis, 2 had confirmed food allergy (egg and milk), and 5 had controlled mild intermittent allergic asthma. A known family history of atopy was recorded in 54 patients.

Total IgE

A quantitative analysis of total IgE was performed in order to evaluate the baseline atopic status of the study population. Total IgE ranged from 10.28 IU/mL to 998.9 IU/mL, with a median value of 523.65 IU/mL (Table 2). The median total IgE value was higher in younger patients than adults (728 IU/mL vs 396 IU/mL).

Prevalence and IgE reactivity of D pteronyssinus, B tropicalis, Der p 1, Der p 2, Der p 10, Der p 23, and Blo t 5 serum IgE in HDM-Allergic Patients

sIgE was positive (≥0.35 kU/L) in 58 and 59 patients 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 patients were exclusively sensitized to the crude extract of *B tropicalis* (0.5 to >100 kU/L). Concerning individual allergens, Der p 1, Der p 2, and Der p 23 were the most prevalent, and 91.67% of patients were sensitized to group 1 and/or group 2 and/or Der p 23. sIgE was positive to Der p 1 and/or Der p 2 in 53 patients (83.33%), Der p 1 in 45 (75%), Der p 2 in 48 (80%), Der p 23 in 48 (80%), and Der p 10 in 5 (8.33%). Two patients (3.33%) were sensitized exclusively to Der p 2 or Der p 23 sIgE, and 1 patient had sIgE to Der p 1. No patients were solely sensitized to Der p 10. Interestingly, 2 patients (#42 and 45) had a positive sIgE response to the crude

Table 2. Detailed Data of the 63 Selected Patients With Cosensitization to *Dermatophagoides pteronyssinus* and *Blomia tropicalis*

Patient	SPT Der p[a]	SPT Blo t[a]	Total IgE, IU/mL	sIgE Der p, kU_A/L	sIgE Blo t, kU_A/L	sIgE Der p 1, kU_A/L	sIgE Der p 2, kU_A/L	sIgE Der p 10, kU_A/L	sIgE Der p 23, kU_A/L	sIgE Blo t 5, ISU-E
1	4	3	156.7	7.68	1.29	2.62	1.39	0.01	0.68	
2	4	2	122.3	16.6	1.15	4.7	5.67	0	1.15	
3	1	1	10.28	0	0	0	0	0	0.01	
4	5	4	342.1	3.25	13.7	0.16	0.47	0.04	0.93	
5	4	3	389.7	76.6	8.42	83.8	0.04	0.02	32.2	
6	5	5	331	10.9	10	4.31	4.8	0.01	0.71	
7	6	4	501.6	88.1	23.3	3.31	90.6	0.02	19.2	
8	6	5	965.3	89	22.7	44	80.8	0.06	0.02	19
9	6	4	1281	94.4	27.6	52.6	71.3	0.04	9.41	
10	5	5	256.2	66.8	26.9	19.8	51.1	0	3.33	
11	5	3	257.7	30.8	2	8.17	19.1	0	1.37	
12	6	4	977.2	>100	13.3	100	100	0.03	30.5	
13	6	4	559.4	64	8.7	4.13	100	0.05	24.8	<0.1
14	5	3	353	61.2	1.34	21.4	32.8	0.01	12.3	
15	6	5	2878	>100	>100	100	100	0.06	22.6	
16	6	6	797.1	>100	>100	35.4	53.1	0.04	44.2	
17	6	6	744.9	>100	45.1	77.3	100	0.02	26.1	
18	4	2	127.6	3.49	1.44	1.28	30.5	0	0.06	
19	5	5	235.4	9.9	38.5	2.1	1.72	0	3.53	
20	5	6	295	11	39.9	1.99	6.57	0.01	1.2	0.3
21	3	1	135.6	13.8	0	9.83	0.23	0.1	9.42	
22	5	5	1194	57.9	76.4	1.05	1.17	10.4	29.4	4.3
23	6	6	2581	>100	>100	100	100	0.07	28.1	<0.1
24	6	4	612	87.8	25.3	28	44.2	0.04	15.6	
25	5	5	745.1	36.6	59.6	2.32	29.2	0.55	2.63	0.2
26	5	4	499.6	86.6	26.5	52.6	85	0.02	18	
27	6	5	1707	>100	21.9	46.8	55.5	0.02	12	
28	5	4	137	2.18	4.43	0.37	0.6	0	0.64	
29	5	2	225	26.1	4.84	17.9	19.4	0.11	9.66	
30	5	2	667	45.3	0	2.1	38.8	0.02	0.68	96
31	3	4	596.6	26	14.8	0.02	16.3	0.02	3.25	
32	5	4	1047	75.2	30.2	45.1	60.6	0.02	14.3	
33	2	3	27.16	<0.1	2.43	0	0	0	0.02	
34	6	4	514.6	>100	36.9	62.2	100	0.02	12.3	37
35	4	5	229	7.18	25.7	0.33	1.22	0.01	1.14	
36	1	1	127.2	<0.1	0.5	0.05	0.03	0.02	0.01	
37	2	1	17.1	3.41	1.25	0.2	3.29	0	0.05	
38	1	1	46.92	1	0.35	0.06	0	0	2.21	
39	5	2	871	99	2.09	56.1	85.3	0.02	19.7	<0.1
40	4	4	153.8	3.03	19.5	0.04	2.01	0.02	0	
41	4	3	322.6	5.59	3.59	0.02	1.21	0.01	2.06	<0.1

(Contd.)

Table 2. Detailed Data of the 63 Selected Patients With Cosensitization to *Dermatophagoides pteronyssinus* and *Blomia tropicalis*

Patient	SPT Der p[a]	SPT Blo t[a]	Total IgE, IU/mL	sIgE Der p, kU_A/L	sIgE Blo t, kU_A/L	sIgE Der p 1, kU_A/L	sIgE Der p 2, kU_A/L	sIgE Der p 10, kU_A/L	sIgE Der p 23, kU_A/L	sIgE Blo t 5, ISU-E
42	4	4	189.8	14.9	24.8	0.02	0	0	0.01	<0.1
43	3	2	263.3	32	1.01	0.06	0.85	0.03	13.2	18
44	4	5	998.9	28.4	>100	0.06	0	1.93	0.66	20
45	2	1	83.39	0.38	1.51	0.01	0.03	0.01	0.03	14
46	6	3	1554	>100	20.5	52.6	66.6	3.05	21.7	0.1
47	2	2	56.81	<0.1	1.74	0.01	0	0.06	0.39	1.1
48	5	4	742.5	>100	13.5	5.38	58.8	1.32	23.4	
49	4	3	83.64	7.43	4.73	2.79	3.48	0.03	0.48	
50	4	3	20.02	5.35	1.09	2.82	4.77	0.02	1.68	<0.1
51	5	1	199	53	1	40.60	12.20	0	9.71	<0.1
52	4	2	211.5	21.8	2.89	2.05	1.31	0.01	0.17	<0.1
53	4	4	571.3	14.6	38.9	4.77	6.19	0.03	7.89	<0.1
54	6	4	965.3	>100	22.3	1.39	0.01	0.04	9.38	
55	3	2	100.4	2.93	12	0.02	20.80	0	0	
56	5	2	292.8	46.4	5.16	0.01	32.60	0.01	0.03	
57	6	1	156.8	47.7	0.43	8.76	0.02	0.01	5.48	
58	2	2	181.7	3.11	2.66	46.60	68.20	0	1.5	
59	4	2	240.5	19	1.87	1.86	0.95	0.01	4.42	
60	5	2	42.68	3.39	0.42	36.70	25.40	0	0.42	
61	4	2	293	6.53	1.8	51.30	23.50	0.01	1.08	
62	3	3	61.55	1.99	5.93	0.86	0.00	0	0	
63	4	5	433.9	10.2	30	12.40	8.12	0.02	0	

Abbreviations: SPT, skin prick test.
[a]0, wheal <3 mm; 1, wheal 3–4 mm; 2, wheal 4–5 mm; 3, wheal 5–6 mm; 4, wheal >6 mm.

extract of *D pteronyssinus*, although no result was detected for any of the 4 individual allergens (Table 2).

The median values of sIgE (kU_A/L) against Der p 1, Der p 2, Der p 10, and Der p 23 were significantly different (3.72, 10.16, 0.02, and 2.42, respectively). The median ratio of sIgE (kU_A/L) against Der p 2 relative to sIgE (kU_A/L) against the crude extract was significantly higher (55.50) than those observed for Der p 1 (28.31), Der p 10 (0.05), and Der p 23 (16.54). Furthermore, the median ratio of sIgE (kU_A/L) against Der p 2 relative to total IgE (IU/mL) was higher (3.67) than those observed for Der p 1 (1.23), Der p 10 (0.0), and Der p 23 (0.76). The association between serum IgE levels of Der p 1, Der p 2, Der p 10, and Der p 23 and correlations between the sIgE levels of HDM extract and single-component Der p 1, Der p 2, Der p 10, and Der p 23 are shown in Figure 1.

Concerning *B tropicalis* major allergen, the measurements for sIgE to Blo t 5 in 20 patients ranged from 0.1 to 96 ISU-E (mean value: 10.5 ISU-E). Specific IgE to Blo t 5 (≥0.3 ISU-E) was not detected in most of the serum samples (55%).

Relation of Sensitization Profile to sIgE With Age

The median sIgE values for crude extracts of *D pteronyssinus* and *B tropicalis* were 39.57 and 15.33 kU_A/L, respectively, with a higher median level of sIgE to both mites in the younger patients (*D pteronyssinus*, 49.01 and 33.16; and *B tropicalis*, 21.64 and 11.91). Again, the median level of sIgE to single allergens was higher in younger than in older patients (Figure 2), as follows: Der p 1, 4.54 and 2.07 kU_A/L; Der p 2, 12.65 and 7.34 kU_A/L; and Der p 23, 8.65 and 1.17 kU_A/L.

SDS PAGE/IgE Western Blot

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

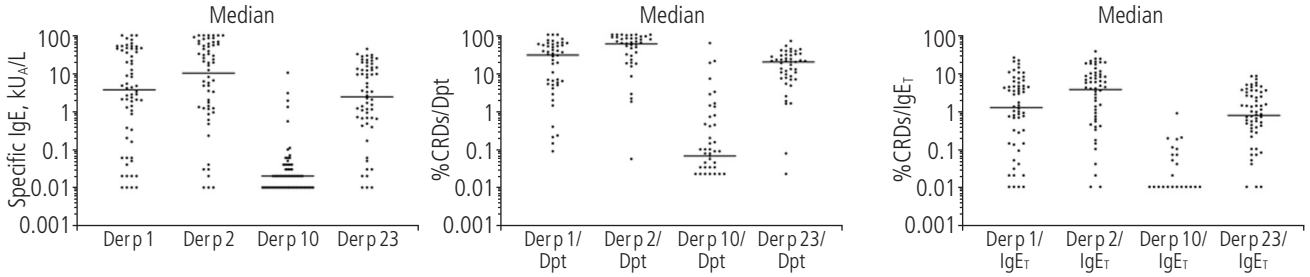

Figure 1. Serum IgE Levels of Der p 1, Der p 2, Der p 10, and Der p 23 and correlations between the sIgE levels of house dust mite extract, total IgE, and single-component Der p 1, Der p 2, Der p 10, and Der p 23.

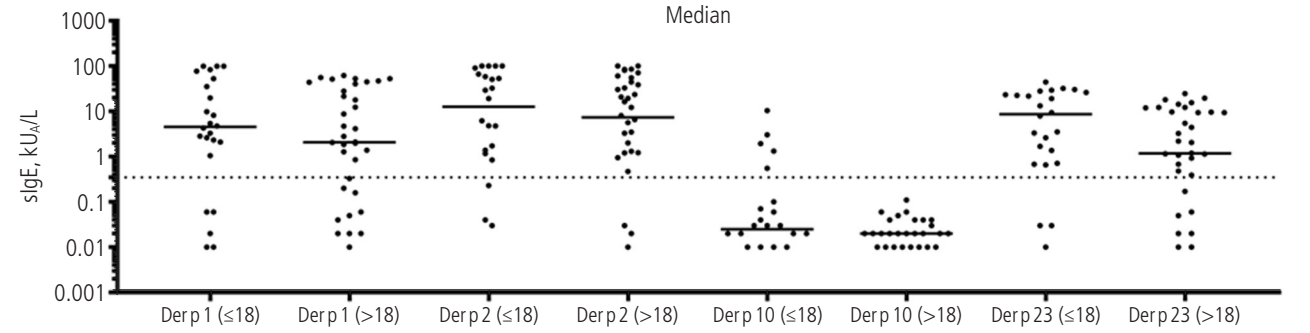

Figure 2. Serum IgE Levels of Der p 1, Der p 2, Der p 10, and Der p 23 for younger and older patients.

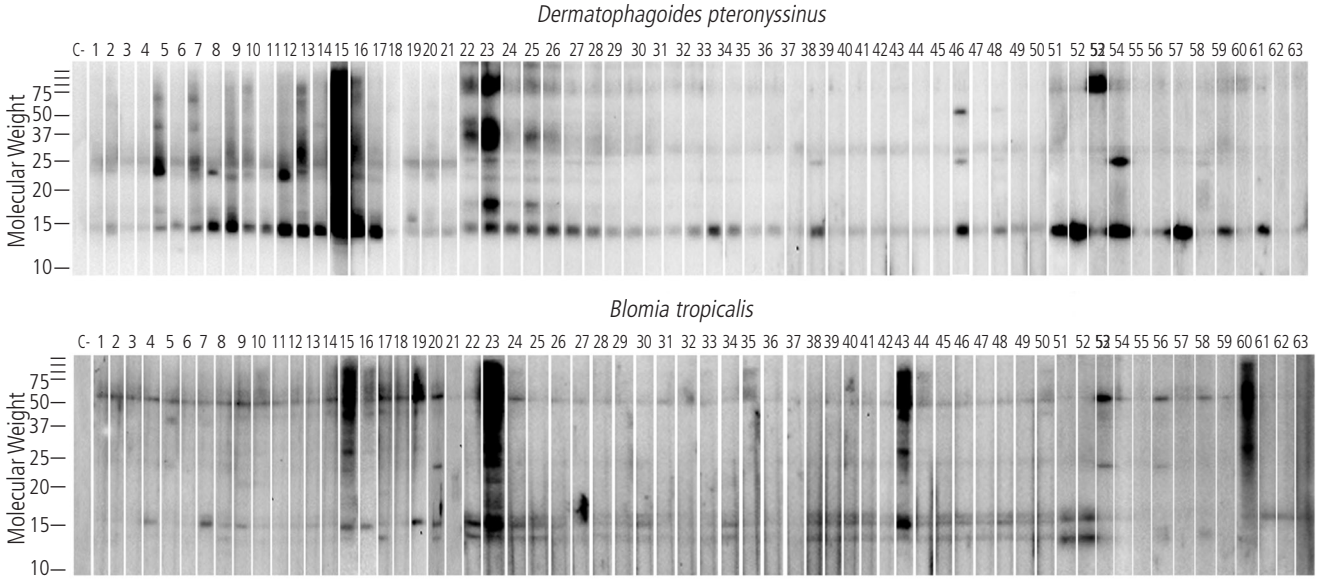

Figure 3. Western blot of selected patients with dual sensitization to *Dermatophagoides pteronyssinus* and *Blomia tropicalis*.

Discussion

A key feature of mite sensitization in the tropics is the larger repertoire of specific mite allergens that atopic individuals are sensitized to, possibly owing to the presence of a more diverse group of mites that are codominant in the environment (eg, the concurrent presence of both *B tropicalis* and *Dermatophagoides* species) [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 immune responses in more temperate regions (with more than 70% and 80% of HDM-allergic patients having specific IgE to these allergens, respectively) [35]. Variations in terms of geographical and

populational mite allergen serodominance may have direct implications for the design, production, and standardization of HDM allergen immunotherapy extracts [5,28,36].

In the current investigation, we confirmed a dominant role for sIgE sensitization to Der p 2 (80%), Der p 23 (80%), and Der p 1 (75%) among a selected population with severe AR in this subtropical part of Spain. Our finding is in line with previous reports from different parts of the world [37], showing a higher prevalence of IgE responses to Der p 1 and Der p 2 than those reported for Australia [33] (up to 77%) and Singapore [38] (63%), probably because of the high perennial exposure to HDM favored by the local weather conditions. Batard et al [5] reported that in temperate regions, between 20% and 47% of 1302 HDM-allergic patients also had sIgE to allergens from groups 4, 5, 7, 13, 15, 21, and 23.

The close correlation between titers of serum IgE against Der p 1, Der p 2, and Der p 23 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, 5 of whom (8.33%) had detectable levels of IgE against this allergen, showing a higher prevalence than reported in earlier studies from China [39,40]. The IgE response to Der p 2 and Der p 23 represented a larger fraction than for Der p 1, as shown by previous reports in Spain [41]. Mean specific IgE responses to Der p 2 and Der p 1 allergens were more marked than those elicited for Der p 23 in terms of quantity.

Several studies indicate that the proteolytic activity of Der p 1 is more important at an early age and that Der p 2—a lipopolysaccharide coreceptor for Toll-like receptor 4—takes the lead later in life, thus indicating that exposure to Der p 1 in house dust is generally more pronounced than exposure to Der p 2 [42,43]. Interestingly, although younger individuals had higher mean levels of sIgE to crude extracts of *D pteronyssinus* and *B tropicalis* and single allergens than elderly patients, we found that the sIgE response to Der p 2 was more prevalent and quantitatively higher than sIgE to Der p 1 and Der p 23 in both children and adults.

Previous investigations by Kidon et al [33] and Zheng and Sun [39] have shown that sIgE responses to an increased number of distinct mite allergens correlate with the complexity of the allergic phenotype, and that both Der p 1 and Der p 2 are risk factors for multisystemic phenotypes of allergic diseases in Asia. Our study found different IgE profiles to Der p 1, Der p 2, Der p 23, and Der p 10 within the same organ and for the same degree of the underlying disease (ie, severe AR), excluding other atopic conditions such as asthma or atopic dermatitis. In this regard, more specific disease-centered 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 et al [14], who found positive results for Der p 23 alone in 5 out of 16 patients, Weghofer et al [44] found that, in 6 of the 158 HDM-allergic patients, IgE reactivity was exclusively to Der p 23 but not to any of the other HDM allergens tested (ie, Der p 1, Der p 2, Der p 5, Der p 7, Der p 10, and Der p 21). Despite the high prevalence of sIgE to Der p 23, only 2 patients (3.33%) were exclusively sensitized to Der p 23 in our sample. The high prevalence of Der p 23 sIgE (80%) confirmed the importance of this allergen component as a major HDM allergen. This finding is 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 observed in the individual responses, suggesting that molecular diagnostics may be useful in formulating personalized immunotherapy [28].

The prevalence of tropomyosin (sIgE to Der p 10) was low (8.33%) and was only recorded in younger patients. In contrast to previous studies, our findings do not support the proposal that sensitization to tropomyosin indicates true food allergy, independently of mite-induced respiratory disease, as no clinical correlation with shrimp allergy was found in any of those individuals [47]. None of the patients 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 (1046.9 kU/L) than those patients without IgE to Der p 10 (480.0 kU_A/L) [48]. This observation was also reported in grass pollen–allergic patients, thus indicating a close connection between wider sensitization profiles and higher IgE levels [23]. As observed by Posa et al [49,50], sensitization to Der p 10 may be taken as a marker for broad sensitization, with the result that the immune response is more susceptible to modification in the first, asymptomatic, mono-oligomolecular stages. Early monitoring of the IgE response at the molecular level may be helpful when starting allergen-specific treatment (immunotherapy).

Twenty sera with positive specific IgE to the crude extract of *B tropicalis* (ranging from 0.35 to >100 kU_A/L) were also selected, and although measurements of specific IgE to Blo t 5 revealed values ranging from 0.1 to 96 ISU-E (median value, 10.5 ISU-E), specific IgE to Blo t 5 (≥0.3 ISU-E) was only detected in 45% of the serum samples studied. In this regard, Barber et al [47] observed that in the Canary Islands, *B tropicalis*—as determined by its major allergen Blo t 5—was only relevant locally on the island of Gran Canaria, but not on Tenerife, where the present study was performed. In the same study, some of the patients who were sensitive to Lep d 2—the major allergen for the storage mite *Lepidoglyphus destructor*—reacted to *B tropicalis* in the SPT, thus suggesting the existence of as yet uncharacterized Lep d 2–like allergens. Although the patterns of sensitization to *B tropicalis* differed, and quantification of relevant allergens for *B tropicalis* (ie, Blo t 4 and Blo t 21) was not commercially available, stain intensities designate mite amylase Blo t 4—a minor allergen from *B tropicalis*—as the most prevalent profile (>90%) in our sample compared with the major allergens Blo t 5 and Blo t 21 (Figure 3). Similarly, Blo t 4 (28%) has also been described as a local serodominant allergen with an unusually higher frequency than the major allergen Blo t 5 (22%) in allergic persons from China [51] and subtropical Spain [52].

In a recent paper by Huang et al [53], the underrepresentation of certain allergens and/or competition from 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 sensitization to HDM with more specific molecular tests. The current investigation is limited by the fact that almost 10% and 55% of the patients sensitized, respectively, to the crude extract of *D pteronyssinus* and *B tropicalis* could not be identified through the proposed CRD panel. Our findings

showed that a panel combining the major allergens Der p 1, Der p 2, and Der p 23 could identify more than 90% of patients with severe AR among *D pteronyssinus*–allergic patients in our area. In contrast, specific IgE to Blo t 5 was only detected in 45% of the serum samples of individuals sensitized to the crude whole extract of *B tropicalis*. While this CRD panel approach may be sufficient to make a precise diagnosis of severe AR caused by *D pteronyssinus* mite. Its performance was very limited in patients who were specifically sensitized to *B tropicalis*. The measurement of *B tropicalis*–specific IgE levels could be enhanced either by adding recombinant allergen molecules to crude allergen extracts or by displaying molecular panels containing the most important allergens, as previously described for HDM [54]. Improvements in CRD and further research into the prevalence and clinical relevance of serodominant allergens are needed to achieve a genuine diagnosis that leads to tailored mite allergy–specific immunotherapy in the near future.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Ruperto González-Pérez

Allergy Department
Hospital Universitario de Canarias
38320 Tenerife, Spain
E-mail: glezruperto@gmail.com