Depiction of Major Mite Allergens in Severe Allergic Rhinitis with High Mite Perennial Exposure

Original Investigation ▶

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Abstract ▶

Objective: Airway diseases, including allergic rhinitis, are prompted by specific IgE antibodies against airborne allergens and notably those derived from mites. The presented study focused on the specific IgE immediate response to *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) mite major allergens and the corresponding pertinence of molecular diagnosis in patients bothered with severe persistent rhinitis.

Methods: Individuals exhibiting confirmed sensitization to *D. pteronyssinus* along with a clinical diagnosis of coexisting severe allergic rhinitis were included in the study. In vivo investigations encompassed intradermal testing with a battery of standardized allergenic extracts, concurrent with *in vitro* specific IgE to the crude extract of *D. pteronyssinus*, and associated individual molecular allergens were assessed.

Results: Fifty-five out of 59 subjects showed sero-dominant IgE responses to the major allergens Der p 1, Der p 2 and Der p 23. Both Der p 2 and Der p 23 reached a prevalence above 80%, while group 10 allergen tropomyosin was scarcely depicted (<10%) and exclusively present in younger individuals.

Conclusion: The proposed component-resolved diagnosis panel accurately recognized 93.22% of *D. pteronyssinus* allergic patients. The raised seroprevalence of IgE response to Der p 23 also confirmed this constituent as a major *D. pteronyssinus* allergen in severe allergic rhinitis. A molecular approach appears to be essential to frame a more precise diagnosis and eventually a personalized immunotherapy regarding this allergic condition.

Keywords: Allergen, allergic rhinitis, *Dermatophagoides pteronyssinus*, component-resolved diagnosis, house dust mite

Introduction

Airborne allergens sensitize and favor the development of allergic rhinitis, asthma, and atopic disease in genetically predisposed individuals (1, 2). The European house dust mite (HDM) *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) is of significant clinical importance as a major elicitor of asthma, allergic rhinitis, and atopic dermatitis (3). Mite allergens are present in mite bodies, in secretion and excretion, with fecal pellets of the HDM as the main sources of indoor allergens worldwide. Although mite allergens can be detected in many areas of the home, beds are the perfect habitat for mites since they provide the ideal temperature, food and moisture for their proliferation and the allergens they produce accumulate inside mattresses and pil-

lows (4). The prevalence of sensitization to mites rises to 90%, with geographical variations, in the respiratory allergic population (5, 6). The dominant causative allergen in a specific community may be locally dissimilar among subjects and the intended underlying atopic disease (7, 8).

Purified allergens are given standardized names by a WHO/International Union of Immunological Societies committee based on the order of their discovery and their sequence homology. To date, 39 *Dermatophagoides spp.* (Pyroglyphidae) allergens have been documented through specific IgE (sIgE) binding or skin test reactivity (9), with noticeable differences in the distribution (seroprevalence) of the major Der p 1 and Der p 2 allergens across regions (9, 10). Two types

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of HDM allergen bioactivity, namely proteolysis—Der p 1, a 24 kDa cysteine protease, with significant consequences for allergic sensitization—and peptidolipid/lipid binding as Der p 2 acting as a ligand to TLR-4, elicit IgE and stimulate immune-responses to unrelated allergens (11). Moreover, group 10 muscle protein tropomyosin, which has been involved in immunological cross-reactions among invertebrates—mainly mollusks and arthropods—have also exhibited varying profiles of sIgE response to Der p 10 (12). Der p 23—a 14 kDa peritrophin from the mite gut is present in the outer membrane of mite feces—has been described as the latest major HDM allergen inducing persistent respiratory symptoms when the aforementioned Der p 1, Der p 2 and Der p 10 are not recognized (13). Although different repertoires of specific HDM allergen sensitization have been investigated in asthma (14, 15), the role of molecular diagnosis in persistent allergic rhinitis has received little attention in the literature. Severe allergic rhinitis (SAR) is defined as a highly prevalent condition affecting both children and adults, leading to a deleterious impact on people´s daily lives (16, 17), and moreover, favoring the further development of asthma (18). The perennial character of SAR is closely linked to the more temperate regions while the bi-annual rhythmicity is frequent in the Mediterranean areas, related to the correspondent HDM lifecycle (19).

Component-resolved diagnosis (CRD) allows the comprehensive assessment of the patient's allergen-specific IgE (sIgE) binding to a panel of individual allergens (20, 21). Although *Dermatophagoides spp.* group 1, 2 and 23 allergens are immunodominant—referring to allergens that quantitatively make the most important contribution to the IgE responses—the association among major allergens and evolving clinical phenotypes has not been assessed (22, 23). The presented study investigates the IgE sensitization repertoire to *D. pteronyssinus* and the outcome of CRD in subjects with SAR and a sharp perennial exposure to HDM (24). Herein, a specific customized molecular panel for *D. pteronyssinus* was comprised including Der p 1, Der p 2, the recently described peritrophin-like protein Der p 23 and Der p 10 as a cross-reacting biological marker between HDM and seafood allergy.

Methods

Subjects

The presented study was previously evaluated and authorized by the domestic Ethical Committee of the Nuestra Señora de Candelaria University Hospital (Approval Date: March 28, 2017; Approval Number: P.I.-14/17), and corresponding informed consent documents were properly signed by all participants. Inclusion criteria required a signed informed consent, a previous clinical diagnosis of present SAR, and a positive immediate intradermal test (SPT) with sIgE ≥0.35 kU_A/L to *D. pteronyssinus* in both cases. Pregnant and breast-feeding women were excluded.

Patients were exclusively recruited from the Allergy Outpatient facility, with a consistent diagnosis of SAR according to the ARIA guidelines (17). Disease severity was evaluated by means of patient self-assessment over a validated scale score of six individual nasal and ocular symptoms (sneezing, rhinorrhea, nasal pruritus, nasal congestion, ocular pruritus and watery eyes) on a scale of 0-3 on the day of the visit. The sum of the six symptom points yielded the rhinoconjunctivitis total symptom score (RTSS) on the day of the visit ranging from 0 to 18 points (25, 26).

Blood specimens were collected, identified with code label, stored at -40°C and immediately thawed in preparation for *in vitro* analysis.

Skin Prick Test and Mite Allergenic Extracts

Percutaneous tests were performed according to the European standards (27) with standardized allergenic extracts of *D. pteronyssinus* (Diater, Madrid, Spain). Saline (0.9%) and histamine (10 mg/mL) were respectively included as negative and positive controls. Antihistamines were stopped seven days in advance to each SPT, with wheal diameters >3 mm considered positive after a 20-minute immediate reading. Concerning allergenic HDM extracts, proteins from mite bodies of *D. pteronyssinus* were extracted into phosphate buffered saline buffer 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 obtained supernatants were then ultrafiltrated for purified water, sterile filtered, frozen and finally lyophilized.

Main Points

- Today, it is recognized that atopic sensitization is an important risk factor in respiratory conditions (i.e., allergic asthma) in both adults and children. However, the role of allergy concerning certain asthma phenotypes—such as severe asthma—is still under debate.

- Allergic rhinitis is a highly prevalent condition worldwide, affecting children and adults, and in the severe form of the disease leading to noticeable morbidity and impaired life quality, thus serving as a risk factor for the development of asthma.

- Even though different repertoires of specific house dust mite allergen (HDM) sensitization have been described in persistent asthma, there is a lack of studies involving a wide diagnostic panel (or specific IgE repertoire) of HDM allergens in severe allergic rhinitis.

- Geographic variation complicates the picture: although HDM allergy is consistently found in Western nations, diversity (allergen serodominance) between countries and regions is notable.

- Our results propose that a component-resolved diagnosis panel facilitates a more precise *Dermatophagoides pteronyssinus* molecular diagnosis, especially in severe rhinitic subjects with a—climate conditioned—high-mite allergen exposure, heightening the prevalence of Der p 2 and Der p 23 in this condition.

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Serological Workup, SDS PAGE & IgE Western Blot

Total IgE levels, sIgE to the whole *D. pteronyssinus* extract, and four individual molecular allergens—Der p 1, Der p 2, Der p 10 and Der p 23—were measured by ImmunoCAP and Immuno Solid-phase Allergen Chip (ISAC) multiplexing (ThermoFisher Scientific, Uppsala, Sweden) following the endorsed manufacturer´s guidelines. The total IgE titers were quantified in international units per unit volume (kU/L) while sIgE levels were quoted in kU_A/L with a cut-off value ≥ 0.35 kU_A/L. In relation to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and IgE Western blot (WB) assays, proteins from *D. pteronyssinus* allergenic extracts were analyzed in 15% polyacrylamide gels under reducing conditions as elsewhere detailed (28). Proteins were stained by Coomassie Brilliant Blue R-250 and transferred to polyvinylidene difluoride (Transblot turbo Biorad®, California, US) while IgE-binding was shown by BioRad Diversity database software. The specific binding of IgE to allergens was performed by WB, through individual participant´s sera against anti-human IgE peroxidase-conjugate (Southern Biotech, US) and chemiluminescence reagents (Western lightning®, Perkin Elmer, Massachusetts, US) were carried out following supplier's indications.

Statistical Analysis

Demographic features were quoted following standard descriptive means, including the corresponding minimum-maximum ranks, and medians were quoted with standard deviations if continuous variables, or percentages when categorical variables through the IBM Statistical Package for Social Sciences for Windows version 25.0 (IBM SPSS Corp.; Armonk, NY, USA). To quantify deviations in variance, Kruskal-Wallis, or chi-square test were used for parametric continuous, nonparametric continuous, and categorical variables. A p-value of less than 0.05 was considered statistically significant.

Results

Demographic Characteristics of Patients

We finally selected 59 subjects from the outpatient allergy office, 35 females and 24 males; median age 25.8 years, ranging from 9 to 70 years that met the inclusion criteria (Table 1). Majority of the subjects (81.35%, 48 out of 59 individuals) had previous clinically confirmed diagnosis of SAR for more than five years, and none of them was formerly treated with allergy immunotherapy (AIT). Participants' median score of individual symptoms on the first outpatients visit was 16. Despite a diagnosis of SAR, only 66.1% of the patients (39 out of 59) were properly taking their medication as prescribed by the physician at the time of inclusion in the study. More than

Table 1. Descriptive statistics

	Total (n)	<18 y.o.	>18 y.o.
n	59	22 (37.28%)	37 (62.71%)
Age (y.o.)	25.8 (9-70)	13.7 (9-18)	36.4 (19-70)
Sex (F/M)	35/24	(15/10)	(20/14)
SAR	59	22 (100%)	37 (100%)
Atopic dermatitis	6	4 (66.6%)	2 (33.3%)
Asthma	5	3 (60%)	2 (40%)
Food allergy	3	2 (66.6%)	1 (33.3%)
Total IgE (UI/mL)	326.8 (17.1-2878)	459 (83.39-2878)	335.2 (17.1-1707)
Family Hx of atopy	51	20 (90.9%)	31 (83.78%)

Median and corresponding rank values are shown.
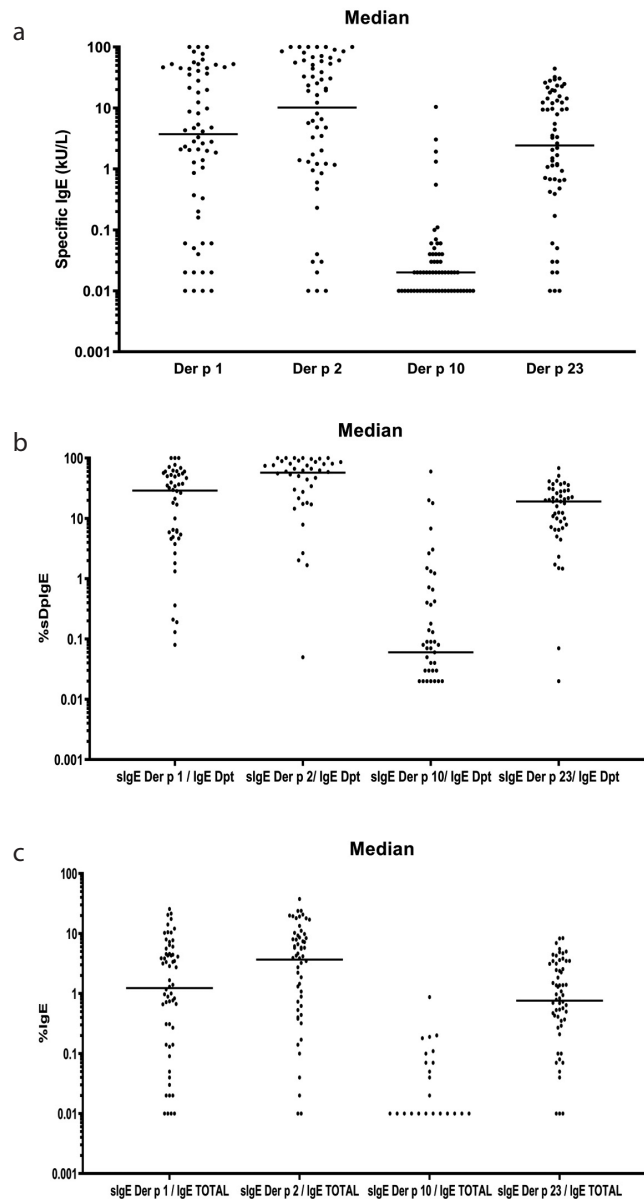SAR: severe allergic rhinitis; y.o.: years old; F: female; M: male

Figure 1. a-c. (a) Serum specific IgE levels of Der p 1, Der p 2, Der p 10 and Der p 23, and correlations between the specific IgE levels of *Dermatophagoides pteronyssinus* (crude extract), (b) total IgE and (c) single-component Der p 1, Der p 2, Der p 10 and Der p 23

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Table 2. Serological analysis of total IgE and corresponding specific IgE responses to the crude extract and molecular allergens of *Dermatophagoides pteronyssinus*

Patient # ID	Total IgE (UI/mL)	sIgE D. pter. (kU/L)	sIgE Der p 1 (kUA/L)	sIgE Der p 2 (kUA/L)	sIgE Der p 10 (kUA/L)	sIgE Der p 23 (kUA/L)	Patient # ID	Total IgE (UI/mL)	sIgE D. pter. (kU/L)	sIgE Der p 1 (kUA/L)	sIgE Der p 2 (kUA/L)	sIgE Der p 10 (kUA/L)	sIgE Der p 23 (kUA/L)
1	156.7	7.68	2.62	1.39	0.01	0.68	32	1047	75.2	45.1	60.6	0.02	14.3
2	122.3	16.6	4.7	5.67	0	1.15	34	514.6	>100	62.2	100	0.02	12.3
4	342.1	3.25	0.16	0.47	0.04	0.93	35	229	7.18	0.33	1.22	0.01	1.14
5	389.7	76.6	83.8	0.04	0.02	32.2	37	17.1	3.41	0.2	3.29	0	0.05
6	331	10.9	4.31	4.8	0.01	0.71	38	46.92	1	0.06	0	0	2.21
7	501.6	88.1	3.31	90.6	0.02	19.2	39	871	99	56.1	85.3	0.02	19.7
8	965.3	89	44	80.8	0.06	0.02	40	153.8	3.03	0.04	2.01	0.02	0
9	1281	94.4	52.6	71.3	0.04	9.41	41	322.6	5.59	0.02	1.21	0.01	2.06
10	256.2	66.8	19.8	51.1	0	3.33	42	189.8	14.9	0.02	0	0	0.01
11	257.7	30.8	8.17	19.1	0	1.37	43	263.3	32	0.06	0.85	0.03	13.2
12	977.2	>100	100	100	0.03	30.5	44	998.9	28.4	0.06	0	1.93	0.66
13	559.4	64	4.13	100	0.05	24.8	45	83.39	0.38	0.01	0.03	0.01	0.03
14	353	61.2	21.4	32.8	0.01	12.3	46	1554	>100	52.6	66.6	3.05	21.7
15	2878	>100	100	100	0.06	22.6	48	742.5	>100	5.38	58.8	1.32	23.4
16	797.1	>100	35.4	53.1	0.04	44.2	49	83.64	7.43	2.79	3.48	0.03	0.48
17	744.9	>100	77.3	100	0.02	26.1	50	20.02	5.35	2.82	4.77	0.02	1.68
18	127.6	3.49	1.28	30.5	0	0.06	51	199	53	40.60	12.20	0	9.71
19	235.4	9.9	2.1	1.72	0	3.53	52	211.5	21.8	2.05	1.31	0.01	0.17
20	295	11	1.99	6.57	0.01	1.2	53	571.3	14.6	4.77	6.19	0.03	7.89
21	135.6	13.8	9.83	0.23	0.1	9.42	54	965.3	>100	1.39	0.01	0.04	9.38
22	1194	57.9	1.05	1.17	10.4	29.4	55	100.4	2.93	0.02	20.80	0	0
23	2581	>100	100	100	0.07	28.1	56	292.8	46.4	0.01	32.60	0.01	0.03
24	612	87.8	28	44.2	0.04	15.6	57	156.8	47.7	8.76	0.02	0.01	5.48
25	745.1	36.6	2.32	29.2	0.55	2.63	58	181.7	3.11	46.60	68.20	0	1.5
26	499.6	86.6	52.6	85	0.02	18	59	240.5	19	1.86	0.95	0.01	4.42
27	1707	>100	46.8	55.5	0.02	12	60	42.68	3.39	36.70	25.40	0	0.42
28	137	2.18	0.37	0.6	0	0.64	61	293	6.53	51.30	23.50	0.01	1.08
29	225	26.1	17.9	19.4	0.11	9.66	62	61.55	1.99	0.86	0.00	0	0
30	667	45.3	2.1	38.8	0.02	0.68	63	433.9	10.2	12.40	8.12	0.02	0
31	596.6	26	0.02	16.3	0.02	3.25							

D. pter.: Dermatophagoides pteronyssinus

86% (51 out 59 subjects) had a family history of atopy and related comorbidities, five patients were afflicted with mild atopic dermatitis, three had confirmed food allergy (egg, milk and tree nuts, respectively) four had associated intermittent asthma.

Quantification of IgE Serum Responses to the Crude Extract of *D. Pteronyssinus*, HDM Molecular Profile and Total IgE

All 59 patients were sIgE positive (≥0.35 kU/L) against *D. pteronyssinus* (whole extract), with a mean value of 32.21±31.06 kU/L, ranging from 0.38 to >100 kU/L. Globally, up to 93.2% of the patients (55 out of 59) were sensitized to major allergens (i.e., either group 1 and/or 2 and/or Der p 23). Regarding individual molecular allergens, Der p 2 was

most frequently found in 50 out of 59 patients (84.74%), followed by Der p 23 in 48 of 59 (81.35%) patients, while 46 (77.96%) patients were positive for Der p 1, and only five out of 59 sera (8.47%) exhibited a sIgE to Der p 10. In addition, four patients (6.77%) were exclusively sensitized (single-molecular responders) to Der p 2 sIgE, with just one patient (1.69%) showing an individual sIgE response to either Der p 1 or Der p 23. By contrast, Der p 10 elicited no exclusive IgE responses in any of the investigated sera samples.

Quantitative median values (ranging in all four individual allergens from 0.01 to >100 kU/L) of sIgE were significantly (p=0.01) the highest for Der p 2 (17.7) to Der p 1 (4.5), Der p 23 (3.29) and the lowest for Der p 10 (<0.35). Median ratios of Der p 2 related to total IgE (Der p 2/total IgE)

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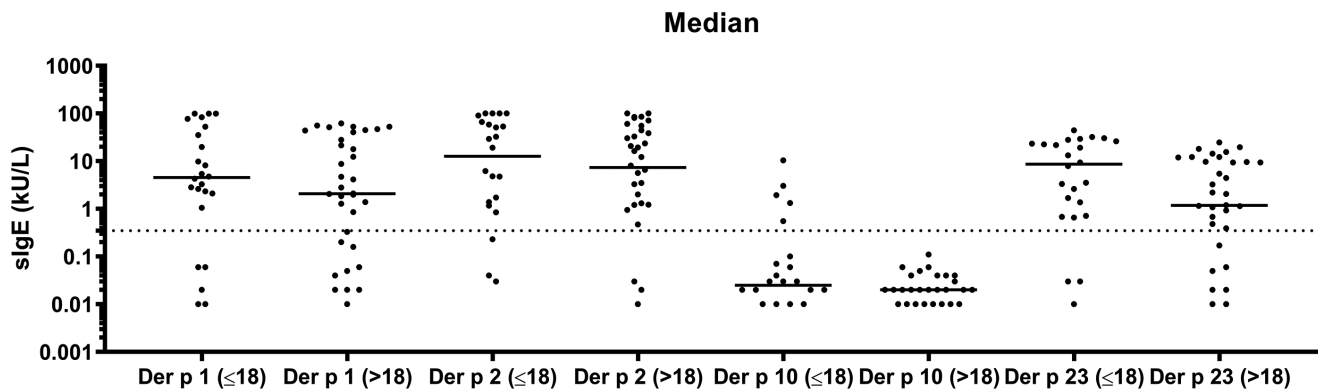Molecular Diagnosis in Mite Allergic Rhinitis 159

Figure 2. Serum specific IgE levels of Der p 1, Der p 2, Der p 10 and Der p 23 from younger subjects compared to elder patients (>18 years of age)
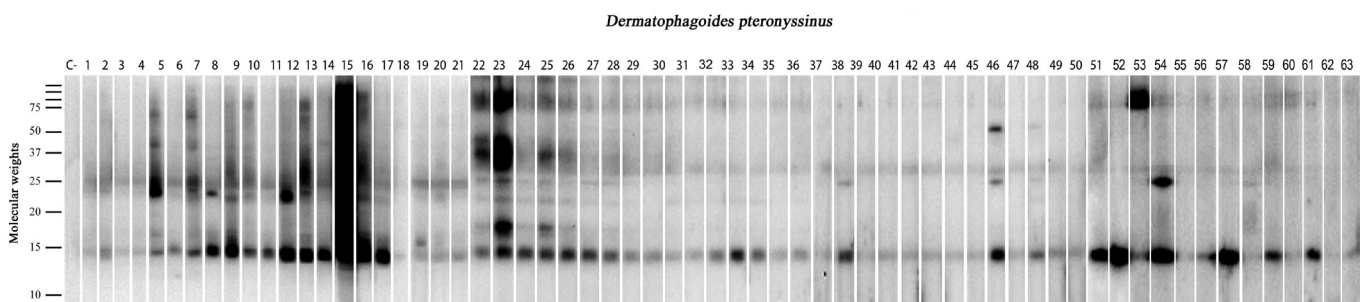

Figure 3. Western blot of selected patients sensitized to *Dermatophagoides pteronyssinus* with severe allergic persistent rhinitis

and sIgE to *D. pteronyssinus* whole extract (Der p 2/sIgE *D. pteronyssinus*) were again the highest compared to the rest of single individual allergens (i.e., Der p 1, Der p 23 or Der p 10), as displayed in Figure 1.

Considering the relation of the sensitization profile of total IgE and sIgE in relation to age, total IgE and median value of 326.8 UI/mL were found in younger patients (<18 years of age), showing a higher median total IgE than adults, denoting a marked atopic phenotype in the investigated population (Table 2).

Considering the relation of the sensitization profile of total IgE and sIgE in relation to age, higher median IgE and sIgE values were found in the younger patients (<18 years of age) compared to adults, denoting a marked atopic phenotype in the investigated population (Table 2).

Moreover, concurrent sIgE responses to *D. pteronyssinus* (whole extract) were displayed with a significantly (p=0.01) higher median level of sIgE in younger subjects (*D. pteronyssinus:* 32.37 to 5.16, ranging 0.01 to >100 kU/L in both cases) and a higher median level of sIgE to all major allergens, except for Der p 10 in comparison with their elder counterparts (Figure 2).

SDS PAGE/IgE Western Blot
Western blot of selected individuals sensitized to *D. pteronyssinus,* including 4 subjects #3, 33, 36 and 47 with no de-

tectable sIgE to the whole extract of *D. pteronyssinus,* showed different repertoires of IgE response (Figure 3). A notable stain intensity was found at 14-15 kDa for *D. pteronyssinus* in almost all subjects (>95%), followed by protein staining at 24 kDa.

Discussion
The evidence that single allergen components assemble specific additions against a complete mite IgE response suggests that biological functions of particular mite allergens contribute to their allergenicity (2). In our investigation, a prevailing capacity for sIgE sensitization to Der p 2 (>80%), Der p 23 (>80%) and Der p 1 (>75%) was confirmed amongst a population with SAR under subtropical climate conditions. These findings are coincident with former reports, showing a higher incidence of IgE responses to both Der p 1 and Der p 2 than those addressed in Australia (77%) and Singapore (63%) (29). The difference can probably be explained by a persistent higher exposure to HDM, with relative humidity as the ultimate feature, to patronize HDM prevalence (30, 31). The tight correlation among IgE responses to Der p 1, Der p 2 and Der p 23, and *D. pteronyssinus,* upholds the significance of these allergens in the IgE recognition profile to HDM in our region. In addition, the recorded IgE response against Der p 2 and Der p 23 represented a larger fraction compared to Der p 1, as shown by previous reports in Spain (32). Der p 10 exhibited noticeable titers of IgE in 8.47% in the studied sample, showing higher prevalence compared to

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previous works from China (33, 34). The elevated prevalence (81.35%) of Der p 23 sIgE–close to Der p 2 (84.74%)–further confirmed the serodominance of this peritrophin-like protein in SAR, supporting investigations from Europe and Asia (35, 36).

The biological proteolytic activity of Der p 1 appears to play a more influential role during the early years of life, while Der p 2 takes the lead in later ages, proposing a higher exposure to Der p 1 rather than Der p 2 (37, 38). By contrast, our observations found that although younger individuals showed a higher mean level of sIgE to *D. pteronyssinus* and to individual allergens compared to elderly patients, the sIgE against Der p 2 was more intense regarding prevalence and individual quantification than Der p 1 and Der p 23 in both infants and adults. Interestingly, despite the high sIgE response to Der p 23, only one subject (1.69%) was exclusively sensitized to this allergen—confirming the polyclonal sIgE HDM profile of the current sample—in contrast to previous Austrian data (39) showing 10% of allergic patients solely responding to Der p 23, excluding otherwise responses to a broad panel of HDM allergens.

Also, previous works from Asia have elegantly confirmed the association among those sIgE profiles enclosing a greater number of HDM allergens, and the intricacy of the evolved allergic clinical endotype (31, 33). In addition to these findings, herein we describe multiple and complex IgE repertoires to Der p 1, Der p 2, Der p 23 and Der p 10, within the same underlying disease and degree of severity—i.e., persistent SAR—excluding further atopic conditions such as asthma or eczema.

Concerning HDM minor allergens, the prevalence of IgE response to the muscular protein tropomyosin was scarce (<10%) and limited to younger patients, with no subjects exclusively sensitized to Der p 10 in our observations. As previously reported, those individuals with a sensitization to the HDM group 10 allergens exhibited significantly higher total IgE levels (1,025.2 kU/L) compared to those without a response to Der p 10 (432.7 kU/L), thus enduring Der p 10 as a biomarker to extensive sensitization concerning susceptible allergic population (40, 41).

The presented research has a potential bias as four out of 59 (6.77%) subjects sensitized to the crude extract of *D. pteronyssinus* were not identified through the composed CRD panel. In this regard, as some HDM allergens may not be sufficiently represented—thus exhibiting lower IgE titers to the HDM raw extract ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden) the inclusion of more specific tests, adding further recombinant molecular allergens to the complete HDM source or even depicting molecular panels containing the most prevalent allergens, may be suitable to overcome this duty (42, 43).

Conclusion

Our findings showed that the currently proposed HDM molecular panel, including Der p 1, Der p 2 and Der p 23, accurately recognized the large majority (>90%) of *D. pteronyssinus*-allergic subjects afflicted with SAR in an area with a high load of mite allergen exposure. Diverse mite allergens dominate the immediate sIgE response in SAR, with patient variability evolving into individual pleomorphic responses, CRD arises critical to formulate a further precise molecular diagnosis, and eventually a personalized and tailored HDM immunotherapy.

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