Sensitisation to Lep d 2- *Lepidoglyphus destructor* allergy in asthma and rhinitis

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Summary

Background. Mites are responsible for allergic diseases, such as asthma and rhinitis, in nearly 1.5% of the world population. It is known that nowadays, besides House Dust Mites (HDM), Storage Mites (SM) are important sensitisers in urban non-occupational dwellings, predominantly in the Mediterranean area. The main objective of our study was to analyse the clinical relevance of the most prevalent SM, *Lepidoglyphus destructor*, by assessing the relationship between sensitisation to the major molecular allergen Lep d 2 and allergic respiratory disease phenotype in an urban population monosensitised to this molecular allergen.

Methods. Cross-sectional study which included consecutive patients evaluated in our Allergy Department between 2012 and 2018, from urban non-occupational setting, with rhinitis and/or asthma, perennial symptoms and proven sensitisation to SM Lep d 2, tested using ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC) technology, which detects 112 allergens.

Results. A total of 37 allergic patients were included (23 female), aged 20.1±12.8, min-max 5-58 years-old. The molecular sensitisation profile showed 18.9% of *L. destructor* mite monosensitised patients, having only sIgE to Lep d 2. This subset of patients mainly had allergic rhinitis, with 28.6% also being asthmatic. Regarding severity, most patients showed a persistent moderate

phenotype of respiratory disease. The mean value of Lep d 2 sIgE was 8.3±9.8 ISU-E, lower than in mite polysensitised patients (21.7±21.5, p=0.049).

Conclusions. Our proof-of-concept study focused on Lep d 2 monosensitisation, showed that SM may have clinical relevance in perennial allergic asthma and rhinitis, and should be taken into account when assessing and treating allergic respiratory disease.

Key words

Storage mites; *Lepidoglyphus destructor;* Lep d 2; asthma; rhinitis.

Impact statement: Molecular sensitisation profiling of allergic patients showed, through Lep d 2 monosensitisation, that *Lepidoglyphus destructor* may have clinical relevance in allergic rhinitis and asthma in urban non-occupational settings.

Introduction

Allergy to House Dust Mites (HDM), such as *Dermatophagoides pteronyssinus*, is well known and has been long described (1). Yet, respiratory allergic disease related to mite sensitisation other than HDM is not equally well addressed. Storage mite (SM), particularly *Lepidoglyphus destructor*, *Tyrophagus putrescentiae* and *Acarus siru*, among others, have mainly been related to occupational settings, like farming or grain/cattle handling (2-4). However, in the last two decades, SM sensitisation has also been demonstrated as preeminent in non-occupational urban environments (5-8).

Despite that, only recently some evidence has been emerging related to SM immune responses taking part in the pathogenesis of rhinitis and asthma allergic phenotype in general populations (9-13). Some studies have shown that *Lepidoglyphus destructor,* one of the most prevalent SM in the Mediterranean area, may contribute to greater disease severity, including moderate-to-severe asthma and allergic rhinitis (9, 12). However, the prevalence of co-sensitisation between SM and HDM may vary depending on the geographic area, the SM species studied, and the methods used. In northern Europe, the population-based cohort from European Community Respiratory Health

Survey (ECRHS) showed a prevalence of co-sensitization between SM and HDM of 44% [10]. Other cohorts mainly from Mediterranean area reported higher values, with more than 90% of the patients sensitised to SM also being sensitised to HDM [12].

Most southern Europe countries are dust complex areas with high prevalence of mite allergen polysensitisation and allergic perennial symptoms (14, 15). Understanding which mites are important contributors to the disease phenotype may be difficult in those regions. Allergen challenges, like Nasal Provocation Tests (NPT), are a useful tool to study clinical relevance (13, 16). Nonetheless, Component-Resolved-Diagnostics (CRD) allows profiling molecular allergen sensitisation and distinguishing between genuine species-specific sensitisation and cross-reactivity, which relates with allergen clinical impact on symptoms (17-19).

Numerous studies have been shown very limited cross-reactivity between *D. pteronyssinus* and *L. destructor*, particularly concerning group 2 allergens – Der p 2 and Lep d 2 (20-24). In contrast, a high cross-reactivity exists within the group 2 of the SM - Lep d 2, Gly d 2, Tyr p 2 (24, 25).

IgE against major allergen Lep d 2 is usually identified in more than 70% of individuals who exhibit sensitisation to *Lepidoglyphus destructor* (12, 26). Sensitisation to Lep d 2 has been frequently associated with asthma and is also linked to symptom severity, but always in HDM-SM co-sensitised patients (9, 12, 14). However, SM monosensitised patients may allow a better understanding of the real importance of those mites in respiratory allergic diseases.

Thus, the aim of our study was to analyse the clinical impact of *L. destructor* monosensitisation (single reactivity to Lep d) in a population of respiratory allergic patients without occupational or rural context.

Materials and Methods

Study design and patient population

In this cross-sectional study, a total of 37 patients were studied in the Department of Allergy and Immunology of Centro Hospitalar de Setúbal, Portugal, between 2012 and 2018. The inclusion criteria were: (1) medically confirmed diagnosis of allergic asthma and/or allergic rhinitis; (2)

presence of perennial respiratory allergic symptoms; (3) residency in an urban area; (4) absence of occupational exposure to SM (e. g. stored grains, hay and straw, poultry and livestock feed, barn dust); and (5) positive specific IgE to Lep d 2 (> 0.30 ISU-E) measured by ImmunoCAP™ ISAC (Thermo Fisher Scientific, Vienna, Austria). Patients co-sensitised with HDM and/or SM can be included.

The study was based on anonymised data and approved by the Local Ethics Committee. All patients (or their legal guardian if underaged) provided their written informed consent for the detailed procedures, as well as for the use of their data for scientific reasons.

Operational definitions and Variables

Demographic and clinical characteristics included age, sex, type of respiratory allergic disease (rhinitis, asthma or rhinitis with concomitant asthma), frequency of symptoms (perennial with or without concomitant seasonal complaints), and severity of the allergic disease. Other collected data included concomitant food allergy, presence of domestic animals (cats and dogs) and indoor humidity (presence of mold growth or water condensation on the inside of the windows).

Asthma definition was based on a medically confirmed diagnosis, and its severity was determined according to Global Initiative for Asthma (GINA) recommendations (27).

Rhinitis definition was based on a medically confirmed diagnosis, and its features were classified according to Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines (28).

Allergen sensitisation was determined by ImmunoCAP Immuno Solid-phase Allergen Chip™ (ISAC) multiplex array (Thermo Fisher Scientific, Vienna, Austria) that detects 112 molecular components from airborne and food allergen sources, and it is considered positive if > 0.30 ISU-E.

The assessed molecular components of HDM were major allergens Der p 1 and Der p 2 from *D. pteronyssinus*, Der f 1 and Der f 2 from *D. farinae* and Blo t 5 from *Blomia tropicalis*. For SM, the only molecular component present in this multiplex panel is Lep d 2, major allergen from *L. destructor*.

Mite-monosensitisation was considered in this study when only a single mite source on the ImmunoCAP™ ISAC panel (Der p 1/Der p 2 or Der f 1/ Der f 2, or Lep d 2 or Blo t 5) showed positive IgE results, and mite-polysensitisation when two or more of these molecular mite allergens were positive.

Sensitisation to other less prevalent and cross-reactive HDM or SM was not assessed, as also happened with sensitisation to minor molecular allergens not available in this multiplex panel.

Descriptive and statistical analysis

Descriptive statistics included frequency analysis (percentages) for categorical variables and medians, interquartile ranges, and ranges for continuous variables. Categorical variables were analysed using Fisher's exact test or $\chi 2$ test, whenever necessary. For continuous variables, comparisons were determined by Mann-Whitney U test, as data failed normality testing with D'Agostino & Pearson test. Statistical significance was considered for p-value ≤ 0.05. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA, version 26.0).

Results

Demographic Characteristics of Patients

The analysis included 37 patients, whose demographic and clinical characteristics are summarised in Table I.

The presence of rhinitis with asthma (62.2%), was more frequent than rhinitis alone (37.8%), with no patients having isolated asthma. Concerning severity of the disease, almost half of the patients had a more severe phenotype of both diseases: 17 (45.9%) with moderate-severe persistent rhinitis and moderate-severe persistent asthma. None had received allergen immunotherapy (AIT) for mite allergy.

Molecular sensitisation profile and its clinical relevance

Table II shows the mite molecular allergen sensitisation profile from each included patient, assayed with the ImmunoCAP™ ISAC multiplex array.

- **Lep d 2 monosensitised patients**

Seven of the 37 patients were only sensitised to Lep d 2, with absence of IgE response to the other mite components (Der p1, Der p 2, Der f 1, Der f 2 and Blo t 5), representing 18.9% of the patients. Considering this group of patients who were Lep d 2 mite-monosensitised, all had rhinitis and two of them also had asthma (28.6% versus 70%, in mite-polysensitised patients) (Table III). Besides perennial symptoms, all patients had a moderate or severe, persistent disease.

As displayed in Figure 1, monosensitised Lep d 2 patients showed significantly lower sIgE values for this allergen than mite-polisensitised patients (8.3±9.8 versus 21.7±21.5 ISU-E, p=0.049).
Some of the mite-monosensitised patients, with IgE response to Lep d 2 and to no other mite allergens, were allergic to other allergen sources, like pet, mold, and food allergens (Table IV). Considering other potential causes of respiratory perennial symptoms, the group of Lep d 2 mite-monosensitised patients had 3 patients who were also sensitised to pets' allergens, but only one of those had repeated contact with pets. Another patient also had positive IgE against mold allergen but information regarding its exposure was not available.
More than half of this group of Lep d 2 mite-monosensitised patients (57,1%) had food allergy, all related with fruits and vegetables, without known cross-reactive components with Lep d 2. None of those 7 patients presented with atopic dermatitis.

- **Overall mite sensitised population**

Regarding the overall studied population, the most prevalent sensitisations, besides Lep d 2, were homologous allergens from group 2 - Der p 2 and Der f 2, present in 73% of the patients. From those, 6 patients were exclusively sensitised to these three components.
IgE to major allergen Der p 1 occurred in 62.3% of the patients.

Conversely, only one patient showed positivity to Der p 10. This patient reported seafood allergy and also exhibited also with other IgE positivity for tropomyosins (Ani s 3, Bla g 7, Pen m 1) – a shellfish-HDM-cockroach cross-reactivity syndrome.

An IgE response to Blo t 5 was found in 29,7% of patients, all of whom being mite-polysensitised and without personal history of residing in tropical geographic regions (Figure 2A).

Considering concentrations of specific IgE responses, in the overall studied population, mean values of sIgE (mean±SD, ISU-E) against mite allergens were: Der p 1: 16.8±16,8, Der p 2: 25.9±23.8, Der f 1: 9.4±8.4, Der f 2: 22.2±24.1, Blo t 5: 20.8±18.5 and Lep d 2: 19.1±20.4 (Figure 2B).

When age subgroups were compared (i.e., children vs adults), only sIgE to Der p 1 was different between paediatric and adult patients, being significantly higher in children (p=0,036) (data not shown).

Regarding the spectrum of IgE-recognised mite components, patients with asthma and rhinitis had a significantly higher number of positive sIgE against major allergens, compared with patients who only had rhinitis (p=0.0052) (Figure 3).

Nonetheless, IgE levels were also compared between patients with and without asthma (only rhinitis), but no statistical difference was found for any of those molecular components in this population.

Discussion and Conclusions

In this study, respiratory allergic patients without occupational dust exposure or rural context and Lep d 2 sensitisation, were assessed to search for Lep d 2 monosensitisation in multiplex microarray analysis.

From this allergic cohort, 18.9% of patients displayed monosensitisation to Lep d 2, a value slightly higher than previously reported in other cohorts for single SM Lep d 2 sensitisation, with prevalences around 10-14% (10, 12, 15). Our Lep d 2 monosensitised patients presented with

significantly lower sIgE levels for Lep d 2 when compared with mite polysensitised patients. However, there is no published information about values of sIgE to Lep d 2 in monosensitised patients.

Regarding clinical symptoms, our subgroup of Lep d 2 monosensitised patients presented mainly with moderate and persistent phenotype of respiratory disease, in accordance with other studies of *L. destructor* allergic patients (12-14). Nonetheless, monosensitised Lep d 2 patients were never addressed concerning clinical characteristics before.

An initial approach for the entire studied population shows these were mostly mite polysensitised patients, with 81.1% of SM-HDM co-sensitisation, which is in line with many other research studies in southern Europe (12-14, 29, 30). Considering the molecular sensitisation profile, the most prevalent components besides Lep d 2 were Der p 2 and Der f 2., with more than 70% of positive IgE response. On the other hand, prevalence of sensitisation to group 1 – Der p 1, Der f 1, was relatively low, around 60%. Similarly to our results, in areas with high exposure to SM in north of Spain and in France, percentages of IgE response to Der p 2 were clearly higher than those to Der p 1 (31, 32, 21). In contrast, in areas with the exclusive presence of HDM, there were more sera with positive sIgE to Der p 1 than to Der p 2 (31).

When analysing all included patients in terms of the levels of specific IgE against mite molecular components, again Der p 2 and Der f 2 allergens showed the highest sIgE values, and this did not change when considering children and adult separately. These results are in accordance with other recent studies, that mention allergens within group 2 being highly cross-reactive and the most prevalent from HDM repertoire in many geographic regions around the world (33, 34, 26, 15).

Interestingly, and in line with previous studies, when looking at the clinical relevance of sensitisation profiles, patients with more severe respiratory disease, with concomitant asthma, exhibited significantly higher sensitisation rates than those with rhinitis only. Indeed, many studies have shown that, despite no specific patterns of molecular sensitisation having been associated with an exact type of allergic disease, a broader spectrum of molecular mite IgE

responses may be related to increased complexity and severity of the clinical phenotype (17, 26, 34-36).

Also, subjects with asthma usually present with higher mite molecular sIgE concentrations than patients with rhinitis without concomitant asthma (13, 34, 37). However, our set of asthmatic patients didn´t displayed significantly higher sIgE concentrations when compared with non-asthmatic ones. This discrepancy with other studies may be related to the small number of patients included in our cohort.

Overall, our data are in accordance with previously published information that support that *L. destructor* single sensitisation should be closely assessed to understand its true clinical relevance in urban non-occupational settings, giving the high prevalence of HDM-SM co-sensitisation. For several years, many other groups have been stating that SM like *L. destructor* may represent an important cause of respiratory allergic symptoms outside rural places (6-8, 22, 30).

In recent years, with the advent of Precision Medicine and CRD, a comprehensive panel of molecular components for diagnosis allowed to better acknowledge true species-specific reactivity versus cross-reactivity in SM (22). Although in a short group of patients, our results may lead to a proof-of-concept: true allergy to L. destructor, supported by positive sIgE to Lep d 2 and no other major HDM components, can be responsible for allergic respiratory inflammation in the general population. These findings, also in line with recent investigations in Europe (9-13), somehow suggest that the classification of *L. destructor* as a "storage mite" might be shortly revised.

Finally, our study has some limitations. In the group of mite Lep d 2 monosensitised patients, three of them were also sensitised to pet allergens and one of them also to molds. Therefore, we cannot exclude the possibility that IgE reactivity to both groups of allergens might be the cause of perennial rhinitis and asthma. NPT could be useful in those cases to confirm the clinical relevance of each allergen. However, the remaining patients had perennial symptoms of

respiratory allergy that were most probably due to sensitisation to Lep d 2, thereby highlighting the potential clinical impact of this allergen.

Another limitation is that, at the date of the study, the version of the ISAC test available included fewer allergens that those available in the current version. Also, other multiplex arrays are now available, as ALEX^2 (38), with broader spectrum of allergen molecules from different mite species. Therefore, IgE reactivity to other HDM allergens not represented on the chip, like Der p 23 or other mid-tier allergens, should not be assumed. Nonetheless, many previous studies have shown that cross-reactivity is practically non-existent between HDM molecules and L. destructor allergens (22-24); thus Lep d 2 should be the probable cause of allergy in those patients.

Furthermore, other highly cross-reactive SM (*Glycyphagus domesticus, Tyrophagus putrescentiae, Acarus siro*) are not represented in ImmunoCAP™ ISAC chip and were not assessed in the studied population. Thus, true monosensitisation to L. destructor might not really exist, but instead, a cross-reactive polysensitisation to SM (22-24). Even so, the importance of those so-called SM in urban areas should not be underestimated.

Effectively, the low number of patients included in our study must also be acknowledge as a limitation, even considering that the study inclusion period was extended to 6 years. In fact, the unavailability of commercial singleplex tests for Lep d 2 sIgE reduces our capacity to identify sensitisation to this molecular component, and we were only able to select these patients after multiplex microarray analysis.

Additionally, selection bias may have occurred, since the clinical reason for each medical doctor to have ordered ISAC ImmunoCAP™ multiplex may had been diverse and not related to mite respiratory allergy. Thus, the absence of proper randomization makes it difficult to extrapolate the prevalence data to general population. Notwithstanding, our group did address the existence of *L. destructor* mite monosensitisation, as well as its clinical impact in perennial allergic asthma and rhinitis.

The main topic of our study is the individual contribution of sensitisation to Lepidoglyphus destructor for perennial asthma and rhinitis symptoms. Once co-sensitisation HDM-SM is highly prevalent in southern European countries, the single clinical impact of each mite is difficult to interpret without performing NPT (which is invasive and time consuming in daily clinical practice). For an accurate AIT prescription, identification of each clinically significant allergen is mandatory. With a small but well characterized cohort of Lep d 2 monosensitised patients, our study showed that *Lepidoglyphus destructor* is clinically relevant for developing asthma and rhinitis symptoms.

Therefore, we suggest that assessment of IgE against major allergen of *Lepidoglyphus destructor,* Lep d 2, should be performed in order to evaluate if this allergen source should be considered for precision therapy with AIT. Major molecular component Lep d 2 singleplex assays should be soon available for more regular assessment of its sIgE response, in respiratory allergic patients with perennial symptoms.

The future challenge lies in integration of this multidimensional interplay - better comprehension of molecular sensitisation profiles to SM; specific allergen challenge tests to assess individual clinical relevance; understanding multifactorial nature of the exposome. Those are certainly major determinants for understanding the importance of SM in the course of the allergic disease.

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Conflict of interests

None of the authors has any financial relationships, political, intellectual or religious conflict of interest related to this manuscript.

Contributions of each author.

FMS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. ET: Data curation. CM: Writing – review & editing. LTB, FI: Supervision, Validation, Writing – review & editing.

Informed Consent Statement and Ethics

Informed consent was obtained from all subjects involved in the study. Ethical approval was obtained by the Ethical Board of the Hospital involved in the study.

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Figure 1. Values for sIgE to Lep d 2 in patients with molecular monosensitisation to Lep d 2 and in mite polisensitised patients. Patients Monosensitised to Lep d 2 present lower values of Lep d 2 sIgE compared with patients polisensitised to mites (p=0,049). Independent-Samples Mann-Whitney U Test was used for this comparison. Boxes indicate the interquartile ranges, horizontal lines indicate the median values, and whiskers indicate maximum and minimum values, excluding outliers (showed as dots).

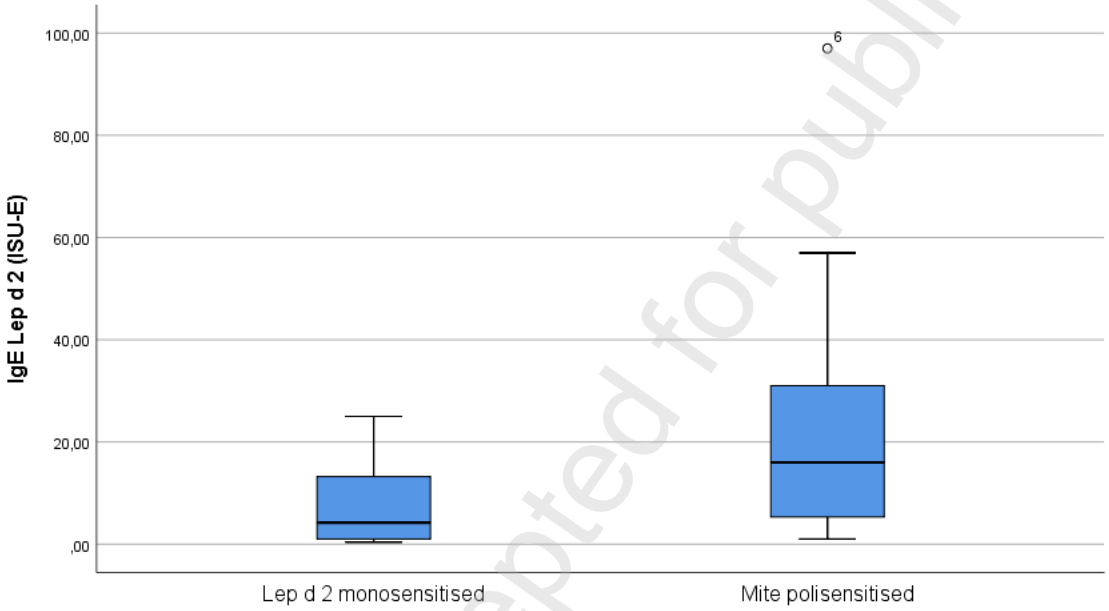


Figure 2. Characterisation of the sensitisation profile in patients with documented mite allergy. **a)** Frequencies of IgE responses to molecular mite allergens (all patients being sensitised to Lep d 2). Prevalence of IgE sensitisation (y-axis: percentages) to individual mite molecular allergens (x-axis). **b)** Levels of sIgE for molecular mite allergens (y-axis). Boxes indicate the interquartile ranges, horizontal lines indicate the median values, and whiskers indicate maximum and minimum values, excluding outliers (º - outlier; * - extreme outlier).

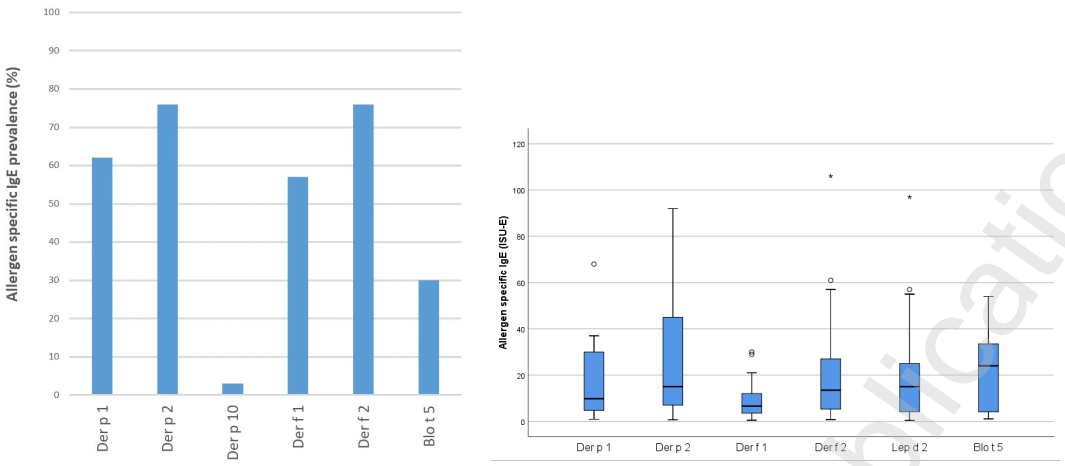
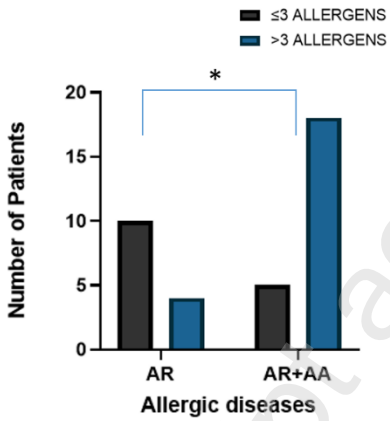


Figure 3. Sensitisation count of mite molecular components regarding respiratory allergic diseases. AR – Allergic Rhinitis; AA - Allergic Asthma; allergens - mite molecular components assessed with microarray ImmunoCAP™ ISAC multiplex (Der p 1, Der p 2, Der f 1, Der f 2, Blo t 5, Lep d 2). * p = 0.0052, Fisher's exact test;



	Number of mite molecular allergens recognised					Total
	1	3	4	5	6	
AR	5	5	1	1	2	**14**
AA+AR	2	3	2	8	8	**23**
Total	**7**	**8**	**3**	**9**	**10**	**37**

Table I. Baseline characteristics of study patients

Demographic characteristics	Total (n=37)
Gender, No. (%)	
Male	14 (37.8%)
Female	23 (62.2%)
Age, years, mean (SD), min-max	20.1 (12.8), 5-58
Age groups, No. (%)	
Children (<18 years)	21 (56.8%)
Adults (≥18 years)	16 (43.2%)
Clinical characteristics	
Frequency of symptoms, *No. (%)*	
Perennial with seasonal complaints	13 (35.1%)
Perennial without seasonal complaints	24 (64.9%)
Respiratory allergic disease, No. (%)	
Rhinitis	14 (37.8%)
Rhinitis + Asthma	23 (62,2%)
Severity of rhinitis[1], No. (%)	n=37
Mild Intermittent	4 (10.8%)
Mild Persistent	4 (10.4%)
Moderate-severe Intermittent	2 (5.4%)
Moderate-severe Persistent	27 (73.0%)
Severity of asthma[2], No. (%)	n=23
Mild Persistent	3 (13.0%)
Moderate Persistent	19 (82.6%)
Severe Persistent	1 (4.4%)
Food allergy, No. (%)	17 (45.9%)
Atopic dermatitis, No. (%)	5 (13.5%)

[1] in accordance with ARIA guidelines; [2] in accordance with GINA recommendations; min – minimum, max – maximum.

Table II. Specific mite IgE profiles tested with microarray ImmunoCAP™ ISAC multiplex. Profiles are ordered by the patient code.

Patient code	Lep d 2	Der p 1	Der p 2	Der p 10	Der f 1	Der f 2	Blo t 5
1	•	•	•	•	•	•	
2	•	•	•		•	•	
3	•		•			•	
4	•	•	•		•	•	
5	•	•			•		
6	•						
7	•		•			•	•
8	•	•	•		•	•	•
9	•	•	•		•	•	
10	•						
11	•	•	•			•	
12	•	•	•		•	•	•
13	•	•	•		•	•	
14	•	•	•			•	
15	•	•	•		•	•	•

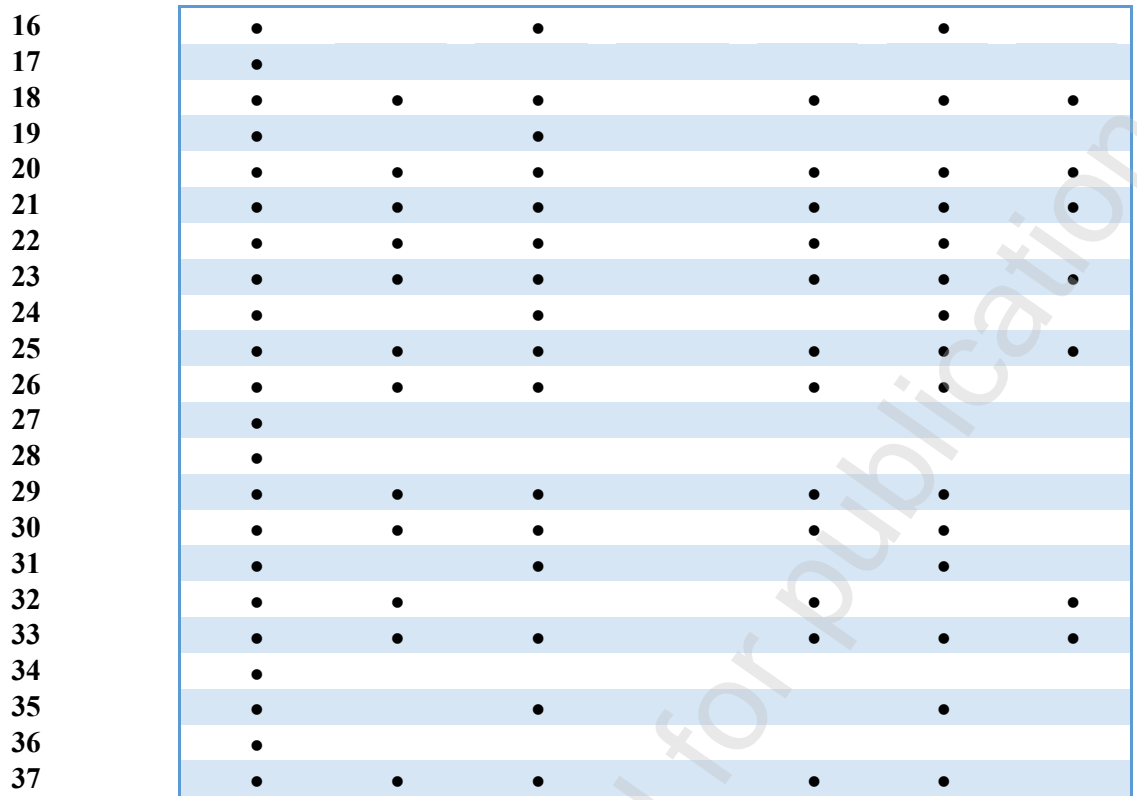


Table III. Demographic and clinical data of patients with Lep d 2 mite-monosensitised patients and mite-polisensitised patients

Demographic characteristics	**Lep d 2 monosensitised (n=7)**	**Mite polisensitised (n=30)**
Gender, No. (%)		
Male	4 (57.1)	19 (63.3)
Female	3 (42.9)	11 (36.7)
Age, years, mean (SD), min-max	19.7 (15.9), 5-44	20.2 (12.3), 7-58
Clinical characteristics		
Respiratory allergic disease, No. (%)		
Rhinitis	5 (71.4%)	9 (30%)
Rhinitis + Asthma	2 (28.6%)	21 (70%)
Severity of rhinitis[1], No. (%)	n=7	n=30
Mild Intermittent	0	4 (13.3)
Mild Persistent	1 (14.3)	3 (10)
Moderate-severe Intermittent	0	2 (6.7)
Moderate-severe Persistent	6 (85.7)	21 (70)
Severity of asthma[2], No. (%)	n=2	n=21
Mild Persistent	-	3 (10)
Moderate Persistent	2 (100)	17 (56.7)
Severe Persistent	-	1 (3.3)

[1] in accordance with ARIA guidelines; [2] in accordance with GINA recommendations; min – minimum, max – maximum.

Table IV. Detailed evaluation of the 7 mite-monosensitised *L. destructor* allergic patients

Patient code	Gender	Age (years)	Rhinitis (severity)	Asthma (severity)	ISAC Lep d 2 (ISU-E)	Food allergy
6	female	5	Moderate/severe persistent	Moderate	1,20	no
10	male	6	Moderate/severe persistent	Moderate	7,40	yes
17	male	12	Mild persistent	-	,80	yes
27	male	13	Moderate/severe persistent	-	4,20	no
28	female	18	Moderate/severe persistent	-	25,00	yes
34	male	40	Moderate/severe persistent	-	19,00	yes
36	female	44	Moderate/severe persistent	-	,40	no

Patient code	SS Mold	SS GrassPolen	SS TreePolen	SS Pet	CC Tropomyosin	CC Polcalcin	CC LTP	CC Profilin
6	no	no	no	no	no	no	no	no
10	no	no	no	yes	no	no	no	no
17	no	no	no	no	no	no	yes	no
27	no	no	no	no	no	no	no	no
28	no	yes	no	yes	no	no	yes	no
34	yes	yes	no	yes	no	no	no	yes
36	no	yes	yes	yes	no	no	no	yes

All the 7 patients had a positive response for Lep d 2, without positive sIgE against other mites (Der p 1, Der p 2, Der f 1, Der f 2 and Blo t 5), assessed by microarray ImmunoCAP™ ISAC multiplex

SS: species specific components; CC: cross-reactive components