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Evaluation and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens using a new multiplex assay: a real-life experience on an Italian population

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Impact statement

Assessment to a comprehensive profile of HDM allergens defines serological rectivity profiles that seem associate with different clinical presentations.

Summary

Background. *House dust mites (HDM) are among the most important allergen sources worldwide, representing a major cause of perennial allergic rhinitis and asthma.* ***Aim.*** *To evaluate the prevalence of IgE responses towards a comprehensive panel of HDM allergens and to evaluate the implications of molecular sensitization profiles on respiratory symptoms.* ***Methods.*** *155 consecutive HDM-allergic patients (mean age: 27.5 years; range: 1-62; female: 63), 86 affected by rhinitis and 68 by asthma, were enrolled. Specific IgE reactivity to Der f 1, Der p 1, Der f 2, Der p 2, Der p 5, Der p 7, Der p 10, Der p 11, Der p 20, Der p 21 and Der p 23 was tested in patients' sera using the last version of the multiparametric assay Allergy Explorer[2] (ALEX[2]).* ***Results.*** *In all, major and minor allergens were positive, respectively, in 96.8% and 50.9% of the patients. Prevalence and IgE levels of Der f 1, Der f 2, Der p 1 and Der p 20 were significantly higher in asthmatic patients ($p < 0.05$), whereas subjects negative for minor allergens resulted more frequently suffering from rhinitis ($p = 0.0001$). Asthmatic patients had IgE reactivity to a larger number of HDM allergens (mean 5.4; SD ± 2.3) than patients with only rhinitis (mean 4.2; SD ± 2.5) ($p = 0.003$), whereas no differences in the number of HDM positive molecules and in the specific IgE levels were found among different ages.* ***Conclusions.*** *This study confirms that the assessment of IgE to a comprehensive panel of HDM allergens defines different serological reactivity profiles that seem associated with different clinical presentations.*

Introduction

House dust mites (HDM) are among the most important allergen sources worldwide representing a major cause of perennial allergic rhinitis and asthma. Up to 85% of asthmatic patients are sensitized to *Dermatophagoides (D.) pteronyssinus* and/or *D. farinae* (1), which are the two most important HDM species (2) present in human habitats around the world. Since 1980, when the first allergen of *D. pteronyssinus* was described (3), many other HDM allergens have been identified; some are not only immunogenic but have also proteolytic and immunomodulatory activity (4). Thirty-six allergens for *D. farinae* and thirty allergens for *D. pteronyssinus* have been detected so far (www.allergen.org), even if only group 1 (Cysteine protease: Der p 1, Der f 1), group 2 (NPC2 protein family; Der p 2 and Der f 2) and Der p 23

(Peritrophin-like protein) represent the major and serum-dominant allergens (5). However, other minor allergens such as Der p 4 (α-amylase), Der p 5 (unknown biochemical function), Der p 7 (Lipid-binding protein), and Der p 21 (unknown biochemical function) seem clinically relevant (5, 6). Most allergen extracts obtained from natural sources contain mainly group 1 and group 2 allergens, whereas other important molecules are present in small amounts or are missing, as shown in extracts for skin testing (7). This may have some important consequences not only for diagnosis but also for immunotherapy, since we can speculate that patients with different profiles of IgE reactivity to HDM may respond differently to immunotherapy, as recently shown by Rodriguez-Dominguez *et al*. (8). In addition, other authors, using a comprehensive panel of HDM allergens, revealed that some serological reactivity profiles might help to discriminate asthmatic and non-asthmatic children or might be able to predict the development of asthma (6, 9, 10).

Until now, only group 1 and 2, Der p 23 and Der p 10 (tropomyosin) allergens have been available for the HDM component resolved diagnosis (CRD) in the clinical practice. A more extended panel of HDM allergens using the immunoCAP ISAC technology has been available for research use only. Recently, a new multiparametric assay containing an extended panel of HDM allergens (including Der f 1, Der p 1, Der f 2, Der p 2, Der p 5, Der p 7, Der p 10, Der p 11 (paramyosin), Der p 20 (arginine-kinase), Der p 21, Der p 23) was launched on the marked and can be used in daily practice for HDM CRD.

Our study aimed to evaluate, the prevalence of IgE responses towards this comprehensive panel of HDM allergens and to evaluate the implications of molecular sensitization profiles on the respiratory symptoms (rhinitis and asthma) in a cohort of Italian HDMs allergic patients.

Materials and methods

Patients

155 consecutive HDM-allergic patients (mean age: 27.5 years; range: 1-62; female: 63), diagnosed by a clinical history of perennial rhinitis and/or asthma and positive skin prick tests (SPT) (Stallergenes, Antony, France) and/or *in vitro* assay (Thermofisher Diagnostics, Uppsala, Sweden) with extracts of both *D. pteronisyinus* and *D. farinae,* were enrolled in two allergy Units (Pordenone and Rome) from June 2019 to March 2020. Eighty-seven (mean age: 26.15 years; range: 8-48; female: 36) were affected by rhinitis, whereas 68 were affected by asthma (mean age: 25.5 years; range: 1- 62; female 26) associated (58; 85.3%) or not (10; 14.7%) to rhinitis. Following the GINA guidelines (11), 53 subjects (77.9%) were classified as having mild asthma and 15 (22.1%) as having moderate/severe asthma. No patients underwent to previous HDM specific immunotherapy or declared previous reactions to crustaceans or shellfish.

In vitro assays

IgE specific for Der f 1, Der p 1, Der f 2, Der p 2, Der p 5, Der p 7, Der p 10, Der p 11, Der p 20, Der p 21 and Der p 23 were tested in sera of all patients using the last version of the multiparametric assay Allergy Explorer^2 (ALEX^2) (Macro Array Diagnostics, Wien, Austria). In this system, the allergens are spotted onto a nitrocellulose membrane in a cartridge chip, which is incubated with 0.5 mL of a 1:5 dilution of serum under agitation. After two hours of incubation, the chip is extensively washed and a pre-titred dilution of anti-human IgE labelled with alkaline phosphatase is added and incubated for 30 minutes. Following further washing, the enzyme-substrate is added, and after eight minutes the reaction is completed. The membrane is dried and the intensity of color reaction for each allergen is measured by a coupled-charged device camera. A dedicated software digitalizes the images and produces a report listing components and they score in kUA/L (range 0.3-50 kUA/L). Values above 0.35 kUA/L were considered positive.

IgE specific for extracts of both *D. pteronissinus* and *D. farinae* were also measured using the monoplex ImmunoCAP assay (Thermofisher Diagnostics, Uppsala, Sweden), following the manufacturer's instructions. Values above 0.35 kUA/L were considered positive.

All tests were performed during routine care, and the samples were anonymized, since no personal data, except for age and sex, was available. The Institutional Review Board of IDI-IRCCS confirmed that ethical approval was not required in this case (n. 493.1).

Statistical analysis

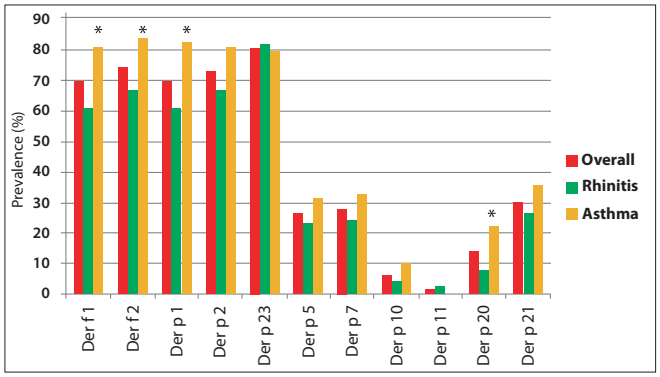
The prevalence of IgE sensitization to the 11 different HDM molecules was evaluated in all subjects and separately in subjects with only rhinitis and with asthma. Differences in specific IgE prevalence between the groups were compared by χ^2 test for categorical variables. Differences of IgE levels between groups were analyzed with the Mann-Whitney U test. A P-value < 0.05 was considered statistically significant. All statistical analysis was performed using MedCalc statistical software, version 10.4.5 (Mariakerke, Belgium) and GraphPad Software (La Jolla, CA).

Results

150 out of 155 evaluated patients were positive for at least one on the 11 HDM allergens, whereas five patients, positive on SPT or on *vitro* test with extracts of *D. pteronissinus* and/or *D. farinae*, were negative for all the tested molecules. No patient negative to the major allergens reacted to the minor allergens. The prevalence of IgE reactivity to individual HDM allergens in all subjects tested, and in subjects with rhinitis and with asthma is shown in **figure 1**. With the only exception of Der p 23, the prevalence of positive results was higher in subjects with asthma, but only Der f 1, Der f 2, Der p 1 and Der p 20 reached statistically significant levels.

Odds ratio (OR) analysis showed that the risk of being asthmatic was more than 2-fold higher in patients in the presence of IgE reactivity to Der f 1 (OR 2.71; 95% CI: 1.20-5.70; p = 0.008), Der f 2 (OR 2.59; 95% CI: 1.18-5.68; p = 0.017) and Der p 1 (OR 2.71; 95% CI: 1.29- 5.70; p = 0.008), and more than 3-fold higher in patients with IgE reactivity to Der p 20 (OR 3.23; 95% CI: 1.23- 8.46; p = 0.001). In contrast, comparing patients with mild and moderate/severe asthma, only reactivity to Der p 21 was significantly associated with moderate/severe asthma (mild asthma: 20%, moderate/severe asthma: 46.4%; p < 0.05), with a 3-fold higher risk of having moderate/severe asthma in Der p 21 positive subjects (OR 3.46; 95% CI: 1.18-10.14; p = 0.02).

Figure 1 *- Prevalence of IgE positivities to individual HDM allergens in subjects with rhinitis (green columns), with asthma (yellow columns) and in combined groups (red columns).*



*Statistically significant differences (p < 0.05).

Concerning the IgE reactivity to major allergens, number and percentage of positivity for a single group of HDM allergens and their combination are shown in **table I**. Interestingly, the presence of the mono-sensitization to Der p 23 was more frequent in subject with rhinitis (p < 0.05).

On the other hand, the minor allergens Der p 5, Der p 7, Der p 10, Der p 11, Der p 20 and Der p 21 resulted positive respectively in 26.4%, 27.7%, 6.45%, 1.3%, 14.2%, and 30.3% of all the patients evaluated, and in 23.0%, 24.1%, 3.5%, 2.35, 8.0% and 25.4% of the patients with rhinitis, and in 30.9%, 32.4%, 10.3%, 0%, 22.1% and 35.3% of the patients with asthma. Patients negative for minor allergens had a probability of 63% of having rhinitis and of 37% of having asthma (p = 0.0001), whereas positive patients had a higher probability of having asthma (52.5%) than negative patients (37%) (p < 0.05) (**figure 2**). 43.5% and 60% of patients with rhinitis and asthma were positive to at least 1 minor allergen (p < 0.05), 24.7% and 41.4% to more than one minor allergen (p < 0.05), and 3.5% and 7.14% to more than 3 minor allergens (p = ns), respectively. In the last case, the absence of statistical significance is probably due to the low number of positive cases.

Concerning allergen specific IgE levels, they resulted higher in asthmatic than in non-asthmatic patients (**figure 3**), but only for Der f 1, Der f 2, Der p 1, Der p 2 and Der p 20 a significant level was reached (p < 0.05). No significant differences in the percentage of positivity and in the IgE specific levels were found comparing patients of different age classes (< 15 years, n = 33; 16-25 years, n = 44; 26-45 years, n = 55; > 45 years, n = 23), as well as no differences in the number of HDM allergens reactivity were shown (**figure 4**). On the contrary, asthmatic patients had IgE reactivity to more HDM allergens (mean 5.4; SD ± 2.3) than patients with only rhinitis (mean 4.2; SD ± 2.5) (p = 0.003) (**figure 4**).

Table I *- Number and percentage of sensitization to the major HDM allergens and their combinations.*

Molecules	Overall n = 155	Rhinitis n = 87	Asthma n = 68	Rhinitis *vs* Asthma
Der p 1$^+$/Der f 1$^+$	104 (67.1%)	51 (58.6%)	53 (77.9%)	p = 0.018
Der p 2$^+$/Der f 2$^+$	114 (73.5%)	58 (66.7%)	56 (82.4%)	p = 0.043
Group 1 + 2	131 (84.5%)	68 (78.2%)	63 (92.6)	p = 0.025
Group 1 alone	14 (9.0%)	8 (9.2%)	6 (8.8%)	p = ns
Group 2 alone	19 (12.3%)	13 (14.9%)	6 (8.8%)	p = ns
Group 1 and 2 + Der p 23	150 (96.8%)	84 (96.6%)	66 (97.1%)	p = ns
Der p 23 alone	18 (11.6%)	16 (18.4%)	3 (4.4%)	p = 0.016
Der p 1$^+$/Der f 1$^-$	3 (1.9%)	2 (2.9%)	1 (1.2%)	p = ns
Der f 1$^+$/Der p 1$^-$	4 (2.6%)	2 (2.3%)	2(2.9%)	p = ns
Der p 2$^+$/Der f 2$^-$	0 (0%)	0 (0%)	0 (0%)	-
Der f 2$^+$/Der p 2$^-$	1 (0.6%)	0 (0%)	1 (1.5%)	p = ns
All negative	5 (3.2%)	3 (3.4%)	2 (2.9%)	p = ns

Figure 2 *- Percentage of subjects with rhinitis (green columns) and asthma (yellow columns) with or without reactivity to HDM minor allergens.*

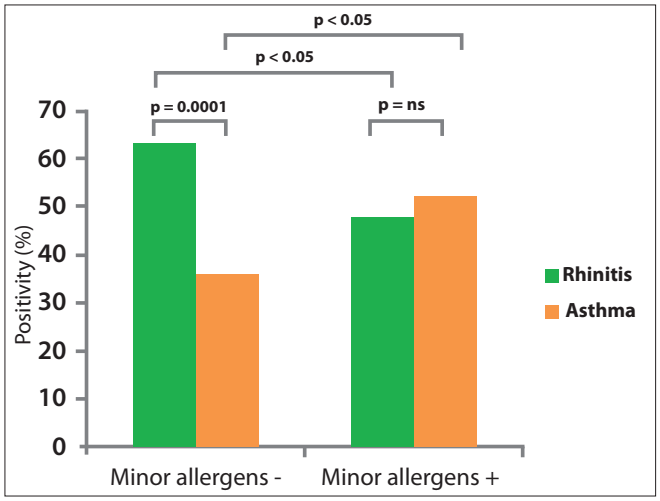
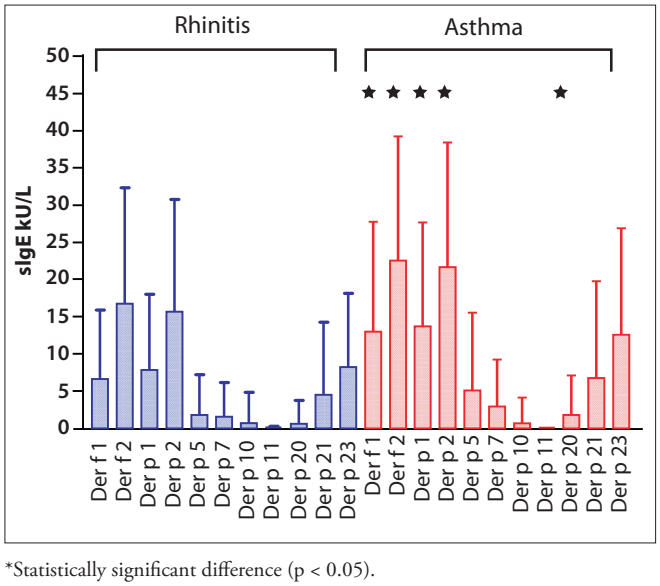


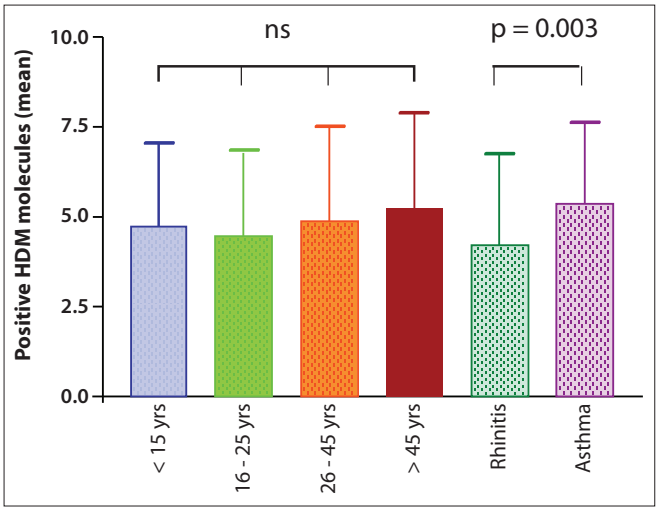
Figure 3 *- Comparison of allergen-specific IgE levels in subjects with rhinitis (blue columns) and with asthma (red columns).*



*Statistically significant difference ($p < 0.05$).

Finally, the level of IgE to extract of *D. ptenonissinus* was strictly related to the le level of Der p 1 ($r = 0.916$, $p < 0.0001$) and Der p 23 ($r = 0.870$; $p < 0.0001$). Lower were the correlations with Der p 5 ($r = 0.504$; $p < 0.0001$), Der p 7 ($r = 0.696$; $p < 0.0009$) and with Der p 21 ($r = 0.690$, $p = 0.003$). Similarly, the level of IgE to extract of *D. farinae* was strictly related to the level of Der f 1 ($r = 0.832$; $p < 0.0001$) and Der f 2 (0.777; $p < 0.0001$).

Figure 4 *- Sum of IgE reactivity to HDM allergens (mean) in different age classes and in subjects with rhinitis only and with asthma.*



ns = p no significant.

Discussion

Our study confirms that among the HDM allergens, group 1, group 2 and Der p 23 are the most important ones in term of prevalence in keeping with other studies of literature (6, 12-16). Most patients show IgE reactivity to group 1 and/or group 2 allergens from both *D. pteronyssinus* and *D. farinae*, but similarly to the data obtained by Batard and coworkers (15) about 5% of them have reactivity to only one of those mite species, confirming that despite a well-known amino acid sequence homology, each of the group 1 (Der p 1 and Der f 1) and Group 2 (Der p 2 and Der f 2) molecules bears species-specific IgE epitopes. This may have some practical consequences both in the diagnostics and in the preparation of extracts for immunotherapy. Der p 23 scored positive in a high percentage of HDM sensitized patients (about 80%), and in 11.6% it was the only positive allergen, a value slightly exceeding that observed in a previous Italian study (16). However, as a difference from the study by Celi and coworkers (16), in which Der p 23 monosensitized patients were more frequently affected by asthma and in particular by severe asthma, in our study Der p 23 monosensitized patients showed a higher prevalence of rhinitis. These differences could be explained by the smaller size of our HDM sensitized cohort, as well as differences in the patients' selection and in co-sensitization to allergens different from HDM (data not evaluated). On the other hand, however, results of our study agree with those of the Manchester Asthma and Allergy Study (MAAS) (9) where children with a more complex molecular pattern of IgE sensitization showed the highest risk of asthma and a significant

higher level of exhaled nitric oxide. Anyway, further studies in large cohorts of HDM hypersensitized subjects are needed to confirm our findings. Considering the other major allergens, the prevalence of Der p 1, Der f 1 and Der f 2 was significantly higher in asthmatic than in nonasthmatic subjects, as well as their specific IgE levels. Also, specific IgE Der p 2 levels were higher in asthmatic patients. These data confirm the results obtained by Resch and coworkers (6).

The good correlations between group 1, group 2 and Der p 23 allergens IgE levels and the IgE levels to extracts *of D. pteronyssinus* and *D. farinae,* confirm that these allergens are not only seroprevalent but also serodominant, representing allergens that quantitatively make the most important contribution to HDM IgE response (5).

Nevertheless, the most interesting novelty offered by the new multiparametric assay used in this study is the possibility to evaluate in daily practice also the IgE responses toward some minor HDM allergens. In the evaluated subjects, 79/155 (50,9%) scored positive for at least 1 minor allergen with a higher prevalence for Der p 5 (overall 26.4%), Der p 7 (27.7%), Der p 20 (14.2%) and Der p 21 (30.3%); these percentages are very similar to those obtained in previous studies using other diagnostic methods (6, 15). Instead, the rate of IgE reactivity to Der p 10 and Der p 11 was very small (6.45%, and 1.3%, respectively). The differences in IgE responses to the minor allergens evaluated might be explained by different routes of sensitization, since Der p 5, Der p 7, Der p 20 and Der p 21 are mainly present in faecal particles and sensitize preferentially through the respiratory tract, whereas Der p 10 and Der p 11 are mainly present in HDM body and therefore may sensitize through the skin or the gut in the case of Der p 10 because of the cross-reactivity with homologous food allergens. It is interesting to remark that some authors reported specific IgE to Der p 11 up to 60% of cases in subject with atopic dermatitis (17), thus representing a major allergen in this clinical condition. However, as we did not consider the co-presence of atopic dermatitis, we cannot confirm these findings. Anyway, the availability of an assay including Der p 11 makes it now possible to design studies on large cohorts of atopic dermatitis subject to confirm the role of this allergen. The prevalence and the level of specific IgE to Der p 5, Der p 7, Der p 20 and Der p 21 were higher in asthmatic patients, though the difference was significant only for Der p 20. Otherwise, the prevalence of Der p 21 resulted significantly higher in the moderate/severe asthma group than in mild asthma. These results are partially different from those obtained by Resch and coworkers (6), who reported that Der p 5 and Der p 7 are more often recognized in asthmatic subjects. However, they did not test Der p 20. Differences between the two studies could be explained by differences in the patients' selection (children in the study of Resch *et al*. and prevalent adults in our study). However, both the studies showed that asthmatic patients with HDM allergy recognize a larger spectrum of molecules, whereas sensitization to fewer components is more related to rhinitis, suggesting that polysensitization to HDM allergens might have functional consequences.

Finally, no differences in the number of HDM positive molecules and in specific IgE levels were found among the different age classes confirming the results of the longitudinal study of Posa *et al*. (10), showing that during the first decade of life the IgE response to HDM components seems to show plasticity, whereas afterwards IgE recognition profiles are more established.

However, some limits of this study have to be underlined. First of all, sample size is quite small for a final conclusion on the prevalence of sensitization profiles and their association with diseases and severity of symptoms. Secondly, the observational design of the study implies weaker and less standardized selection criteria.

Conclusions

The results of this study confirm that the assessment of IgE responses toward a comprehensive panel of HDM allergens defines different serological reactivity profiles that seem associated with different clinical presentations. The recent availability in the daily practice of a multiplex assay, able to detect specific IgE toward 11 different HDM components, will allow design large real-life studies to confirm the role of different serologic HDM profiles both in predicting the clinical evolution and the outcome of specific immunotherapy.

Fundings

None.

Conflict of interests

The authors declare that they have no conflict of interests.

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