

House dust mite allergy in Italy—Diagnostic and clinical relevance of Der p 23 (and of minor allergens): A real-life, multicenter study

To the Editor,

House dust mites (HDM) are a major cause of respiratory allergy and of perennial asthma worldwide. Thirty-two allergens for *Dermatophagoides farinae* (D2) and 21 for *Dermatophagoides pteronyssinus* (D1) have been detected so far, and novel allergens are still being reported.¹ Der p 23, a gut-derived peritrophin present in the outer membrane of mite feces,² has been recognized as a major allergen.^{2,3} Der p 1, Der p 2, Der p 23, and Der p 10 (tropomyosin) are the only allergens currently available for the component-resolved diagnosis of HDM allergy on ImmunoCAP. The clinical relevance of Der p 23 is only partially defined, and the prevalence and relevance of the exclusive sensitization to allergens other than groups 1, 2, 10, and 23 has received little attention so far. We addressed these aspects in a large multicenter study.

Seventeen Italian allergy centers (Figure 1 in Data S1) participated in this real-life, cross-sectional study. Consecutive HDM-allergic patients, diagnosed on the basis of history of perennial rhinitis with or without asthma and of positive SPT with commercial extracts of either D1 or D2, were enrolled between September 1, 2017, and June 30, 2018. Rhinitis and asthma were classified following the ARIA⁴ and GINA⁵ guidelines, respectively. Patients underwent SPT also with commercial extracts of an array of other airborne allergens (see Data S1). IgE specific for D1, D2, Der p 1, Der p 2, Der p 10, and Der p 23 was measured by ImmunoCAP (Thermo-Fisher Scientific, Uppsala, Sweden). Levels > 0.35 kU/L were considered positive. Sera from patients scoring D1/D2-positive but negative for all 4 allergens underwent immunoblot analysis at Lofarma (Milan, Italy) (details in Data S1); the 100 kDa allergen recognized by one of these patients was characterized by mass spectrometry (details in Data S1). Statistical methods as well as ethical issues are detailed in the Data S1; probability levels < 5% were considered statistically significant.

A total of 519 patients (M/F: 256/263; mean age 28.4 years, range 4-79) were studied; 221 were monosensitized to HDM, while 298 were co-sensitized to other airborne allergens. Two hundred and ten (40.5%) had asthma. The prevalence of rhinitis and/or asthma did not show gender differences although both severe rhinitis and moderate/severe asthma prevailed in males. Asthma prevalence was similar in the four age groups (0-19, 20-39, 40-59, and 60-79 years), and asthmatic and nonasthmatic patients showed a similar mean age. Patients co-sensitized to other allergens showed

a higher prevalence of moderate/severe rhinitis (171/298 [57%] vs 104/221 [47%]; $P < 0.05$) and of asthma of any severity (132/298 [44.3%] vs 78/221 [35.3%]; $P < 0.05$) than HDM monosensitized ones.

In vitro, 411/457 (89.9%) scored D1/D2-positive, 16 (3.5%) and 5 (1.1%) monosensitized to D1 or D2, respectively, and 23 (5%; F/M 12/11; 9 with asthma; 8 monosensitized to HDM; 1 with shrimp allergy) both D1- and D2-negative. In positive patients, the median IgE level was 38.3 and 44.6 kU/L for D1 and D2, respectively. Rhinitis severity did not correlate with IgE levels, while asthma severity did ($P < 0.0001$ for both D1 [Figure 1] and D2). IgE to Der p 1, Der p 2, Der p 10, and Der p 23 was detected in 58.6%, 67.9%, 11.9%, and 59.8% of patients, respectively. Sixty-seven (12.9%; 53 and 67 positive for D1 and D2, respectively, 35 with moderate/severe rhinitis, 23 with asthma, and 3 with shrimp allergy) patients did not recognize any molecule, whereas 17 (3.3%), 48 (9.3%), 13 (2.5%), and 42 (8.1%) were monosensitized to Der p 1, Der p 2, Der p 10, and Der p 23, respectively. Mean IgE levels to Der p 1 (19.5 kU/L) and Der p 2 (24.1 kU/L) were significantly higher than IgE to Der p 23, which were nonetheless substantial (9.7 kU/L; $P < 0.001$) (for complete data and comparisons, see Table 2 in Data

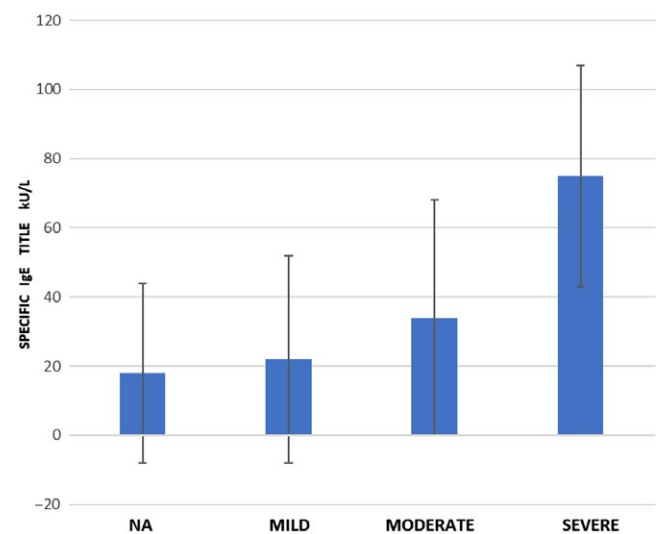


FIGURE 1 Asthma severity and mean level of IgE to *Dermatophagoides pteronyssinus*. NA, nonasthmatic patients

S1). Der p 23 sensitization prevalence dropped significantly with age, being 73%, 55%, 49%, and 35% in the four subgroups, respectively; accordingly, Der p 23 reactors were younger than negative ones (mean age [SD]: 25.6 [14.8] vs 32.6 [17.2]; $P < 0.0001$). Of the 23 D1/D2-negative patients, 12 reacted to one component: Der p 23 in 10 cases and Der p 2 in 2 cases; none bound more than one component. Sensitization to either Der p 1, Der p 2, or Der p 23 prevailed in central and southern Italy ($P < 0.01$), while patients scoring negative for all four molecules prevailed in the north (48/240 [20%] vs 19/279 [7%]; $P < 0.0005$; details in Table 2, Data S1). Mean D1 IgE level was strictly related to the number of recombinant allergens recognized ($P < 0.0001$) and to the level of IgE to Der p 1 ($r = 0.791$), Der p 2 ($r = 0.83$), and Der p 23 IgE ($r = 0.66$; $P < 0.0001$ in all three cases). In patients negative for all 4 components studied, mean D1 IgE level was significantly lower than in patients reacting to at least 1 molecule (2.91 ± 6.97 kU/L vs 25.75 ± 30.47 kU/L; $P < 0.0001$). Asthma prevalence did correlate with the number of recombinant molecules recognized, being 30.5%-34.3%, 50.6%, and 64.7% in patients sensitized to <3, 3, or all 4 molecules, respectively ($r: 0.844$; $P < 0.05$). Patients negative for all 4 molecules had less asthma than patients sensitized to at least 1 allergen (23/67 [34.3%] vs 183/452 [40.5%]; $P < 0.01$). Further, asthma severity also depended on the number allergen components recognized (no asthma < mild < moderate < severe asthma; [$r = 0.166$; $P = 0.0001$]).

The association between number of molecules recognized and asthma was confirmed in patients monosensitized to HDM. Asthmatics recognized on average 2.26 molecules vs 1.70 in nonasthmatics ($P < 0.005$), and the number of components recognized by nonasthmatics, patients with mild asthma, or patients with moderate/severe asthma was 1.79 (SD 1.16), 2.06 (1.14), and 2.58 (0.99), respectively ($P < 0.05$ for moderate/severe vs mild asthma and $P < 0.001$ for moderate/severe asthmatic vs nonasthmatic patients).

In monosensitized patients, asthma was strongly associated with Der p 23 hypersensitivity (58/78 [74%] vs 66/143 [46%]; $P < 0.0005$; [OR: 3.38 at 95% CI]); such association lacked for the other 3 allergens (Figure 2). Further, asthma severity was associated with Der p

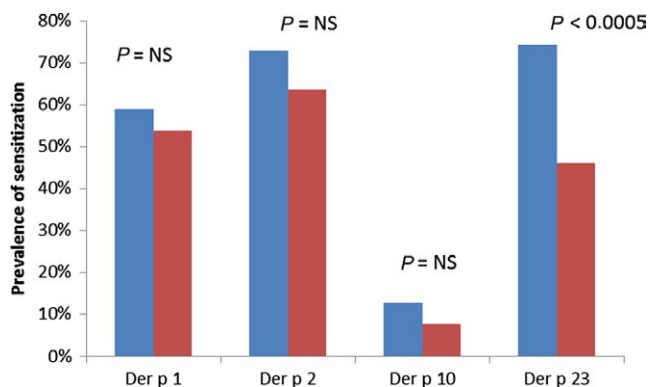


FIGURE 2 Association between asthma and hypersensitivity to specific molecules in patients monosensitized to house dust mites. A clear association with Der p 23 hypersensitivity is observed. Left column: asthmatic patients; right column: nonasthmatic patients

23 IgE levels ($P < 0.0001$), which were 3.9 kU/L (SD 9.5), 5.9 kU/L (10.4), 11.0 (17.1), and 17.8 (20.3) in nonasthmatic patients, and patients with slight, moderate, or severe persistent asthma, respectively. On immunoblot (performed using all 27 available sera out of 67 patients who did not react to any component), most sera reacted to high mw HDM allergens ranging between 80 and 220 kDa (Figure in Data S1); the allergen detected by LC-MS-MS in one single patient was identified as paramyosin (mw, about 100 kDa).

This study shows that asthma prevails in multisensitized patients suggesting an increased inflammation of lower airways in this subpopulation. Further, it confirms that asthma prevalence and severity depend on the number of HDM molecules recognized.^{6,7} The higher severity of rhinitis and asthma in males needs further investigation. In vitro studies show that measuring both D1 and D2 IgE is needed to diagnose HDM allergy, as 5% are monosensitized. However, D1/D2-negative patients are often Der p 23 reactors confirming that Der p 23 is underrepresented in HDM extracts.⁹ Further, HDM allergy diagnosis cannot rely on the current panel of components only as 13% of patients react to currently unavailable high mw allergens, one of them being the muscle protein, paramyosin, as previously observed.¹⁰ Der p 23 is a major allergen, is clearly associated with asthma and its prevalence drops with age; long-term, follow-up studies in young HDM-allergic patients will clarify whether sensitization is lost with age or if Der p 23 is a novel allergen that has become relevant in recent years.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.