ORIGINAL ARTICLE

Sensitization to aeroallergens and asthma severity in the tropics

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Summary

Background. The allergic asthma phenotype is the most extensively investigated. However, few studies have explored the association between sensitization to different aeroallergens, asthma severity and blood eosinophil counts, an important biomarker of Type 2 inflammation. This study aims to evaluate associations between sensitization to aeroallergens, severity of asthma, eosinophilic phenotypes and age of onset of symptoms. **Methods.** This was a cross-sectional study involving a tropical urban population sample. We included 450 individuals with mild asthma (MA) and 441 with moderate to severe asthma (MSA). Serum-specific IgE to nine aeroallergens was measured by the ImmunoCap™. We considered a cut-off point of 0.70 KU/L for mites and 0.35

KU/L for all other allergens. Blood eosinophilia was considered if the count was > 260 cells/mm^3, and the age of onset was defined as early (EO < 12 years) and late (LO ≥ 12 years). **Results.** Patients with asthma sensitive to dust mites, *B. germanica* and those with polysensitization are 1.6 to 1.8-fold more likely to have MSA, while in cases of sensitization to *A. fumigatus* this probability increases to 3-fold. Regarding the eosinophilic phenotype, individuals with MA sensitized to dust mites, *B. germanica*, dog epithelium and polysensitized individuals are more likely to present the eosinophilic phenotype (p < 0.05). However, we did not observe any association between sensitization to different aeroallergens and age of symptom onset (p > 0.05). **Conclusions.** We conclude that polysensitization or sensitization to mites, *B. germanica*, and mainly to *A. fumigatus*, are associated with the severity of asthma. Only in individuals with mild asthma, sensitization to dust mites, *B. germanica*, dog epithelium and polysensitization is associated with eosinophilic asthma. No sensitization profile was associated with the age of onset of asthma symptoms.

Key words

Allergic asthma; eosinophilia; IgE antibodies specific; polysensitization; severe asthma

Impact statement

Asthma is a disease with diverse phenotypes. The association between sensitization to perennial aeroallergens and most specifically *A. fumigatus* with the severity of asthma indicates they may be relevant risk factors for severe asthma and should inform personalized clinical management.

INTRODUCTION

Asthma is a disease that has a chronic inflammatory nature, in general, being characterized by narrowing of the airways, thickening of the basement membrane, increased production and secretion of mucus, and bronchial hyperresponsiveness, which can reduce lung function over the years (1, 2). Commonly, the patient with asthma presents a clinical history of respiratory symptoms such as wheezing, shortness of breath, chest tightness, and cough

that vary in intensity and over time. Asthma severity can range from a mild condition, with almost imperceptible symptoms, to a severe condition, with persistent and troublesome symptoms that significantly impair the patient's quality of life.

Despite the diagnosis being mostly clinical and supported by lung function measurements, previous studies have reported the complexity and heterogeneity of asthma as a disease comprising several phenotypes (3). Asthma phenotypes often overlap and include atopic asthma, non-atopic asthma, late-onset asthma, fixed airflow-limited asthma, asthma with obesity, eosinophilic asthma, neutrophilic asthma (1, 3). Because of these phenotypic characteristics, it was noted that asthma management should not be tailored only to the severity of the disease but individualized and modified according to the patients response to treatment and guided by the inflammatory phenotype alongside with several clinical and behavioral traits (4, 5).

The allergic asthma phenotype is the most frequent among the phenotypes observed in asthma (6, 7). Its response is predominantly Type 2, with production of IL-4, IL-5, and IL-13 cytokines and specific IgE antibodies. Such molecules will contribute to the pathogenesis of the disease with the production of mucus, inflammation, bronchoconstriction, and bronchial hyperreactivity, as reviewed in Lambrecht and Hammad, 2021 (8).

Approximately 65 to 80% of the patients diagnosed with asthma have some type of sensitization to aeroallergens, with sensitization to indoor aeroallergens being more frequent, such as dust mites, dog and cat epithelium, insects (cockroaches), fungi, and some rodents (9-11). Furthermore, this sensitization to indoor airborne allergens have been associated with greater severity and morbidity of asthma in areas of temperate climate (12, 13).

Thus, our objective was to evaluate the association between sensitization to different aeroallergens with the severity of asthma in a tropical environment, to understand which sensitization profile might correlate with the severity of the disease, as well as to investigate possible associations between the sensitization profile, blood eosinophilia and age of onset of symptoms.

METHODS

Study population

This was a cross-sectional study involving a tropical urban population conducted between January 2013 and July 2015 at the ProAR Central Clinic in Salvador, Bahia, Brazil. We included individuals with mild asthma (MA) and moderate to severe asthma (MSA), of both sexes, aged between 18 and 60 years. Individuals with MSA were part of the Program for Asthma Control in Bahia (ProAR), a referral clinic for severe asthma in Salvador, Bahia, Brazil. We did not include pregnant women, patients with poor understanding and verbal communication, patients with incomplete or inconsistent information or data. Furthermore, patients with a history of stroke, congestive heart failure, myopathy, pulmonary disease (besides asthma), or other diseases that could interfere with the assessment of asthma severity and control were excluded, as well as those who were over 60 years old. Since aging is associated with physiological changes in lung function and a higher prevalence of the aforementioned comorbidities, immunosenescence might also bias the results of specific IgE measurements (14, 15).

Patients with MSA were classified according to the presence of any of the following criteria at their enrollment in ProAR: (i) report of daily symptoms, (ii) frequent exacerbations or nighttime symptoms, (iii) limitation in their physical activities, (iv) reduced lung function (FEV1 or Peak Expiratory Flow ≤ 60%) or variability in FEV1 or Peak Expiratory Flow >30%. At the time of blood collection, at least 6 months after enrollment, all individuals with MSA were under treatment and were using a combination of medium or high-dose inhaled corticosteroids (800mcg to 1600mcg of Budesonide or equivalent) and a long-acting beta-2 agonist (12 or 24 mcg of formoterol or 100 mcg of salmeterol daily). These individuals with MSA were classified according to the criteria established by GINA 2012 (16). Individuals with MA were invited to volunteer through advertisements at health care centers and public transport and had their diagnosis validated by an expert in the field, according to the criteria of GINA 2012 (16). They were not under regular treatment with inhaled corticosteroids. In addition, the asthma diagnosis of all patients with MSA was validated by two specialists, and the opinion of a third specialist was also requested in case of disagreement regarding the diagnosis.

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A blood sample was also collected from the patients to perform the complete blood count and measure specific IgE (sIgE).

The study was approved by the Ethics Committee of Maternidade Climério de Oliveira, Federal University of Bahia, Salvador, Bahia, Brazil (License Number: CONEP n°15782, CEP 095/2009 and 095/2012). All individuals who were recruited and agreed to participate in the study signed an informed consent form.

Confounding variables

Potential confounding variables considered in this study included age, sex, exposure to wood stove smoke, diagnosis of rhinitis, gastroesophageal reflux disease (GERD), and obesity. The diagnosis of rhinitis and GERD were obtained during a consultation with a specialist, considering reports of symptoms and previous medication use. Obesity was defined by a body mass index (BMI) ≥ 30kg/m2 and abdominal circumference measurement (≥ 88cm for women and ≥ 102cm for men) (17, 18). These variables were collected to account for potential confounding effects in the association analyses between asthma and aeroallergen sensitization. Age and sex are fundamental demographic variables that may influence a wide range of clinical outcomes, including asthma severity and specific IgE levels (11, 14, 15). Environmental exposures, such as wood-stove smoke, have been associated with airway inflammation and inadequate asthma control (19). Comorbidities, including rhinitis and GERD, are common in asthma patients and may contribute to increased disease severity, while obesity is consistently associated with poorer asthma control (11, 20). Given these established associations, these variables were included in the model to minimize confounding effects. It is noteworthy that we did not include tobacco smoke exposure due to the low frequency of active and passive smoking in our study population (11, 21).

Measurement of specific IgE (sIgE), definition of eosinophilia and age at onset of asthma symptoms

Sensitization to aeroallergens was determined by the presence of sIgE in serum by the fluorescence solid phase immunoassay (ImmunoCAP™) technique (Thermo Fisher Scientific, Uppsala, Sweden). The main aeroallergens evaluated

were *Blomia tropicalis, Dermatophagoides pteronyssinus, Dermatophagoides farinae, Aspergillus fumigatus, Penicillium notatum, Periplaneta americana, Blatella germanica*, dog epithelium, and rat epithelium and urine. Cat epithelium was not included in this study because it has similar clinical relevance to dog epithelium among adult individuals with asthma in Brazil (22), in addition to project cost constraints. We considered a cut-off point of 0.70 KU/L for mites and 0.35 KU/L for all other allergens, as recommended by the manufacturer. The cut-off of ≥0.70 kU/L for mites sIgE was chosen because in tropical regions such as Brazil, low levels of dust mite-specific IgE may reflect cross-reactivity with helminth antigens rather than true allergic sensitization, thus reducing specificity in the definition of atopy (23-27). Furthermore, Hong et al. (2018) demonstrated, using ROC curve analysis, that the optimal point for *Dermatophagoides pteronyssinus* is approximately 0.69 kU/L (28), very close to our chosen cut-off.

In the present study, monosensitization was defined as sensitization to a single aeroallergen, whereas polysensitization was defined as sensitization to two or more aeroallergens, regardless of whether they were from different categories (e.g., mites and cockroach) or from the same type (e.g., different mite species). The criterion we established for eosinophilic asthma was a peripheral blood eosinophil count greater than 260 eosinophils/mm^3 (29). Regarding the age of onset of asthma symptoms, the 12-year limit was used to define the two phenotypes: early age of onset (EO <12 years) and late age of onset (LO≥12 years) (30, 31).

Statistical analysis

Statistical analyzes were performed using SPSS 26.0. After performing the Kolmogorov-Sminorv normality test, we noticed our data had a non-parametric distribution. Thus, we used the Mann-Whitney test to compare two groups. For analysis of categorical data, we performed Fisher's exact test. Regarding logistic regression, we adjusted for confounding variables such as age, sex, exposure to wood stove smoke, diagnosis of rhinitis, gastroesophageal reflux disease (GERD), and obesity. Moreover, each logistic regression model was built including one sensitization variable and adjusted for confounders. No

simultaneous comparison of all sensitization categories was performed. The continuous variables were expressed in median (1st quartile – 3rd quartile). All statistical tests were two-tailed and a p<0.05 was considered statistically significant.

RESULTS

Characteristics of the study population

We evaluated 891 individuals, 450 with mild asthma (MA) and 441 with moderate to severe asthma (MSA), of both sexes, aged between 18 and 60 years. Table I shows the characteristics of the study population. We observed that the median age of individuals with MSA was higher when compared to individuals with MA. Most participants were female, with no difference between groups. Regarding the age of onset of asthma symptoms, we noticed that the median was higher in individuals with MSA than in individuals with MA. Additionally, the frequency of obesity, rhinitis, GERD and total IgE levels were higher in individuals with MSA than individuals with MA. Regarding lung function, analyzed through the assessment of pre-bronchodilator forced expiratory volume in the 1st second (Pre-BD FEV1), we observed that FEV1 in individuals with MSA was lower than in the MA group. We did not observe a significant difference in eosinophil counts nor the frequency of eosinophilic asthma in comparing MA and MSA.

Association between sensitization aeroallergens with asthma severity and phenotypes

Table II shows the association between sensitization to relevant aeroallergens and asthma severity. Initially, we observed no significant differences in monosensitization to mites in general (such as *B. tropicalis, D. pteronyssinus*, and *D. farinae*), cockroach (*B. germanica*), or polysensitization between individuals with MA and MSA, nor any association between these sensitizations and disease severity. However, after adjusting for confounding variables, individuals with these sensitization profiles were 1.6- to 1.8-fold more likely to have MSA. Conversely, sensitization to *A. fumigatus* was significantly more

frequent in the MSA group than in the MA group. After adjustment, asthmatic individuals sensitized to this fungus had a three-fold higher likelihood of having MSA.

Furthermore, we also assessed associations with eosinophilia and age at onset of symptoms. Individuals with MA sensitized to the three mites are 3-fold more likely to present the eosinophilic phenotype (Table III). When sensitized to *Blatella germanica* and dog epithelium, the chances of presenting this phenotype are 1.7 and 2.5-fold greater, respectively, while the possibilities increase to 4-fold more when these individuals are polysensitized.

For individuals with MSA, we did not observe any association between sensitization to different aeroallergens and eosinophilia (Table IV).

Regarding the age of onset of symptoms, there was no significant association with sensitization (Tables V and VI). Individuals with MA sensitized to *D. pteronyssinus* and individuals with MSA sensitized to any of the three mites or polysensitized were more likely to have early-onset asthma, but after adjustments for confounding variables this association was not statistically significant (Tables V and VI).

DISCUSSION AND CONCLUSIONS

This study explored the relationship between sensitization to relevant aeroallergens and asthma phenotypes in a tropical city. Overall, our results indicate that sensitization to aeroallergens was significantly associated with asthma severity. Among patients with MA, sensitization was related with the eosinophilic phenotype, but not with the age of symptom onset.

Initially, we looked for associations between sensitization to aeroallergens and severity of asthma. Among the nine aeroallergens evaluated, asthmatic individuals sensitive to dust mites (*B. tropicalis, D. pteronyssinus, D. farinae*) and the *Blatella germanica* cockroach were more likely to present more severe disease. Some studies have observed that increased exposure and sensitization to aeroallergens such as the *D. pteronyssinus* mite and cockroaches in children diagnosed with asthma may be a factor that contributes to the severity and increased morbidity associated with this disease (32, 33). Li et al. evaluated 3,780 asthmatics between 5 and 65 years old in China, and they noted that the severity

of the disease increases as the frequency of sensitization to *D. pteronyssinus, D. farinae,* and *B. tropicalis* increases (34).

Our study evaluated only adults over 18 years of age, these aforementioned associations were observed only after adjusting for confounding variables. Among all tested variables, age was the only factor that modified these associations (data not shown). A plausible explanation for this finding is that specific IgE levels to aeroallergens, particularly to dust mites, tend to decrease significantly with increasing age, as previously reported by Tosca et al. (2017) (14). This age-related decline in IgE reactivity likely attenuated the differences in sensitization to these aeroallergens between the MA and MSA groups, which may explain the absence of significant differences in the unadjusted analyses. Thus, as described in studies with younger populations, adults sensitized to dust mites also appear more likely to develop moderate to severe asthma. Furthermore, it is important to highlight that we found a strong association between sensitization to *A. fumigatus* and asthma severity, confirming the findings of other studies that found that fungal sensitization is indeed associated with asthma severity, mortality, and hospital admission (35, 36).

Regarding sensitization to the epithelium of dogs and rats, we found no association with asthma severity. Our data agrees with Cacheiro-Llaguno et al (2024) who observed an association between sensitization to dog epithelium and the severity of rhinitis symptoms, but not asthma (37). In addition, Schleich et al (2023) found a low proportion of sensitization to the epithelium of dogs and rats in individuals with severe asthma, similar to our study, and they did not observe an association of this sensitization with the severity of the disease neither (10).

The number of sensitizations is another relevant aspect to be considered, since both our study and others have observed a significant association between polysensitization and asthma severity (38, 39). This makes prescribing specific immunotherapy more challenging (37, 40), as well as taking care to reduce exposure to these aeroallergens.

Atopy is generally associated with the phenotypes of eosinophilic asthma and early-onset asthma(41). For this reason, we also investigated association between sensitization to different aeroallergens with eosinophilic phenotypes and age at onset of symptoms. We observed that individuals with MA sensitized to *D. pteronyssinus, D. farinae, B. tropicalis, B. germanica*, dog epithelium and those

polysensitized are more likely to present an eosinophilic phenotype than a non-eosinophilic one. However, regardless of the profile and number of sensitizations, in individuals with moderate to severe asthma, the chances of presenting the eosinophilic or non-eosinophilic phenotype are similar.

In relation to the age at onset of symptoms, although atopy is generally associated with onset asthma in childhood, our data showed no sensitization profile or number of sensitizations (i.e., monosensitization or polysensitization) associated with early onset, regardless of asthma severity (i.e., MA or MSA). This shows that, regardless of the severity of asthma, an individual presenting the first asthma symptoms in childhood or adulthood may have the same chances of being sensitized to any of the aeroallergens evaluated.

Our study has limitations. We did not perform direct comparisons between excluded and included individuals, since excluded participants had incomplete or inconsistent data or limited verbal communication, which could have affected the assessment of asthma severity and control. The cut-off count used to define eosinophilic asthma is arbitrary; however, it has been adopted in previous studies (29). Similarly, the definitions of early and late onset are also arbitrary, but they have been used by other authors (30, 31). Our analysis was not designed to directly compare all sensitization categories, thus the possibility of a Type I error cannot be excluded. Additionally, interaction terms were not explored, and model fit was not formally assessed, which we recognize as limitations to be addressed in future studies. One must also remember that being a variable disease, the classification of asthma severity is not entirely precise. Any of these limitations or a combination of them, may generate biases that may influence the results observed.

As far as we know, this is the first study to evaluate sensitization to different aeroallergens and various phenotypes in individuals with mild and moderate to severe asthma. Furthermore, our study is one of the few that provides this more detailed approach to atopy and asthma phenotypes in the adult population. Our findings suggest that sensitization to dust mites, *B. germanica, A. fumigatus* and polysensitization may be associated with asthma severity in adult asthmatic individuals. In individuals with mild asthma, but not in moderate to severe asthma, sensitization to dust mites, *B. germanica*, dog epithelium and polysensitization may be associated with eosinophilic phenotype asthma. No sensitization profile

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was associated with the age of onset of asthma. Due to the cross-sectional nature of this study in an urban tropical population, these associations of sensitization patterns with asthma severity and phenotypes may not have external validity to different populations and environments.

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Table I: Sociodemographic and clinical characteristics of the study sample.

	Mild Asthma (MA) n= 450	Moderate to Severe Asthma (MSA) n= 441	P
Age[*][a]	35 (26.0-47.0)	52 (43.0-61.0)	**<0.0001**
Female sex n (%)[**]	349 (77.5)	354 (80.3)	0.32
Age of onset of asthma[*][a]	7.0 (1.0-19.0)	10.0 (2.0-26.0)	**<0.01**
Obesity n (%)[**][b]	106 (23.6)	176 (39.9)	**<0.0001**
Rhinitis n (%)[**]	406 (90.2)	416 (94.3)	**<0.05**
GERD n (%)[**]	288 (64)	311 (70.5)	**<0.05**
FEV$_1$ Pre-BD (%)[*][a]	82.2 (72.0-90.8)	61.0 (49.1-71.9)	**<0.0001**
Total IgE (UI/mL)[*][a]	212.0 (86.2-529.4)	449.3 (144.3-499.3)	**<0.01**
Eosinophils/mm^3[*][a]	235.0 (131.0-394.0)	248.5 (143.0-392.3)	0.21
Eosinophils >260 cells/mm^3 n (%)[**]	209 (46.4)	203 (46.0)	0.94

*Mann Whitney test; **Fisher's Exact Test; [a]median (1st quartile-3rd quartile); [b]Considering body mass index (BMI) ≥30kg/m^2 and measurement of abdominal circumference (≥88cm for women and ≥102cm for men); Bolded results are statistically significant at P < 0.05. FEV1 pre-BD, forced expiratory volume in one second pre-bronchodilator; GERD, gastroesophageal reflux disease.*

Table II: Association between sensitization to the relevant aeroallergens and asthma severity.

	MA (n=415)	MSA (n=396)	P	OR Unadjusted	OR Adjusted*
B. tropicalis	239 (57.6%)	202 (51.0%)	0.06	0.76 (0.58 – 1.01)	**1.61 (1.13 – 2.29)**
D. pteronyssinus	213 (51.3%)	189 (47.7%)	0.32	0.86 (0.65 – 1.14)	**1.76 (1.23 – 2.52)**
D. farinae	227 (54.7%)	198 (50.1%)	0.20	0.83 (0.63 – 1.09)	**1.74 (1.22 – 2.47)**
A. fumigatus	13 (3.1%)	30 (7.6%)	**0.007**	**2.53 (1.30 – 4.93)**	**3.21 (1.41 – 7.26)**
P. notatum	15 (3.6%)	25 (6.3%)	0.10	1.79 (0.93 – 3.45)	1.70 (0.75 – 3.81)
Blatella germanica	113 (27.2%)	125 (31.6%)	0.19	1.23 (0.91 – 1.66)	**1.84 (1.26 – 2.67)**
Periplaneta americana	67 (16.1%)	60 (15.2%)	0.70	0.92 (0.63 – 1.35)	1.33 (0.83 – 2.12)
Dog epithelium	60 (14.5%)	65 (16.4%)	0.49	1.16 (0.79 – 1.70)	1.44 (0.91 – 2.29)
Rat epithelium and urine	4 (1.0%)	4 (1.0%)	1.00	1.04 (0.26 – 4.21)	0.58 (0.11 – 3.06)
Monosensitization	27 (6.5%)	48 (12.2%)	**0.007**	**1.97 (1.20 – 3.23)**	1.55 (0.83 – 2.84)
Polysensitization	244 (59.1%)	222 (56.2%)	0.43	0.88 (0.67 – 1.17)	**1.82 (1.27 – 2.60)**

Odds ratio adjusted for the covariates: age, sex, exposure to wood stove smoke, chronic rhinitis, gastroesophageal reflux disease and obesity. Bolded results are statistically significant at P < 0.05. MA, mild asthma; MSA, moderate to severe asthma; Monosensitization: sensitization to a single aeroallergen; OR, odds ratio; Polysensitization: sensitization to two or more aeroallergens

Table III: Association between sensitization to relevant aeroallergens and mild eosinophilic asthma.

	MA Eosinophilic (n= 188)	MA Non - Eosinophilic (n= 227)	P	OR Unadjusted	OR Adjusted*
B. tropicalis	141 (75%)	98 (43.1%)	**0.000**	**3.94 (2.59 – 6.02)**	**3.39 (2.16 – 5.32)**
D. pteronyssinus	128 (68%)	85 (37.4%)	**0.000**	**3.56 (2.37 – 5.35)**	**2.87 (1.86 – 4.43)**
D. farinae	139 (73.9%)	88 (38.76%)	**0.000**	**4.48 (2.94 – 6.87)**	**3.82 (2.45 – 5.96)**
A. fumigatus	8 (4.2%)	5 (2.2%)	0.26	1.97 (0.84 – 7.47)	1.26 (0.37 – 4.26)
P. notatum	10 (5.3%)	5 (2.2%)	0.11	2.50 (0.36 – 1.90)	2.92 (0.85 – 9.96)
Blatella germanica	64 (34%)	49 (21.5%)	**0.006**	**1.87 (1.21 – 2.90)**	**1.71 (1.08 – 2.71)**
Periplaneta americana	36 (19.1%)	31 (13.6%)	0.14	1.49 (0.88 – 2.53)	1.31 (0.75 – 2.27)
Dog epithelium	40 (21.2%)	20 (8.81%)	**0.000**	**2.79 (1.57 – 4.98)**	**2.58 (1.41 – 4.72)**
Rat epithelium and urine	3 (1.59)	1 (0.44%)	0.33	3.68 (0.38 – 35.7)	4.71 (0.43 – 50.5)
Monosensitization	10 (5.3%)	17 (7.5%)	0.43	0.70 (0.31 – 1.57)	0.82 (0.35 – 1.92)
Polysensitization	147 (78.2%)	97 (42.7%)	**0.002**	**5.05 (3.25 – 7.84)**	**4.27 (2.68 – 6.80)**

Table IV: Association between sensitization to relevant aeroallergens and moderate to severe eosinophilic asthma.

	MSA Eosinophilic (n= 203)	MSA Non - Eosinophilic (n= 234)	P	OR Unadjusted	OR Adjusted*
B. tropicalis	99 (48.7%)	103 (44%)	**0.05**	**1.49 (1.00 – 2.22)**	1.18 (0.77 – 1.82)
D. pteronyssinus	89 (43.8%)	100 (42.7%)	0.31	1.25 (0.84 – 1.86)	0.98 (0.63 – 1.50)
D. farinae	95 (46.7%)	103 (44%)	0.12	1.37 (0.92 – 2.05)	1.05 (0.68 – 1.62)
A. fumigatus	14 (6.8%)	16 (6.8%)	0.84	1.11 (0.52 – 2.35)	1.03 (0.47 – 2.28)
P. notatum	10 (4.9%)	15 (6.4%)	0.83	0.83 (0.36 – 1.90)	0.70 (0.29 – 1.66)
Blatella germanica	59 (29%)	66 (28.2%)	0.44	1.19 (0.78 – 1.82)	0.99 (0.63 – 1.57)
Periplaneta americana	29 (14.2%)	31 (13.2%)	0.48	1.21 (0.70 – 2.11)	1.05 (0.59 – 1.89)
Dog epithelium	36 (17.7%)	29 (12.3%)	**0.05**	**1.71 (1.00 – 2.92)**	1.55 (0.88 – 2.71)
Rat epithelium and urine	2 (0.98%)	2 (0.85%)	1.00	1.26 (0.17 – 9.07)	0.72 (0.93 – 5.57)
Monosensitization	16 (7.8%)	32 (13.6%)	0.12	0.59 (0.31 – 1.13)	0.64 (0.33 – 1.25)
Polysensitization	105 (51.7%)	117 (50%)	0.15	1.35 (0.90 – 2.02)	1.06 (0.69 – 1.64)

Table V: Association between sensitization to relevant aeroallergens and early-onset mild asthma.

	MA- EO (n= 255)	MA - LO (n= 147)	P	OR Unadjusted	OR Adjusted*
B. tropicalis	156 (61.2%)	76 (51.7%)	0.07	1.47 (0.98 – 2.22)	0.98 (0.62 – 1.55)
D. pteronyssinus	143 (56.1%)	64 (43.5%)	**0.017**	**1.66 (1.10 – 2.49)**	1.17 (0.74 – 1.84)
D. farinae	149 (58.4%)	72 (48.9%)	0.07	1.46 (0.97 – 2.20)	1.01 (0.64 – 1.59)
A. fumigatus	8 (3.1%)	5 (3.4%)	1.00	0.92 (0.29 – 2.86)	0.66 (0.19 – 2.29)
P. notatum	9 (3.5%)	6 (4.0%)	0.79	0.86 (0.30 – 2.48)	0.95 (0.29 – 3.09)
Blatella germanica	70 (27.4%)	40 (27.2%)	1.00	1.01 (0.64 – 1.59)	0.85 (0.52 – 1.37)
Periplaneta americana	44 (17.2%)	23 (15.6%)	0.78	1.12 (0.65 – 1.95)	0.84 (0.47 – 1.52)
Dog epithelium	35 (15.5%)	23 (15.6%)	0.66	0.86 (0.48 – 1.52)	0.74 (0.39 – 1.36)
Rats epithelium and urine	1 (0.3%)	1 (0.3%)	1.00	0.58 (0.36 – 9.29)	0.69 (0.04 – 13.4)
Monosensitization	16 (6.2%)	9 (6.1%)	1.00	1.03 (0.44 – 2.40)	1.15 (0.46 – 2.85)
Polysensitization	157 (61.5%)	80 (54.4%)	0.14	1.37 (0.91 – 2.07)	0.95 (0.59 – 1.51)

Table VI: Association between sensitization to relevant aeroallergens and early-onset moderate to severe asthma.

	MSA- EO (n= 229)	MSA - LO (n= 210)	P	OR Unadjusted	OR Adjusted*
B. tropicalis	120 (52.4%)	81 (38.5%)	**0.002**	**1.90 (1.27 – 2.83)**	1.31 (0.84 – 2.04)
D. pteronyssinus	114 (49.7%)	75 (35.7%)	**0.002**	**1.92 (1.28 – 2.86)**	1.52 (0.97 – 2.38)
D. farinae	118 (51.5%)	79 (37.6%)	**0.002**	**1.92 (1.29 – 2.87)**	1.39 (0.89 – 2.18)
A. fumigatus	12 (5.2%)	18 (8.5%)	0.18	0.59 (0.27 – 1.26)	0.63 (0.27 – 1.50)
P. notatum	9 (3.9%)	16 (7.6%)	0.14	0.49 (0.21 – 1.15)	0.48 (0.18 – 1.25)
Blatella germanica	69 (30.1%)	56 (26.6%)	0.38	1.21 (0.79 – 1.85)	1.04 (0.64 – 1.68)
Periplaneta americana	33 (14.4%)	27 (12.8%)	0.67	1.15 (0.66 – 2.01)	1.09 (0.59 – 2.02)
Dog epithelium	35 (15.2%)	30 (14.2%)	0.78	1.09 (0.64 – 1.87)	0.90 (0.50 – 1.63)
Rat epithelium and urine	1 (0.47%)	3 (1.3%)	0.35	0.30 (0.03 – 2.96)	0.31 (0.25 – 4.00)
Monosensitization	24 (10.4%)	24 (11.4%)	0.87	0.92 (0.50 – 1.68)	1.15 (0.59 – 2.23)
Polysensitization	128 (55.8%)	93 (44.2%)	**0.006**	**1.75 (1.17 – 2.62)**	1.27 (0.81 – 1.99)

*Odds ratio adjusted for the covariates: age, sex, exposure to wood stove smoke, chronic rhinitis, gastroesophageal reflux disease and obesity. Bolded results are statistically significant at P < 0.05. EO, early onset; LO, late onset; MSA, moderate to severe asthma; Monosensitization: sensitization to a single aeroallergen; OR, odds ratio; Polysensitization: sensitization to two or more aeroallergens.