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## Predictors of airway hyperreactivity in house dust mite allergic patients

### Abstract

**Introduction:** Airway hyperresponsiveness (AHR) is a cardinal feature of asthma. Asthma is a heterogeneous disorder which consists of different phenotypes and endotypes. Mechanisms leading to AHR may differ in different asthma subtypes. Allergy to perennial allergens, including house dust mites (HDM) is a major risk factor for asthma development. The aim of this study was to determine predictors of AHR in a well-characterized population of HDM-allergic patients.

**Material and methods:** In a retrospective analysis 843 patients with HDM-allergic rhinitis with/without asthma were evaluated. The following parameters were included in the analysis: serum concentration of total (t)- and Dermatophagoides pteronyssinus (Dp)-specific IgE, fractional exhaled nitric oxide concentration (FeNO), lung function tests, bronchial challenge with histamine, age sex, and body mass index (BMI). Linear regression analysis was used to determine predictors of AHR.

**Results:** In a simple linear regression analysis baseline lung function results expressed as either forced expiratory volume in 1 s (FEV<sub>1</sub>) or maximal expiratory flow at 50% of the forced vital capacity (MEF<sub>50</sub>), FeNO, tIgE, DpIgE, age and BMI affected AHR. A multiple regression analysis demonstrated that in the whole group of HDM-allergic patients the most important, independent predictors of AHR were MEF<sub>50</sub>, FeNO and DpIgE.

**Conclusion:** Even in a well-characterized asthma phenotype several processes participate in development of AHR. Major, independent predictors of AHR: lung function parameters, FeNO and DpIgE indicate possible targets for therapeutic intervention in a population of HDM-allergic patients.

**Key words:** airway hyperresponsiveness, asthma, house dust mite, nitric oxide, IgE

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### Introduction

Airway hyperresponsiveness (AHR) is a cardinal feature of asthma [1]. However, it is present not only in asthma patients but in patients with other respiratory illnesses as well [1, 2]. Nevertheless, moderately-severe AHR is virtually pathognomonic for asthma. Although the exact mechanism of AHR has not been fully elucidated it is known that AHR results from a complex interaction between several mechanisms including bronchial inflammation and airway remodeling [1–3].

Association of AHR with asthma is even more complex due to heterogeneity of asthma. Asthma is not a homogenous disease but consists of several phenotypes and endotypes which differ in

type of airway inflammation, clinical course and response to different treatment modalities [4]. The heterogeneity of airway inflammation may affect assessment of its intensity using conventional, noninvasive methods such as evaluation of exhaled nitric oxide concentration [5]. This in turn may interfere with attempts aimed at determining the role of individual factors in AHR. Among all asthma phenotypes, allergic asthma is the most frequently encountered [4]. In a general population, allergic patients are more likely to suffer from asthma than non-atopic individuals [6]. Moreover, greater intensity of atopy seems to be associated with greater risk of AHR and asthma [7].

Allergic asthma is characterized by eosinophilic airway inflammation and clinical favorable response to anti-immunoglobulin E (anti-IgE)

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therapy and allergen immunotherapy which support the role of aeroallergens and IgE in its pathogenesis [8]. In a great majority of allergic asthma patients involvement of upper respiratory tract, manifested as allergic rhinitis, can be demonstrated, while in a substantial part of allergic rhinitis patients clinical asthma or AHR can be found [6, 9]. Various aeroallergens exhibit varying degree of association with asthma and AHR with house dust mite (HDM) allergens, including *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df) demonstrating the greatest association [10–19].

Sensitization to Dp/Df has been shown in the vast majority of allergic asthma patients in different countries, different climates and different populations including populations of adults and children [10–19]. Moreover, a relationship between sensitization to HDM and AHR and/or asthma severity has been demonstrated [13–15]. This was clearly shown in a population of young adults in whom AHR was more frequent HDM-allergic patients than in those sensitized to other allergens [16]. In HDM-allergic patients exposure to HDM allergens triggers asthma symptoms and a linear relationship between the risk of appearance of asthmatic symptoms and the concentration of the main Dp allergen — Der p 1 in house dust has been demonstrated [17]. Moreover, prolonged exposure to house dust is associated with more severe asthma and seasonal changes in exposure to house dust are reflected in changes of the level of AHR in patients with HDM-allergic asthma [18, 19]. These data argue for an important function of both exposure and sensitization to HDM allergens in the pathogenesis of asthma and AHR.

Therefore it was of interest to determine factors affecting AHR in a large, homogenous population of HDM-allergic patients.

## Material and methods

This is a retrospective analysis of 843 house dust mite allergic patients (HDM-AP). The patients who underwent a routine evaluation in the outpatient Allergy Clinic of the University Hospital of Białystok over a period of 11 years.

All included patients reported rhinitis and/or asthma symptoms which were triggered by exposure to house dust and had positive skin prick tests with Dp and Df allergens. Asthma diagnosis was established according to the Global Initiative for Asthma (GINA) guidelines [20] by an allergy/pulmonary specialist. The diagnosis was based on a typical medical history, including asth-

ma symptoms triggered by exposure to house dust and if possible demonstration of airway obstruction with significant improvement after inhalation of 400 mcg of salbutamol within 1 year prior to the study. Patients treated with systemic or inhaled corticosteroids, allergen immunotherapy or biologics were not included in the study. Lung function tests with reversibility test, if indicated, were performed as a routine evaluation in allergy clinic.

In addition to routine tests total and Dp specific IgE (tIgE and DpIgE, respectively), histamine bronchial challenge and exhaled nitric oxide concentration were evaluated.

## Skin testing

All persons were skin tested using the prick methodology with a screening panel of aeroallergens (Allergopharma, Reinbek, Germany) including the following allergen extracts: 1) *Dermatophagoides pteronyssinus* (Dp); 2) *Dermatophagoides farinae* (Df); 2) trees: including birch, alder, hazel and a tree mix; 3) grass mix; 4) mugwort; 5) cat and dog allergens and 6. *Alternaria tenuis*. The reaction was considered positive when the longest wheal diameter induced by an individual allergen was equal to or greater than that induced by histamine control having at least 3 mm.

## Exhaled nitric oxide concentration

Fractional concentration of nitric oxide in the expired air (FeNO) was evaluated “on-line” using a chemiluminescence analyzer NOATM 280i (Sievers, Boulder, CO, USA). The measurements were performed according to ATS recommendations as described before [21]. Briefly, each patient exhaled against the fixed expiratory resistance of 16 cm H<sub>2</sub>O, which resulted in a constant flow of 50 mL/s. Both NO concentration and flow rate were displayed on the screen. A plateau of NO concentration in the exhaled air at the selected exhalation rate was automatically selected by the computer software according to the ATS recommendations. The NO measurements were repeated 3 times and the mean value was used for analysis.

## Lung function assessment

Lung function tests were performed using MasterScreen Pneumo (Erich Jaeger, Germany). All tests were done between 7 and 10 A.M. Forced expiratory volume in 1 s (FEV<sub>1</sub>) or maximal expiratory flow at 50% of the forced vital capacity (MEF<sub>50</sub>) expressed as percentage of the predicted values were used for further analysis.

Histamine bronchial challenge was performed as previously described elsewhere [21].

**Table 1. Patients characteristics**

	All (n = 843)	AR (n = 422)	AA (n = 421)	P
Age (years)	27 (22–36)	25 (21–33)	30 (23–40)	< 0.001
Height (cm)	172 (164–180)	172 (164–180)	171 (164–180)	> 0.05
Weight (kg)	72 (61–81)	70 (60–80)	74 (64–82)	< 0.001
BMI	24 (21.6–26.3)	23.4 (21.1–25.8)	24.7 (22.2–27.5)	< 0.001
FEV <sub>1</sub> (% predicted)	101 (89.8–110.6)	107.5 (100–116)	93.5 (81.9–103)	< 0.001
MEF <sub>50</sub> (% predicted)	83.5 (63.7–105.4)	100.3 (84.8 – 120)	66.3 (51.96–83)	< 0.001
PC <sub>20</sub> (mg/ml)	7.2 (1.6–31)	31 (16–64)	1.6 (0.38–3.49)	< 0.001
FeNO (ppb)	47.5 (25.8–88)	30.3 (19.2–53.2)	76 (43–121)	< 0.001
tIgE (kU/L)	231 (101–432)	146.5 (83–305)	335 (167–551)	< 0.001
DpIgE (kU/L)	9.4 (2.2–31.8)	5.04 (1.72–17.3)	17.3 (3.3–44)	< 0.001
Sex (% males)	52.6	50.9	54.2	> 0.05

Results are presented as medians with interquartile range.

AA — allergic asthma; AR — allergic rhinitis; BMI — body mass index; DpIgE — Dermatophagoides pteronyssinus (Dp) specific IgE; FeNO — fractional exhaled nitric oxide; FEV<sub>1</sub> — forced expiratory volume in 1 s; MEF<sub>50</sub> — maximal expiratory flow at 50% of forced vital capacity; PC<sub>20</sub> — histamine provocative concentration causing a 20% fall in FEV<sub>1</sub>; tIgE — total immunoglobulin E

Briefly, all patients inhaled doubling concentrations of histamine starting from a concentration of 0.062 mg/mL. Aerosol was generated using a DeVilbiss No. 646 nebulizer attached to a Rosenthal-French dosimeter. All subjects performed 5 inspiratory capacity breaths of given histamine concentration. Forced expiratory maneuvers were performed 90 s after each fifth inhalation. The procedure was continued until either at least a 20% fall of FEV<sub>1</sub> or a histamine concentration of 32 mg/mL was reached. Bronchial reactivity to histamine was expressed as histamine concentration causing a 20% fall of FEV<sub>1</sub> (PC<sub>20</sub>). In patients, in whom the maximal histamine concentration produced decrease of FEV<sub>1</sub> of more than 10% but less than 20% PC<sub>20</sub> was calculated by extrapolation. In patients with negative histamine challenge the PC<sub>20</sub> concentration of 64 mg/ml was used for statistical analysis.

### Immunological assays

The concentration of tIgE and DpIgE were evaluated in serum using the Pharmacia CAP system (Phadia Diagnostics, Uppsala, Sweden) according to the manufacturer instructions.

### Statistical analysis

All results are presented as medians with 95% confidence intervals (95% CI) unless stated otherwise. Comparison of individual parameters between the studied groups was performed using the Mann-Whitney U test. The Shapiro-Wilk test was used for evaluation of normal distribution of individual parameters.

Serum concentration of tIgE and DpIgE, PC<sub>20</sub>, and FeNO were logarithmically transformed for statistical analysis. The Pearson correlation coefficient was calculated for evaluation of association between two variables. Simple and multiple linear regression analyses were used to estimate association between AHR and other parameters studied.

Statistical significance was determined at the  $P < 0.05$  level.

## Results

Patients characteristics is presented in Table 1. There was no significant difference of height and sex distribution between allergic rhinitis (AR) and allergic asthma (AA) patients. Asthmatic patients were older and were characterized by greater body mass index (BMI), tIgE, DpIgE, FeNO, but lower FEV<sub>1</sub>, maximal expiratory flow at 50% of forced vital capacity 50% (MEF<sub>50</sub>) and PC<sub>20</sub> than AR patients.

In simple linear regression analysis baseline lung function results (reflected as either FEV<sub>1</sub> or MEF<sub>50</sub>), log<sub>FeNO</sub>, log<sub>tIgE</sub>, log<sub>DpIgE</sub>, age and BMI affected AHR expressed as log<sub>PC20</sub> (Table 2). The strongest association was demonstrated for FEV<sub>1</sub>, MEF<sub>50</sub> and log<sub>FeNO</sub> ( $r^2 = 0.261, 0.350$  and  $0.274$ , respectively). The associations remained significant when multiple regression analysis was applied. Using multiple regression analysis AHR expressed as log<sub>PC20</sub> can be best described using the following formula:  $\log_{PC20} = 0.015 \times \text{MEF}_{50} - 1.003 \times \log_{\text{FeNO}} - 0.171 \times \log_{\text{DpIgE}} - 0.114 \times \text{BMI} + 1.53$  ( $R^2 = 0.548$ ;  $p < 0.001$ ). When the whole group was divided

**Table 2. Linear regression analysis between  $\log_{PC20}$  and the individual parameters in the whole group of HDM-APs (n = 843)**

	$\beta$	$r^2$	95% CI	P-value
FEV <sub>1</sub>	0.031	0.261	0.027 to 0.034	< 0.001
MEF <sub>50</sub>	0.018	0.350	0.016 to 0.020	< 0.001
Log <sub>FeNO</sub>	-1.324	0.274	-1.470 to -1.180	< 0.001
Log <sub>tIgE</sub>	-0.70	0.110	-0.56 to -0.31	< 0.001
Log <sub>DpIgE</sub>	-0.460	0.105	-0.55 to -0.367	< 0.001
Age	-0.02	0.040	-0.024 to -0.012	< 0.001
BMI	-0.045	0.034	-0.061 to -0.030	< 0.001
Sex	0.115	0.002	-0.013 to 0.243	> 0.05
Asthma	-1.489	0.616	-1.570 to -1.409	< 0.001

BMI — body mass index; FEV<sub>1</sub> — forced expiratory volume in 1 s; HDM-APs — house dust mite allergic patients; MEF<sub>50</sub> — maximal expiratory flow at 50% of forced vital capacity

**Table 3. Linear regression analysis between  $\log_{PC20}$  and the studied parameters in allergic rhinitis (n = 422)**

	$\beta$	$r^2$	95% CI	P-value
FEV <sub>1</sub>	0.0004	-0.0022	-0.0021 to 0.0029	> 0.05
MEF <sub>50</sub>	0.0019	0.0209	0.0007 to 0.0031	< 0.01
Log <sub>FeNO</sub>	-0.2947	0.078	-0.390 to -0.199	< 0.001
Log <sub>tIgE</sub>	-0.0947	0.0122	-0.169 to -0.2	< 0.05
Log <sub>DpIgE</sub>	-0.119	0.045	-0.171 to 0.068	< 0.001
Age	-0.0026	0.0022	-0.0064 to -0.0011	> 0.05
BMI	-0.0117	0.0122	-0.0209 to -0.0025	< 0.05
Sex	0.0177	-0.0017	-0.0483 to 0.0837	> 0.05

BMI — body mass index; FEV<sub>1</sub> — forced expiratory volume in 1 s; MEF<sub>50</sub> — maximal expiratory flow at 50% of forced vital capacity

according to the clinical asthma diagnosis FeNO and DpIgE demonstrated the strongest association with AHR in both AR and AA, while lung function expressed as FEV<sub>1</sub> or MEF<sub>50</sub> demonstrated strong association in AA but in AR only MEF<sub>50</sub> ( $p < 0.01$ ) but not FEV<sub>1</sub> ( $p > 0.05$ ) was associated with AHR (Table 3 and 4).

To address a possible interactions of age and BMI correlation between those factors and functional and immunological parameters was performed. In HDM-AA lung function parameters MEF<sub>50</sub> ( $r = -0,3178$ ; 95%CI -0,3772 to -0,2557;  $p < 0,0001$ ) and FEV<sub>1</sub> ( $r = -0,1405$ ; 95% CI -0,2060 to -0,0736;  $p < 0,0001$ ) correlated with age (Figure 1). Similarly significant correlation between age and log<sub>DpIgE</sub> ( $r = -0,1349$ ; 95% CI -0,2006 to -0,0680;  $p = 0,0001$ ) could be demonstrated (Figure 1). Neither log<sub>FeNO</sub> ( $r = 0,0474$ ; 95% CI -0,0202 to 0,1145;  $p > 0,05$ ) nor log<sub>tIgE</sub> ( $r = 0,0232$ ; 95% CI -0,0444 to 0,0906;  $p > 0,05$ ) correlated with age (not shown). Similarly lung function parameters

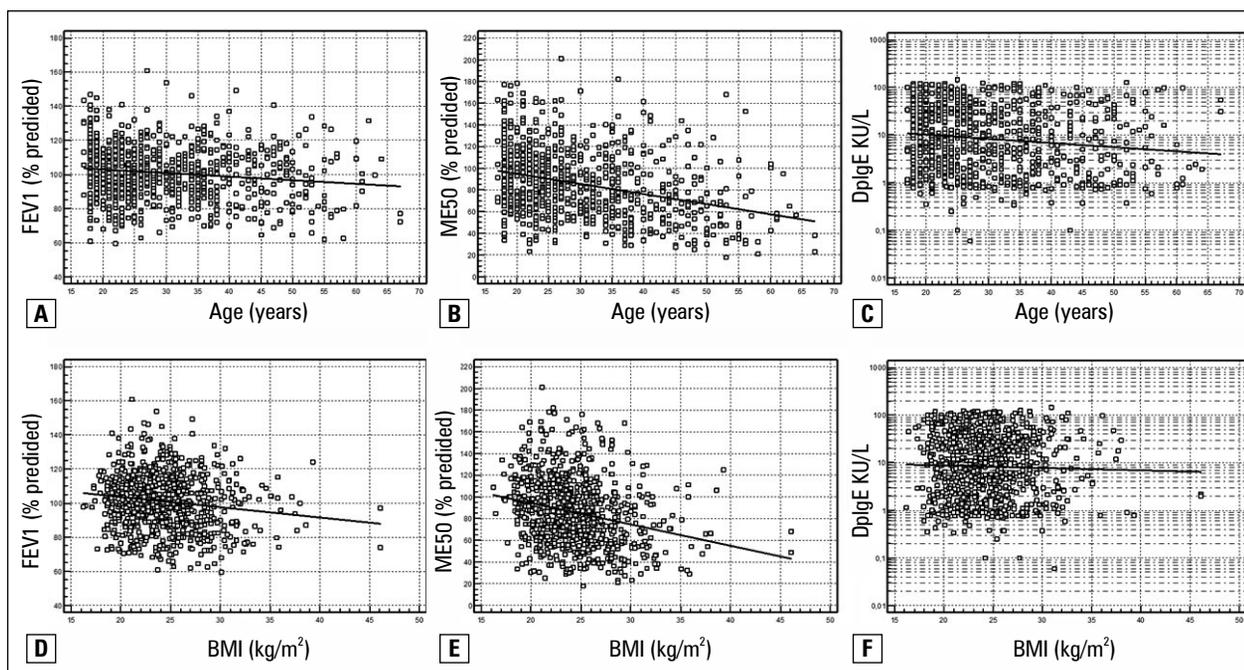
including MEF<sub>50</sub> ( $r = -0,2475$ ; 95% CI -0,3099 to -0,1830;  $p < 0,0001$ ) and FEV<sub>1</sub> ( $r = -0,1477$ ; 95% CI -0,2131 to -0,0810;  $p < 0,0001$ ) correlated inversely with BMI (Figure 1). However neither log<sub>FeNO</sub> ( $r = 0,0526$ ; 95% CI -0,0149 to 0,1197;  $p > 0,05$ ) (Figure 1) nor log<sub>DpIgE</sub> ( $r = -0,0299$ ; 95% CI -0,0972 to 0,0377;  $p > 0,05$ ) nor log<sub>tIgE</sub> ( $r = 0,0595$ ; 95% CI -0,0080 to 0,1266;  $p > 0,05$ ) correlated with BMI (not shown).

## Discussion

Our study evaluated predictors of AHR among several functional and immunological parameters frequently assessed in allergic patients. In this study we demonstrated that baseline lung function expressed as MEF<sub>50</sub> in combination with a marker of airway inflammation FeNO are two main independent predictors of AHR in HDM-allergic patients. The presented results seem to indirectly support the concept of the pathogenesis

**Table 4. Linear regression analysis between  $\log_{PC20}$  and the studied parameters in allergic asthma (n = 421)**

	$\beta$	$r^2$	95% CI	P-value
FEV <sub>1</sub>	0.017	0.106	0.013 to 0.022	< 0.001
MEF <sub>50</sub>	0.013	0.164	0.01 to 0.016	< 0.001
Log <sub>FeNO</sub>	-0.748	0.109	- 0.952 to 0.545	< 0.001
Log <sub>ttlgE</sub>	-0.375	0.041	-0.546 to -0.205	< 0.001
Log <sub>DpIgE</sub>	-0.245	0.046	-0.349 to -0.141	< 0.001
Age	-0.035	0.0006	-0.01 to -0.003	> 0.05
BMI	-0.0113	0.0015	-0.029 to -0.006	> 0.05
Sex	0.114	0.0032	-0.032 to 0.259	> 0.05



**Figure 1.** Correlation of age (A–C) and BMI (D–F) with FEV<sub>1</sub> (A, D), MEF<sub>50</sub> (B, E) and  $\log_{DpIgE}$  in house dust mite allergic patients

of AHR in which airway remodeling and airway inflammation play major additive roles. Assessment of FeNO was used as a surrogate marker of eosinophilic inflammation in the airways. It allows for relatively simple and noninvasive evaluation of intensity of airway inflammation and therefore is useful in large, epidemiological studies. It has already been demonstrated that the number of eosinophils in induced sputum strongly correlates with  $\log_{FeNO}$  [22]. Moreover, the intensity of airway eosinophilic inflammation either demonstrated as number of eosinophils in induced sputum or FeNO correlates with AHR particularly in patients with relatively short duration of asthma [22]. However, in asthmatic patients with longer duration of the disease structural changes in the airways seem to be

more important determinants of AHR [22]. This is consistent with our study since the selected patients were mostly young adults and therefore significant association of FeNO with AHR could be demonstrated [22]. Airway inflammation triggered by allergen exposure is reflected by increase in FeNO [21, 22]. Interestingly FeNO was associated with AHR in the whole HDM-allergic population and also in both subgroups of ARs and AAs. Moreover, FeNO did not correlate with age which indicates that airway inflammation is related to AHR independently of the duration of this process. On the other hand, lung function in particular MEF<sub>50</sub> or FEV<sub>1</sub> was significantly correlated with age indicating that impaired lung function develops with the progression of the disease or possibly with duration of allergen

exposure. This is consistent with a study which evaluated longitudinally over a period of 10 years the prevalence of atopy and AHR in 8-10 years old children [23]. The study demonstrated that atopy is a risk factor not only for current presence but also for future development of AHR. In fact, allergic rhinitis is a risk factor for developing asthma and AHR [24]. Greater risk for developing asthma in ARs was demonstrated in both children and adults [24]. In some populations as many as 50% of ARs not treated with allergen immunotherapy developed asthmatic symptoms over time [24]. In our study the association of baseline lung function expressed as FEV<sub>1</sub> with AHR was seen only in AAs but not in ARs. This indicates that in a subgroup of ARs baseline lung function parameters are less important determinants of AHR than in AA patients. One may speculate that the effect may partially depend on the duration of allergic process as patients with asthma were older than rhinitis patient. However, significant association of MEF<sub>50</sub> with AHR seen in both subgroups indicate that involvement of distal airways may be important in both ARs and AAs. This is further supported by correlation of MEF<sub>50</sub> with age in both ARs and AAs. Our study indicates that airway structural changes and inflammation play an important role in the pathogenesis of AHR as demonstrated by independent association of MEF<sub>50</sub> and FeNO with AHR.

In line with our results is a study which showed in a large group of allergic rhinitis patients that forced expiratory flow 25–75% (FEF<sub>25–75</sub>) was a predictor of AHR [25]. This is consistent with our observations which demonstrate in ARs a significant association of AHR with MEF<sub>50</sub> but not FEV<sub>1</sub>.

In addition our study shows that allergen specific IgE is an independent risk factor for AHR in house dust mite allergic patients and its effect is significant both in AA and AR patients. Moreover, in AR patients allergen specific IgE concentration is a major factor determining AHR. Atopy has been recognized as a risk factor for asthma and the intensity of atopy correlates with risk of asthma [26]. Sensitization to individual allergens imposes different risk for developing AHR [10–13]. Patients sensitized to perennial allergens have greater risk for developing AHR and asthma than those sensitized to seasonal allergens [10–13]. In order to avoid those confounding factors we evaluated a homogenous group of patients allergic to HDM. House dust mite allergy is a major risk factor for asthma development among children and young adults [12]. Our study is consistent with

the above mentioned publications indicating that IgE-mediated effects affect AHR in an independent of eosinophilic airway inflammation way. It is tempting to speculate that our analysis support the concept of interference with IgE as a therapeutic strategy which should affect AHR. Moreover, it may explain why in allergic asthmatics allergen immunotherapy or omalizumab may have synergistic effect with therapeutic options interfering with eosinophilic inflammation such as inhaled corticosteroids. In addition our study indicates that at different stages of allergic diseases different components play a dominant role in development of AHR. A recently published experimental study seems to support this concept demonstrating IgE independent effect of HDM on development of airway inflammation and AHR [26].

Finally, in our population BMI was also independently associated with AHR. Association of BMI with AHR has been demonstrated in some but not all studies [27–28]. The effect of BMI on the development of asthma may depend on the age of occurrence of the disease, being significant in young children but not in adults [29]. This however, supports the direct rather than indirect effect of obesity on asthma development. An experimental asthma model provided evidence that independent risk factors obesity and allergy to HDM synergized in development of asthma phenotype resistant to corticosteroids [30].

In summary our study demonstrates that even in a well-characterized population of HDM-allergic patients lung function parameters, FeNO and DpIgE are major, independent predictors of AHR indicating targets for therapeutic intervention and emphasizes possible synergistic effect of anti-IgE and anti-eosinophilic therapy.

### Conflict of interest

Krzysztof Kowal has received speaker fee from Alk Abello, Astra Zeneca, Berlin Chemie, Chiesi, Hal Allergy, Lekam, Meda Pharma and royalties from UpToDate.

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