



## Original research article

Sensitization to major *Dermatophagoides pteronyssinus* allergens in house dust mite allergic patients from North Eastern Poland developing rhinitis or asthmaKrzysztof Kowal<sup>a,b,\*</sup>, Agnieszka Pampuch<sup>b</sup>, Grzegorz Siergiejko<sup>c</sup>, Zenon Siergiejko<sup>d</sup>, Ewa Swiebocka<sup>e</sup>, Caleb R. Schlachter<sup>f</sup>, Maksymilian Chruszcz<sup>f</sup>, Alain Jacquet<sup>g</sup><sup>a</sup> Department of Experimental Allergology and Immunology, Medical University of Białystok, Białystok, Poland<sup>b</sup> Department of Allergology and Internal Medicine, Medical University of Białystok, Poland<sup>c</sup> Department of Department of Pediatrics, Gastroenterology, Hepatology, Nutrition and Allergology, Medical University of Białystok, Poland<sup>d</sup> Laboratory of Respiratory Diagnostics and Bronchoscopy, Medical University of Białystok, Białystok, Poland<sup>e</sup> Children's Clinical Hospital, Medical University of Białystok, Poland<sup>f</sup> Department of Chemistry and Biochemistry, University of South Carolina, Columbia, United States<sup>g</sup> Center of Excellence in Vaccine Research and Development, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

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## ABSTRACT

**Purpose:** Recognition of individual allergens by IgE is crucial for triggering symptoms in allergic rhinitis (AR) or asthmatic (AA) patients. House dust mite (HDM) allergy is frequent around the world, the sensitization profile to individual HDM allergens varies in individual HDM-allergic patients (APs). The aim of this study was to evaluate the pattern of IgE sensitization to three major *Dermatophagoides pteronyssinus* (Dp) allergens among patients from North Eastern Poland suffering from HDM-AR and/or AA.

**Patients and methods:** The study was performed on 323 HDM-AR and/or AA patients and 106 controls (CG) including 30 healthy non-atopic subjects, 32 AR patients not sensitized to Dp and 44 non-atopic asthmatics. IgE levels to natural (n)Der p 1, nDer p 2, recombinant (r)Der p 2.0101 and rDer p 23 allergens were measured by ELISA.

**Results:** The majority of HDM-APs were sensitized to nDer p 1 (72.1%), nDer p 2 (81.7%), rDer p 2.0101 (78.3%) and rDer p 23 (70.9%). The frequency of positive results to individual allergens depended on clinical manifestations and the level of IgE to the whole Dp extract. In HDM-AA patients, reactivity to nDer p 1 and rDer p 23 was detected more frequently than in HDM-AR patients. The whole Dp extract completely inhibited IgE binding to nDer p 1 and nDer p 2 but only partially to rDer p 23.

**Conclusions:** HDM-APs from North-Eastern Poland display sensitization profile to major allergens which is similarly observed in western Europe. HDM-based diagnostic and therapeutic products should include all major allergens.

## 1. Introduction

House dust mite (HDM) allergy is one of the most prevalent worldwide [1–3]. The most common clinical manifestations developed in HDM-allergic patients (HDM-APs), include allergic rhinitis (AR) and asthma (AA) [1–3]. Moreover, it has been reported that high serum HDM-specific IgE titers were associated with greater risk for acute wheezing among asthmatic children [4]. While more than 35 groups of *Dermatophagoides* sp. allergens have already been identified [5], group 1

(Der p 1, Der f 1) and 2 (Der p 2, Der f 2) allergenic proteins are considered to be the unique major HDM allergens [6–8]. Moreover, association between sensitization to these allergens and morbidity among HDM-APs was demonstrated [9].

The diagnosis of HDM sensitizations in allergic patients, through the use of well characterized recombinant proteins, evidenced Der p 23 as a third major HDM allergen [10]. Since this discovery, allergen specific IgE response to Der p 23 has been measured among different cohorts of HDM-APs from Western Europe, United States and Thailand [10–12].

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The IgE reactivity to Der p 23 ranged from about 50 to 87%, depending on the population studied [10–13]. These differences seemed to be related to geographical factors and clinical presentation of HDM-APs [10–13]. Longitudinal studies confirmed the importance of Der p 23 as together with Der p 1 and Der p 2 it represents the allergens which initiate the immune response to HDM [14].

Appropriate characterization of the sensitization pattern to the whole spectrum of individual HDM allergens is crucial for future development of personalized medicine: setting effective vaccines for allergen immunotherapy based only on the sensitizing molecules.

The aim of this study was to evaluate the pattern of IgE sensitization to three major *Dermatophagoides pteronyssinus* (Dp) allergens among HDM-AR and -AA patients from three Allergy Clinics in Białystok, North-Eastern Poland.

## 2. Patients and methods

### 2.1. Patients

The study was performed on a cohort of 323 adult HDM-APs referred to three allergy clinics due to respiratory symptoms. The patients reported upper and/or lower respiratory symptoms related to exposure to house dust. Only patients who had not been treated with allergen immunotherapy were included in the study. The diagnosis was made by experienced allergists as previously described [15]. Allergy to HDM was confirmed by positive skin prick test results and the presence of specific serum IgE to Dp extract (D1) at a concentration 0.7 kU<sub>A</sub>/L or greater by ImmunoCAP (Phadia, Uppsala, Sweden). In addition, 106 subjects including 30 healthy non-atopic subjects (HCs), 32 AR patients not sensitized to Dp or *Dermatophagoides farinae* (Df) (ARCs) and 44 non-atopic asthmatics (ACs) were included as a control group (CG).

### 2.2. Ethical issues

All participants of the study signed informed written consent. This study which involves human participants is in compliance with the 1964 Helsinki Declaration and its later amendments and it was approved by the local Bioethics Committee of the Medical University of Białystok, Poland (approval number: R-I-007/161/2016).

### 2.3. Skin prick test

All patients were tested with a panel of aeroallergens including Dp, Df, grass pollen mix, birch pollen, mugwort pollen, *Alternaria*, *Cladosporium*, cat and dog allergens. The results were considered positive when the wheal diameter was equal to or greater than that of histamine standard with the latter being at least 3 mm. Only patients with positive skin prick test results to Dp extract were included in the study.

### 2.4. Exhaled nitric oxide measurements

Fractional concentration of exhaled nitric oxide (FeNO) was evaluated “on-line” using a chemiluminescence analyzer NOA™ 280i (Sievers, USA). The measurements were performed at an exhalation flow of 50 mL/s according to the American Thoracic Society recommendations as previously described [16]. Both FeNO and flow rate were displayed on the screen and a plateau of FeNO at the selected exhalation rate was automatically selected by the computer software. The measurements were repeated 3 times and the mean value was used for analysis.

### 2.5. Der p 2.0101 expression and purification

Der p 2 was purchased from ATUM (formerly DNA 2.0, Newark, CA, USA) in a codon-optimized *Escherichia coli* vector pJExpress411 with

kanamycin (KAN) resistance. The plasmid was designed with a purification poly-histidine tag as described by Offermann et al. [17]. For protein expression, the plasmid was transformed into *E. coli* BL21(DE3) cells. Cells were grown at 37 °C to an optical density at 600 nm (OD<sub>600</sub>) of 0.8 in 1.0 L of Lysogeny Broth (LB) with KAN (50 µg/mL), induced with 0.4 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG), cooled to 16 °C, and grown further for 16–18 h. Cell pellets were resuspended in lysis buffer (50 mM Tris-HCl, 500 mM NaCl, 10 mM imidazole, 2% glycerol, pH 7.4) and lysed by sonication. The sonicated mixture was centrifuged and the supernatant was loaded onto a Bio-Rad Econo-Pac chromatography column containing HisPur Ni-NTA IMAC resin equilibrated with equilibration buffer (50 mM Tris, 500 mM NaCl, 10 mM imidazole, 2% glycerol and pH 7.4). The protein was eluted using elution buffer (50 mM Tris-HCl, 50 mM NaCl, 250 mM imidazole, 2% glycerol and pH 7.4).

Elutions containing protein were determined by SDS-PAGE and were dialyzed overnight by using Pierce SnakeSkin Dialysis Tubing (3.0 kDa molecular weight cut off) in dialysis buffer (10 mM Tris-HCl, 150 mM NaCl and pH 7.4). The dialyzed protein was concentrated using a 3.0 kDa molecular weight cut off Millipore Amicon Ultra concentrator and was further purified by size exclusion by using a GE Healthcare AKTA-Pure fast protein liquid chromatography (FPLC) and HiLoad Superdex 200 column. Elutions containing protein were concentrated and Der p 2 concentration was determined at 280 nm (extinction coefficient 11,830 M<sup>-1</sup>cm<sup>-1</sup> determined by ProtParam online tool) [18] using a Nanodrop, and purity was analyzed by SDS-PAGE. The yield was 3.0 mg Der p 2 per liter of culture. The purification tag was removed using a protocol described previously with tobacco etch virus (TEV) protease [19].

### 2.6. Immune assays

Serum concentrations of IgE to individual allergenic sources was evaluated using extract-based ImmunoCAP (Phadia, Uppsala, Sweden).

Purified natural (n) Der p 1 and nDer p 2 allergens were obtained from Indoor Biotechnologies Ltd (Cardiff, UK). Recombinant (r) Der p 23 was produced and purified as previously described [12].

Serum levels of allergen specific IgE were measured by direct ELISA using nDer p 1, nDer p 2, rDer p 2.0101 and rDer p 23 as coating antigens and according to a previously described protocol [17]. Briefly, 96-well MaxiSorp ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at 4 °C with the different individual allergens (100 ng/well) diluted in coating buffer (25 mM carbonate/bicarbonate buffer pH = 9.4). After washing with Tris buffered saline and Tween-20 (TBST), the plates were blocked with TBST + 1% bovine serum albumin (BSA) for 1 h at room temperature (RT). The plates were subsequently washed with TBST and incubated with patients' sera diluted at 1:5 in TBST + 1%BSA for 2 h at RT. The plates were thoroughly washed with TBST and incubated with a monoclonal anti-human IgE antibody conjugated to alkaline phosphatase (BD Pharmingen, Heidelberg, Germany) diluted at 1:1000 in TBST + 1%BSA or 2 h at RT. Following washing, the immune complexes were detected by addition of p-nitrophenyl phosphate (Sigma Fast p-nitrophenyl phosphate tablet sets; Sigma-Aldrich, St. Louis, MO, USA). OD was determined at 405 nm using a microplate reader. To evaluate specificity of the test, negative controls were used including: no allergen immobilized on the plate, no serum applied, irrelevant allergen immobilized (rBet v 2) on the plate or no anti-IgE added. To evaluate reproducibility of the test, the same positive serum was run on each plate as a reference. The serum contained IgE against Der p 1, Der p 2 and Der p 23 at concentrations of 5.6 kU/L, 7.1 kU/L and 3.4 kU/L, respectively, as determined by ImmunoCAP (Phadia, Uppsala, Sweden). All samples were assayed in triplicate and the mean value was used for analysis. The ELISA results are presented as OD in optical units (OU). The cut-off for positive values was considered as the OD above the 95% confidence interval (CI) for 30 HCs sera.

For the ELISA inhibition, patients' sera were preincubated for 30 min at RT with serial two-fold dilution of *Dp* extract (Allergopharma, Reinbek, Germany) dissolved in TBST supplemented with 1%BSA. The concentration of Der p 1 and Der p 2 in the original dilution of *Dp* extract was 2.5 µg/mL and 7.5 µg/mL, respectively [20]. The samples were then loaded on a plate, and the procedure was continued as described for the ELISA above.

## 2.7. Statistical analysis

Normal distribution was evaluated using Wilk-Shapiro test. Data with normal distribution are presented as means with standard deviation (SD). Data not normally distributed are presented as means with 95%CI. Comparison of continuous variables was performed using the Mann-Whitney *U* test. Linear correlation by Pearson was used to estimate the correlation between the studied parameters. Values of  $p < 0.05$  were considered to be statistically significant. All computations were carried out using the Statistica 13.1 software (StatSoft Poland).

## 3. Results

There was no difference in age ( $31.6 \pm 11.6$  vs.  $34.2 \pm 11.9$ ;  $p = 0.06$ ) and sex (males 52% vs. 50%;  $p = 0.73$ ) distribution between HDM-APs and the CG. HDM-AAs were characterized by greater serum concentration of D1 IgE ( $p < 0.01$ ) than those without asthma (Table 1).

In the CG, the mean OD of IgE ELISA was 0.006 OU (95%CI 0.0048 to 0.0068), 0.0106 OU (95%CI 0.0078 to 0.0133), 0.08 OU (95% CI, 0.0057 to 0.0101), 0.0045 OU (95%CI 0.0035 to 0.0054) for nDer p 1, nDer p 2, rDer p 2.0101 and rDer p 23, respectively. No significant difference of the mean ODs of IgE ELISA to individual Der p allergens in individual subgroups of the CG was demonstrated (data not shown); therefore, the whole CG was used for further comparisons. The mean OD of IgE ELISA for nDer p 1, nDer p 2, rDer p 2.0101 and rDer p 23 in HDM-APs were statistically significantly greater than those in the CG ( $p < 0.001$  for each) (Fig. 1). The mean OD of IgE ELISA for an irrelevant allergen rBet v 2 did not differ significantly between HDM-APs and the CG (Fig. 1).

In the whole group of HDM-APs, positive ELISA was demonstrated in 72.1%, 81.7%, 78.3% and 70.9% for nDer p 1, nDer p 2, rDer p 2.0101 and rDer p 23, respectively (Table 2). Positive IgE ELISA to either nDer p 1 or nDer p 2 was demonstrated in 90.4% of patients, while the frequency of HDM-APs developing sensitization to any of the studied allergens reached 95.0% of patients (Table 2). IgE reactivity to rDer p 2.0101 was observed only in patients positive to nDer p 2 and less than 5% of subjects sensitized to nDer p 2 were negative for rDer p 2.0101 (Fig. 2). AAs were characterized by positive ELISA to a broader spectrum of the major Der p allergens: 72.9% of AAs were co-sensitized to Der p 1, Der p 2 and Der p 23, whereas this co-sensitization frequency decreased to 47.8% in AR patients ( $p < 0.01$ ) (data not shown). The frequency of positive IgE ELISA to any of the studied allergens did not differ significantly between ARs and AAs (Table 2). The spectrum of IgE response to the three major *Dp* allergens differed significantly depending on the IgE levels to the whole *Dp* extract. In patients classified

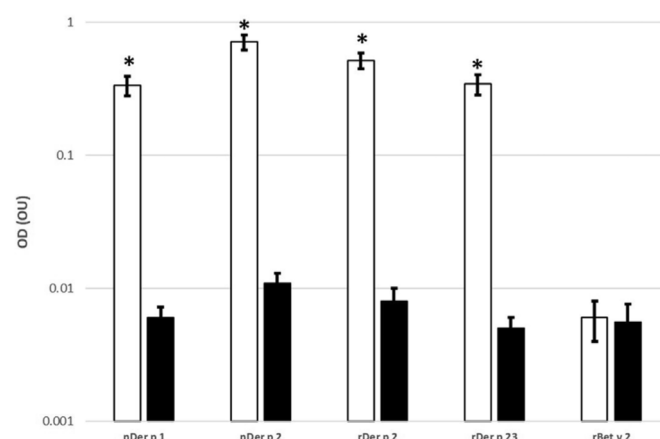


Fig. 1. IgE ELISA results with individual major *Dp* allergens nDer p 1, nDer p 2, rDer p 2, rDer p 23 and irrelevant birch allergen rBet v 2 used as negative control. The results are presented as means with 95% confidence intervals. White bars – house dust mite allergic patients. Black bars – control group. \* - significantly greater than in the control group ( $p < 0.001$ ).

Abbreviations: OD - optical density; OU – optical units.

as class 5 and 6 by D1 IgE ImmunoCAP, the prevalence of IgE binding frequencies to nDer p 1, nDer p 2 and rDer p 23 was each greater than 85% (Table 3). This is in contrast to patients with class 2 D1 IgE ImmunoCAP in whom Der p 1 and Der p 23 recognition pattern was below 40% (Table 3).

Among HDM-APs who had never been treated with inhaled corticosteroids ( $n = 118$ ), a relatively high correlation between serum IgE ELISA to rDer p 23 and FEV<sub>1</sub> was evidenced ( $r = -0.192$ ; 95%CI -0.3605 to -0.0103;  $p = 0.032$ ) (Fig. 3). No statistically significant correlation between FEV<sub>1</sub> and sensitizations to nDer p 1 ( $p > 0.05$ ), nDer p 2 ( $p > 0.05$ ) or rDer p 2.0101 ( $p > 0.05$ ) could be demonstrated (data not shown). Moreover, FeNO level positively correlated with serum IgE ELISA to nDer p 1 ( $r = 0.295$ ; 95% CI 0.121 to 0.451;  $p = 0.001$ ), nDer p 2 ( $r = 0.271$ ; 95% CI 0.094 to 0.43;  $p = 0.003$ ), and Der p 2.0101 ( $r = 0.305$ ; 95% CI 0.132 to 0.461;  $p = 0.0008$ ) in HDM-APs not taking inhaled corticosteroids (Fig. 4). The relatively higher correlation was demonstrated for rDer p 23 ELISA results ( $r = 0.495$ ; 95% CI 0.345 to 0.62;  $p < 0.0001$ ).

Pre-treatment of patients' sera with serial concentrations of commercially available *Dp* extract resulted in complete inhibition of IgE binding to nDer p 1 and nDer p 2 (Fig. 5). However, full inhibition of IgE binding to rDer p 23 was never achieved, the maximal inhibition with the highest concentration of *Dp* extract reaching only 55%.

## 4. Discussion

The present study evaluated the IgE response to the three major *Dp* allergens in a large group of HDM-APs who were referred to outpatient allergy clinics in North-Eastern Poland. To the best of our knowledge this is the first study reporting sensitization pattern to all three major *Dp* allergens among a cohort of HDM-APs in Poland. The patients included in the study represent a population of HDM-APs who are

Table 1  
Patient characteristics.

|               | CG (n = 106) | HDM-APs          |                  |                               |
|---------------|--------------|------------------|------------------|-------------------------------|
|               |              | All (n = 323)    | ARs (n = 170)    | AAs (n = 153)                 |
| Age (years)   | 34.2 ± 11.9  | 31.6 ± 1.6       | 32 ± 11.3        | 31.3 ± 11.6                   |
| Sex (% males) | 50           | 52               | 52               | 52                            |
| D1 IgE (kU/L) | < 0.35       | 29.1 (25.3–32.9) | 19.7 (15.4–23.9) | 38.5 (32.5–44.6) <sup>a</sup> |

<sup>a</sup> - significantly greater than in ARs.

**Table 2**  
Frequency of IgE reactivity to major HDM allergens in relation to clinical symptoms.

|     | nDer p 1           | nDer p 2 | rDer p 2.0101 | rDer p 23          | nDer p 1 or nDer p 2 | rDer p 23 only | Any   |
|-----|--------------------|----------|---------------|--------------------|----------------------|----------------|-------|
| All | 72.1%              | 81.7%    | 78.3%         | 70.9%              | 90.4%                | 4.6%           | 95.0% |
| AA  | 83.0% <sup>a</sup> | 83.3%    | 82.4%         | 82.4% <sup>a</sup> | 90.8%                | 4.5%           | 95.3% |
| AR  | 62.5%              | 80.0%    | 74.7%         | 60.7%              | 90.0%                | 4.7%           | 94.7% |

<sup>a</sup> - significantly greater than in ARs.

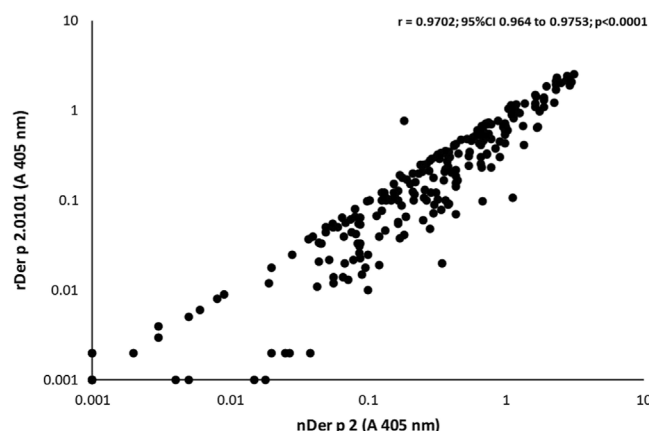


**Fig. 2.** Correlation between IgE ELISA results (absorbance at 405 nm) with nDer p 2 and rDer p 2.0101.

**Table 3**  
Frequency of IgE reactivity to major HDM allergens in relation to the levels of HDM extract-specific IgE determined by Dp ImmunoCAP.

|           | ImmunoCAP         |                    |                   |                   |                   |
|-----------|-------------------|--------------------|-------------------|-------------------|-------------------|
|           | Class 2<br>n = 55 | Class 3<br>n = 112 | Class 4<br>n = 86 | Class 5<br>n = 43 | Class 6<br>n = 27 |
| nDer p 1  | 21 (38.2%)        | 79 (70.5%)         | 72 (83.7%)        | 38 (88.4%)        | 23 (85.2%)        |
| nDer p 2  | 33 (60%)          | 88 (78.6%)         | 80 (93%)          | 40 (93%)          | 23 (85.2%)        |
| rDer p 23 | 20 (36.4%)        | 78 (69.6%)         | 70 (81.4%)        | 37 (86%)          | 24 (88.9%)        |

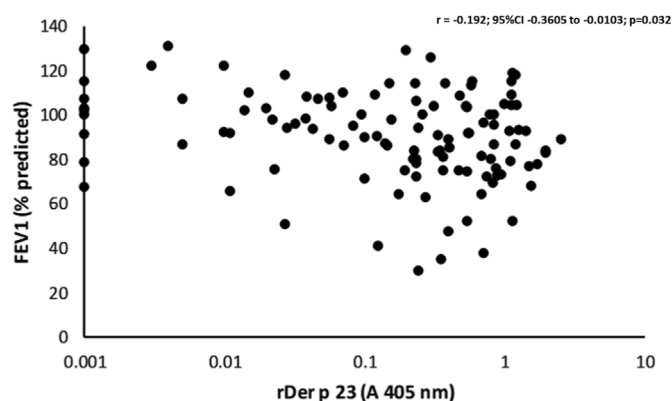


**Fig. 3.** Correlation between IgE ELISA results (absorbance at 405 nm) with rDer p 23 and lung function expressed as forced expiratory volume within the first second (FEV<sub>1</sub>).

potential candidates for allergen immunotherapy based on clinical history and HDM extract-based *in vivo* and *in vitro* positive testing. In the population studied, the frequency of sensitization to major *Dp* allergens Der p 1, Der p 2 and Der p 23 was in general similar to that previously reported using different cohorts of HDM-APs from Western Europe, USA and Thailand [10–14]. However, whereas a multi-cohort

study showed that IgE binding frequency to Der p 23 was lower than those to Der p 1 and Der p 2 [13], our data evidenced comparable levels of IgE reactivity to Der p 23 and Der p 1, and the Der p 23 sensitization being slightly less frequent than that to Der p 2. Interestingly, the previously reported frequency of positive IgE response to Der p 23 varied with the selected cohorts [10–14]. This variability suggested that several factors, including geographic region, age and clinical manifestations of HDM allergy, could significantly affect the level of serum IgE reactivity to Der p 23, while Der p 1 or Der p 2-specific IgE titer was less affected [10–14]. Notably, these previously reported studies used different criteria of HDM-sensitization to select HDM-APs. Some studies included patients with at least class 2, while other studies included also those with class 1 by D1 IgE ImmunoCAP [10–14,21,22]. Interestingly, the frequency of IgE positive test for major *Dp* allergens was greater in the former than in the latter cohorts [10–14,21,22]. This is consistent with our observations which indicate that IgE response to individual major allergens is encountered much less frequently in patients with lower classes of D1 IgE ImmunoCAP [11]. Moreover, a correlation between the level of D1 IgE and the number of allergens recognized has been recently shown in a multicentre Italian study [23]. Our results are in agreement with the results of Celi et al. [23] who indicated that in HDM-APs greater levels of IgE to the whole *Dp* extract are associated with recognition of more major allergen components. Interestingly, an increasing body of evidence indicates that in many patients with low titers of serum IgE to the whole *Dp* extract the positive results are due to nonspecific binding and/or are associated with asymptomatic sensitization [24]. Therefore, analysis of IgE response to individual allergen components may be helpful in evaluation of patients with HDM allergy.

Many studies clearly demonstrated that the presence of IgE to Der p 1 and Der p 2 is correlated with some clinical parameters [6–9]. IgE responses to Der p 1 and Der p 2 are commonly associated with severity of AR or development of AA [6–9]. A possible clinical implication of sensitization to Der p 23 have been recently reported [10,25,26]. In one of those studies, sensitization to Der p 23 has been shown to differentiate symptomatic from asymptomatic HDM-APs [25]. Interestingly, in that study, it was sensitization to Der p 23 but not Der p 1 or Der p 2 which was characteristic for symptomatic HDM allergy. A recent report demonstrated that the presence of serum IgE to Der p 23 is associated with asthma in HDM-APs [22]. Our results are consistent with those observations indicating inverse correlation between IgE ELISA to rDer p 23 and lung function parameter - FEV<sub>1</sub>. Moreover, the intensity of airway inflammation reflected by FeNO showed the higher correlation with rDer p 23 ELISA. Finally, sensitization to Der p 23 has been recently proposed as a marker of ineffective allergen immunotherapy in HDM-APs [22,25]. This may be caused by underrepresentation of Der p 23 in HDM extracts [27]. In fact, using very sensitive methods such as mass spectrometry analysis, only tiny amounts of Der p 23 could be demonstrated in *Dp* extracts [13]. These observations are corroborated by our study which demonstrated weak inhibition of IgE to rDer p 23 by commercially available *Dp* extract. A detailed analysis of sensitization to individual allergen components seem to be particularly important in AA patients as they represent a real challenge for allergen immunotherapy from both efficacy and safety points of view [28]. Comparison of IgE response to nDer p 2 and rDer p 2.0101 suggested that recombinant form of this single Der p 2 isoform could substitute the natural allergen for the detection of specific IgE. Of note, at least 15

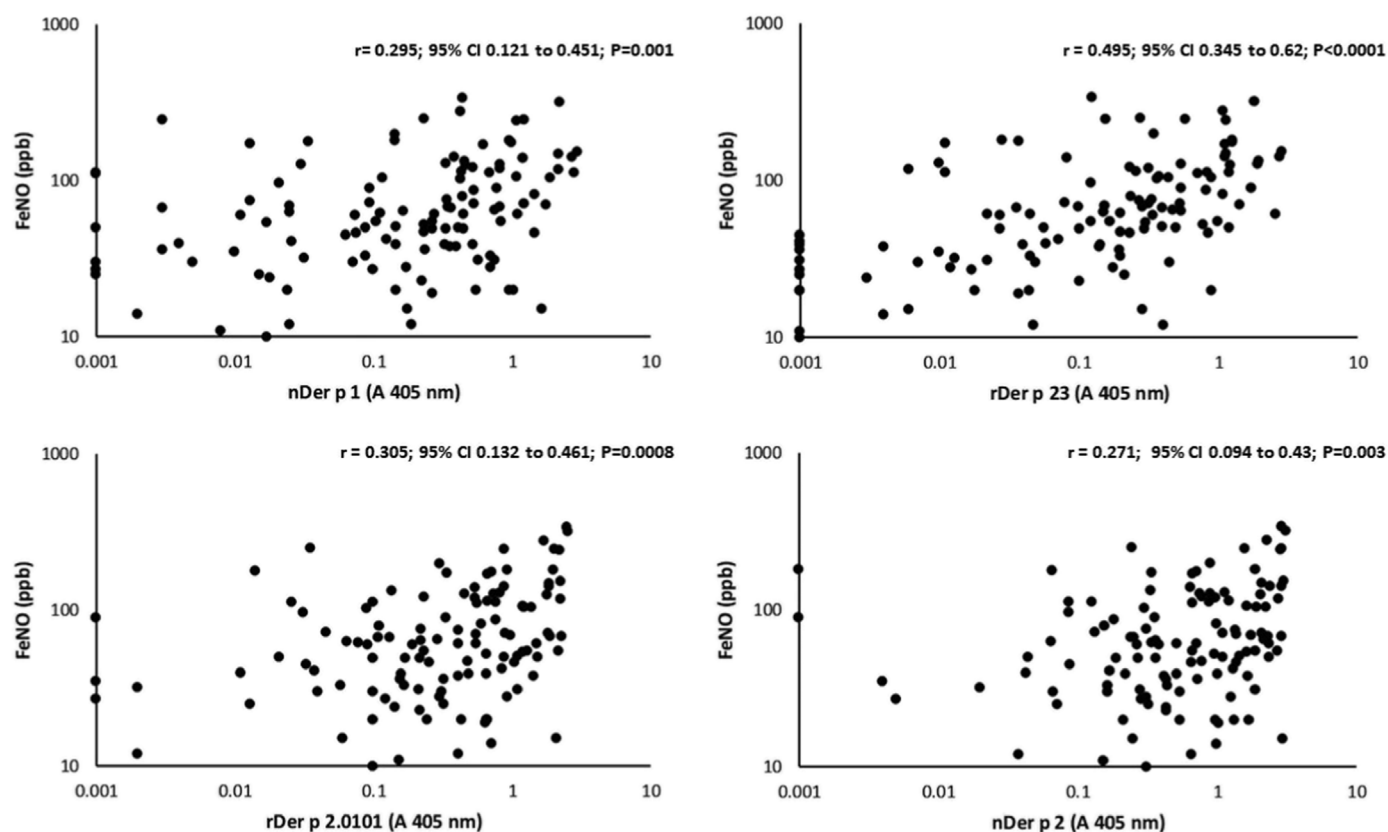


**Fig. 4.** Correlation between serum IgE ELISA results (absorbance at 405 nm) against major *Dp* allergens and intensity of airway inflammation expressed as exhaled nitric oxide concentration (FeNO).

isoallergens of Der p 2 have been identified and further studies are warranted to clarify the performance of application of other isoallergens in Der p 2 diagnosis [29,30]. Presently, only one Der p 23

isoallergen has been identified and, therefore, it is not known whether evaluation of IgE response to a single Der p 23 isoallergen reflects true sensitization rate to that protein.

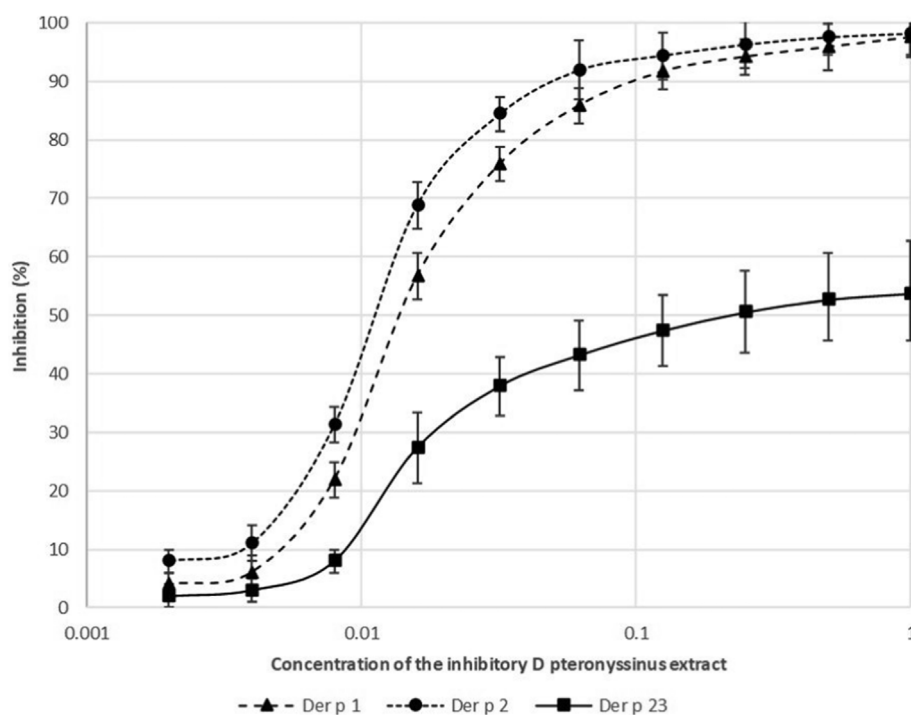


**Fig. 5.** Inhibition ELISA of serum IgE binding to nDer p 1, nDer p 2 and rDer p 23 allergens with *Dp* extract. The maximum concentration of *Dp* extract contained Der p 1 and Der p 2 at 2.5  $\mu\text{g/mL}$  and 7.5  $\mu\text{g/mL}$ , respectively.

## 5. Conclusions

In summary, our study indicates that high levels of sensitization to Der p 1, Der p 2 and Der p 23 are commonly measured in HDM-APs from North-Eastern Poland. Due to underrepresentation of some proteins in HDM extracts, component-resolved diagnosis is helpful in evaluating HDM-APs qualified for allergen immunotherapy.

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## Declaration of competing interest

The authors declare no conflict of interest.

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