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The Role of Der p 23 Sensitization: An Analysis of 474 Patients Sensitized to Mite

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Abstract
Introduction: House dust mite contains several allergen components and causes perennial allergy. Lately, a new major allergen, Der p 23, was described with relatively high sensitization rates in different European Countries. In addition, Der p 23 is supposed to cause asthmatic disease. ***Objective:*** We would like to question the prevalence and clinical impact of specific immunoglobulin E to Der p 23 in a large patient sample in southern Bavaria, Germany. ***Methods:*** 474 patients from southern Bavaria, who visited the allergy department within the Department of Oto-Rhino-Laryngology of a university hospital, with sensitization to *Dermatophagoides pteronyssinus* were retrospectively compared regarding their sensitization profile to Der p 1, Der p 2, and Der p 23 and their clinical characteristics. ***Results:*** Among *D. pteronyssinus*-sensitized patients, the overall sensitization rate to Der p 23 was 42% in southern Bavaria. Most likely, patients were simultaneously sensitized to Der p 1, Der p 2, and Der p 23. Der p 23-sensitized patients reported more frequently asthma and showed higher prevalence of poly-sensitization towards 3 additional allergen groups and higher prevalence of double-sensitization to Der p 1 and Der p 2 compared to patients with missing sensitization to Der p 23. Considering the results of allergen provocation tests, neither IgE sensitization against Der p 23 nor levels of specific immunoglobulin E to Der p 23 allow a clear prediction of the clinical relevance of the sensitization. ***Conclusion:*** With a sensitization rate of 42%, Der p 23 closely misses the criterion of a major allergen in our southern Bavarian patient collective. A higher prevalence of polysensitization and self-reported asthma was the only clinical feature found in Der p 23-sensitized patients.

Introduction

House dust mite (HDM), especially *Dermatophagoides pteronyssinus* (*D. pter.*), is one of the most important indoor allergen sources causing perennial allergy. Various allergen components have been described, and sev-

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eral groups have investigated the prevalence of various components worldwide showing a strongly varying pattern of sensitization profiles depending on the region [1–10]. Der p 1 and Der p 2 are described as the most important major allergen components with high sensitization rates in central Europe. Weghofer et al. [7] described a prevalence of Der p 1 and Der p 2 sensitization in Austria, France, and Italy of >90%, whereas the sensitization rates in Sweden were lower (Der p 1 85% and Der p 2 63%). Sensitization rates for Der p 1 and Der p 2 from an Italian multicentre study were 59 and 68%, respectively [9]. Data from the German Multicenter Allergy study displayed a 61% sensitization rate against Der p 1 and 77% for Der p 2 [11]. Prevalence in southern Bavaria lately was described as >70% for specific immunoglobulin E (sIgE) to Der p 1 and Der p 2 as the 2 major allergens [1]. Furthermore, many years after the characterization of Der p 1 and Der p 2, Der p 23, a relatively newly available allergen component, was identified as a new major allergen with a prevalence much higher than 50% [12]. Der p 23 is a peritrophin-like protein and can be found in the posterior midgut and faeces of HDM. Due to the rather late identification of Der p 23, there are only few studies with relatively small patient numbers investigating the prevalence and clinical impact of Der p 23 sensitization. The sensitization rate in HDM allergic patients in Austria was as high as 70% (n = 67), in France 80% (n = 55), and in Italy 87% (n = 67) [12]. Celi et al. [9] reported a much lower sensitization rate of 60% for Der p 23 in Italy (n = 519). A recent study from Tenerife, Spain, reported a prevalence of 80% Der p 23 sensitivity in 59 patients with severe allergic rhinitis [13]. In a Thai cohort, only 54% of 222 patients with HDM-related allergic rhinitis or asthma showed sIgE reactivity to Der p 23 [14]. Data from the German Multicenter Allergy study showed a prevalence of just 51% for sIgE to Der p 23 in 97 patients [11].

Compared to Der p 1 and Der p 2, Der p 23 is supposed to cause stronger immune reactions at relatively low allergen concentrations [12, 15]. In a comparison of sensitization profiles to HDM components in asthmatic and non-asthmatic children, Resch et al. [16] found reactivity to an enlarged repertoire of HDM allergen components as well as higher sIgE levels for different components among asthmatic children. Data from the German Multicenter Allergy study could confirm that broader IgE sensitization patterns were correlated to a significantly higher risk of HDM-related allergic rhinitis and asthma. Also, sIgE to Der p 23 at the age of 5 or less predicted asthma at school age [11]. Furthermore, Celi et al. [9] reported a higher prevalence of allergic rhinitis and asthma in poly-sensitized patients compared to HDM-mono-sensitized patients, as well as a strong association of asthma with Der p 23 hypersensitivity in HDM-mono-sensitized patients (odds ratio 3.38, $p < 0.0001$). Also, Jiménez-Feijoo et al. [10] demonstrated in a paediatric patient collective that patients with persistent moderate/severe Asthma significantly higher recognized Der p 23. Zidarn et al. [17] could show a statistically significant difference in the prevalence of sensitization to Der p 23 in asymptomatic patients (26.3%, n = 19) versus patients that were allergic to HDM proved by nasal provocation test (NPT) (70.6%, n = 17).

Der p 23 is the fourth single allergen component commercially available for testing in clinical routine. After the first studies with relatively high prevalences for sIgE against Der p 23 in Austria, France, Spain, and partially Italy, but lower prevalence of 51% in a relatively small patient collective in Germany, the major aim of the study was to challenge if Der p 23 performs as a major allergen component in southern Germany tested in a larger patient collective. Second, it should be questioned if Der p 23 could close the diagnostic gap in patients with missing sensitization to Der p 1 and Der p 2 despite sensitization to mite extract. Further, more evidence concerning the clinical impact and relevance of Der p 23 sensitization is required [10].

Materials and Methods

Patient Data

The allergy database of the Department of Oto-Rhino-Laryngology, Head and Neck Surgery of the Ludwig Maximilian University of Munich contains all information and diagnostic results of patients who visited the allergy department. These patients do not represent the main population, but rather are pre-selected by certain rhinological symptoms that account for the visit in our department. The database was retrospectively scanned between January 2011 and July 2018 for patients who had received a skin prick test (SPT) with *D. pter.*, resulting in 1,227 patients. Inclusion criterion for the study was sIgE ≥0.35 kU/L to *D. pter*. This led to 482 patients. Exclusion criteria were sIgE <0.35 kU/L, unavailable data for sIgE reactivity against *D. pter.*, or no available blood serum. Total serum IgE was available for all patients. We identified 8 patients who had taken blood samples at 2 different time points and excluded the older results. Consequently, 474 patients sensitized to *D. pter.* were included in the study. Laboratory tests were performed for sIgE to Der p 1, Der p 2, and Der p 23 for all patients. All laboratory tests were performed along current guidelines and are described in detail below.

All patients visiting the department receive a questionnaire at the day the blood sample is taken, which is based on the German validated version of the "Rhinoconjunctivitis Quality of Life Questionnaire RQLQ" by Juniper et al. [18, 19]. In addition, patients are

asked for comorbidities, asthma, and environmental and social aspects. Anamnestic patient information was taken from this questionnaire.

Next to investigating the total study population, we divided the total study population into children aged ≤12 years and teens and adults according to the finding that the HDM-specific sensitization profile of a patient develops within the first 10–12 years and does not change later on [11]. Also, the total patient collective was divided into Der p 23-positive and Der p 23-negative patients.

The use of data from routine clinical practice was approved by the local ethics committee of the Ludwig Maximilian University, Germany, and the local data protection commissioner with the project number 18-448 UE. All patients provided written informed consent for the use of their parameters for scientific research and gave consent to publish these results.

Nasal Provocation Test

298 of the patients mentioned above had received NPT with mite allergen. NPT was performed in accordance with the current guidelines [20, 21]. First, rhinomanometry (RhinoSys; Happersberger Otopront GmbH, Hohenstein, Germany) was performed as baseline measurement after the administration of allergen-free solution (LETI Pharma GmbH, Ismaning, Germany) and, second, with the intranasal challenge test solution for *D. pter.* (100 HEP/mL; LETI Pharma GmbH), each by a nasal spray pump. In addition, patients reported on their symptoms according the guideline: The symptoms registered were secretion (0 = no secretion, 1 = little secretion, and 2 = plenty of secretion), irritation (0 = 0–2× sneezing, 1 = 3–5× sneezing, and 2 = >5× sneezing), and remote symptoms (0 = no remote symptoms, 1 = lacrimation and/or itching of palate and/or itching of ears, and 2 = conjunctivitis and/or chemosis and/or urticaria and/or coughing and/or dyspnoea) [20]. Testing was considered positive with a decrease in rhinomanometry >40% at 150 Pa on the allergen-challenged side, as well as a symptom score >3, or a decrease in intranasal airflow >20% in combination with a symptom score >2. According to the result of NPT, we refer to patients with positive NPT as patients with allergy and patients with negative NPT as patients with clinically irrelevant sensitization.

Skin Prick Test

A standardized SPT with *D. pter.*, *Dermatophagoides farinae*, grass mix, cultivated rye, birch, hazel, alder, ash, mugwort, *Parietaria*, ragweed, cat, dog, *Alternaria*, *Cladosporium*, *Aspergillus*, and positive and negative control (ALK-Abelló, Wedel, Germany) was available for 458 out of 474 (97%) patients and taken as basis to divide the study population into mono-sensitized to HDM, oligo-sensitized to 1–2 other allergens, or poly-sensitized to 3 or more allergen groups (grasses, trees, herbs, animals, and moulds) in addition to HDM. The SPT was considered positive with a wheal ≥3 mm in diameter (I = ≥3–4, II = ≥4–5, III = ≥5–6, and IV = ≥6) in combination with histamine dihydrochloride solution at 1 mg/mL as positive control and allergen-free saline solution as negative control. It was read 20 min after application. The procedure and classification were in line with European standards and published guidelines [22, 23].

Fluorescence Enzyme Immunoassay

The fluorescence enzyme immunoassay (FEIA) method (UniCAP-FEIA; Thermo Fisher Scientific, Freiburg, Germany) was used to detect sIgE reactivity to *D. pter.* and allergen components Der p 1, Der p 2, and Der p 23 with a commercially available test kit (Thermo Fisher Scientific, Freiburg, Germany). All procedures were in accordance with the manufacturer's instructions. The results are given as CAP class (1: ≥0.35–0.70 kU/L; 2: 0.71–3.50 kU/L; 3: 3.51–17.50 kU/L; 4: 17.51–50.00 kU/L; 5: 50.01–100.00 kU/L; and 6: >100.00 kU/L). Alternatively, concentrations are given as ratio of total IgE or as ratio of sIgE to *D. pter*. The positive cut-off value was ≥0.35 kU/L as suggested by the manufacturer.

Statistical Analyses

SigmaPlot (Jandel Corp., San Rafael, CA, USA) and Excel (Microsoft, Redmond, WA, USA) were used for the majority of statistical analysis. Group differences in prevalence were compared by the χ^2 test. To describe sIgE values, the median was used since all data failed normality testing (Shapiro-Wilk). Correlation between the sum of sIgE to Der p 1, Der p 2, and Der p 23 and sIgE to *D. pter.* was calculated by Spearman rank-order correlation with absolute values of specific IgE in kU/L. To statistically compare median values of sIgE to Der p 23, the Mann-Whitney rank sum test was used. A p value <0.05 was considered significant. Logistic analysis and linear regression analysis were performed in IBM SPSS Statistics.

Results

The study's patient collective included 474 patients sensitized to HDM proven by sIgE to *D. pter*. Table 1 summarizes the patient data in terms of gender, age, sensitization to other allergens, and self-reported asthma (Table 1).

Within the total study population, we found a slight predominance of male patients (56 vs. 44% female patients) with a median age of 28 years (range of 4–85 years). According to potential co-sensitizations towards grasses, trees, herbs, animals, and moulds, patients were divided into mono-sensitized, oligo-sensitized (1–2 additional allergen groups), and poly-sensitized (3 or more additional allergen groups): 15% of patients were mono-sensitized and did not show any positive SPT besides to HDM, 33% were oligo-sensitized, and 49% of patients were poly-sensitized. In 3% of patients, we did not have SPT data concerning other allergens than HDM and could not identify the sensitization profile. Table 1 summarizes sensitization rates towards other allergens. Sixty-five percentage of patients suffered from perennial symptoms and 38% from self-reported asthma. Dividing the study population into 2 groups, children with an age from 4 to 12 years and patients with an age from 13 years on, we did not find any difference in clinical characteristics except the gender distribution showing a higher male predominance among the children. In addition, we divided the patient collective into Der p 23-positive and Der p 23-negative patients. There, we found a statistically significant difference in the rate of self-reported asthma (p = 0.019). Also, the higher

Table 1. Demographics and characteristics of patients with sensitization to house dust mite

	Total	>12 years	≤12 years	Der p 23+	Der p 23–
Patients, *n* (%)	474 (100)	377 (80)	97 (20)	**198 (42)**	**276 (58)**
Male, *n* (%)	267 (56)	204 (54)	63 (65)	**116 (59)**	**151 (55)**
Female, *n* (%)	207 (44)	173 (46)	34 (35)	**82 (41)**	**125 (45)**
Age					
Range	4–85	13–85	4–12	**4–60**	**5–85**
Median	28	32	9	**25**	**31**
Mono-sensitized, *n* (%)	70 (15)	56 (15)	14 (14)	**20 (10)**	**50 (18)**
Oligo-sensitized, *n* (%)	158 (33)	122 (32)	36 (37)	**63 (32)**	**95 (34)**
Poly-sensitized, *n* (%)	230 (49)	190 (50)	40 (41)	**108 (55)**	**122 (44)***
No data available, *n* (%)	16 (3)	9 (2)	7 (7)	**7 (4)**	**9 (3)**
Co-sensitization to, *n* (%)					
Grasses	295 (62)	233 (62)	62 (64)	138 (70)	157 (57)
Trees	286 (60)	239 (63)	47 (48)	129 (65)	157 (57)
Herbs	135 (28)	110 (29)	25 (26)	56 (28)	79 (29)
Animals	279 (59)	231 (61)	48 (49)	130 (66)	149 (54)
Moulds	103 (22)	85 (23)	18 (19)	48 (24)	55 (20)
Self-reported perennial symptoms, *n* (%)	308 (65)	250 (66)	58 (60)	**132 (67)**	**176 (64)**
Self-reported asthma, *n* (%)	179 (38)	140 (37)	39 (40)	**87 (44)**	**92 (33)****

Values are number of patients in total (percent of each evaluated group). Age is given as range and median. A p value <0.05 was considered significant. Bold type denotes significance. * Difference in poly-sensitization between Der p 23+ and Der p 23– is statistically significant ($p = 0.033$). ** Difference in self-reported asthma between Der p 23+ and Der p 23– is statistically significant ($p = 0.019$).

ratio of poly-sensitized patients in the Der p 23-positive group was statistically significant ($p = 0.033$) (Table 1). Performing logistic regression with regard to Der p 23 positivity, the odds ratio of oligo- versus mono-sensitization was 1.712 ($p = 0.096$) and of poly- versus mono-sensitization 2.122 ($p = 0.015$) adjusted for self-reported asthma (odds ratio 1.434, $p = 0.082$) and age (odds ratio 0.967, $p < 0.001$). This could be confirmed by higher sensitization rates towards grasses, trees, moulds, and animals but not herbs in Der p 23-positive patients compared to lower sensitization rates in patients without specific IgE towards Der p 23 (Table 1).

Dividing the total study population into patients with and without self-reported asthma, there was a statistically significant difference in percentage of mono-sensitized patients (7% mono-sensitized with self-reported asthma vs. 19% without asthma, $p < 0.001$) and in the percentage of poly-sensitized patients (63% poly-sensitized with self-reported asthma vs. 40% without asthma, $p < 0.001$). In addition, individual sensitization rates to other allergens than mite were significantly higher in patients with self-reported asthma (data not shown).

Table 2 shows the laboratory characteristics in terms of prevalence of sIgE to *D. pter.* and recombinant components Der p 1, Der p 2, and Der p 23 for the individual groups and the median CAP class. In the total study population, only 42% of patients showed sIgE to Der p 23, whereas 61% were positive for Der p 1 and 76% for Der p 2. Prevalence rates in the children group were higher than those in the >12 years old group and showed a statistically significant difference for Der p 1 and Der p 23 with $p < 0.001$ and $p = 0.003$, respectively. CAP classes by trend were higher as well for Der p 2, but this difference was not statistically significant. We also found a statistically significant higher prevalence of sIgE to Der p 1 and Der p 2 in Der p 23-sensitized patients compared to Der p 23-negative patients ($p < 0.001$): sIgE to Der p 1 was measurable in 82% of Der p 23-sensitized patients versus 45% in Der p 23-negative patients, and sIgE to Der p 2 was visible in 85% of Der p 23-sensitized patients and in 67% of Der p 23-negative patients (Table 2).

As shown in Table 3, two-thirds of patients in each group had received NPT to HDM. In all evaluated groups, the number of allergic patients was slightly higher than clinically silent sensitized patients, highest in the children group. None of the differences showed any statistically significant result (Table 3).

Table 4 summarizes the sensitization rates towards Der p 1, Der p 2, and Der p 23 in patients with positive and negative NPT. Whereas sensitization towards Der p

Table 2. Sensitization profiles of different groups to *D. pter.*, Der p 1, Der p 2, and Der p 23

	Total	>12 years	≤12 years	Der p 23+	Der p 23–
D. pter. positive, *n* (%)	474 (100)	377 (100)	97 (100)	**198 (100)**	**276 (100)**
CAP class	3 (1–6)	3 (1–6)	4 (1–6)	**3 (1–6)**	**2 (1–6)**
Der p 1 positive, *n* (%)	287 (61)	212 (56)	75 (77)*	**163 (82)**	**124 (45)****
CAP class	2 (0–6)	2 (0–6)	3 (0–6)	**3 (0–6)**	**0 (0–6)**
Der p 2 positive, *n* (%)	358 (76)	279 (74)	79 (81)	**168 (85)**	**190 (67)****
CAP class	3 (0–6)	2 (0–6)	4 (0–6)	**3 (0–6)**	**2 (0–6)**
Der p 23 positive, *n* (%)	198 (42)	144 (38)	54 (56)*	**198 (100)**	**0 (0)**
CAP class	0 (0–6)	0 (0–6)	2 (0–5)	**2 (1–6)**	**na**

Values of serum diagnostic approaches are number of patients in total (percent of each evaluated group). CAP classes are given as median (range). A p value <0.05 was considered significant. Bold type denotes significance. * Differences between >12 years and ≤12 years are statistically significant (Der p 1 $p < 0.001$; Der p 23 $p = 0.003$). ** Differences between Der p 23+ and Der p 23– are statistically significant ($p < 0.001$).

Table 3. Patients with nasal provocation testing to house dust mite divided into clinically irrelevant sensitization and allergy

	Total	>12 years	≤12 years	Der p 23+	Der p 23–
Patients with provocation testing, *n* (%)	298 (63)	236 (63)	62 (64)	**117 (59)**	**181 (66)**
Allergy, *n* (%)	159 (53)	120 (51)	39 (63)	**67 (57)**	**92 (51)**
Sensitization, *n* (%)	139 (47)	116 (49)	23 (37)	**50 (43)**	**89 (49)**

Values are number of patients in total (percent of each evaluated group). A p value <0.05 was considered significant. Bold type denotes significance.

1 ($p = 0.001$) and Der p 2 ($p < 0.001$) was significantly higher in allergic patients compared to patients with negative NPT, sensitization towards Der p 23 was higher in allergic patients (42% compared to 36%), but this difference was not statistically significant.

All patients included in this study had sIgE to *D. pter.* Regarding the sensitization to the single components Der p 1, Der p 2, and Der p 23, we found a distribution shown in Figure 1 with predominance of 31% with sIgE to all 3 components. Twenty percentage of patients had sIgE to Der p 1 and Der p 2, and another 20% to Der p 2 alone. Only 3% of patients were positive for Der p 23 alone; furthermore, 12% of patients did not show any sIgE to Der p 1, Der p 2, or Der p 23 (Fig. 1).

The sum of sIgE to Der p 1, Der p 2, and Der p 23 correlated highly with sIgE to *D. pter.* (Spearman correlation coefficient = 0.942 [$p < 0.001$]).

Concerning the power of sIgE level to Der p 23 to differentiate between allergic versus clinically silent sensitized patients, we evaluated 117 of Der p 23-sensitized patients who had received NPT to HDM. Fifty-seven percentage of those showed a positive NPT. We compared the ratio of sIgE to Der p 23/total IgE in allergic and clinically irrelevant sensitized patients and did not find a statistically significant difference (Fig. 2a), whereas comparing sIgE to Der p 23/*D. pter.* of allergic versus clinically irrelevant sensitized patients revealed a statistically sig-

Table 4. Sensitization profile with regard to sensitization towards Der p 1, Der p 2, and Der p 23 in patients with nasal provocation testing to house dust mite divided into clinically irrelevant sensitization and allergy

	Total	Der p 1+	Der p 2+	Der p 23+
Allergy, *n* (%)	159 (53)	110 (69)	136 (86)	67 (42)
Sensitization, *n* (%)	139 (47)	70 (50)	90 (65)	50 (36)
p value	0.381	0.001	<0.001	0.333

Values are number of patients in total (percent of each evaluated group, allergy/sensitization). The p value of the χ^2 test is to evaluate difference in rates.

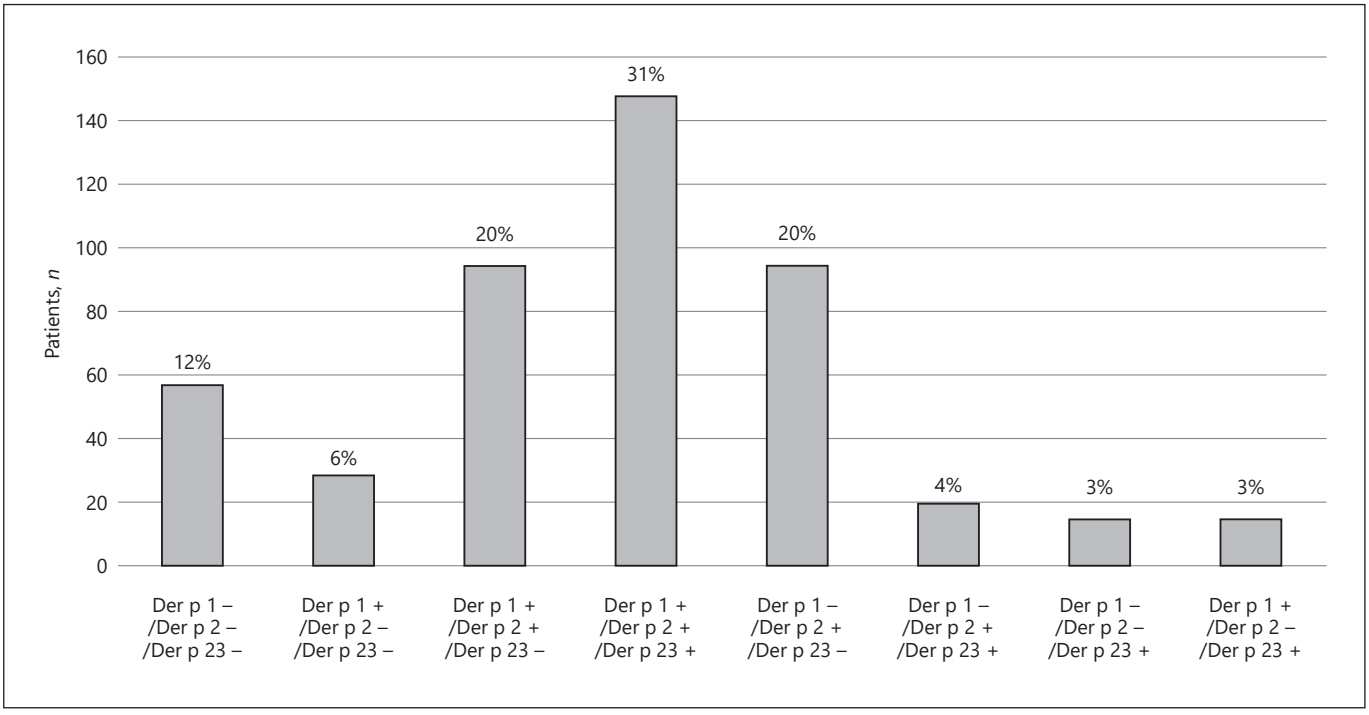

Fig. 1. Number of patients and prevalence rates for different sIgE-profiles (combinations of Der p 1, Der p 2, and Der p 23) in 474 patients tested positive for sIgE to *D. pter*.

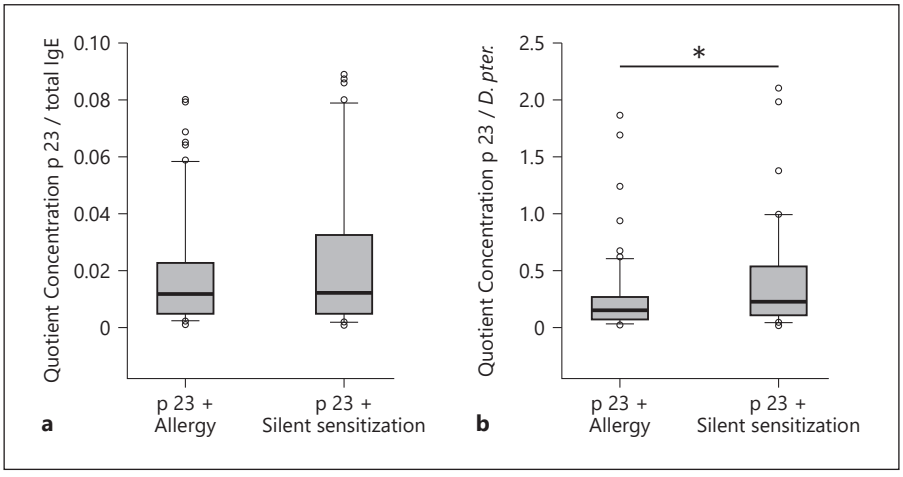

Fig. 2. Concentration of sIgE to Der p 23 in patients, who received nasal provocation testing. According to the result patients were separated in patients with allergic symptoms to house dust mite (n = 67) and patients with clinically silent sensitization (n = 50) (**a** as ratio with total serum IgE, p = 8.867, **b** as ratio with sIgE to *D. pter.*, p = 0.026). Data is given in box plots with the median, the twenty-fifth and seventy-fifth percentile as boundary of the box, and the tenth and ninetieth percentile as dots above and below the box.

nificant difference with p = 0.026 (Fig. 2b). Nevertheless, Figure 2b clearly visualizes that the difference in values of allergic and clinically silent sensitized patients is not great enough to define clear cut-off values for sIgE to Der p 23 in allergic patients. Linear regression was performed to analyze clinical relevance on this finding. Adjusting for age, sensitization profile, and asthma, linear regression was not statistically significant.

Discussion/Conclusion

The aim of the study was to investigate the prevalence of IgE reactivity to Der p 23 in a relatively large sample of patients in southern Bavaria based on the evidence that Der p 23 could be a major allergen of HDM, as already published for other regions [9, 10, 12–15]. Furthermore, it is discussed if testing for Der p 23 sensitization should

be implemented in routine clinical practice for HDM-sensitized patients.

Sensitization profiles for HDM allergens strongly vary depending on the geographic area. Therefore, diagnostic approaches could be individually selected depending on the region of exposure in order to treat and manage the patient in an optimal way. Our study collective included 474 patients that visited our allergy department in Munich, Bavaria, Germany, and were sensitized to HDM proven by sIgE to *D. pter*. Therefore, this group clearly does not represent the main German population but includes higher-order-care level patients with possibly allergy-induced symptoms, probably leading to higher sensitization rates and number of allergic patients compared to population-based data. Regarding the HDM-specific sensitization profile of our study population, we found an overall prevalence for sIgE to Der p 23 of 42% and of 38% in patients older than 12 years (Table 2). Therefore, in southern Bavaria Der p 23 does not fulfil the criteria of a major allergen component (prevalence >50%) as reported, for example, for nearby Austria with a Der p 23 prevalence of 74% [12] or other countries. However, most studies with higher prevalence rates only included allergic patients or did not provide detailed information about the patient collective. Our patient collective was exactly described as patients sensitized to *D. pter.* and in a subgroup of patients additionally divided into allergic versus clinically irrelevant sensitized patients by NPT as seen in Table 3. Therefore, we argue that the prevalence rate of sIgE reactivity to Der p 23 of around 40% (42% in the total collective of *D. pter.*-sensitized patients, and 39% in the subgroup of HDM NPT patients) is a fair reference value for southern Germany.

As outlined in the section Materials and Methods and seen in Tables 1–3, we divided the total study population into children aged ≤12 years and teens and adults. The subgroup of patients older than 12 years certainly can stand for a representative HDM-sensitized collective in southern Bavaria. However, we assume a selection bias affecting the differing prevalence of IgE reactivity to Der p 1, Der p 2, and Der p 23 in children. Rates were higher for all 3 allergen components in children, although only higher sensitization rates for Der p 1 and Der p 23 were statistically significant with $p < 0.001$ and $p = 0.003$, respectively (Table 2). Also, CAP classes were higher in children. Primarily, especially in younger children, there might be a certain restraint in matters of partially invasive diagnostic approaches such as SPT or taking blood. Also, children with suspected allergic rhinitis might not be only seen in our allergy department but can also be seen solely by a local paediatrician or in the paediatric allergy department. Only complex paediatric patients or children with severe clinical symptoms are then transferred to our department for interdisciplinary management. Both reasons could result in putative higher prevalence rates of allergen components and higher CAP classes in children. This bias could be confirmed by differing serum levels of total IgE. Children showed a mean level of 698.4 kU/L, whereas patients >12 years only showed a mean level of 315.6 kU/L total IgE. Additionally, we saw a higher percentage of allergic children versus children with clinically silent sensitization compared to patients >12 years (Table 3), although this difference was not statistically significant ($p = 0.121$). On the other hand, we did not find a higher percentage of poly-sensitized patients in the children group. Furthermore, Celi et al. [9] also found a higher prevalence of Der p 23 reactivity in younger patients compared to older patients and mentioned that future studies have to show if Der p 23 simply is a novel allergen or whether sensitization is lost with age.

In addition, we divided the total study population into patients with and without IgE reactivity to Der p 23. The difference seen here is the higher number of patients reporting asthma and the number of poly-sensitized patients in the group of patients with sensitization towards Der p 23 (Table 1). Logistic regression analysis could confirm the relationship between Der p 23 positivity and poly-sensitization and to a lesser extent to asthma. Weghofer et al. [12] hypothesize that especially Der p 23 itself and not HDM-specific poly-sensitization in general induces asthma to a greater extent than other HDM allergen components because of its high allergenic activity. However, Celi et al. [9] could find a correlation between the prevalence of asthma and the number of recombinant molecules recognized within HDM. Furthermore, this finding was confirmed in HDM-mono-sensitized patients. But also, asthma was strongly associated with Der p 23 hypersensitivity itself, and asthma severity was associated with Der p 23 IgE levels [9]. On the other hand, Resch et al. [16] could not find a positive predictive value indicating asthma for sIgE reactivity to Der p 23 but also reported higher sIgE to Der p 23 in asthmatic patients versus patients without asthma. This finding could be lately confirmed in a children's collective [10]. In our study, patients with self-reported asthma also showed a higher percentage of co-sensitizations towards other allergen groups (63% poly-sensitized with self-reported asthma vs. 40% poly-sensitized without asthma, $p < 0.001$).

Regarding the HDM-specific sensitization profile of patients with sIgE to Der p 23 (Table 2), we could see a

statistically significant higher prevalence of sIgE to Der p 1 and Der p 2 (Der p 1: 82% in the Der p 23-positive group vs. 45% in the Der p 23-negative group, Der p 2: 85 vs. 67%; $p < 0.001$). Therefore, a patient with sIgE to Der p 23 is more likely to have sIgE to other components, for example, Der p 1 and Der p 2 as well. Following, in our study collective not only poly-sensitization in general is more likely in Der p 23-positive patients, but also poly-sensitization within HDM components.

Along the lines of Zidarn et al. [17], we suspected a higher ratio of allergic patients versus clinically irrelevant sensitized patients in the Der p 23-positive collective compared to the Der p 23-negative collective. Around two-thirds of our patients received NPT, but we could not find a statistically significant difference in the ratio of allergic versus clinically irrelevant sensitized patients within the Der p 23-positive versus Der p 23-negative collective (Table 3), concluding that at least Der p 23 sensitization itself in our study collective does not necessarily cause allergic disease. On the other hand, sensitization rates towards Der p 1 and Der p 2 were significantly higher in allergic patients compared to patients with negative NPT. Also, we compared the rate of allergic patients who received NPT in Der p 1/Der p 2/Der p 23-sensitized patients (63%) with the rate of allergic patients in the Der p 1/Der p 2/Der p 23-negative group (19%) and could see a statistically significant difference ($p < 0.001$). Consequently, we argue that poly-sensitization within HDM components is a risk factor for allergic disease as already shown by Posa et al. [11].

Regarding the distribution of sIgE to Der p 1, Der p 2, and Der p 23 in *D. pter.*-sensitized patients in general (Fig. 1), sensitization to all 3 components was most frequent (31%). Equally frequent was sensitization to Der p 1 and Der p 2 and sensitization to Der p 2 alone (both 20%). Twelve percentage of patients did not have sIgE to Der p 1, Der p 2, or Der p 23. This distribution pattern is in line with data from the German Multicenter Allergy study. Posa et al. [11] found 36% of patients having sIgE to Der p 1, Der p 2, and Der p 23, 23% with sIgE to Der p 1 and Der p 2, and 13% with sIgE to Der p 2 alone. Furthermore, it was shown that 9% of patients did not have sIgE to Der p 1, Der p 2, or Der p 23. All other sIgE combinations were rare.

Although Weghofer et al. [7] could show that 97–100% of patients depending on the European country have at least sIgE to Der p 1 and/or Der p 2, our gap of 15% Der p 1/Der p 2-negative patients could be confirmed by Resch et al. [16] in a German multicentre study collective, who investigated 11% Der p 1/Der p 2-negative patients. Thirty-three percentage of these patients showed sIgE to Der p 23. In our study, only 21% of patients without sensitization to Der p 1/Der p 2 showed sIgE to Der p 23. Mono-sensitization to Der p 23 was as low as 3% and also comparable to Weghofer et al. [12] showing that 4% of patients were mono-sensitized to Der p 23. Also, González-Pérez et al. [13] reported a mono-sensitization of 3% towards Der p 23 in Spain. Although mono-sensitization seems to be a rare event, Matos Semedo et al. [24] argue that Der p 23 itself has clinical relevance and should probably be considered in terms of HDM-specific immunotherapy extract composition. Concluding, testing for sIgE to Der p 23 in patients with sensitization to HDM/*D. pter.* could not identify the majority of patients from southern Germany without sensitization to the 2 major allergens Der p 1 and/or Der p 2 and, therefore, is not suitable to close a diagnostic gap that arises when exclusively testing with components. On the other hand, specific knowledge of the exact sensitization profile is indispensable for the decision for allergen immunotherapy and its probable success [25, 26]. Furthermore, Jiménez-Feijoo et al. [10] reported that all elven HDM allergic patients of their collective who did not respond to immunotherapy were sensitized towards Der p 23.

Regarding the differentiation between allergy and clinically irrelevant sensitization, it was shown by 2 groups that levels of sIgE to Der p 1 and Der p 2 have the potential to replace provocation testing [27, 28]. On the other hand, Gellrich et al. [29] indeed could confirm a significant difference in the amount sIgE in clinically irrelevant and symptomatic HDM-sensitized patients but far from the definition of clinically potent cut-off values. This data shows that the amount of sIgE to Der p 23 not is suitable to hint at symptomatic versus clinically irrelevant sensitization (Fig. 2). Although we see a statistically significant difference in the amount of sIgE to Der p 23 in allergic patients compared to clinically irrelevant patients if related to the amount of sIgE to *D. pter.*, this could not be confirmed by linear regression models. Also, sIgE to Der p 23 in relation to the amount of total IgE was not statistically significant (Fig. 2a). Furthermore, Figure 2b illustrates that the difference found in levels of sIgE to Der p 23 is far from defining clear ranges to differentiate between allergy and clinically irrelevant sensitization.

Finally, we would like to discuss several limitations of the study. First, as outlined in the beginning of the discussion, the data set does not represent the main population of southern Germany. Rather, patients were pre-selected by airway symptoms possibly leading to higher rates of

allergic and sensitized patients. This could possibly result in a higher percentage of Der p 23 sensitization. However, Der p 23 sensitization still did not meet the criteria of a major allergen. Second, this study is based on retrospectively analyzed data, does not include follow-up information, and therefore does not allow potential predictive evaluation or conclusion. In addition, classification of patients with an allergy versus clinically silent sensitization was based on a subset of patients who had received nasal provocation testing, possibly leading to another selection bias favouring allergic patients in general. Nasal provocation was performed along the German guideline back then [20, 21]; however, in the meantime, there has been implemented a new European guideline [30] with a major change to provoke bilaterally with control and allergen solution consecutively. Of course, the nasal cycle could theoretically affect results when testing with control and allergen solution is performed single-sided. On the other hand, the time course of nasal provocation is rather short compared to the nasal cycle, and for many years this procedure was applied in routine clinical practice. Last, the differentiation of patients with and without asthma was not based on clinical examination and lung function tests, but only on patients' report. Of course, this is a major limitation. Unfortunately, the data of this study derive from a Department of Otorhinolaryngology, where the focus of interdisciplinary handling of allergy patients does not lie on asthma; therefore, this study focuses on the sensitization profile of mite components in different groups of patients in general. On the other hand, we did not want to ignore the possible impact of Der p 23 on asthma and decided to include reported asthma as one of several criteria.

In summary, Der p 23 does not serve as a major allergen with an overall prevalence of 42% in southern Bavaria, Germany. Nevertheless, testing for Der p 23-specific IgE response in *D. pter.* positively tested patients can identify a low percentage of patients who are not sensitized to the 2 major allergens Der p 1 and Der p 2, but a relevant number of patients are still overlooked. Polysensitization within HDM allergen components is more frequent in allergic patients, but Der p 23 sensitization itself in our study is not found in a higher percentage of allergic patients. Clearly, Der p 23 does not serve as a marker to distinguish allergy to HDM from clinically silent sensitization. However, this data suggests that Der p 23 may play a specific role in asthmatic patients. Concluding, additional testing for Der p 23 could be crucial in HDM allergic patients for more precise clinical profiling and estimation on the course in allergic disease and with regard to a possible selection of therapeutic strategy. But, more research and follow-up studies are needed to define the predictive value of Der p 23 positivity.

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Statement of Ethics

The retrospective use of data from routine clinical practice was approved by the local ethics committee and the local data protection commissioner. All patients provided written informed consent for the use of their parameters for scientific research and gave consent to publish these results.

Disclosure Statement

Moritz Gröger has received speaker honoraria from ALK-Abelló, Allergy Therapeutics, HAL, Leti, Thermo Fisher Scientific, and Stallergenes, received financial support for attending symposia from ALK-Abelló, Allergopharma, Allergy Therapeutics, HAL, Lofarma, Thermo Fisher Scientific, and Shire, received research grants from ALK-Abelló, GSK, Sanofi, and Thermo Fisher Scientific, and is a member of the advisory board of ALK-Abelló. Sven Becker has received speaker honoraria from ALK-Abelló, Bencard Allergy, HAL, Thermo Fisher Scientific, and Allergopharma, received financial support for attending symposia from ALK-Abelló, Allergopharma, HAL, and Bencard Allergy, received research grants from Ambu, and is a member of the advisory board of Bencard Allergy, Ambu, and Sanofi Genzyme. Donata Gellrich has received speaker honoraria from ALK-Abelló and Bencard Allergy and financial support for attending symposia from HAL Allergy, Phadia diagnostics, and Shire. Petra Zieglmayer is a medical director at Thermo Fisher Scientific, received lecture fees from Alk Abello, Allergopharma, Allergy Therapeutics, HAL, Leti, Meda, Merck, Novartis, Stallergenes, and Thermo Fisher Scientific, received research grants from Allergopharma, Allergy Therapeutics, Biomay, Calistoga, GSK, HAL, Marinomed, MSD, Ono, Oxagen, RespiVert, Stallergenes, and VentirX, and is advisory board member for Alk Abello, Bencard, Meda, Merck, Sigmapharm, and Stallergenes. All other authors declare that they have no conflicts of interest.

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Author Contributions

K.E. participated in the study design, analyzed the data, performed statistical analysis, interpreted the data, and wrote the manuscript. M.G. participated in the study design, collected the data, was a major contributor in writing the manuscript, provided critical revisions, and holds overall responsibility for the project. D.G. collected the data, gave critical advice in interpreting the data, and critically revised the manuscript. S.B. and P.Z. advised the study design, provided critical advice in analyzing the data, and revised the manuscript. All authors read and approved the final manuscript.

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