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Value of Component Resolved Diagnostics to *Aspergillus fumigatus* in Patients with Upper Airway Complaints

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Keywords

Aspergillus fumigatus · Molecular diagnostics · Mold allergy · Allergic rhinitis · Recombinant allergens · Allergic asthma

Abstract

Introduction: Sensitization to *Aspergillus fumigatus* is a risk factor for severe asthma. However, little is known about its presence, appearance, and impact on allergic rhinitis. Herein, we investigated the usefulness of component resolved diagnostics in patients sensitized to *Aspergillus fumigatus* protein extract. ***Methods:*** Seventy-eight patients with suspected allergic rhinitis and elevated IgE levels toward *Aspergillus fumigatus* protein extract were retrospectively evaluated regarding their total and *Aspergillus*-specific IgE levels and their skin prick test. Furthermore, they were tested for specific IgE antibodies against Asp f 1, 2, 3, 4, and 6. ***Results:*** Skin prick test missed 6 patients (7.7%) with elevated IgE toward *Aspergillus fumigatus* protein extract. Fifty percent of patients ($n = 39$) were sensitized to at least one component. Even though monosensitization affected all components, all patients with positivity toward more than one component were sensitized to Asp f 1. There was a statistically significant increase of *Aspergillus*-specific IgE with increasing number of components affected by sensitization. Many patients were oligo- (34.6%) or polysensitized (51.3%). There was a high prevalence of sinusitis (61.8%). ***Conclusions:*** Component resolved diagnostic testing toward the major allergen Asp f 1 was less sensitive than skin prick test and serology to *Aspergillus fumigatus* protein extract. However, sensitivity of component resolved diagnostics might be underestimated. Diagnostics of the species-specific allergens Asp f 1, 2, and 4 might allow to differentiate between genuine and cross-reactive sensitization. In the clinical routine, skin prick test and serology to crude extract remain the methods of choice.

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Introduction

Fungi such as *Aspergillus fumigatus*, *Alternaria alternata*, and *Cladosporium herbarum* can cause various health problems, including infections, IgE-mediated allergies, non-IgE-mediated hypersensitivity, and toxicity. From the approximately 1.5 million existent species of fungi, numerous fungal species have been described as

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causes of allergic diseases in the literature [1]. There seems to be a high prevalence of mold allergy ranging from 6% [2] to 24% [3] in the general population and reaching up to 44% in atopics [4].

Alternaria and *Cladosporium* species are considered important outdoor allergens, and sensitization and exposure to species of these genera are related to the development of asthma and rhinitis, as well as epidemics of asthma exacerbation. *Aspergillus fumigatus*, next to causing allergic rhinitis (AR) and allergic chronic sinusitis, has a high capacity to colonize the bronchial tract of asthmatic patients, causing severe persistent asthma and low lung function and sometimes leading to allergic bronchopulmonary aspergillosis. When comparing patients with AR caused by molds to patients with AR due to pollen or mites, they had a significantly greater predisposition for asthma and chronic sinusitis [5].

Aspergillus fumigatus is a complex fungus – the WHO/IUIS Allergen Nomenclature Sub-Committee has acknowledged over 20 allergens so far (www.allergen.org). Asp f 1 is a major allergen produced by the mycelia of *Aspergillus fumigatus*. It is not present in spores and can be used as a specific marker for the detection of germination of this fungus [6]. Asp f 1 is a species-specific allergen, related to ribotoxins, which are inhibitors of eukaryotic protein synthesis [7]. Asp f 2 is a fibrinogen-binding protein and, same as Asp f 1 and Asp f 4, is species-specific. Yet, the biological function of Asp f 4 is unknown so far [7–9]. In contrast to Asp f 1, 2, and 4, Asp f 3 (peroxismal membrane protein), Asp f 6 (manganese superoxide dismutase), and others (Asp f 8/12/22/27) exhibit high similarity and identity with homologous proteins from fungi of genera other than *Aspergillus*[10–13].

Similar to other allergens, it is still unclear if single recombinant proteins or a mix of recombinant allergens are superior to crude fungal protein extracts for serological detection of specific IgE antibodies or for skin testing in patients with potential sensitization or allergy to *Aspergillus fumigatus*. A standardization of extracts is a difficult task due to the high number of different *Aspergillus fumigatus* allergens. Asp f 1 levels showed a great variability when comparing allergen products from different manufacturers [14, 15]. The broad cross-reactivity of some allergens with taxonomically unrelated fungi makes interpretation difficult. Asp f 1, Asp f 2, and Asp f 3 are the major allergens of *Aspergillus fumigatus*, while Asp f 4 and Asp f 6 proved to be specific for patients with allergic bronchopulmonary aspergillosis [9, 16, 17]. The pathogenic capacity of *Aspergillus fumigatus* is related to its pronounced thermotolerance [18], which allows growth at human body temperature, and its small spore size [19], which enables transfer to the terminal airways.

Table 1. Demographics and characteristics of patients with sensitization to native *Aspergillus fumigatus* extract

	Sensitization to native *Aspergillus* (n = 78)
Male	47 (60.3%)
Female	31 (39.7%)
Age (years)	44 (5–87)
Monosensitized	11 (14.1%)
Oligosensitized	27 (34.6%)
Polysensitized	40 (51.3%)
Cosensitization against	
Grasses	38 (48.7)
Trees	31 (39.7)
Herbs	25 (32.1)
Animals	27 (34.6)
Mites	32 (41.0)
Other molds	39 (50.0)
Alternaria	35 (44.9)
Cladosporium	20 (25.6)
Asthma	32 (41.0%)
CRSsNP	26 (33.8%)
CRSwNP	21 (27.3%)
Oral allergy syndrome	21 (26.9%)
Atopic dermatitis	19 (25.0%)

Values are numbers of patients and the percent of the evaluated group. Age is given as median with range in parentheses. CRSsNP/CRSwNP, chronic rhinosinusitis sine/with nasal polyps.

The aim of this study was to determine whether component diagnostics to *Aspergillus fumigatus* can replace or upgrade the performance of extract-based serology and skin prick test (SPT) in clinical routine.

Patients and Methods

The department of Otorhinolaryngology, Head and Neck Surgery of the Ludwig-Maximilians-University, in Munich runs a database, where all relevant patient information and allergy diagnostics are stored. From this database, consecutive patients with elevated specific IgE to *Aspergillus fumigatus* protein extract were extracted. We scanned the database for patients presenting at our department from 1999 to 2019. A total of 95 patients resulted. We excluded patients where not enough serum was left for further component diagnostics, resulting in a total of 78 patients being included in this study. Patients had been suspected for suffering from AR and underwent routine in vivo tests such as SPT (ALK-Abelló, Wedel, Germany) and in vitro tests such as total IgE and allergen-specific IgE in serum. An allergy-specific questionnaire, including information on nasal, ocular, and dermal symptoms, food allergy, as well as the presence of other atopic diseases, was

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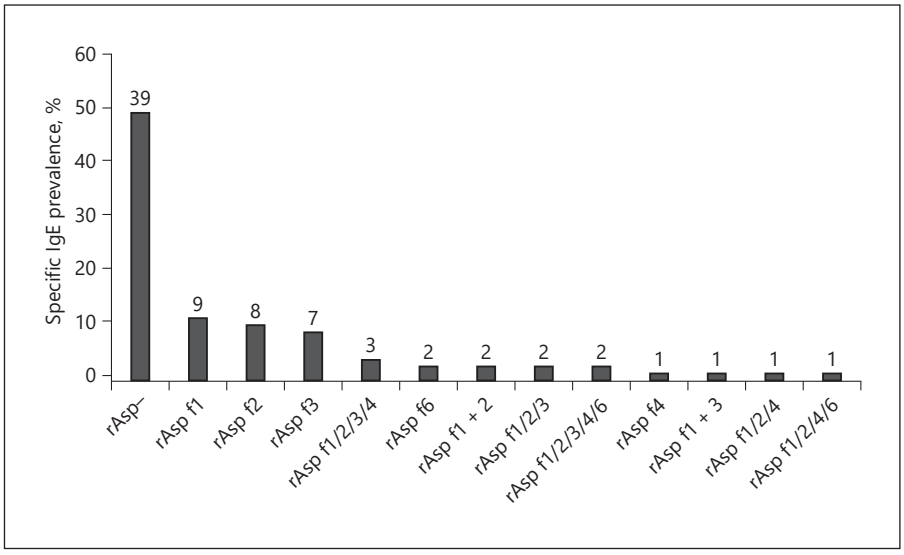

Fig. 1. Sensitization profiles of the 78 investigated patients (in percent and total number).

available for all patients. Information on cosensitizations was extracted from the database – we distinguished between patients being monosensitized to *Aspergillus fumigatus*, patients oligosensitized to 1–2 additional perennial or seasonal allergens, and patients polysensitized to 3 or more additional allergen groups apart from *Aspergillus fumigatus*. Focus was put on cosensitization to other fungi. Regarding nasal symptoms, all patients were asked about nasal obstruction, secretion, itching, and sneezing (scale from 0 [no symptoms] to 3 [maximal symptoms]). Afterward, the single values were summed up. Additionally, in all selected patients, diagnostic for the allergen components rAsp f 1, rAsp f 2, rAsp f 3, rAsp f 4, and rAsp f 6 was performed.

The study was approved by the local ethics committee and the local data protection commissioner. All patients gave written informed consent.

Skin Prick Test

The SPT solution for *Aspergillus fumigatus* (m3) by ALK-Abelló as well as the SPT solution for *Alternaria alternata* (m6) and *Cladosporium herbarum* (m2), by the same manufacturer, were used. The SPT was considered positive with a wheel ≥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. The results were read out 20 min after application. The procedure was in line with European standards and published guidelines [20–23].

Fluorescent Enzyme Immunoassay

For measuring IgE reactivity to *Aspergillus fumigatus* protein extract (m3) and allergen components, the fluorescent enzyme immunoassay (FEIA) method (UniCAP FEIA; Thermo Fisher Scientific, Freiburg, Germany) was used with a commercially available test kit according to the manufacturer's instructions. Initially, specific IgE antibodies to the components rAsp f 1, rAsp f 2, rAsp f 3, rAsp f 4, and rAsp f 6 have not been routinely measured. Therefore, sera of the patients with positive IgE reactivity to *Aspergillus fumigatus* extract were defrosted, and component diagnostics was completed for the 5 components (UniCAP FEIA; Thermo Fisher Scientific, Freiburg, Germany). Results are given in concentrations (kU/L) as well as CAP classes from 0 to 6. The positive cutoff value was ≥0.35 kU/L as suggested by the manufacturer.

Statistical Analysis

Statistical analysis was performed with Excel (Microsoft, Redmond, WA, USA) and SigmaPlot (Jandel Corp., San Rafael, CA, USA). All of the data failed normality testing (Shapiro-Wilk). Therefore, we used median values for descriptive statistics and the Kruskal-Wallis test for testing statistically significant differences between the groups followed by pairwise multiple comparison procedures (Dunn's Method) in case of statistically significant results. The degree of relationship between quantitative variables studied was analyzed using Spearman's rank order correlation. A value of $p < 0.05$ was considered significant.

Results

The database query resulted in 95 patients with a proven sensitization to *Aspergillus fumigatus*. In 78 patients, enough serum was left for further component diagnostics including rAsp f 1, rAsp f 2, rAsp f 3, rAsp f 4, and rAsp f 6. The relevant patients' demographic and clinical data are summarized in Table 1.

We analyzed different diagnostic tools. SPT was available for 71 patients: 65/71 (91.5%) showed a positive SPT to *Aspergillus fumigatus* (median I, range 0–IV), 40/71 (56.3%) to *Alternaria alternata* (median I, range 0–IV), and 23/68 (33.8%) to *Cladosporium herbarum* (median I, range 0–IV). Three patients without sensitization to rAsp f 1–4 or rAsp f 6, 2 patients with sensitization to rAsp f 1, and 1 patient with sensitization to rAsp f 3 showed a neg-

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Table 2. Sensitization profile to native *Aspergillus fumigatus* extract and different components of *Aspergillus fumigatus*

	Sensitization
m3 (native *Aspergillus*)	78 (100.0%)
CAP class	2 (1–6)
IgE, kU/L	1.67 (0.35–100.0)
rAsp f 1	21 (26.9%)
CAP class	0 (0–5)
IgE, kU/L	0.11 (0.04–62.5)
rAsp f 2	19 (24.4%)
CAP class	0 (0–3)
IgE, kU/L	0.08 (0–17.5)
rAsp f 3	15 (19.2%)
CAP class	0 (0–4)
IgE, kU/L	0.05 (0–23.5)
rAsp f 4	8 (10.3%)
CAP class	0 (0–4)
IgE, kU/L	0.06 (0–22.3)
rAs f 6	5 (6.4%)
CAP class	0 (0–4)
IgE, kU/L	0.04 (0–18.2)

Values are numbers of patients total and percent of the evaluated group. CAP classes and IgE are given as median with range in parentheses.

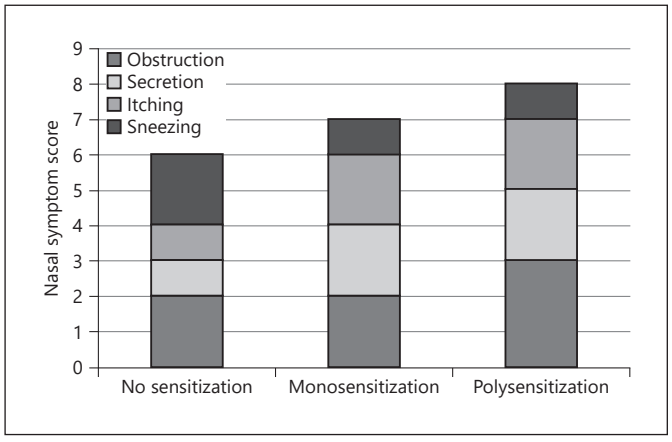

Fig. 2. Nasal symptom score for patients without sensitization, with monosensitization, or with polysensitization (≥2) to the components rAsp f 1–4 or rAsp f 6. The score is composed of nasal obstruction, secretion, itching, and sneezing (scale from 0 [no symptoms] to 3 [maximal symptoms]) and summed up to a maximum of 12 points.

ative SPT to *Aspergillus fumigatus*. The component diagnostics resulted in a variety of sensitization profiles as shown in Figure 1. Prevalence of specific IgE against *Aspergillus fumigatus* protein extract was 100%, as it has been an inclusion criterion for this study. Thirty-nine patients (50%) did not show any sensitization to rAsp f 1–4 or rAsp f 6. The prevalence of sensitization to rAsp f 1, rAsp f 2, rAsp f 3, rAsp f 4, and rAsp f 6 was 26.9, 24.4, 19.2, 10.3, and 6.4%, respectively. Detailed information is given in Table 2. Of the 39 patients with proven sensitization to one of the tested components, 27 patients showed a sensitization to 1, 3 to 2, 3 to 3, 4 to 4, and 2 to 5 components. Among the 27 monosensitized patients, monosensitization affected all tested components (rAsp f 1–4 and rAsp f 6). However, all patients being positive for more than one component showed a sensitization to the major allergen rAsp f 1. Age and *Aspergillus*-specific IgE titer showed a moderate correlation (rho = 0.45, $p < 0.001$).

We compared 3 groups with regard to laboratory and clinical characteristics: (1) patients showing no sensitization to rAsp f 1–4 and rAsp f 6 ($n = 39$, 50%), (2) patients being sensitized to one single *Aspergillus fumigatus* component ($n = 27$, 34.6%), and (3) patients being sensitized to at least 2 of the tested *Aspergillus fumigatus* components ($n = 12$, 15.4%). The detailed serological results of these subgroups are given in Table 3, while Table 5 shows the relevant clinical data. The median titers of total IgE and *Aspergillus*-specific IgE increased with the number of *Aspergillus fumigatus* components sensitized to. However, this trend was only statistically significant regarding the specific IgE antibodies toward the *Aspergillus fumigatus* protein extract ($p < 0.001$ for group 3 vs. group 1, $p = 0.008$ for group 3 vs. group 2, and $p = 0.029$ for group 1 vs. group 2), while we found no statistically significant difference in the titers of the total IgE ($p = 0.094$). When looking at the ratio of *Aspergillus*-specific IgE/total IgE, this ratio increased with increasing number of components affected by sensitization. All patients with sensitization toward one or several of the tested *Aspergillus fumigatus* components – regardless of the number of affected components – showed a statistically significant higher ratio of *Aspergillus*-specific IgE/total IgE compared to those patients with negative component-resolved diagnostics despite positive IgE reactivity against *Aspergillus fumigatus* protein extract ($p < 0.001$ for group 3 vs. group 1, $p = 0.008$ for group 2 vs. group 1, and $p = 0.298$ for group 3 vs. group 2). Both cosensitization to other molds and number of sensitizations to allergens of other allergen families did not show a significant difference among groups (see Table 3).

When looking at the clinical data (Table 5), it becomes apparent that both the prevalence of asthma and sinusitis are increasing and showing the highest level in patients

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Table 3. Total and *Aspergillus fumigatus*-specific IgE as well as cosensitization to other fungal and nonfungal allergens for patients without sensitization to Asp f 1–4 and Asp f 6, with monosensitization, or with polysensitization (≥2 components) to Asp f 1–4 and/or Asp f 6

Sensitization to	*N* (%)	Total IgE, kU/L	Asp IgE, kU/L	Asp IgE/ total IgE, %	Sensitization to				
					allergen families			other molds	
					mono	oligo	poly	Alt	Cla
0 components (Asp f 1–4, Asp f 6)	39 (50)	379 (41–3,558)	0.67 (0.35–15.8)	0.18 (0.02–4.21)	4 (10%)	14 (36%)	21 (54%)	20 (51%)	11 (28%)
1 component (Asp f 1–4, Asp f 6)	27 (34.6)	432 (46–1,407)	2.89 (0.37–85.8)	0.97 (0.07–8.88)	6 (22%)	9 (33%)	12 (45%)	10 (37%)	4 (15%)
≥2 components (Asp f 1–4, Asp f 6)	12 (15.4)	1,233 (105–5,000)	24.75 (3.18–100)	4.29 (0.06–7.68)	1 (8%)	4 (33%)	7 (59%)	5 (42%)	5 (42%)

Values are numbers of patients with percent in parentheses. IgE levels are given as median with range in parentheses. Mono, monosensitized; oligo, oligosensitized; poly, polysensitized; Asp, *Aspergillus*; Alt, *Alternaria*; Cla, *Cladosporium*.

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Table 4. Total and *Aspergillus fumigatus*-specific IgE as well as cosensitization to other fungal and nonfungal allergens for patients without sensitization to Asp f 1–4 and Asp f 6 or with sensitization to Asp f 1, Asp f 2, Asp f 3, Asp f 4, or Asp f 6

Sensitization to rAsp f	1	2	3	4	6	*N* (%)	Total IgE, kU/L	Asp IgE, kU/L	Asp IgE/ total IgE, %	Sensitization to				
										allergen families			other molds	
										mono	oligo	poly	Alt	Cla
1						39 (50)	379 (41–3,558)	0.67 (0.35–15.8)	0.18 (0.02–4.21)	4 (10%)	14 (36%)	21 (54%)	20 (51%)	11 (28%)
2	✓					21 (26.9)	519 (46–5,000)	7.73 (0.38–100)	3.06 (0.06–7.68)	3 (14%)	6 (29%)	12 (57%)	8 (38%)	6 (29%)
3		✓				19 (24.4)	874 (105–5,000)	22.2 (0.99–100)	4.05 (0.06–8.88)	2 (11%)	8 (42%)	9 (47%)	10 (53%)	6 (32%)
4			✓			15 (19.2)	876 (164–5,000)	5.42 (0.37–100)	1.99 (0.07–7.14)	3 (20%)	3 (20%)	9 (60%)	5 (33%)	7 (47%)
5				✓		8 (10.3)	1,483 (138–5,000)	19.47 (0.67–100)	2.52 (0.06–7.14)	0 (0%)	2 (25%)	6 (75%)	4 (50%)	4 (50%)
6					✓	5 (6.4)	1,995 (268–5,000)	3.18 (0.41–80.8)	0.11 (0.06–4.05)	0 (0%)	1 (20%)	4 (80%)	3 (60%)	2 (40%)

Values are numbers of patients with percent in parentheses. IgE levels are given as median with range in parentheses. Mono, monosensitized; oligo, oligosensitized; poly, polysensitized; Asp, *Aspergillus*; Alt, *Alternaria*; Cla, *Cladosporium*.

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Table 5. Demographics and characteristics for patients without sensitization to Asp f 1–4 and Asp f 6, with monosensitization, or with polysensitization (≥2 components) to Asp f 1–4 and/or Asp f 6

Sensi-tization to		*N* (%)	Age, years	M:F	Rhinitis	Conj	Asthma	CRS		Atopic derm
								sNP	wNP	
1	0 components (rAsp f 1–4, rAsp f 6)	39 (50.0)	38 (7–69)	28:11	28 (97%), 10 n.k.	22 (71%), 8 n.k.	14 (36%)	11 (28%)	11 (28%)	12 (32%) 1 n.k.
2	1 component (rAsp f 1–4, rAsp f 6)	27 (34.6)	46 (5–87)	13:14	23 (92%), 2 n.k.	10 (44%), 4 n.k.	12 (44%)	10 (37%)	6 (22%)	3 (11%)
3	≥2 components (rAsp f 1–4, rAsp f 6)	12 (15.4)	47 (31–59)	6:6	11 (100%), 1 n.k.	6 (55%), 1 n.k.	6 (50%)	5 (46%), 1 n.k.	4 (36%), 1 n.k.	4 (36%), 1 n.k.

Values are numbers of patients with percent in parentheses. Age is given as median with range in parentheses. M:F, male:female; CRSsNP/CRSwNP, chronic rhinosinusitis sine/with nasal polyps; conj, conjunctivitis; atopic derm, atopic dermatitis; n.k., not known.

Table 6. Demographics and characteristics for patients without sensitization to Asp f 1–4 and Asp f 6 or with sensitization to Asp f 1, Asp f 2, Asp f 3, Asp f 4, or Asp f 6

Sensi-tization to rAsp f	1	2	3	4	6	*N* (%)	Age, years	M:F	Rhinitis	Conj	Asthma	CRS		Atopic derm
												sNP	wNP	
1						39 (50)	38 (7–69)	28:11	28 (97%), 10 n.k.	22 (71%), 8 n.k.	14 (36%)	11 (28%)	11 (28%)	12 (32%), 1 n.k.
2	✓					21 (26.9)	47 (31–62)	9:12	19 (95%), 1 n.k.	6 (35%), 2 n.k.	12 (57%)	9 (45%), 1 n.k.	6 (30%), 1 n.k.	5 (25%), 1 n.k.
3		✓				19 (24.4)	55 (31–87)	8:11	18 (100%), 1 n.k.	9 (56%), 3 n.k.	9 (47%)	6 (33%), 1 n.k.	8 (44%), 1 n.k.	4 (22%), 1 n.k.
4			✓			15 (19.2)	49 (25–70)	10:5	13 (100%), 2 n.k.	8 (57%), 1 n.k.	6 (40%)	6 (40)	3 (20%)	3 (20%)
5				✓		8 (10.3)	42 (5–59)	6:2	8 (100%)	4 (50%)	4 (50%)	2 (25)	3 (38%)	4 (50%)
6					✓	5 (6.4)	31 (18–55)	2:3	4 (80%)	2 (50%), 1 n.k.	3 (60%)	2 (40)	1 (20%)	4 (100%), 1 n.k.

Values are numbers of patients with percent in parentheses. Age is given as median with range in parentheses. M:F, male:female; CRSsNP/CRSwNP, chronic rhinosinusitis sine/with nasal polyps; conj, conjunctivitis; atopic derm, atopic dermatitis; n.k., not known.

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with polysensitization to the components rAsp f 1–4 and rAsp f 6. For asthma, the prevalence increased from 36 to 50%, while for sinusitis, when taking together both sinusitis with and without nasal polyps, the prevalence increased from 56 to 82%. However, these differences were not significant. Also, the prevalence of atopic dermatitis did not show a significant difference among groups. Further, the single values of the nasal symptom scores, which were taken from the patients' questionnaires, were summed up: patients without sensitization to any *Aspergillus* component showed a median nasal symptom score of 6 (range 0–12), patients being sensitized to one of the components rAsp f 1–4 or rAsp f 6 exhibited a median score of 7 (range 0–11), and patients being sensitized to at least 2 of the components rAsp f 1–4 or rAsp f 6 showed a median score of 8 (range 2–12), as shown in Figure 2. There was no statistically significant difference.

With regard to laboratory and clinical parameters, we also compared the following 6 groups: patients with no sensitization to components and patients being sensitized to rAsp f 1, rAsp f 2, rAsp f 3, rAsp f 4, or rAsp f 6 (Tables 4, 6). The ulterior motive was to find out if a certain component shows a characteristic serological or clinical phenotype. As the groups would have been too small for comparisons, if only patients with monosensitization to one single component had been included, we pooled all patients being sensitized to a certain component into groups. As there are patients with multiple sensitizations to components, there is a relevant number of patients being part of more than one group. Tables 4 and 6 show all relevant serological and clinical data when looking at the single components. When comparing the groups, patients without sensitization to any tested *Aspergillus fumigatus* component showed a significantly lower *Aspergillus*-specific IgE, when compared to all other groups except patients being sensitized to rAsp f 6 (rAsp f 1: $p < 0.001$, rAsp f 2: $p < 0.001$, rAsp f 3: $p = 0.012$, rAsp f 4: $p = 0.003$, and rAsp f 6: $p = 1.0$; see Table 5). Analogous to the groups above, all other comparisons regarding clinical features revealed a value of $p > 0.05$ and thus showed no statistically significant differences among groups (Table 6).

Discussion

The aim of this study was to evaluate whether component resolved diagnostic testing with rAsp f 1, rAsp f 2, rAsp f 3, rAsp f 4, and rAsp f 6 in patients with suspected AR can replace or add any medical value to the performance of extract-based diagnostic analysis with SPTand serology with *Aspergillus fumigatus* protein extract in routine clinical practice. Our study collective consisted of 78 patients with a median age of 44 years. The majority of patients were polysensitized to seasonal and/or perennial aeroallergens (Table 1). A high number of cosensitization to other fungi, nonfungal allergens, and even food allergens has been described before for molds [24, 25]. The high number of polysensitized patients makes the attribution of coexisting symptoms and diseases to the *Aspergillus fumigatus* sensitization difficult. Additionally, due to missing data on provocation testing (which is not routinely performed due to the missing recommendation for an allergen-specific immunotherapy according to German guidelines), it is not possible to differentiate between silent sensitization and allergy. There is little known about the role of *Aspergillus fumigatus* in AR [1, 5]; thus, studying both the clinical as well as the serological profile of patients with AR and sensitization toward *Aspergillus fumigatus* remains interesting.

Clinical symptoms and coexisting disease were assessed with a questionnaire. Even though this procedure might not be as precise as a full diagnostic workup, the results are well worth to be discussed. Forty-one percent of the investigated patients reported about asthma. This is a high prevalence, when compared to the German population, where a lifetime prevalence for asthma of 8.6% has been identified [26]. However, when comparing this number to the preselected group of patients presenting in our allergy department of a university hospital, it seems even quite low. All patients presenting in our allergy department from 2009 to 2013 because of AR had a prevalence of self-reported asthma of 50.3%. Also, compared with the current literature, the prevalence is rather low. In a Polish study on 1,750 patients, patients sensitized to molds, especially *Aspergillus fumigatus* and *Alternaria alternata*, were more frequently diagnosed with asthma (57%) than patients sensitized to other aeroallergens [5]. When comparing patients without sensitization to any of the tested components of *Aspergillus fumigatus* to those with sensitization to any of the components of rAsp f 1–4 or rAsp f 6, it becomes apparent that the prevalence of both asthma and sinusitis is higher in patients with sensitization to tested components, even though this difference was not statistically significant (Table 5). The overall prevalence of sinusitis in our study population was very high (62%) – higher when compared to that in the current literature (25%) [5]. The presence of sinusitis was assessed by clinical diagnosis including nasal endoscopy and questionnaires. Because of ethical considerations, due to radiation exposure, no CT-scans were performed

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to address this question and confirm the diagnosis. Therefore, especially for chronic sinusitis without nasal polyps, the reported prevalence might be overestimated. Nevertheless, an association between mold allergy and sinusitis has been extensively reported before. For many years, molds have been discussed to play an important role in causing chronic sinusitis [27]. Especially *Aspergillus fumigatus* seems to cause allergic fungal sinusitis frequently. Elevated IgE levels against native *Aspergillus fumigatus* have been detected, and hyphae resembling *Aspergillus fumigatus* have been extracted in mucin of sinusitis patients [28]. In recent years, however, the discussion is controversial, and there are more arguments that suggest that fungi might rather have a disease modifying than a causative role in chronic sinusitis with or without nasal polyps [29].

Looking at nasal symptoms, the nasal symptom score increased with increasing sensitization to tested components, reaching a maximum median of 8 (out of 12) in patients with ≥2 sensitizations to components (Fig. 2). Yet, the difference was not statistically significant. Kolodziejczyk et al. [5] found a significantly higher level of obstruction and itching in patients with allergy to molds than in patients with allergy to pollen or to multiple allergies. As we do not have a mold-negative control group, this statement can be neither confirmed nor disproved. However, both obstruction and itching increased from patients without sensitization to any of the tested components to patients being sensitized to ≥2 components. Nevertheless, it remains unclear how much the sensitization to *Aspergillus fumigatus* amounts to the total nasal symptom score, as most patients were also sensitized to other molds and/or nonfungal allergens.

As AR is an atopic disease, a higher percentage of patients also showed other diseases of the atopic spectrum (e.g., atopic dermatitis) when compared to the general population [26]. For example, 26.9% of the investigated patients showed an oral allergy syndrome. This prevalence seems reasonable, as about two-thirds of patients with birch-pollen allergy show a birch-pollen-related food allergy, and almost 40% of patients in this study were sensitized to trees [30].

Aspergillus fumigatus is an important mold causing several allergic diseases. There is a high prevalence of sensitization in the population. In a Mexican study, >50% of children with any allergic symptoms showed a sensitization toward molds. About 20% were sensitized against at least 2 different molds [31]. A high prevalence of cosensitization to other fungi was also seen in the present study (Tables 3, 4), which is in accordance with the current literature [31–33]. To our knowledge, there is no described cross-reactivity between the here evaluated components of *Aspergillus fumigatus* (Asp f 1–4 and Asp f 6) and components of *Alternaria alternata* or *Cladosporium herbarum*. However, apart from the here evaluated components, there is a big cross-reactivity between different molds (including *Alternaria alternata* and *Cladosporium herbarum*) as well as to allergens from nonfungal species, resulting in patients showing cross-reactive and not genuine sensitizations. For example, the acid ribosomal protein Asp f 8 (*Aspergillus fumigatus*), Cla h 5 (*Cladosporium herbarum*), and Alt a 5 (*Alternaria alternata*) show cross-reactivity. Cla h 8, an NADP-dependent mannitol dehydrogenase, has 75% sequence similarity with Alt a 8. Asp f 22, an enolase of *Aspergillus fumigatus*, shows cross-reactivity with Pen c 22 (*Penicillium citrinum*), Alt a 6 (*Alternaria alternata*), and Cla h 6 (*Cladosporium herbarum*) [1]. Additional to that, there are various cosensitizations also to other fungal species. Asp f 3 shows cross-reactivity to 2 peroxisomal membrane proteins of *Candida boidinii* [10], Mal f 5 (*Malassezia furfur*) [11], and Pen c 3 (*P. citrinum*) [12]. However, *Aspergillus fumigatus* does not only cross-react to other molds – Asp f 6, a manganese superoxide dismutase (MnSOD), was described as a cross-reactive allergen to Hev b 10 (*Hevea brasiliensis*), which belongs to the "latex-mold" group of latex allergens. Furthermore, it is a candidate for primary sensitization in patients allergic to the panallergen MnSOD [34]. As within this study sensitization to *Aspergillus fumigatus*, *Alternaria alternata*, and *Cladosporium herbarum* was tested with both SPT and serology to crude protein extract, a cross-reactivity is possible. Therefore, sensitization rates to *Aspergillus fumigutus* might be overestimated if only looking at SPT and serology to *Aspergillus fumigatus* protein extract.

Patients were selected to be included in this study when specific IgE toward *Aspergillus fumigatus* protein extract was elevated. Most patients also underwent SPT. This resulted positive in 65 of 71 patients. The remaining 6 patients with negative SPT were representative for the whole study population. Discrepancies between in vivo and in vitro tests have been described previously for mold allergy [35]. Eventually, an intradermal skin test in those patients suggestive for mold allergy could detect some more cases [36]. On the other hand, due to this circumstance with 6 patients being missed by SPT, it seems important to combine both SPT and in vitro test, at least with the *Aspergillus fumigatus* protein extract.

Component-resolved diagnostics would have detected 50% of the patients with elevated IgE to *Aspergillus fu-*

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migatus in this study. There are 2 possible explanations for it: firstly, as already mentioned above, there are various cosensitizations among fungi and nonfungal allergens. Thus, an elevated IgE to *Aspergillus fumigatus* protein extract or a positive SPT might be caused by cross-reactivity and not by genuine sensitization. Secondly, there might be other components of *Aspergillus fumigatus*, than the ones tested, leading to sensitizations. All patients being sensitized to more than one component showed a sensitization toward Asp f 1 (see Fig. 1). This suggests that Asp f 1 is the major allergen of *Aspergillus fumigatus* and that the additional sensitizations occur later as a result of molecular spreading. This assumption is supported by the fact that age and IgE level toward native *Aspergillus fumigatus* show a positive correlation. For the remaining cases with monosensitization to components other than Asp f 1, it is unclear if they are caused by (yet unknown) cross-reactions, as in genuine sensitization it is assumed that the sensitizations to other components follow the one toward the major allergen. This, at least, seems to be the case for pollen [37, 38]. On the other hand, Asp f 1 does not even fulfill the requirement of 50% prevalence to be called a major allergen in the present study [39].

When comparing patients without sensitization to rAps f 1–4 or rAsp f 6 to patients being either monosensitized or polysensitized (≥2) to multiple *Aspergillus* components, it becomes apparent that especially IgE levels to *Aspergillus fumigatus* protein extract are increasing. This difference was statistically significant. Also, the ratio of *Aspergillus*-specific IgE and total IgE is increasing analogous to the aforementioned, whereas the distribution of patients being mono-, oligo-, or polysensitized to various aeroallergens is similar among groups. These serological results are strong arguments for the assumption that patients without sensitization to Aps f 1–4 or Asp f 6 are (at least in parts) sensitized to the *Aspergillus fumigatus* protein extract due to cross-reactivity and not due to genuine sensitization. On the other hand, patients with sensitization to at least one of the 3 species-specific allergens Asp f 1, Asp f 2, and Asp f 4 ($n = 30$; 38.46%) can be considered as being definitely genuinely sensitized to *Aspergillus fumigatus*.

Besides the *Aspergillus fumigatus* specific IgE level, also the total IgE level is increasing throughout groups – yet, the difference was not significant (see Table 3). Vincent et al. [40] described a generally elevated total IgE level for patients sensitized to *Aspergillus fumigatus*, when compared to patients sensitized to other molds or nonfungal allergens.

When looking at the different components, it becomes apparent that patients without sensitization to rAsp f 1–4 or rAsp f 6 showed a significantly lower IgE level toward *Aspergillus fumigatus* protein extract, when compared to all other groups except patients being sensitized to rAsp f 6. Even though the ratio of *Aspergillus*-specific IgE and total IgE level increased, the difference did not reach statistical significance (Table 4). However, the attribution of clinical and serological data to single components is definitely limited, as there are, on the one hand, many patients with sensitization to ≥2 components, thus impeding selectivity, and, on the other hand, many patients with sensitizations to other fungi and nonfungal allergens.

Conclusion

The present study investigated patients with sensitization to *Aspergillus fumigatus* protein extract. There was a high prevalence of oligo- and polysensitized patients and no provocation testing available, thus making a differentiation between silent sensitization and allergy toward *Aspergillus fumigatus* impossible. This also impedes the attribution of clinical symptoms to the *Aspergillus* sensitization. Yet, from a clinical point of view, especially the high prevalence of sinusitis was noticeable, whereas the prevalence of asthma was not higher than in patients being sensitized to other aeroallergens. Regarding diagnostics, SPT missed 6 patients being positive in the in vitro diagnostics toward the crude protein extract. Component diagnostics with rAsp f 1–4 and rAsp f 6 resulted positive in 50% of patients. All patients being sensitized to more than one component showed sensitization toward the major allergen Asp f 1. However, monosensitization affected all tested components. The rather low number of patients showing a sensitization toward one of the tested components might at least partly be explained by cross-reactivity of fungal components of the *Aspergillus fumigatus* protein extract to other molds or nonfungal allergens, resulting in a relevant number of component-negative patients not being genuinely sensitized to *Aspergillus fumigatus*. Thus, the sensitivity of component-resolved diagnostics might be underestimated within this study. As Asp f 1, Asp f 2, and Asp f 4 can be accounted as specific markers for genuine *Aspergillus fumigatus* sensitization, another possible lecture of the results is that at least 30 patients (38.46%), who showed sensitization to Asp f 1, Asp f 2, or Asp f 4, were truly genuinely sensitized to *Aspergillus fumigatus*. To conclude, in the diagnostical workup of patients with AR and sensitization to *Aspergil-*

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lus fumigatus, extract-based diagnostic analysis with SPT and serology remain the methods of choice. Component-resolved diagnostics, especially toward the species-specific allergens Asp f 1, Asp f 2, and Asp f 4, might be useful to differentiate cross-reactive from genuine sensitization. Thus, component-based diagnostics in the context of AR should be reserved as an add-on for special questionings beyond routine clinical practice.

Statement of Ethics

The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The study was approved by the local ethics committee and the local data protection commissioner. All patients gave written informed consent.

Conflict of Interest Statement

D.G. has received speaker honoraria from ALK-Abelló and Bencard Allergy and financial support for attending symposia from HAL Allergy, Phadia diagnostics, and Shire. M.G. has received speaker honoraria and financial support for attending symposia from ALK-Abelló, Allergopharma, Bencard Allergy, HAL Allergy, Phadia diagnostics, Shire, and Stallergenes and is a member of the advisory board of ALK-Abelló. However, all these potentially conflicting interests did not have any influence on the study. There were no further financial or nonfinancial competing interests.

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The authors have no funding sources to declare.

Author Contributions

V.V. has analyzed and interpreted data and drafted the manuscript. J.L. has analyzed and interpreted data. M.G. was responsible for the conception of the work and interpretation of data. All authors acquired data, revised the manuscript critically, and gave final approval for publication. All authors agree to be accountable for all aspects of the work.

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