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ORIGINAL RESEARCHES

Sensitization to Fungal Allergens in Patients with Respiratory Allergy – Accuracy in Diagnostic Process

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Introduction. In Bulgaria, mold allergies are common, and sensitization to different fungal species is found in many patients with atopy.
The objective of this study is to explore sensitization to the most widespread mold species in Bulgaria, and to determine the extent of sensitization to Alt a1, a major allergen of *Alternaria alternata*, by using component-resolved diagnosis.
Materials and Methods. 21 patients (14 males and 7 females, age range 5–40 years), with respiratory allergy participated in the study. All patients are sensitized to mix of fungal allergens containing: *Alternaria alternata, Aspergillus sp., Cladosporium herbarum, Penicillium notatum,* proved by *in vivo* and *in vitro* methods. All patients underwent the ImmunoCAP test and were assessed for sIgE to individual fungal allergens: m1 *Penicillium notatum,* m2 *Cladosporium herbarum,* m3 *Aspergillus fumigatus* and m6 *Alternaria alternata.* The component-resolved diagnosis to Alt a1 was performed for 10 patients with increased sIgE to m6 *Alternaria alternata*.
Results. All studied patients (100%) had elevated sIgE to *Alternaria alternata.* Eight (38%) patients were sensitized to *Penicillium notatum.* 11 (52%) and 10 (48%) patients were sensitized to *Cladosporium herbarum* and to *Aspergillus fumigatus*, respectively. Ten patients (48%) were monosensitized to *Alternaria alternata*. Nine (90%) patients with sensitization to *Alternaria alternata* demonstrated elevated levels of IgE to Alt a1.
Conclusion. *Alternaria alternata* most often causes sensitization in patients with respiratory allergy. The component-resolved diagnosis using Alt a1 is a precision marker to prove species-specific sensitization to *Alternaria alternata.*

Keywords: *mold allergy; allergy to Alternaria alternata; Alt a1 component-resolved diagnosis.*

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Сенсибилизация к аллергенам плесневых грибов у пациентов с респираторной аллергией. Оптимизация диагностического процесса

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Актуальность. В Болгарии часто встречается аллергия на плесневые грибы, сенсибилизация к различным аллергенам грибов обнаруживается у многих пациентов с атопией.
Цель данного исследования — установить чувствительность к наиболее распространенным видам плесневых грибов в Болгарии и определить с помощью компонентной диагностики наличие сенсибилизации к Alt a1 — одному из главных аллергенов *Alternaria alternata*.
Материалы и методы. В обследование включен 21 пациент с респираторной аллергией в возрасте 5–40 лет. Сенсибилизация к микстам грибковых аллергенов, содержащих *Alternaria alternata, Aspergillus sp., Cladosporium herbarum, Penicillium notatum,* у всех пациентов доказана посредством диагностики *in vivo* и *in vitro*. Кроме того, все пациенты тестированы с помощью «ImmunoCAP» на наличие аллергенспецифических IgE к отдельным грибковым аллергенам: m1 *Penicillium notatum,* m2 *Cladosporium herbarum,* m3 *Aspergillus fumigatus* и m6 *Alternaria alternata.* У 10 из обследованных пациентов с повышенным sIgE к m6 *Alternaria alternata* проведена компонентная диагностика с помощью Alt a1.

Результаты. Все обследованные пациенты имели повышенный уровень sIgE к *Alternaria alternata. И*з них 10 (48%) пациентов проявили чувствительность только к *Alternaria alternata*. У 8 (38%) пациентов выявлена сенсибилизация к *Penicillium notatum*, у 11 (52%) — к *Cladosporium herbarum* и у 10 (48%) — к *Aspergillus fumigatus;* 9 (90%) пациентов с сенсибилизацией к *Alternaria alternata* имели повышенный уровень IgE к Alt a1.

Выводы. *Alternaria alternata* чаще других видов плесневых грибов вызывает сенсибилизацию у пациентов с респираторной аллергией в Болгарии. Компонентная аллергодиагностика с использованием Alt a1 — главного аллергена плесени — дает полное доказательство видоспецифической сенсибилизации к *Alternaria alternata.*

Ключевые слова: *аллергия на плесневые грибы; аллергия на Alternaria alternata; компонентная диагностика с помощью Alt a1.*

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Introduction

Fungi are major components of environmental bioaerosols; many mold allergens have been associated with allergic diseases in humans, including allergic rhinitis, conjunctivitis, bronchial asthma and allergic broncho-pulmonary mycoses. Thus, molds have a far greater impact on people's immune system than pollen or other allergenic sources [1].

In Bulgaria, due to favorable climatic conditions, mold allergy is common, and sensitization is found in many patients with atopy. The main mold species, which most often cause sensitization in our country, are: *Cladosporium, Alternaria, Penicillium* and *Aspergillus*. Our previous findings show that at least 6 % of the adults and children evaluated in our department over the last 5 years are mono-sensitized to fungal allergens [2, 3].

Sensitization to fungi can be detected through skin prick and intradermal tests with fungal extracts or in vitro tests for specific IgE antibodies. Unfortunately, cross-reactivity among molds is a commonly observed phenomenon. It reduces the accuracy of the mold allergy diagnosis; therefore, clear algorithms for accurate diagnostic process are required [4–5].

To overcome limitations typical of standard allergy diagnostics relying on crude allergen extracts, European allergists have turned to molecular or component-resolved diagnostics (CRD) using individual recombinant or native allergenic molecules tested on a florescence enzyme immunoassay (ImmunoCAP) or a microarray-based assay platform [6].

The objective of this study is to explore sensitization to the mold species most common in Bulgaria, and to determine the extent of sensitization to Alt a 1 – the major allergen from *Alternaria alternata* using CRD.

Materials and methods

21 patients (14 males and 7 females, age range 5–40 years) participated in the study. All patients suffered from clinically manifested respiratory allergy. Seven of them had allergic rhinitis, 9 patients had bronchial asthma and 5 – bronchial asthma and allergic rhinitis.

All patients had a positive allergy skin test to EI Mixed fungal allergen (Bul Bio NCIPD) containing: *Alternaria alternata, Aspergillus sp., Cladosporium herbarum, Penicillium notatum*.

All patients had elevated allergen-specific IgE (sIgE) – class 2–4 when tested *in vitro* by ImmunoCAP with mx1 Mold mix (Phadia) containing *Penicillium notatum, Cladosporium herbarum, Alternaria alternata* and *Aspergillus fumigatus.*

The patients participating in the study were tested using ImmunoCAP for sIgE to the following individual fungal allergens: m1 *Penicillium notatum,* m2 *Cladosporium herbarum,* m3 *Aspergillus fumigatus* and m6 *Alternaria alternata* (Phadia). The study was performed according to the manufacturer's instructions. The amount of allergen-specific sIgE was calculated in KUA /l. The levels above 0.35 KUA /l were considered as elevated.

10 patients with elevated sIgE to m6 *Alternaria alternata* (5 with monosensitization and 5 with polysensitization to the other studied mold allergens) were evaluated for sIgE to m229 Alt a 1 using ImmunoCAP according to the manufacturer's instructions. The amount of sIgE was calculated in KUA/l, with levels above 0.35 KUA/l being considered as elevated.

The results were statistically processed by using the GraphPad Prism 6.0 software package (GraphPad Software, Inc.). The mean (M) and standard error of

the mean (SEM) were calculated. Spearman's rank-order correlation test was used to measure the strength and direction of the association between two ranked variables – sIgE to m6 *Alternaria alternata* and sIgE to m229 Alt a 1. P values of <0.05 were considered statistically significant.

Results

Summarized results for the sIgE levels to individual allergen extracts from the studied mold species are shown in **Fig. 1**.

All 21 patients (100%) had elevated IgE to *Alternaria alternata*, with the measured amount averaging 15.84 ± 8.52 KUA/l. Eight (38%) patients were sensitized to *Penicillium notatum* with an amount of sIgE of 1.46 ± 0.73 KUA/l. 11 (52%) patients were sensitized to *Cladosporium herbarum*, and 10 (48%) patients were sensitized to Aspergillus fumigatus, with amounts of IgE 2.79 ± 1.83 and 3.08 ± 2.31 KUA/l, respectively.

Based on the analysis of the data for every patient we identified the following profiles of sensitization (**Table**).

According to the results, two main groups of sensitization were identified. Ten (48%) patients were monosensitized to *Alternaria alternata.* The other 11 patients (52%) were sensitized to more than one fungal species and 8 (38%) patients had sIgE to all four tested mold allergens.

Mono-component resolved diagnosis using a recombinant Alt a 1 allergen (rAlt a 1) was performed for 10 patients with sensitization to *Alternaria alternata* (5 with monosensitization and 5 with polysensitization). The results of the study are shown in **Fig. 2**.

The data show that 90% (n=9) of the studied patients with sensitization to *Alternaria alt.* had elevated levels of IgE to rAlt a 1. The Spearman's rank-order correlation test showed a high (rs = 0.9152), positive and statistically significant (p = 0.0003) correlation between serum levels of IgE to *Alternaria* and those to rAlt a 1 in the studied patients.

Discussion

Surveys conducted in various parts of the world demonstrated that sensitization to fungi is common, particularly in patients with respiratory allergy. The exact prevalence of mold sensitization is not known but is estimated to range from 3% to 10% in the general population [7]. The 4 genera most commonly associated with the development of mold allergy are: *Alternaria*, *Cladosporium*, *Penicillium*, and *Aspergillus* [8].

Many studies proved that *Alternaria alternata* is one of the most important allergenic molds in Europe, and up to 70% of mold-allergic patients show positive skin test to *Alternaria alternata*; it is known to be a risk factor for development of asthma [7-9].

Our study also demonstrated that most of the patients with mold allergy were sensitized primary to *Al-*

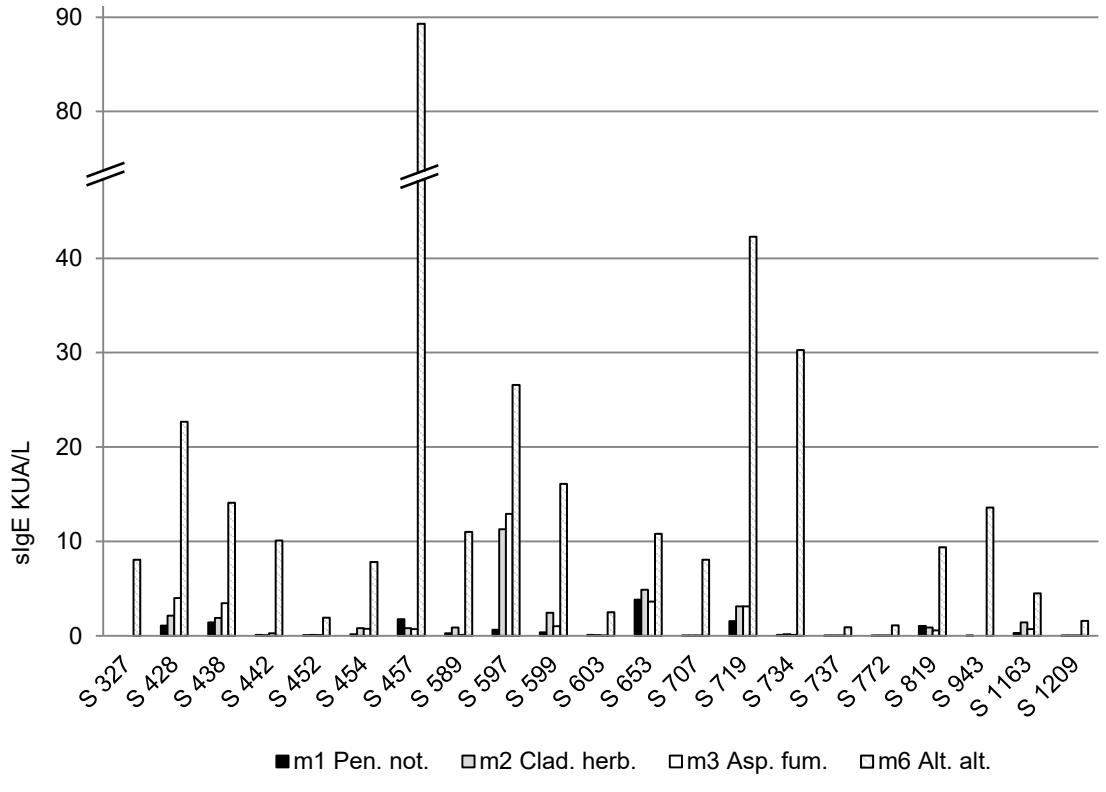

Fig. 1. Determination of sIgE to individual allergenic mold extracts

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Sensitization profiles to the studied fungal allergens in patients with respiratory allergy

No.	Sensitization profile				Number of patients	
	Pen. not.	Clad. herb.	Asp. fum.	Alt. alt.	abs.	%
1	–	–	–	+	10	48
2	+	+	+	+	8	38
3	–	+	+	+	2	9,5
4	–	+	–	+	1	4,5

ternaria alternate, and 48% of patients had monosensitization to *Alternaria alternata.* The other 52% of patients were sensitized to more than one fungal species and 38% of patients had sIgE to all four tested mold allergens. These findings confirm the observation of many researchers that patients allergic to moulds are usually sensitised to several species [3, 4, 8].

Diagnosis of mold allergy, as routinely performed today, is a stepwise process, including anamnesis, determination of total and allergen-specific IgE antibodies, skin tests and, if necessary, other provocation tests [10].

To improve the accuracy of contemporary diagnosis of mold allergy, allergists use CRD with individual recombinant or native allergenic molecules more and more frequently .

Alt a 1 is an allergen molecule that is recognized by IgE antibodies in 80–90% of Alternaria allergic individuals [11]. Thus, Alt a 1 can be seen as a major allergen of *Alternaria alternata*, to which patients are primarily sensitized. As the Alt a 1 molecule is specific only to this fungal species, sensitization to this allergen is defined as species-specific for *Alternaria alternata* [12–13]. Thus, the existing sensitization to Alt a 1 can be used as a precision marker to prove allergy to this fungal species [14–15].

Our results from mono-component resolved diagnosis using a rAlt a 1 show that 90% of the studied patients with sensitization to *Alternaria* have elevated levels of IgE to rAlt a 1, and there is a high (rs = 0.9152), positive and statistically significant (p = 0.0003) correlation between serum levels of IgE to *Alternaria* and those to rAlt a 1.

Recently, the study including 80 European patients showed that rAlt a 1 can be used to diagnose 98% of patients with allergy to Alternaria alternata and that almost all specific IgE in these patients were directed against Alt a 1 [16].

This finding suggests that Alt a 1 can be used as a reliable diagnostic marker allergen for genuine sensitisation to Alternaria and could be alternative to the Alternaria extract in diagnostic panels *in vitro*.

The natural allergenic extract of *Alternaria alternata* also contains other allergenic molecules, some of which can also be identified as major allergens [17]. These are, for example, Alt a 2 (aldehyde dehydrogenase), a species-specific allergen, and Alt a 5 (enolase) – panallergen, which is found in a number of fungal allergenic extracts [18].

The cross-reactivity of *A. alternata* with other airborne fungal species has been extensively described [19] and there is evidence that a significantly high percentage of patients sensitized to *A. alternata* are poly-sensitized to more than one other fungal species and might also be sensitized to other environmental aeroallergen sources such as pollens, mites or even to food allergens [8]. Therefore, it can be assumed that the presence of such a high percentage of patients poly-sensi-

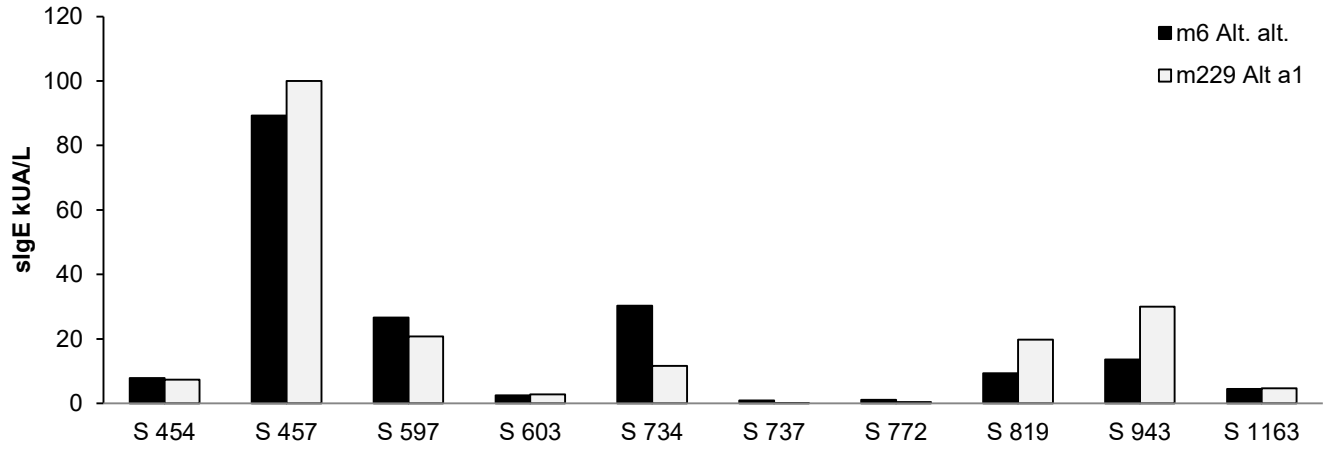

Fig. 2. Comparison of the amounts of sIgE to crude extract of Alternaria alternata (m6) and results of CRD with rAlt a 1 in patients with respiratory allergy.

tized to different mold species in the present study can be explained by cross-reactivity.

Conclusion

Alternaria alternata most often causes sensitization in patients with respiratory allergy. The levels of allergen-specific IgE to this fungal species are the highest. Moreover, this fungal species is the most common cause of the monosensitization.

The component-resolved diagnosis using rAlt a 1 can be used as a precision marker to prove species-specific sensitization to *Alternaria alternata* and could be alternative to the use of *Alternaria* extract in diagnostic panels *in vitro*.

The identification and characterization of the whole array of *Alternaria* allergens and new techniques based on allergenic recombinant proteins will contribute to preparation of higher quality test solutions to improve *Alternaria* allergy diagnosis.

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