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Bayesian analysis suggests independent development of sensitization to different fungal allergens

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ABSTRACT

Background: Fungi are known for their ability to cause allergies, but data on individual sensitization to them are insufficient. The purpose of the study was to carry out a comprehensive analysis of the fungal allergens' sensitization profile in the Ukrainian population and to determine both population and individual sensitivity to these allergens.

Methods: We utilized a set of ALEX allergy test data from 20,033 inhabitants of 17 regions of Ukraine from 1 to 89 years conducted in 2020–2022. A complex of programs in the Python language was developed and Bayesian network analysis was applied to determine the sensitivity combinations in individual patients to various fungal components.

Results: Sensitivity to Alt a 1 dominated and was observed in 79.39% of patients, and 62.17% of them were sensitive solely to Alt a 1. Exclusive sensitivity to Mala s 6 was second in individual patient profiles with a frequency of 4.06%. Combined sensitivity to Alt a 1 – Asp f 3 was third with a share of 3.28%. Pen ch and Cla h extracts stimulated the production of the lowest median sIgE levels. The highest median sIgE levels were for Alt a 1, Mala s 11 and Asp f 6, respectively. Median sIgE levels increased in adults compared to children for all components of *Aspergillus fumigatus*, as well as for Mala s 5 and Mala s 11. In the rest of the cases, they decreased in adults compared to children. The sensitization rates to fungi in general and specifically to *Alternaria* were lower in the western parts of Ukraine, especially in the Carpathian region, situated within the Broad-leaved Forest zone. The results of Bayesian modeling revealed that in the case of Alt a 1, the simultaneous absence of sensitivity to Cla h 8, Mala s 11, Mala s 5 and Mala s 6 molecules could condition the presence of sensitization to the major *Alternaria* allergen with a probability of 92.42%. In all other cases, there was a high probability of absence of sensitivity to particular allergen against the background of absence of sensitivity to other ones, which may indicate the independent development of sensitization to different fungal allergens.

Conclusions: Sensitivity to Alt a 1 dominated in the studied population with a lower rate in the western regions. The highest median sIgE levels were induced by Alt a 1, Mala s 11 and Asp f 6. Bayesian Analysis suggest a high probability of the independent development of sensitization to

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different fungal allergens. The idea that sensitization to one allergen may be protective against sensitization to another one(s) requires further clinical study.

Keywords: Fungal sensitization, *Alternaria*, *Malassezia*, Component-resolved allergy diagnostics, Bayesian modelling

INTRODUCTION

The ability of fungi to cause respiratory tract allergic diseases has become well known in recent years. In particular, fungal allergens can cause an allergic inflammatory response of the lung epithelium,[1] which, in turn, can lead to the development of asthma.[2] What is more, fungal sensitization worsens asthma control[3,4] and severity.[5] Due to the fact that fungi are ubiquitous by their nature,[6] the contact with them is often associated with inflammatory diseases of the respiratory tract, in particular, chronic rhinosinusitis,[7] or keratitis[8] or allergic bronchopulmonary mycoses, allergic fungal sinusitis, and hypersensitivity pneumonitis.[9] Sensitization to fungal spores, along with pollen and house dust mite sensitivity, is defined in the literature as one of the main triggers of allergic diseases.[10,11] Apart from fungal spores, mycelium can cause allergic reactions as well.[12] Similar to fungal spores, it can be present in the air or on human skin. According to research, the allergenicity of fungi depends on the type of fungus, the way and time of its influence on humans.[13]

Nowadays, it is believed that more than 112 fungus genera are associated with the development of allergic sensitization. They belong to 3 fungal phyla specifically relevant to allergic reactions: Zygomycota, Ascomycota, and Basidiomycota.[14] The World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database[15] isolates 120 molecules as allergic reactions triggers, 95 or 79.2% of which belong to the Ascomycota division; 23 molecules (19.2%) belong to the Basidiomycota division and 2 molecules (1.7%) belong to the Zygomycota division.

Data indicate that the following 4 genera are most often associated with the occurrence of allergies: *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus*.[16–18] All of them belong to Ascomycetes. The mentioned Allergen Nomenclature database identifies 12 allergenic molecules of *Alternaria alternata* (Alternaria plant rot fungus); 38 molecules of 6 species of the genus *Aspergillus*, 30 of which belong to *Aspergillus fumigatus* (Common mold),[19] 10 allergens of the genus *Cladosporium*, 8 of which belong to *Cladosporium herbarum* (Plant Fungus or "black mold"), 17 – allergens of the genus *Penicillium* (mainly *Penicillium chrysogenum* and *Penicillium citrinum*).[20] The same source mentions *Malassezia sympodialis* (skin-colonizing yeast) among the fungal allergens with the allergens Mala s 5, Mala s 6, and Mala s 11. The last species is a representative of the Basidiomycota division and is a factor in opportunistic skin infections.[21]

Among the named allergenic molecules, Alt a 1[22,23] belongs to the major allergens, which, in addition to sensitization, can cause chronic allergic asthma.[24] Instead, *Cladosporium*, in addition to allergic rhinitis, can cause the development of pneumonia.[25,26]

However, the issue of characteristics of acquisition and development of both mono- and poly-sensitization to fungal allergens remains studied insufficiently.[24] Therefore, the purpose of the presented study was to carry out a comprehensive analysis of the sensitization profile to fungal allergens in the Ukrainian population; to determine both population and individual sensitivity to these allergens; to establish possible interrelationships between contributory fungal allergens in their ability to promote the development of sensitization, as well

as to analyze sensitization to fungi in people of different age groups.

METHODS

Participants

We utilized a set of ALEX allergy test data from 20,033 inhabitants of 17 regions of Ukraine from 1 to 89 years conducted in 2020–2022.

The study analyzed the data from the patients with a history of allergic rhinitis and/or atopic dermatitis and/or asthma.[27-29] The absence of the mentioned diseases in the anamnesis and the absence of sensitization to fungal allergens were exclusion criteria for the study. We did not aim to collect and analyze too detailed of symptoms of patients as it would be too much to implicate both detailed symptom data and sensitization data for more than 20,000 people in one article. The only data which are available so far for the fungi-sensitive patients of these samples is that 77% of children and 82% of adults were diagnosed with allergic rhino-conjunctivitis, 40% of children and 29% of adults had asthma. Atopic dermatitis was seen in 9% and 5% accordingly. Several pathologies could be observed in 1 person. As we are certainly sure that absence of the detailed symptom analyses was the limitation of our study, we are going to pay more attention to the symptom description in our further papers.

The doctor, to whom the patient presented with the above-mentioned diseases by themselves, made a decision about the appointment of a multicomponent molecular allergy diagnostics for this patient, based on the protocols of the Ministry of Health of Ukraine on the diagnosis and treatment of allergic diseases. This is, in particular, the Unified clinical protocol of primary, secondary (specialized), tertiary (highly specialized) medical care for atopic dermatitis; the Unified clinical protocol of primary, secondary (specialized) medical care for bronchial asthma, Evidence-based clinical guidelines for asthma ARIA, Guidelines on Allergic Rhinitis and its Impact on Asthma (2016 Revision), approved in Ukraine at an extended meeting of state experts in accordance with the orders of the Ministry of Health of Ukraine.

The outcomes of the ALEX test conducted at the DIVERO medical center, Kyiv, Ukraine, were analyzed. According to Alex Diagnostics Ukraine LLC, the exclusive dealer of the ALEX test in Ukraine, a total of 45,700 ALEX tests were performed in Ukraine during the mentioned research period. Data from 43.84% of them were used for analysis in this study.

The aim of the presented study was not to collect and analyze the detailed symptoms of the patients and compare them with the results of the molecular tests. Instead, the main emphasis was placed on the analysis of the epidemiological patterns of age-related, individual and regional sensitization of the population of Ukraine to fungal allergens, as well as factors that can affect this sensitivity.

Variables and data sources

During the study, the sensitization of each patient to the following components of the multiplex allergy test ALEX was determined: allergens *Aternaria alternata* Alt a 1 (major allergen, class not defined) and Alt a 6 (enolase); *Apergillus fumigatus* – Asp f 1 (representative of the mitohyline family), Asp f 3 (peroxisomal protein), Asp f 4 (class not defined), Asp f 6 (manganese superoxide dismutase); *Cladosporium herbarum* – Cla h (extract), Cla h 8 (mannitol dehydrogenase); *Malassezia sympodialis* – Mala s 5 (class not determined), Mala s 6 (cyclophilin), Mala s 11 (manganese superoxide dismutase), as well as *Penicillium chrysogenum* (Pen ch) and *Saccharomyces cerevisiae* (Sac c) extracts. The classes of the allergen molecules are specified according to The World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database.[15]

According to the reference values of the ALEX test, the sensitization threshold was determined at the level of 0.31 kU/L.

To determine age-related IgE reactivity to fungal spores, sensitization was analyzed in groups from 0 to 1 year, 2–3, 4–6, 7–12, 13–18, 19–25, 26–36, 37–44 and 45–60 years old. The division of children by age (up to 18 years) was based on the generally accepted classification, which distinguishes the period of infancy to 1 year, the period of first childhood from 1 to 3 years, preschool – up to 6 years, primary school age (from 6 to 12 years), high school or adolescence (from 12 to 18 years old).

The selected adult age values corresponded to the age of the beginning and end of youth (25 and 44 years, respectively) and the end of middle age (60 years).[30] In addition, the age of 36 years was taken as an intermediate for the analysis.

Data analysis

An algorithm was used to process the input data of patients, which, using sorting, removed from the input sample the records with the value of the sensitivity level to each of the allergens was not lower than the established threshold of 0.31 kU/L. Thus, we obtained a set of patients sensitive to fungal allergens.

The distribution of these patients by age, by the specific IgE (sIgE) level to the above-mentioned fungal spores' components, and by the of sensitivity level to these components in separate age groups was calculated using descriptive statistics in MS Excel 2013.

A complex of programs in the Python language was developed and applied to determine the sensitivity combinations in individual patients to various fungal components. Bayesian network (BN) analysis was used to determine the probability of developing combined sensitivity in individual patients to molecular components[31] of fungi. The significance of using BN is its ability to identify relationships between various factors that may not generally be obvious even to an expert in a particular subject area. Constructing a Bayesian network requires two steps: building a directed acyclic graph (a graph in which there are no paths that start and end at the same vertex) and estimating the distribution at each available node. Each constructed graph allows you to draw a conclusion about the probability of the occurrence of a certain event. In our case, the possibility of developing sensitivity to a certain allergen is analyzed depending on the presence of sensitivity to others. By identifying directional connections among discrete nodes represented by individual molecular components, the Bayesian network allows for the identification of so-called parent, leading nodes that influence other nodes dependent on them.[32]

In our case, a directed acyclic graph was constructed with respect to the Alt a 1 allergen and using the bnlearn library (a library for working with Bayesian networks for the Python language), the values of the conditional probability distributions (CPD) of the connections between individual allergens for each patient and for each investigated component were calculated.

RESULTS

Characteristics of the patients

Sensitivity to at least 1 fungal allergen was established in 3349 patients (2607 children under the age of 18 vs 742 adults), which was 16.71% of the total number of tested persons. The percentage of sensitized children (77.84%) was 3.51 times higher than the one of adults (22.16%). Most of the patients, 94.45% of them, were sensitive to 2 and more allergens, always from a non-fungal group, usually it included pollen and or Fel d 1; the rest 5.55% were sensitive to Alt a 1 only.

The distribution patterns of variables in the data set for each allergen were different from normal (see Appendices), and the median age of the fungal sensitized group was 10 years. The group of children aged 0–8 was the largest in the entire sample. In the group of children, the age of 5–8 years prevailed. Among the adults, the age group of 18–24 years prevailed (Fig. 1).

Characteristics of sensitization to fungal allergens in the studied sample

In the profiles of the patients included in the study, sensitization to the allergen Alt a 1 prevailed. Sensitivity to it was observed either alone or in combination with other allergenic molecules in 2659 (79.39%) patients sensitive to fungal allergens. Additionally, in children this percentage was higher (84.23%), and in adults it was comparable to the value for the entire sample. Alt a 1 was also the component with the highest proportion of patients (62.17%) sensitized solely to this fungal allergen. Separately, in children, the share of those sensitized only to Alt a 1 was slightly higher than this value, and for adults it decreased to 49.73%.

The share of people sensitized to other allergens was much smaller. Allergens Mala s 6 and Cla h 8 with values slightly exceeding 10% for the total group were the next after Alt a 1 in terms of the number of people sensitized to them. In the group of children, the same allergens were in the second

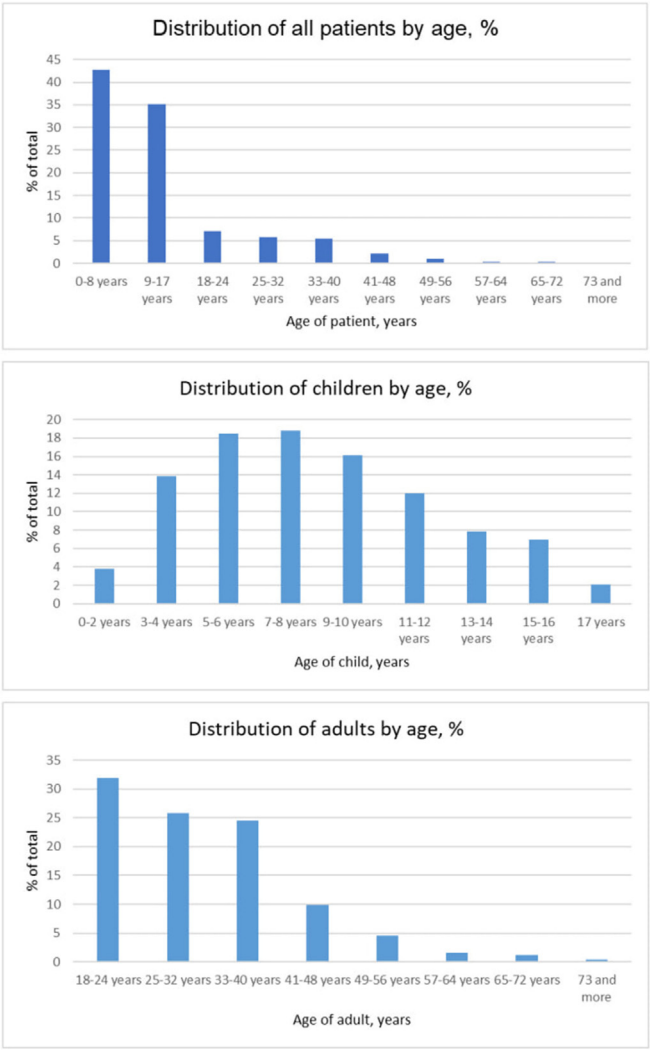

Fig. 1 Distribution of the studied sample by age

and third positions with the number of sensitized also approximately 10%, but in adults, Mala s 11 and Mala s 6 occupied the second and third places respectively with the number of sensitive persons more than 12%.

Allergens of *Malassezia sympodialis* Mala s 6 and Mala s 5 stood next after Alt a 1 in the number of people in the total sample who were sensitive only to this fungal allergen. However, the percentage of such people was low – 4.06% and 2.72%, respectively. Moreover, more than half of patients sensitive to Mala s 5 were sensitized only to this fungal allergen. Mala s 6 and Mala s 5 ranked second and third in the number of children sensitized only to these allergens. Mala s 6 and Cla h 8 took the similar positions for the adults.

Members of the general sample and children were the least sensitized to Pen ch extract and to Asp f 1. In the group of adults, it was Alt a 6 and Pen ch, respectively. Moreover, among children there was no one sensitized solitary to Asp f 1 and only one child was sensitive solitary to Asp f 6. Among adults, the least number of those who were sensitive solitary to this fungal allergen was observed for Asp f 1 and for Sac c.

Pen ch and Cla h extracts were the allergenic fungal components that stimulated the production of the lowest median sIgE levels in the total sample, as well as separately in the adult group and in the children group. In contrast, the highest median sIgE levels in the overall group, as well as in the child and adult groups, were for Alt a 1, Mala s 11

and Asp f 6, respectively. Median sIgE levels increased in adults compared to children for all components of *Aspergillus fumigatus*, as well as for Mala s 5 and Mala s 11. These levels were comparable for Pen ch, in the rest of the cases they decreased in adults compared to children (Table 1).

In all age groups, except those older than 60 years, sensitivity to Alt a 1 prevailed quantitatively. People older than 60 years were sensitive only to Asp f 1 and Cla h 8. In contrast, children aged 1 year, except for Alternaria, were sensitized to a significant number of molecules. The descending order of the number of sensitized children were the following, respectively, Mala s 6, Cla h 8, Mala s 11, Asp f 6, Mala s 5, Asp f 4, and Asp f 1 (Fig. 2).

The residence region was determined for 1765 patients, which was 52.64% of people sensitive to fungi. In all regions, except Transcarpathia (Uzhgorod), sensitivity to *Alternaria* prevailed (Table 2). Using regional data, we determined the quartiles for its distribution and created maps of regional sensitivity of tested population to fungi in general and to *Alternaria* in particular. It was established that sensitization rates in both cases were lover in the western parts of Ukraine, corresponding with the Carpathian region located in the Broad-leaved Forest zone. General tendency to higher sensitization to Alternaria was also seen in the most Southern Steppe zone of Ukraine (Fig. 3).

As we have already noted out, the molecular component Alt a 1 was the undisputed leader in terms of representation in the individual profile of patients. Sensitivity to it solely was observed in 62.17% of test subjects. The people who were sensitive only to Mala s 6 took the second place (4.06%) in the total sample. The next most frequent combinations in individual profiles were the ones of Alt a 1-Asp f 3 and Alt a 1-Cla h 8. The list of the most frequently encountered combinations also included sensitization separately to Mala s 5, Cla h 8 and Mala s 11, the frequency of which ranged from 2.72% to 1.52%, respectively. The frequency of sensitization separately to each of the following components: Asp f 4, Asp f 3, Alt a 6, Sac c and Cla h was also among the most frequent. However, it did not exceed 1% (Fig. 4).

Analysis of probabilistic relationships between sensitization to various molecular components of fungi

Analysis of Bayesian modeling data showed that sensitization to some fungal components is mostly conditioned by the absence of sensitization to others. Alternaria was an exception to this rule. In particular, in the case of Alt a 1, the lack of sensitivity to the molecules Cla h 8, Mala s 11, Mala s 5 and Mala s 6 could determine the presence of sensitization to the major *Alternaria* allergen. In the absence of sensitization to the former ones, the CPD of sensitivity to Alt a 1 was 92.42%.

In other cases, we can speak about the absence of sensitivity to some allergens against the background of the absence of sensitivity to others. For example, absence of sensitization to Alt a 1 determined 78.63–80.69% of the CPD of absence of sensitivity to Asp f 4 (together with absence of sensitivity to Asp f 3), Asp f 1 (together with absence of sensitivity to Asp f 4) and Alt a 6 (together with absence of sensitivity to Cla h 8).

Similarly, absence of sensitization to Asp f 6 entailed a high probability (89.69%) of absence of sensitivity to Mala s 11.

There was a high probability (91.86%) of absence of sensitivity to Cla h extract against the background of absence of sensitivity to Pen ch and Sac c extracts. On the other hand, Cladosporium extract regulated sensitivity to Cla h 8, Mala s 6 and Asp f 3: against the background of absence of sensitivity to Cla h, the probability of absence of sensitization to Cla h 8 was 85.07%, to Mala s 6–83.36%, and to Asp f 3–86.68%. The CPD of absence of sensitivity to Sac c (89.86%) and Mala s 5 (88.47%) was regulated by the absence of sensitivity to Mala s 11 (Supplement, link to dataset for BN construction). Asp f 6 was the only allergen to which sensitization developed independently of others (Fig. 5).

DISCUSSION

The absence of sensitization to a certain fungal allergen against the background of a high probability of the absence of sensitization to other allergens of this group may indicate the independence of the occurrence of sensitivity to

Name of the allergenic component/ biochemical name	Median/ IQR of sIgE in sensitive individuals, kU/L	Number (%) of patients sensitive to the allergen	Number (%) of patients sensitive to this fungal allergen only	Median/ IQR of sIgE in the group of children, kU/L	Number (%) of children sensitive to the allergen	Number (%) of children sensitive to this fungal allergen only	Median/ IQR of sIgE in the group of adults, kU/L	Number (%) of adults sensitive to the allergen	Number (%) of adults sensitive to this fungal allergen only
Alt a 1/class is unknown	20.90/27.99	2659 (79.30)	2082 (62.09)	23.80/ 28.53	2196 (84.11)	1713 (65.61)	10.35/ 22.02	463 (62.40)	369 (49.73)
Alt a 6/enolase	1.45/2.80	126 (3.76)	22 (0.66)	1.60/2.96	99 (3.79)	14 (0.54)	1.20/1.34	27 (1.03)	8 (1.08)
Asp f 1/mitogilins	0.88/1.32	36 (1.07)	14 (0.42)	0.50/1.19	13 (0.50)	0 (0.00)	1.04/1.31	23 (3.10)	14 (0.34)
Asp f 3/Peroxisomal protein	0.82/1.27	228 (6.80)	27 (0.81)	0.81/0.84	164 (6.28)	6 (0.23)	0.93/1.91	64 (8.63)	21 (2.83)
Asp f 4/class is unknown	1.06/1.77	70 (2.09)	29 (0.86)	0.98/1.66	51 (1.95)	24 (0.92)	1.60/3.87	19 (2.56)	5 (0.67)
Asp f 6/Mn superoxidedismutase	3.06/7.89	133 (3.97)	10 (0.30)	2.25/5.91	68 (2.60)	1 (0.04)	4.63/8.51	65 (8.76)	9 (1.21)
Cla h/Extract	0.94/0.54	177 (5.28)	15 (0.45)	0.55/0.51	132 (5.06)	9 (0.34)	0.47/0.64	45 (6.06)	6 (0.81)
Cla h 8/ Magnitoledehydrogenase	1.00/1.96	343 (10.23)	76 (2.27)	1.12/2.23	260 (9.96)	47 (1.80)	0.77/1.16	83 (11.19)	29 (3.91)
Mala s 5/class is unknown	0.87/2.29	165 (4.92)	91 (2.71)	0.63/0.65	107 (4.10)	65 (2.49)	1.92/6.39	58 (7.82)	26 (3.50)
Mala s 6/Cyclophilin	0.86/1.55	354 (10.56)	134 (4.00)	0.96/1.51	261 (10.00)	94 (3.60)	0.64/1.45	93 (12.53)	40 (5.39)
Mala s 11/Mn superoxidedismutase	4.01/14.12	187 (5.58)	51 (1.52)	3.49/ 11.39	93 (3.56)	26 (1.00)	4.61/ 17.00	94 (12.67)	25 (3.37)
Pen ch/Extract	0.54/0.41	42 (1.25)	6 (0.18)	0.53/0.35	28 (1.07)	5 (0.19)	0.56/0.40	14 (1.89)	1 (0.13)
Sac c/Extract	0.70/0.92	104 (3.10)	16 (0.48)	0.70/0.79	72 (2.76)	12 (0.46)	0.67/1.33	32 (4.31)	4 (0.54)

Table 1. Number, percentage and median sIgE values of patients sensitized to molecular components of fungi ***Note:*** *The median tIgE level for the entire fungi-sensitized group was 146.00 kU/L, IQR – 363.50; The median level of tIgE in children was 149.00 kU/L, IQR – 380.50; median tIgE level in adults – 133.62 kU/L, IQR – 293.50.*

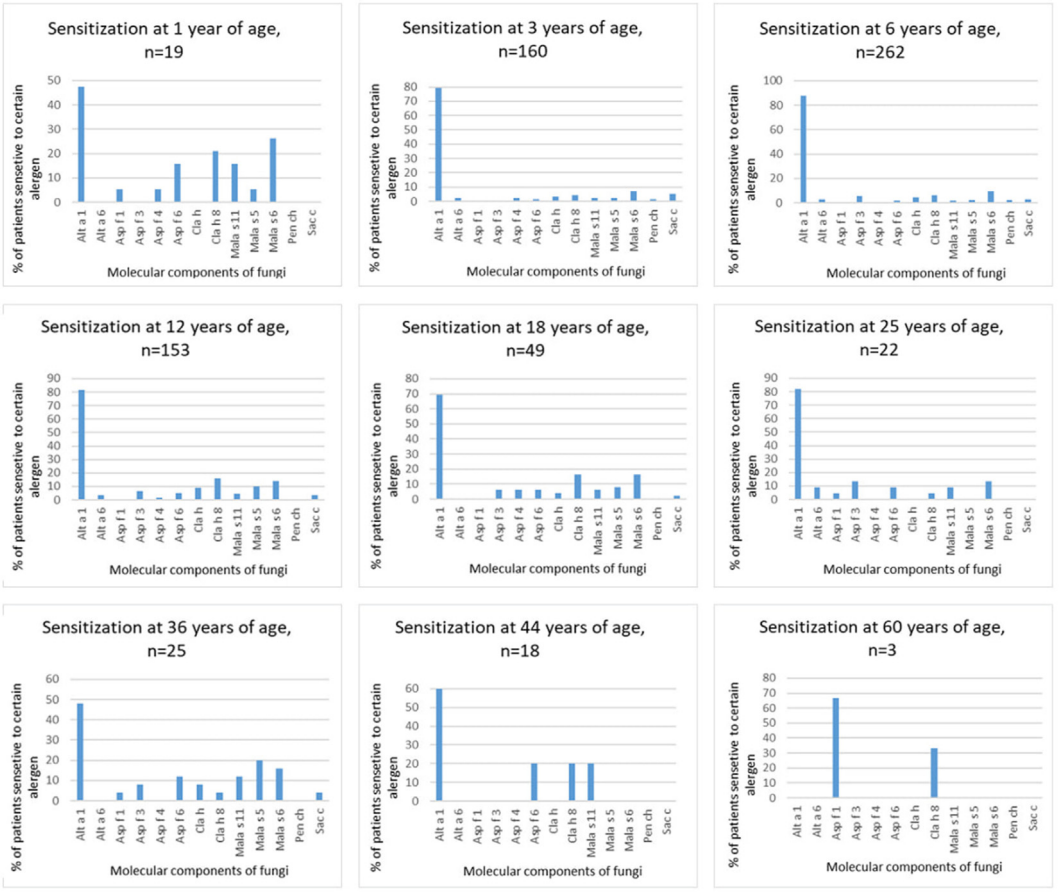

Fig. 2 Characteristics of sensitization to molecular components of fungi in different age groups

different fungal allergens. In particular, molecules Mala s 11 and Asp f 6, which belong to the same group manganese-dependent superoxide dismutase (MnSOD), confirm this.[33]

Sensitivity to them was related to each other according to Bayesian modeling: the absence of sensitization to Asp f 6 caused a high probability of the absence of sensitization to Mala s 11. And since, in general, different allergenic fungal components interact little with each other in the process of causing sensitization to them, multicomponent molecular allergy diagnosis[34,35] is of particular importance as a strategy to avoid misdiagnosis[36] and determine the sensitivity of patients to the largest number of mold allergens. An example of such an allergy diagnosis tool is, in particular, the ALEX molecular multicomponent test, the panel of which currently includes 13 allergenic molecules and fungal extracts. It also contains components of *Alternaria*, *Aspergillus*, *Penicillium* and *Cladosporium* fungi, which, according to the literature,[2,6,37–39] are the most significant factors in the development of severe asthma and rhinitis.

Alternaria, according to our analysis, is the main allergen to which patients in Ukraine are sensitized, which is consistent with data for different populations (Europe, United States, Australia, Asia)[37,40] and the world as a whole. In particular, Sánchez et al showed that 60% of patients, sensitive to fungi, had positive prick test results specifically for *Alternaria*.[41] More than 70% of patients were sensitive to *Alternaria* also in Egypt[42] which goes in line well with the data of our study.

In addition, our data on the tested persons' sensitivity only to the major component of the *Alternaria* Alt a 1, which prevails in all regions and is found in two thirds of the tested persons in

Region	Individuals hypersensitive to fungal allergens		Individuals hypersensitive to *Alternaria* allergens	
	Number	Percentage of total tested	Number	Percentage of those sensitive to fungi
Vinnytsia	20	22,99	15	75,00
Dnipro	357	21,43	304	85,15
Ivano-Frankivsk	38	12,30	24	63,16
Kamianets-Podilskyi	3	20,0	2	66,67
Kyiv	233	13,71	159	68,24
Lviv	121	9,57	71	58,68
Mykolaiv	2	18,18	2	100,00
Odesa	522	17,79	413	79,12
Pavlograd	12	18,46	11	91,67
Poltava	31	16,32	26	83,87
Rivne	21	12,35	15	71,43
Rubizhne	4	14,81	3	75,00
Sumy	62	17,77	50	80,65
Uzhhorod	4	7,69	1	25,00
Kharkiv	278	22,82	230	82,73
Kherson	43	17,00	36	83,72
Cherkasy	14	13,86	8	57,14
Q1		12,35		7,92
Q2		16,66		13,51
Q3		18,46		16,92
Max		22,99		18,88

Table 2. Regional prevalence of the patients sensitive to fungal allergens

Ukraine, coincide with the data of Forkel et al, who found that the majority of patients sensitized to fungi in Germany were monosensitized specifically to *Alternaria* with predominant sensitization of 80–90% to Alt a 1.[43]

Thus, Alt a 1 can be considered as the key target allergen when utilizing AIT, which has shown its effectiveness in sensitization to Alt a 1.[44]

Moreover, the profile of sensitization to Alt a 1, as well as to others, according to our data, is formed in children before the age of 5. Our data completely match to the data of Zhao et al, who found the highest proportion of fungal sensitizers in the 1–17 age group[45] and partially coincide with the data of Schmitz et al, who indicated the highest frequency of people sensitized to fungi at the age of 14–17[46] and of Kwong et al who indicated the highest rates of fungal sensitization for those aged from 9 to 19 in the United States.[40]

The decrease in the frequency of sensitization to fungi with age, which we observed in our population, is also indicated by Zheng & Zou, with Shanghai (China) as an example.[47]

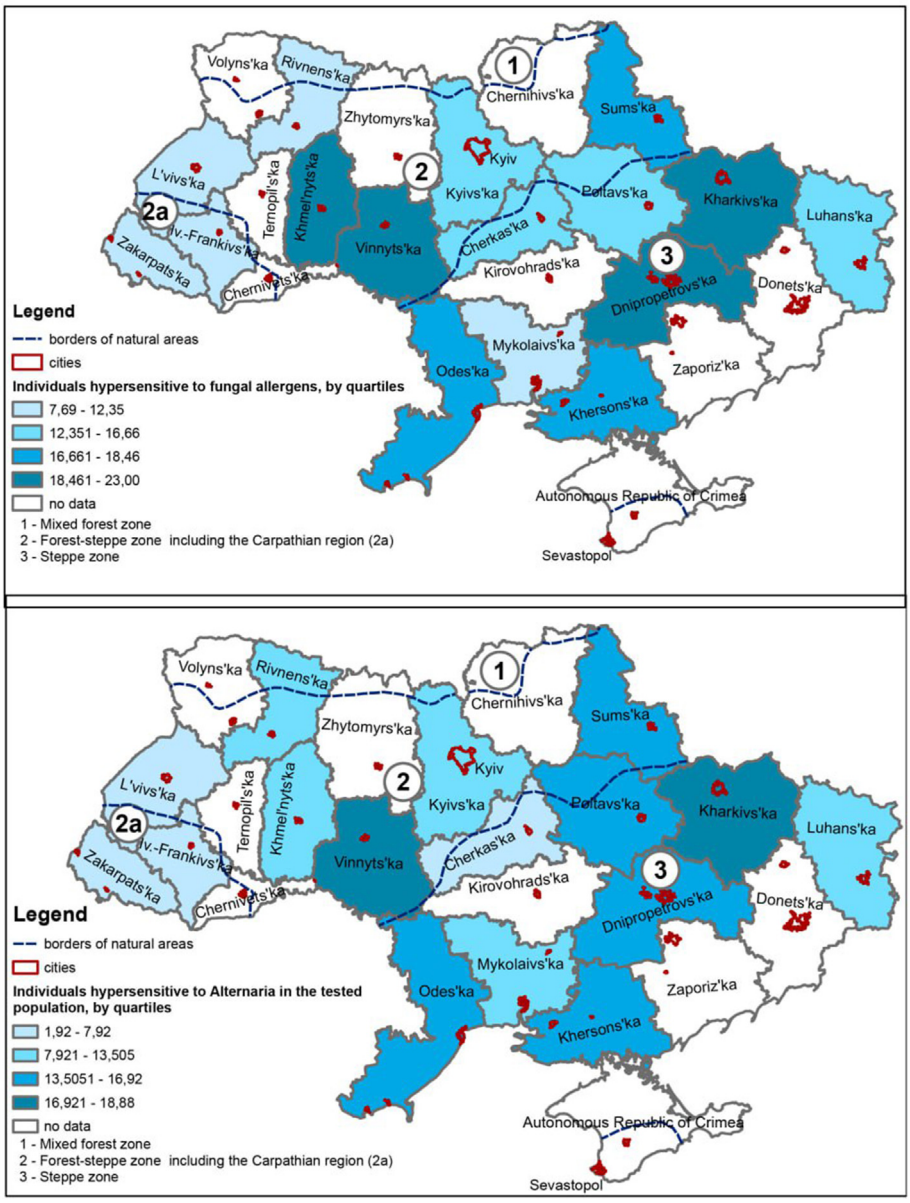

Fig. 3 Regional patterns of fungal sensitization in Ukraine

The findings of our study also match with the data of Forkel et al: 7% of the patients examined by them were sensitive to more than one kind of fungi. In our case, the largest number of patients, which accounted for only 3.28% of the total number of fungi sensitive to allergens, had combined sensitization to Alt a 1 and Asp f 3.

Monosensitivity to Mala s 6, which was only in 4.06%, but took the second place in terms of the frequency of sensitization in the studied sample, may indicate the risk of development of allergic rhinitis symptoms in such patients.[48]

In general, there is practically no literature data indicating combined sensitization in patients to *Alternaria* and other allergens, such as *Malassezia*, which can confirm our Bayesian modeling data, which indicate the practical absence of the probability of such combinations in individual patient profiles. Instead, there are data that sensitivity to Mala s 6, Mala s 11, Sac c, Asp f 6, Cla h, and Cla h 8 (not *Alternaria*) correlates with the severity of atopic dermatitis.[48] In contrast, sensitivity to *Cladosporium herbarum*, *Aspergillus fumigatus* and *Alternaria alternata* prevailed in the group of patients with psoriasis in comparison with a

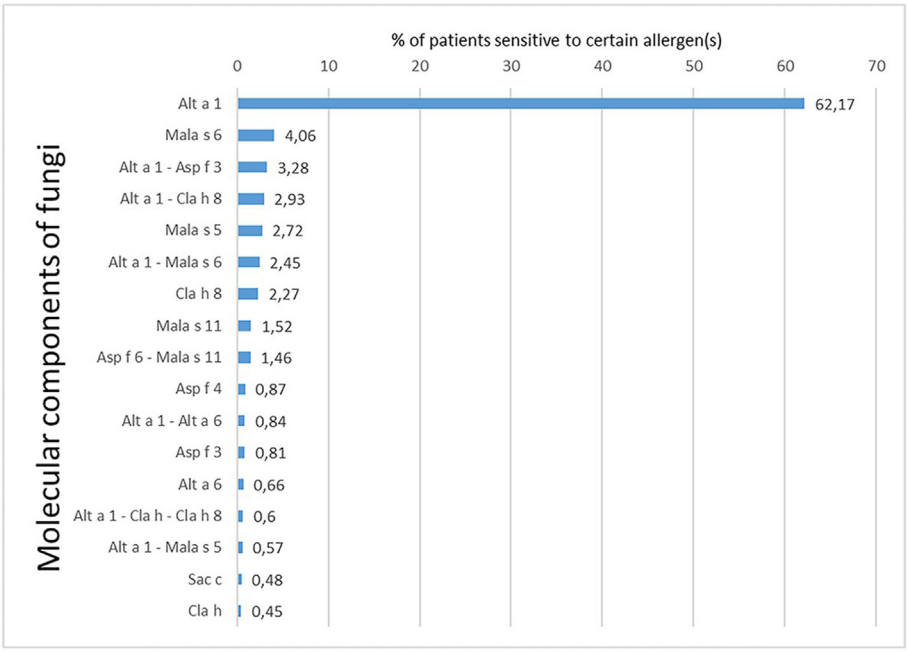

Fig. 4 The most frequent combinations of molecular components of fungi in individual patient profiles

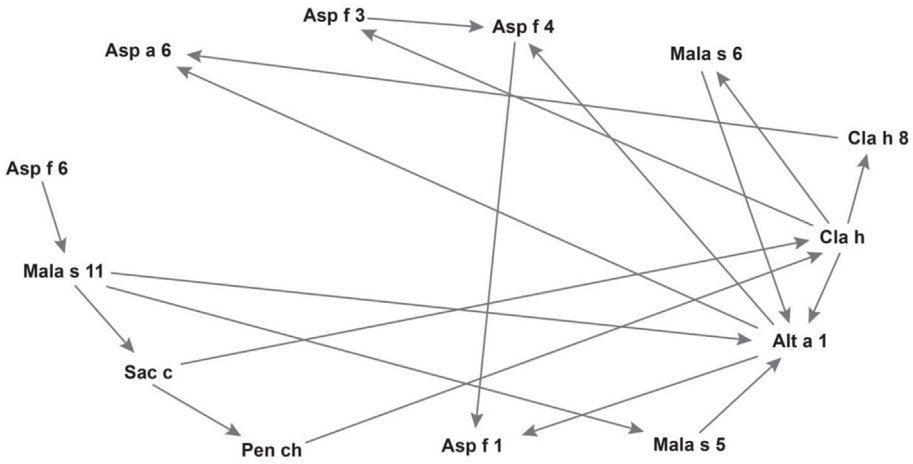

Fig. 5 The resulting Bayesian directed acyclic graph of probabilistic connections between individual allergen components of fungi in the individual patient profiles of the studied sample

group of patients with atopic dermatitis.[49] Being lipid-dependent yeast[49] *Malassezia* can occupy another than *Alternaria* ecological niche.[21] It was established, in support of this hypothesis, that *Aspergillus* sp., *Schizophyllum* sp., *Curvularia* sp., and *Malassezia* sp. but not *Alternaria* are fungi the most frequently detected in the nose. And *Malassezia* sp. predominates here, the most like being coming from the nasal vestibule.[7]

Hence, even monosensitization to fungal allergens, including *Alternaria*,[50] is already associated with a high risk of developing clinical symptoms.[41] However, the symptoms of patients, whose sensitization was analyzed in this study, require further study.

CONCLUSIONS

Among the analyzed patients, sensitivity to Alt a 1 dominated, which accounted for 79.39% of the examined, and 62.17% of the total number of sensitized to fungi were sensitive only to Alt a 1. Sensitivity only to Mala s 6 took second place in the individual profiles of patients with a share of 4.06%. The combined sensitivity to Alt a 1 – Asp f 3 was the third with a share of 3.28%. The sensitization rates to fungi in general and specifically to

Alternaria were lower in the western parts of Ukraine, especially in the Carpathian region, situated within the Broad-leaved Forest zone.

The results of Bayesian modeling revealed that in the case of Alt a 1, the absence of sensitivity to Cla h 8, Mala s 11, Mala s 5 and Mala s 6 molecules could determine the presence of sensitization to the main *Alternaria* allergen with a probability of 92.42%.

In all other cases, there was a high probability of absence of sensitivity to some allergens against the background of absence of sensitivity to others, which may indicate the independent development of sensitization to different fungal allergens.

The idea that sensitization to 1 allergen, for example sensitization to Alt a 1, may be protective when sensitization occurs to another/others (in this case to Cla h 8, Mala s 11, Mala s 5 and Mala s 6) needs further clinical study.

Abbreviations
ALEX, Alergy Explorer test; BN, Bayesian Network; CPD, conditional probability distributions in the Bayesian network; MnSOD, manganese-dependent superoxide dismutase; WHO/IUIS, The World Health Organization and International Union of Immunological Societies

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Availability of data and materials
The original contributions presented in the study are either included in the article/supplementary material or can be seen at https://www.kaggle.com/code/vbmokin/fungi-13-chains-and-bn-learn where Principles of Bayesian Network construction and their results for the given dataset can be found. Further inquiries can be directed to the corresponding author.

Author contributions
Victoria Rodinkova: Formal analysis; conceptualization; writing original draft; review & editing. Serhii Yuriev: Conceptualization; data curation; funding and other resources acquisition; investigation; project administration; writing original draft; review & editing. Vitalii Mokin: Methodology; software; data validation and analyses; review & editing. Dmytro Shmundiak: Formal analysis; descriptive statistics, methodology and visualization. Maria Kryvopustova: Conceptualization; discussion the results and writing original draft. Mykyta Bortnyk: Conceptualization, writing original draft; review & editing. Yevhenii Kryzhanovskyi: Statistical analyses of regional sensitivity data, its mapping; Andrii Kurchenko: Conceptualization; data curation; formal analysis; methodology; project administration and supervision.

Authors' consent for publication
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Ethics approval
All patients signed informed consent before testing. Among others, it included a paragraph about the possible usage of impersonalized patient data for scientific purposes. The Commissions for Bioethical Expertise and Research Ethics at the Bogomolets National Medical University, Kyiv, Ukraine, approved the study protocol on December 29, 2022.

Declaration of competing interest
All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential competing interests.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2024.100908.

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