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ORIGINAL ARTICLE

# Pattern of IgE sensitization to fungal allergens: frequency and co-sensitization with aeroallergens

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## Summary

**Introduction:** Fungal allergens are a significant cause of respiratory allergies, yet sensitization to fungi, as well as co-sensitization with other inhalant allergens, often remains unrecognized.

**Aim:** To determine the pattern of fungal sensitization to optimize routine diagnostic and therapeutic approaches.

**Materials and methods:** Over the period of two years (January 2023–January 2025), using the immunoblot, we analyzed specific IgE against four fungal (*Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium notatum*) and 16 non-fungal inhalant allergens in 200 consecutive adults referred to a tertiary allergy center for the evaluation of suspected respiratory atopy.

**Results:** 101/200 (50.5%) patients were sensitized to inhalant allergens, and 23/101 (22.8%) patients were sensitized to at least one fungal allergen. Among the 20 (87%) patients sensitized to *Alternaria alternata*, 14/20 were monosensitized, and 6/20 were polysensitized (*Penicillium* 5/6, *Aspergillus* 3/6, and *Cladosporium* 3/6). 18/23 (78.3%) fungal-sensitized patients had co-sensitization, most often to *Dermatophagoides spp.*, grass pollen, *Betula* pollen, and dog and cat dander (72.2%, 72.2%, 66.7%, and 55.6%, respectively).

Compared to patients without fungal sensitization, fungal-sensitized patients showed significantly higher frequencies of co-sensitization to *Dermatophagoides spp.*, grass pollen, *Betula* pollen, dog dander ($p<0.01$), and cat dander ($p<0.05$), with approximately fourfold higher odds of co-sensitization (OR 4.06; 95% CI 1.39-11.85).

**Conclusion:** This is the first study to assess fungal sensitization in this part of Europe. *Alternaria alternata* is the dominant fungal allergen. Fungal sensitization is associated with sensitization to common inhalant allergens. These findings support including fungal allergens, particularly *Alternaria alternata*, in the routine diagnostic evaluation of atopic patients.

**Keywords:** *Alternaria alternata,* Atopy, Fungal sensitization, Inhalant allergens

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## INTRODUCTION

Respiratory allergic diseases, including allergic rhinitis and asthma, represent a major global health burden, with increasing prevalence worldwide. Sensitization to inhalant allergens underlies the disease in most affected patients, and accurately identifying the causative allergens is essential for diagnosis, implementing allergen avoidance measures, and considering allergen-specific immunotherapy.

Although pollen, *Dermatophagoides* species (*spp.*), and animal dander are the most frequently investigated inhalant allergens, fungal allergens remain underrecognized despite their well-established role in respiratory allergy. Airborne fungal spores are ubiquitous indoors and outdoors and can trigger immunoglobulin E (IgE)-mediated sensitization, particularly in predisposed individuals. Major fungal species associated with respiratory allergy include *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, and *Penicillium notatum*, each expressing several well-characterized allergens recognized by the World Health Organisation/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Subcommittee (1,2). *Alternaria alternata* contains the major allergen Alt a 1, which is regarded as the molecular marker of genuine *Alternaria* sensitization (1). Similarly, *Aspergillus fumigatus* expresses the clinically significant major allergen Asp f 1. *Cladosporium herbarum* (Cla h 8) and *Penicillium chrysogenum* (Pen ch 13) also have well-characterized major allergenic proteins involved in IgE-mediated respiratory diseases (1). Significantly, sensitization to *Alternaria alternata* is associated with persistent respiratory symptoms, a more severe form of asthma, and an increased risk of exacerbations.

The prevalence of fungal allergen sensitization varies considerably across geographic regions because of differences in climate, environmental exposure, and population characteristics (3). Moreover, fungal sensitization frequently coexists with sensitization to other inhalant allergens, suggesting a broader atopic phenotype. However, data on patterns of monosensitization and polysensitization to fungi, as well as their association with sensitization to common non-fungal inhalant allergens, remain limited. Better characterization of these sensitization profiles could improve the selection of fungal allergens for routine diagnostic panels used to assess atopy.

Atopy is a major risk factor for allergic rhinitis and allergic asthma and is characterized by IgE sensitization to environmental allergens. The prevalence of atopy varies depending on the population studied, geographic area, age, and the diagnostic criteria used to define sensitization (4–6). Approximately 60- 80% of patients with asthma have an atopic phenotype. However, reported prevalence varies by population and definition of atopy, including whether skin prick testing, allergen-specific IgE measurement, or both are used, as well as the range of allergens tested (3,4,7).

The main sources of allergens associated with asthma and other allergic diseases are pollen, *Dermatophagoides spp.*, fungal allergens, and animal dander (2). Fungal allergens are considered the third most common cause of respiratory pathologies (1, 3). Fungal allergens are increasingly recognized as important causes of IgE-mediated respiratory allergy, although they remain underrepresented in routine diagnostic work-up compared with pollen, *Dermatophagoides spp.*, and animal dander. In clinical practice, skin prick tests for fungal allergens are often negative. As an allergen source, fungi are highly complex, unlike pollen or insect venom, they are often overlooked in molecular allergology. Standardizing fungal allergens for skin prick tests is challenging because allergen release is time-dependent, protease activity affects extract stability, batch-to-batch variation occurs, and extraction procedures differ.

Furthermore, fungi contain IgE-binding proteins that may be secreted, cell-bound, or intracellular. Moreover, commercially available fungal allergen solutions for skin prick tests vary significantly in antigen content and IgE-binding capacity. It is not surprising that skin tests using extracts from different manufacturers often differ. Consequently, sensitization to fungal allergens is often neither adequately diagnosed nor treated (8,9).

In large epidemiological studies based on skin prick tests, approximately 19–20% of patients with respiratory allergies were sensitized to at least one fungal allergen, with *Alternaria alternata* being the predominant species causing sensitization. Sensitization to *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Penicillium notatum* was significantly less common (5,10).

This study aimed to determine the frequency and level of IgE sensitization to fungal allergens in patients referred to a tertiary allergy center for evaluation of suspected respiratory allergy, characterize patterns of fungal mono- and polysensitization, and to assess co-sensitization with common non-fungal inhalant allergens. We also sought to identify the most representative fungal allergens for inclusion in routine diagnostic panels for detecting respiratory atopy.

## MATERIAL AND METHODS

This retrospective study included 200 consecutive patients examined at the Clinic of Allergy and Immunology at the University Clinical Center of Serbia over two years. Patients presented to the clinic with symptoms suggestive of allergic disease or were referred from other healthcare facilities to confirm or rule out atopy. We included patients regardless of their inhalant allergen sensitization status. We collected demographic and laboratory data retrospectively from existing medical records. We analyzed IgE results specific to selected inhalant allergens, including fungal allergens, using a standard immunoblot

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method (Atopy screen, Biochemie GmbH). Patients with incomplete or unavailable specific IgE antibody results were excluded from the analysis, as were those with duplicate records or insufficient demographic or clinical data for reliable classification.

The study has been approved by the Ethics Committee of the University Clinical Center of Serbia, protocol number 1880/27 (December 25, 2025).

The profile of specific IgE antibodies against a panel of 20 inhalant allergens was studied in patients who were being evaluated for an atopic constitution.

We analyzed specific IgE to *Alternaria alternata* (M6), *Penicilium notatum* (M1), *Cladosporium herbarum* (M2), *Aspergillus fumigatus* (M3), *Dermatophagoides species (spp.): pteronyssinus* (D1) *and farine* (D2), *Ambrosia elatior* (W1), *Plantago lanceolata* (W9), *Artemisia vulgaris* (W6), alder (T2), *Betula* pollen (T3), hazel (T4), grass pollen mixture (GX)(Including *Lolium perenne, Phleum pratense, Cynodon dactylon, Festuca pratensis, Secale cereale),* ray (G12), cat dander (E1), dog dander (E5), feather mixture (EX8), guinea pig (E6), golden hamster (E84), and rabbit (E82).

We analyzed serum samples from 20 of the 200 patients testing positive for cross-reactive carbochidrate determinants (CCD)-IgE using an anti-CCD absorbent. The absorbent (CCD inhibitor) used to eliminate CCD-IgE consisted of the glycoprotein bromelain, isolated from pineapple. Serum samples selected for retesting were incubated with the anti-CCD absorbent at room temperature for 60 minutes, as directed by the manufacturer.

The cut-off value for positive IgE test results was 0.35 IU/ml. The IgE positivity was categorized quantitatively into six classes: Class 1 (>0.35 to 0.69 IU/l), Class 2 (0.70 to 3.49< IU/l), Class 3 (3.50 to 17.49< IU/l), Class 4 (17.50 to 49.99< IU/l), Class 5 (50.00 to 99.99< IU/l), and Class 6 (>100 IU/l). Conventional criteria considered allergen-specific IgE concentrations above 0.35 IU/ml positive (11). Specific IgE levels for each inhalant antigen fell into the following ranges: 0.35-3.49 IU/ml weakly positive; 3.5-49.9 IU/ml medium positive; >50 IU/ml highly positive.

Statistical analyses were performed using IBM SPSS Statistics software for Windows (version 26; IBM, Armonk, NY, USA). Mean values were used for quantitative variables, and frequencies for qualitative variables. We used the nonparametric Chi-squared and Fisher's exact tests to assess relationships between qualitative variables. Continuous variables were expressed as median (interquartile range) and compared using the Mann-Whitney U test. We calculated odds ratios (OR) with 95% confidence intervals (CI) to estimate the association between fungal sensitization and co-sensitization to non-fungal inhalant allergens. We considered a p-value ≤ 0.05 statistically significant.

## RESULTS

Sensitization to at least one inhalant allergen was identified in 101 of 200 patients (50.5%). Among patients sensitized to inhalant allergens, fungal sensitization was observed in 23/101 (22.7%) patients (female 9/50, 18%; male 14/51, 27.5%), with no significant gender difference (P = 0.343).

The results showed CCD-IgE positivity in 20 of 101 patients (19.8%), and among them, 3 of 101 (3%) lost IgE positivity to all inhalant allergens after the CCD inhibition test.

Demographic characteristics and IgE positivity to inhalant allergens among patients in our study group are presented in **Table 1**.

**Table 1.** Demographic data of IgE-positive patients and the prevalence of IgE positivity to inhalant allergens in the study group

| | |
|---|---|
| Total number of patients | 200 |
| IgE positivity to inhalant allergens n (%) | 101 (50.5) |
| Male n (%) | 51 (25.5) |
| Age (years± SD) | 35.9 ± 13.7 |
| Female n (%) | 50 (25) |
| Age (years ± SD) | 41.3 ± 13.3 |
| IgE positivity to fungal allergens n (%) | 23 (11.5) |
| IgE positivity only to fungal allergens n (%) | 5 (2.5) |
| IgE positivity to fungal and non-fungal inhalant allergens | 18 (9) |
| IgE positivity to non-fungal inhalant allergens | 78 (39.0) |
| No IgE positivity to inhalant allergens | 99 (49.5) |

Among sensitized patients, 23 (22.8%) were sensitized to at least one fungal allergen, representing 11.5% of the total study population **(Table 1)**.

Of the 23 fungal-sensitized patients, 16 (69.6%) exhibited fungal monosensitization, whereas 7 (30.4%) were polysensitized to two or more fungal allergens **(Table 2, Figure 1)**.

*Alternaria alternata* was the predominant fungal allergen in both groups (20/23; 87%, or 10% of the entire study population), accounting for 14 of 16 (87.5%) monosensitized and 6 of 7 (85.7%) polysensitized patients. In contrast, sensitization to *Aspergillus fumigatus* was more frequent among polysensitized patients (4/7; 57.1%) than among monosensitized patients (2/16; 12.5%), with an OR of 9.33 (95% CI: 1.14–76.69; Fisher's exact test p = 0.045).

Sensitization to *Cladosporium herbarum* (4/7; 57.1%) and *Penicillium notatum* (6/7; 85.7%) occurred exclusively in patients with polysensitization to fungal allergens **(Figure 1, Table 2)**.

Five patients (5/23; 21.7%) showed isolated sensitization to fungal allergens (1/10 after CCD inhibition), while 18 patients (18/23; 78.3%) exhibited co-sensitization to both fungal and non-fungal inhalant allergens (5/13 after CCD inhibition).

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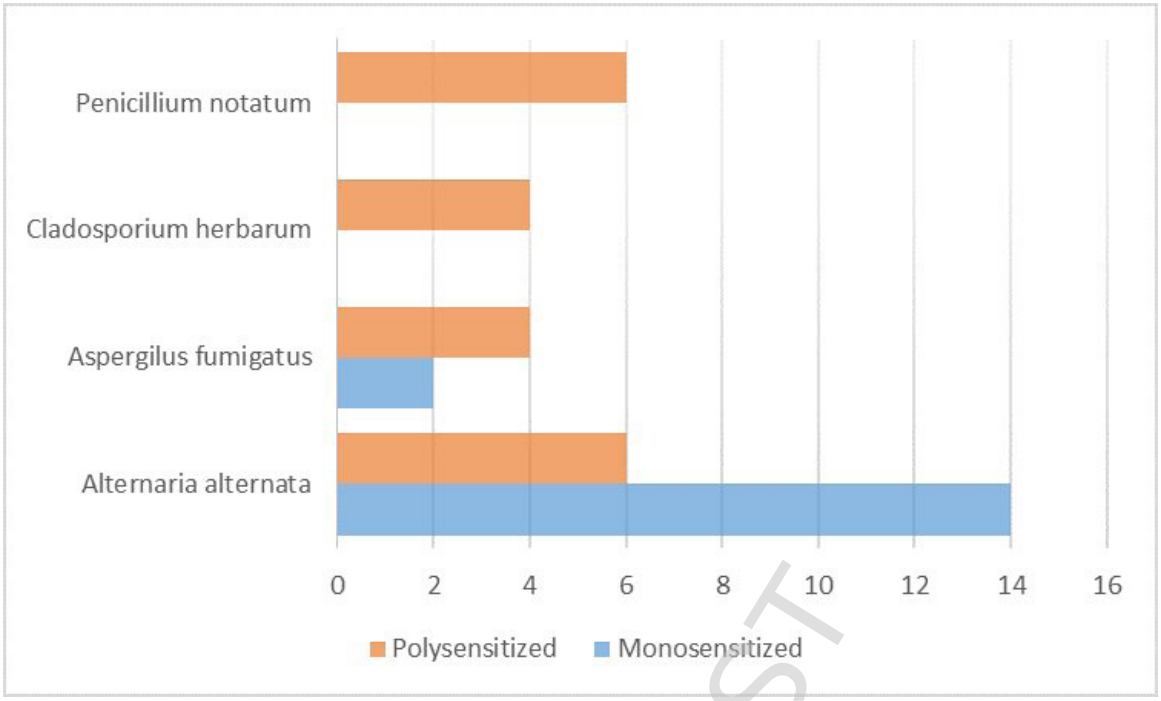



**Figure 1.** Distribution of fungal sensitization in patients with monosensitization and polysensitization to fungal allergens

**Table 2.** Prevalence of fungal sensitization in 23 patients with monosensitization and polysensitization to fungal allergens

| Fungal allergen(s) n | n | % |
|---|---|---|
| *Alternaria aletrnata* only | 14 | 60.9 |
| *Aspergilus fumugatus* only | 2 | 8.7 |
| *Alternaria alternata* + *Aspergilus fumigatus* | 1 | 4.3 |
| *Alternaria alternata* + *Penicillium notatum* | 1 | 4.3 |
| *Alternaria alternata* + *Aspergilus fumigatus* + *Penicillium notatum* | 1 | 4.3 |
| *Alternaria alternata* + *Cladosporium herbarum* + *Penicillium notatum* | 2 | 8.7 |
| *Aspergilus fumigatus* + *Cladosporium herbarum* + *Penicillium notatum* | 1 | 4.3 |
| *Alternarai alternata* + *Aspergilus fumigatus* + *Cladosporium herbarum*+ *Penicillium notatum* | 1 | 4.3 |

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Of the 23 patients sensitized to fungal allergens, 18 (78.3%) were also sensitized to non-fungal allergens. Co-sensitization to *Dermatophagoides spp.* (72.2%), grass pollen (72.2%), and *Betula* pollen (66.7%) was most frequently observed. Compared to patients without fungal sensitization, this group showed a significantly higher prevalence of sensitization to *Dermatophagoides spp.* (p<0.01), grass pollen (P<0.01), and *Betulla* pollen (p<0.01), as well as to dog (p<0.01) and cat dander (p<0.05) **(Table 3)**.

Low levels of specific IgE antibodies predominated for all fungal allergens, whereas moderate and high levels were rare **(Table 4)**. Median specific IgE antibody levels were higher in polysensitized than in monosensitized patients for both *Alternaria alternata* (3.09 vs. 0.70 kU/L; p = 0.353) and *Aspergillus fumigatus* (7.40 vs. 0.61 kU/L; p

**Table 3.** Comparison of co-sensitization to non-fungal inhalant allergens in patients with and without fungal sensitization

| Non-fungal inhalant allergens | Fungal IgE+ n=18 | Fungal IgE- n=78 | OR (95% CI) | p-value |
|---|---|---|---|---|
| *Dermatophagoides spp.*n (%) | 13 (72.2) | 29 (37.2) | 4.38 (1.46 -13.13) | **0.0088** |
| *Ambrosia elatior*, n (%) | 7 (38.9) | 41 (52.6) | 0.57 (0.20 -1.64) | 0.433 |
| *Artemisia vulgaris*, n (%) | 2 (11.1) | 5 (6.4) | 1.82 (0.32 -10.48) | 0.612 |
| *Betula* pollen,n (%) | 12 (66.7) | 23 (29.5) | 4.78 (1.61 -14.20) | **0.0056** |
| Grass pollen, n (%) | 13 (72.2) | 28 (35.9) | 4.63 (1.53 -14.02) | **0.0075** |
| Cat dander, n (%) | 10 (55.6) | 21 (26.9) | 3.39 (1.18 - 9.74) | 0.026 |
| Dog dander, n (%) | 10 (55.6) | 13 (16.7) | 6.25 (2.07 -18.90) | **0.0013** |

P values were obtained using Pearson's chi-square test or Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated from 2x2 contingency tables.

**Table 4.** Distribution of patients according to fungal-specific IgE levels and fungal allergen type

| Fungal allergen | Low, n (%) (0.35-3.49 IU/ml) | Medium n (%) (3.50-49.9IU/ml) | High n (%) (>50IU/ml) | Total IgE-positive |
|---|---|---|---|---|
| *Alternaria alternata* | 18 (90) | 2 (10) | 0 | 20 |
| *Aspergilus fumugatus* | 4 (66.7) | 2 (33.3) | 0 | 6 |
| *Cladosporium herbarum* | 3 (75) | 0 | 1 (25) | 4 |
| *Penicillium notatum* | 5 (83.3) | 0 | 1 (16.6) | 6 |

**Table 5.** Median fungal-specific IgE levels in mono- and polysensitized patients by fungal allergen

| Fungal allergen | Monosensitized Median (kU/L) | Polysensitized Median (kU/L) | p-value |
|---|---|---|---|
| *Alternaria alternata* | 0.70 | 3.09 | 0.353 |
| *Aspergilus fumigatus* | 0.61 | 7.40 | 0.800 |
| *Cladosporium herbarum* | _ | 1.38 | NA |
| *Penicillium notatum* | _ | 3.33 | NA |

Abbreviation: NA - Not applicable.

= 0.800), although none reached statistical significance **(Table 5)**.

## DISCUSSION

The study shows the importance of IgE antibodies to fungal allergens, especially to *Alternaria alternata* in patients sensitized to inhalant allergens. IgE testing for fungal allergens is necessary in patients with an atopic constitution.

Although the skin prick test is a sensitive, first-line method for detecting IgE to inhalant allergens, it may show unexplained variability and inconclusive "false positive" results due to cross-reactivity and "false-negative" results due to unstandardized extracts (8). Because of this, *in vitro* testing is the most important diagnostic step for detecting IgE positivity to specific inhalant allergens (12).

Precise identification of sensitization to the appropriate allergen is important not only for selecting an allergen for specific allergen immunotherapy, but also for proper prevention in patients with allergic skin and respiratory diseases.

This study investigated the prevalence and patterns of fungal sensitization in patients referred for allergy evaluation and reported several clinically relevant findings. First, we identified fungal sensitization in 23 of 101 patients sensitized to inhalant allergens (22.8%), representing 11.5% of the total study population. Second, *Alternaria alternata* was by far the predominant fungal allergen, present in nearly 90% of fungal-sensitized patients. Third, patients sensitized to fungal allergens had significantly higher odds of concomitant sensitization to several common inhalant allergens, including *Dermatophagoides spp., Betula* pollen, grass pollen, and dog and cat dander. Finally, although *Alternaria alternata* predominated, *Aspergillus fumigatus, Cladosporium herbarum*, and *Penicillium notatum* were primarily associated with fungal polysensitization, regardless of the sensitization pattern.

The prevalence of fungal sensitization observed in our study is comparable with that reported in previous European studies, although considerable geographic variability has been described. These differences likely reflect climatic conditions, environmental fungal exposure, study populations, and diagnostic methods. The true prevalence of fungal sensitization remains poorly established, and prevalence data vary across authors and geographic regions. Nevertheless, fungal sensitization is estimated at 3% to 10% of the global population (3). Our findings confirm that fungal sensitization is a clinically relevant component of respiratory allergy and should not be overlooked during routine allergy assessment.

In this study, we observed a higher proportion of fungal sensitization in males. Although fungal sensitization was more frequent in males, the difference was not statistically significant. This observation aligns with previous studies reporting male predominance in fungal sensitization, particularly to *Alternaria alternata*. The lack of statistical significance in our study may reflect the relatively small number of fungal-sensitized patients and, consequently, limited statistical power (3,5,13). Conversely, other investigations have not demonstrated a significant association between sex and fungal sensitization, suggesting that sex may influence results depending on the study population, age distribution, and geographic region.

A particularly striking finding of our study was the overwhelming predominance of *Alternaria alternata*. 87% of fungal-sensitized patients were IgE-positive to *Alternaria alternata*, making it the principal fungal allergen in our population. Moreover, *Alternaria alternata* accounted for nearly all cases of fungal monosensitization (87.5%) and remained highly prevalent among our polysensitized patients (85.7%). The predominance of *Alternaria alternata*, particularly among our monosensitized patients, is consistent with primary sensitization to its major allergen, Alt a 1, a species-specific marker allergen

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with limited cross-reactivity with *Aspergillus fumigatus*, *Penicillium notatum*, and *Cladosporium herbarum*. In contrast, additional *Alternaria alternata* allergens, including Alt a 6 (enolase), Alt a 8 (mannitol dehydrogenase), and Alt a 13 (glutathione-S-transferase), share homologous proteins with other fungal species and may contribute to the broader sensitization patterns observed in polysensitized patients (14,15). These findings are consistent with numerous studies identifying *Alternaria alternata* as the most important outdoor fungal allergen associated with allergic rhinitis and asthma (16–18). The predominance of monosensitization to *Alternaria alternata* in our study population therefore aligns with a primary IgE response directed against Alt a 1. However, component-resolved diagnostics (CRD) would be required to confirm this hypothesis. Consequently, the dominance of *Alternaria alternata* observed in our study therefore likely reflects both its widespread environmental distribution and its high allergenic potential. Although Alt a 1 is the main allergen of *Alternaria alternata*, the diagnostic panel for fungal allergy should also include other allergens, Alt a 6, Alt a 8, and Alt a 13 (17). Molecular diagnostics for allergic diseases, known as CRD, uses recombinant allergens and represents a modern approach to identifying true sensitization to allergens (19).

Although *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Penicillium notatum* are the primary fungal species known to cause allergies, some studies have shown that sensitization to several less-studied fungal allergens is significantly higher than to the four species most commonly tested in clinical practice (3,20). Consequently, testing for fungal sensitization to the above-mentioned "representative" fungi may lead to some patients not being diagnosed (7,18). A causal relationship has been established between asthma exacerbation, on the one hand, and general indoor humidity levels, on the other. Therefore, testing for the presence of IgE antibodies directed against only four fungal species may fail to identify the actual causative agent (7,21).

The literature indicates that *Alternaria alternata* is most commonly associated with allergic respiratory diseases and is found in both outdoor and indoor environments (22–24). Its major allergen, Alt a 1, plays a significant role in asthma pathogenesis by inducing a potent Th2 immune response (5). Upon inhalation, its spores interact with receptors on airway epithelial cells, triggering the release of key pro-inflammatory cytokines IL-25 and IL-33. The resulting cascade involves group 2 innate lymphoid cells (ILC2s), eosinophils, and Th2-associated cytokines (IL-4, IL-5, IL-13), leading to airway IgE-mediated hyperreactivity, mucus hypersecretion, and eosinophilic infiltration (18).

Although *Alternaria alternata* was highly prevalent in our study regardless of the sensitization pattern, the remaining fungal allergens were distributed considerably differently. Sensitization to *Aspergillus fumigatus* was significantly more common in polysensitized patients than in those with monosensitization, while *Cladosporium herbarum* and *Penicillium notatum* were detected exclusively in patients with fungal polysensitization.

These observations suggest that *Alternaria alternata* may be the primary fungal sensitizer, whereas sensitization to *Aspergillus fumigatus*, *Cladosporium herbarum*, and *Penicillium notatum* may indicate broader fungal allergen recognition. Previous studies have reported similar trends, noting that *Aspergillus fumigatus* sensitization occurs more often alongside other fungal allergens than as an isolated cause, most often with *Alternaria alternata* (25,26). Although our sample size was limited, the calculated odds ratio revealed a significantly higher likelihood of sensitization to *Aspergillus fumigatus* among polysensitized patients, supporting this hypothesis. Recent molecular studies further suggest that complex sensitization profiles to *Aspergillus* allergens reflect molecular spreading and are associated with broader IgE responses rather than isolated sensitization (25).

Another important observation regarding our patients concerned the strong association between fungal sensitization and sensitization to non-fungal inhalant allergens. Compared with patients sensitized to non-fungal inhalant allergens, fungal-sensitized individuals were approximately four to six times more likely to co-sensitize to *Dermatophagoides spp.*, grass pollen, *Betula* pollen, and dog and cat dander. Our findings are consistent with previous studies showing that fungal sensitization is often accompanied by sensitization to other non-fungal inhalant allergens (27). Specifically, we observed co-sensitization to *Dermatophagoides spp.*, grass pollen, tree pollen, and animal dander significantly more often in individuals sensitized to fungi. These findings indicate that fungal sensitization is associated with a broader atopic phenotype characterized by multiple IgE responses, rather than an isolated allergy to fungi (27,28). Although most epidemiological studies indicate co-sensitization to tree pollen in general rather than to individual tree species, we found a significantly higher prevalence of sensitization to *Betula* pollen in patients sensitized to fungi. This observation warrants further research, involving larger study groups (29,30).

In some undiagnosed patients, respiratory symptoms caused by fungal allergens can have a poor prognosis. Because fungal allergens co-occur with allergens from *Dermatophagoides spp.*, pollen, and/or cat/dog dander in the environment, fungal hypersensitivity in polysensitized patients may be masked by sensitization to other allergen sources, making fungal allergy diagnosis difficult. The previously mentioned lack of standardized fungal allergen extracts also complicates the diagnosis and treatment of fungal allergies (9,31).

The particularly strong association observed between fungal sensitization and grass pollen sensitization deserves special attention. Experimental studies have shown that *Alternaria alternata* spores may transport

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group 1 grass pollen allergens, potentially prolonging allergen exposure beyond the grass pollen season and enhancing allergic airway inflammation (18,32). Our patients sensitized to *Alternaria alternata* had more than four times the chance of simultaneous sensitization to grass pollen than patients sensitized to non-fungal inhalant allergens. Although our study was not designed to investigate this mechanism directly, these results provide a biologically plausible explanation for the frequent coexistence of *Alternaria alernata* and grass pollen sensitization observed in our patients. Further clinical studies are needed to determine whether this interaction contributes to respiratory symptoms in fungal-sensitized individuals.

Interestingly, recent case reports suggest that ingesting food contaminated with *Alternaria alternata* may rarely trigger anaphylaxis in sensitized individuals (33).

Our findings align with previous evidence indicating that sensitization to fungi often occurs within a broader pattern of polysensitization. In children hospitalized for asthma, researchers found a strong association between sensitization to indoor allergens, including fungal allergens, and sensitization to dog and cat dander, with 68% of children sensitized to three or more allergens (34). Similarly, recent data from Malaysia link sensitization to cat dander and other allergens to allergic diseases, further supporting a broader pattern of polysensitization (35). In our patient group, sensitization to fungi was strongly associated with sensitization to dog and cat dander (OR >6 and 3.3, respectively), indicating a broader atopic sensitization profile among sensitized patients.

Clinically, our findings emphasize the importance of including fungal allergens, particularly *Alternaria alternata*, in the routine diagnostic evaluation of patients with respiratory allergy. Identifying fungal sensitization may not only improve characterization of the patient's sensitization profile but also identify individuals at greater risk of concomitant sensitization to multiple inhalant allergens. Such information may facilitate individualized patient management and improve diagnostic accuracy.

The present study has several limitations. The number of fungal-sensitized patients was relatively small, particularly those with fungal polysensitization. Furthermore, molecular allergen diagnostics were not available, precluding assessment of sensitization to individual fungal allergenic components, such as Alt a 1. Finally, limited data on environmental exposure and a lack of information on the current disease of the analyzed patients', limit the possibility of a complete assessment of atopic constitution. Future studies should include more samples, disease diagnosis, and risk factor in order to better understand the role of fungal allergens in allergic diseases.

## CONCLUSION

In conclusion, among patients sensitized to inhalant allergens, fungal sensitization was observed in 23/101 (22.8%) patients, with no significant difference by gender. *Alternaria alternata* was the predominant fungal allergen in our study (20/23 patients, 87%) and represented the principal pattern of both monosensitization and polysensitization. Sensitization to fungal allergens was strongly associated with sensitization to several common inhalant allergens (*Dermatophagoides spp.*, grass pollen, *Betula* pollen, dog and cat dander), suggesting that fungal allergy may identify a subgroup of patients with a broader atopic profile. These findings support including fungal allergens, particularly *Alternaria alternata*, in the routine diagnostic workup of atopy in this part of Europe. Larger multicenter studies, incorporating molecular diagnostics, are warranted to clarify further the clinical significance of fungal sensitization in respiratory allergy.

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**Conflicts of interest:** The authors have no conflicts of interest to declare.

**Author contribution:** Conceptualization: DJ and BBN; Methodology: DJ; Validation: BL and SR; Formal Analysis: DJ; Data Curation: DJ, BBN, BL, SR, AK, MB; Writing – Original Draft Preparation: DJ; Visualization: BBN and SR; Supervision: BBN.

**Ethical approval:** The study has been approved by the Ethics Committee of the University Clinical Center of Serbia (protocol number 1880/27, date: December 25, 2025). Because the study is retrospective, the Ethics Committee waived informed consent prior to this date.

**Informed Consent:** The Ethics Committee approved the publication of anonymized clinical data and waived the requirement for informed consent because the study is retrospective.

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# OBRAZAC IGE-SENZIBILIZACIJE NA GLJIVIČNE ALERGENE: UČESTALOST I KOSENZIBILIZACIJA SA AEROALERGENIMA

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**Sažetak**

**Uvod:** Alergeni gljiva predstavljaju značajan uzrok respiratornih alergija, a ipak senzibilizacija na gljive kao i kosenzibilizacija sa drugim inhalatornim alergenima često ostaje neprepoznata.

**Cilj:** Utvrđivanje obrasca senzibilizacije na gljive radi optimizacije rutinskih dijagnostičkih i terapijskih pristupa.

**Materijali i metode:** Tokom dvogodišnjeg perioda (od januara 2023. do januara 2025. godine), metodom imunoblota, analizirali smo specifična IgE na četiri gljivična (*Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium notatum*) i 16 inhalatornih alergena koji su negljivičnog porekla kod 200 uzastopnih pacijenata upućenih u tercijarni alergološki centar radi procene sumnje na respiratornu atopiju.

**Rezultati:** 101 od 200 (50, 5%) pacijenata bilo je senzibilisano na inhalatorne alergene, a 23/101 (22, 8%) na najmanje jedan alergen gljive. Od 20 (87%) pacijenata senzibilisanih na *Alternaria alternata*, 14/20 bilo je monosenzibilisano, a 6/20 polisenzibilisano (*Penicillium* 5/6, *Aspergillus* 3/6, i *Cladosporium* 3/6). Od 18/23 (78,3%) senzibilisanih na gljive najčešća kosenzibilizacija bila je na *Dermatophagoides spp*., polene trava, polen breze, perut psa i mačke (72,2%, 72,2%, 66,7%, 55,6% i 55,6%, respektivno). U poređenju sa pacijentima bez gljivične senzibilizacije, pacijenti senzibilizirani na gljivice pokazali su veću učestalost kosenzibilizacije na *Dermatophagoides spp*., polen trava, polen breze, perut psa (p<0, 01) i perut mačke (p<0, 05), uz približno četiri puta veće šanse za kosenzibilizaciju (OR 4, 06; 95% CI 1, 39–11, 85).

**Zaključak:** Ovo je prva studija koja je analizirala senzibilizaciju na gljive u ovom delu Evrope. *Alternaria alternata* je dominantna gljiva. Senzibilizacija na gljive je povezana sa senzibilizacijom na uobičajene inhalatrone alergene. Ovi nalazi podržavaju uključivanje gljivičnih alergena, posebno *Alternaria alternata*, u rutinsku dijagnostičku obradu atopijskih pacijenata.

**Ključne reči:** *Alternaria alternata*, atopija, senzibilizacija na gljive, inhalatorni alergeni

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