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Original Article

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Study toward resolving the controversy over the definition of allergic fungal rhinosinusitis

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Abstract

Dysbiosis of the microbiome on the airway mucosa leads to the development of chronic inflammatory and allergic disorders. The aim of this study was to consider the potential diagnostic criteria for allergic fungal rhinosinusitis (AFRS) and nonallergic fungal rhinosinusitis (FRS), and the role of fungal presence in an environment for the development of AFRS. In this study, 136 patients were divided into two groups: patients with positive specific immunoglobulin E (sIgE) and fungal finding (AFRS group), and patients with negative sIgE and positive fungal finding (FRS group). The study design included: anamnesis data, sIgE, eosinophil count and skin-prick test, rhinology and computerized tomography (CT) observation and mycological finding. Our results showed: (i) the prevalence in Serbia is: AFRS 1.3%, FRS 2.8%; (ii) 30.4% patients with sIgE+ had more often severe and recurrent chronic rhinosinusitis (CRS) ($P = .005$) and the presence of polyps ($P = .025$); (iii) 46.4% patients with sIgE+ had positive fungi on the sinonasal mucosa and were considered as AFRS; (iv) patients with AFRS had more frequent asthma ($P = .024$) and chronicity of CRS >10 years ($P = .000$). The persistent fungal presence and prolonged duration of CRS could be a silent threat for the progression of inflammation and development of FRS. Lavage with hypertonic-NaCl should be included in the everyday hygiene routine in an effort to decrease fungal load and antigenic exposure. The presence of allergological parameters and better response to

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corticosteroid therapy in AFRS patients should be considered as crucial diagnostic criteria for AFRS.

Key words: allergic fungal rhinosinusitis, chronic rhinosinusitis, nasal polyps, fungi, sinonasal mucosa.

Abbreviations

AFRS, allergic fungal rhinosinusitis; FRS, fungal rhinosinusitis; CT, computerized tomography; sIgE, specific immunoglobulin E; CRS, chronic rhinosinusitis; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; Ab, antibodies; BA, blood agar; SDA, Saburoad dextrose agar; PDA, potato dextrose agar; FESS, functional endoscopic surgery of sinuses; ISNS-F, induced sinonasal secretion flow; ISNS-A, aspiration of induced sinonasal secretion; reFESS, repeated functional endoscopic surgery of sinuses; EDTA, ethylene-diamine-tetra-acetic acid; ENT, ear, nose and throat; HS, home sample; F+, positive fungal finding; EFRS, eosinophilic fungal rhinosinusitis; SPT, skin prick test.

Introduction

The human mycobiome, consisting of a diverse collection of fungi, is one of the new perspectives in the research of upper airway diseases. Resident mycobiome interacts extensively with immune cells and epithelium of the airway mucosal surfaces. Dysbiosis of the microbiome on airway mucosa and pathogen-host interaction leads to the development of chronic inflammatory and allergic disorders.[1] Many studies have shown that inflammation of sinonasal mucosa is associated with enrichment of specific sinus pathogens that leads to the development of chronic rhinosinusitis (CRS).[1,2] Researchers reported that microbiome of sinonasal mucosa has a major role in regulating immune cells that are of relevance for allergic diseases.[3] However, there are many gaps in knowledge about the role of fungi modulating local immune response on sinonasal mucosa in these patients. After birth, a child is exposed to fungi present in the environment. After 4 months from birth, fungi can be obtained as normal content from almost everyone's nasal mucosa.[3] On the contrary, there are many reports of sinonasal disease caused by the presence of fungi, termed fungal rhinosinusitis (FRS).[4,5]

Although there are many controversies in the classification of FRS, it is well known that it encompasses a diverse spectrum of disease depending on the immune status of the host. Asymptomatic fungal colonization of sinonasal mucosa is common and requires no treatment, while allergic fungal rhinosinusitis (AFRS) represents a huge challenge for diagnosis and treatment. AFRS is a noninvasive form of FRS with a prevalence of 6–9%, often co-localized with nasal polyps (NP), type immunoglobulin E (IgE)-mediated hypersensitivity, and presence of eosinophilic mucin.[4,5,6] Diagnosis of AFRS is controversial and requires multiple tests for measurement of moulds, specific IgE antibodies (Ab), eosinophil count in blood/tissue, and mycological confirmation of fungal presence.[7] Treatment of AFRS differs from the one for FRS; hence the precise definition of AFRS is crucial.[6,7]

The objective of this study was to consider potential differential diagnostic criteria for AFRS and non-mould-allergic FRS, a pathway of AFRS development, and the role of fungi present in the environment for the development of AFRS.

Methods

Study population

This prospective clinical case-series study was conducted at the Clinic for Allergology and Immunology, Clinical Centre of Serbia and Institute of Microbiology, Faculty of Medicine, University of Belgrade, Serbia, from March 1 to August 1, 2014. The study was approved by the Ethical committee of Clinical Center of Serbia (5030/5) and the Ethical committee of Faculty of Medicine University of Belgrade (29/VI-3).

CRS is clinically defined by three criteria:

1. At least two of following symptoms: nasal blockage/obstruction/congestion, nasal discharge (anterior/posterior nasal drip), facial pain/pressure, reduction or loss of smell for ≥12 weeks.
2. Endoscopic finding of nasal polyps and/or mucopurulent discharge and/or edema/mucosal obstruction.
3. Computerized tomography (CT) finding: mucosal changes within ostiomeatal complex without nasal polyps (CRSsNP) or with nasal polyps (CRSwNP).[8]

Patients with CRS were included in the study if they fulfilled inclusion criteria: (1) >16 years; (2) symptoms ≥12 weeks; (3) no treatment with systemic corticosteroids over last 7 days and local corticosteroids for 3 days before inclusion; and (4) absence of invasive fungal infection (IFI) (screened by serology testing of anti-*Aspergillus* and anti-*Candida* immunoglobulin M [IgM] and immunoglobulin G [IgG] Ab, as well as the concentration of galactomannan and mannan in patients' sera).

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Patients were divided into two groups depending on the class of mould mix specific IgE (sIgE) in sera. Patients with mould-mix sIgE Ab classes 1–6 belonged to group sIgE+, while patients with mould-mix specific IgE Ab class 0 belonged to group sIgE−. After mycological analyses, patients were divided into two groups: patients with positive sIgE and presence of fungi in nasal mucosa (group A/AFRS) and patients with negative sIgE, but the presence of fungi in nasal mucosa (group B/non-moulds-allergic FRS).

AFRS is defined as a presence of the following: (i) type I (IgE) hypersensitivity reaction to moulds (serology), (ii) hyperattenuating signal density visualized by CT scan ("double density" sign), (iii) presence of eosinophilic mucus, and (iv) presence of the fungi in sinus content.[9]

Study design

Study design included: (1) collection of patient's demographics and history data including number of previous functional endoscopic surgery of sinuses (FESS), duration of CRS, previous use of local corticosteroids during three months, co-morbidity data; (2) atopic/allergic examination (mould-mix sIgE in serum, total IgE in serum, absolute eosinophil count in blood and skin prick test/SPT on inhalant allergens); (3) rhinoscopic inspection and evaluation of rhinoscopic score; (4) measurements and evaluation of CT score for fulfillment and opacification of maxillary sinuses; (5) microbiological analysis of induced sinonasal secretion flow (ISNS-F) and induced aspirate; and (6) cultivation of air samples from patients' homes (bedrooms).

Allergy examination

Mould-mix specific IgE Ab

Serological measurement of the level of sIgE was conducted by fluoroenzyme immunoassay using ImmunoCAP-100 (Phadia AB, Uppsala, Sweden). The sIgE was related to mould-mix (Mx1) comprised of *Penicillium chrysogenum* (m1), *Cladosporium herbarum* (m2), *Aspergillus fumigatus* (m3), and *Alternaria alternata* (m6). *A. alternata* allergen contained rAlt a1, while *A. fumigatus* contained rAsp f1, rAsp f2, rAsp f3, rAsp f4 and rAsp f6 antigen components.

Test results were reported as positive or negative. A cut-off value of 0.35 kUA/l was considered as positive. Values $\geq$0.35 kUA/L indicated sIgE to one or more of allergens from mould-mix.[10]

Total serum IgE

A concentration of total IgE Ab in serum was measured by enzyme-linked immunosorbent assay (ELISA; Euroimmun AG, Germany). Results were interpreted as follows: (i) negative (<100 kU/L), (ii) low positive (100–500 kU/l) and (iii) high positive ($\geq$500 kU/l).

Eosinophil count

The blood sample was taken before SPT and put into the tube containing ethylene-diamine-tetra acetic acid (EDTA). Eosinophil counting was performed with Fuchs-Rosenthal counting chamber.[11] Results <350 mm^3 were considered as negative.

Skin prick testing (SPT)

The panel of SPT extracts comprised a variety of allergens: epithelia (cat, dog, horse), pollens (*Betula verucosa, Olea europea, Cupressus arizonica, Phleum pratense, Ambrosia artemisia, Artemisia vulgaris*), dust mite (*Dermatophagoides farinae, D. pteronyssinus*), and fungi (*A. alternata*, *Aspergillus flavus*, *C. herbarum*, *Penicillium notatum*) (ALK-abello, Hørsholm, Denmark). Histamine (10 mg/ml) was used as positive control, and diluent (saline in 50% glycerol) was used as negative control. Wheal reaction was measured as the mean value of longest diameter and diameter perpendicular to it. Reactions with diameter $\geq$3 mm were considered as positive.

Rhinoscopy

All patients were examined by ear, nose, and throat (ENT) specialist at Clinic for Otorhinolaryngology, Clinical Centre of Serbia, Belgrade, Serbia. The ENT specialist performed rhinoscopy with standard rigid rhinoscope and estimated Lund-Mackay score (LMS) by measuring a presence of following parameters: edema, discharge, and presence of NP. Each parameter was scored as 0-absent, 1-mild, 2-severe, and 3-only for parameter "NP" if completely obstructing nose. All parameters were noted separately for left/right side, and all were summed up from 0 to 14.[12] Rhinological scoring index for all observed parameters was suggested as follow: (i) 0–4: "0 index" and mild CRS, (ii) 5–9: "1 index" and moderate CRS and (iii) 10–14: "2 index" and severe CRS.

CT imaging

CT visualization of paranasal sinuses was done in the Department of Radiology, Faculty of Dental Medicine, University of Belgrade, Serbia. To determine the stage of CRS we established "CT-scoring index" measured by fulfilment of maxillary sinus in one section on each side separately: (i) fulfilment 0–33% "score 0", (ii) 33–66% "score 1" and (iii) 66–100% "score 2". Sum of scores from both sides gave cumulative index and determinate the grade of CRS:

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(i) 0 score as "0 index" and mild CRS, (ii) 1–2 scores as "1 index" and moderate CRS, and (iii) 3–4 scores as "2 index" and severe CRS.

Microbiology

Sinonasal secretion flow and aspiration

Before sampling ISNS, nasal cavities were pretreated with cotton swabs in the aim to decrease the possibility of contamination and to increase the probability of sampling proper content directly from sinuses. Patients were sprayed by the sterilized hypertonic 7% NaCl solution into each nostril and forcefully exhaled ISNS-F through nostrils directly on blood agar (BA) and Saburoad dextrose agar (SDA), separately for each nostril. Afterward, each patient did the inhalation by PARI-SINUS nebulizer with 5 ml of hypertonic-NaCl solution for 10 minutes in the quadruped position (PARI, Starnberg, Germany). Quadruped position facilitates drainage of the content from paranasal sinuses.[13] After the inhalation, mucin was collected from the sinuses by aspiration in a supine position with head tilted backward at a 30° angle. ISNS-A sampling was done by use of the mucus extractor (ULTRAMED, Asyut, Egypt), and the obtained sample was additionally processed in ultrasound cleaner (BlueWave Ultrasonic, Davenport, IA, USA). ISNS-F and processed ISNS-A were incubated on SDA at 25°C for 7 days.

The patient was considered as fungal positive if: (i) ISNS-F was positive twice within an interval of 7 days with an isolation of the same fungi in both samples or (ii) ISNS-A was positive once.

Air sampling

Patients were asked to leave open petri dish with potato dextrose agar (PDA) for 1 h at room temperature in their bedrooms. Petri dishes containing air-samples were fixed with parafilm (Sigma-Aldrich, USA) and deposited at the laboratory for further analysis. Plates were incubated at 25°C for 7 days. Identification of fungi was based on macroscopic and microscopic characteristics using standard mycological methods.[14]

Statistical analysis

Descriptive and inferential statistical analyses were used for evaluation of data using Statistical Package for Social Science (SPSS 17.0) (SPSS, Chicago, IL, USA). Data were expressed as mean±standard deviation (SD) and counts or percentages, where appropriate. Mann–Whitney test was used for nonparametric data, and χ^2 test was used for categorical variables. All differences were considered significant at $P < .05$.

Results

Clinical characteristics and sociodemographic data of CRS patients

Out of 985 consecutive outpatients with any allergic disease examined during 5 months, 136 patients (13.8%) had clinically confirmed the diagnosis of CRS by criteria proposed by the European Position Paper on Rhinosinusitis and Nasal Polyps.[8] Out of 136 consecutive patients with CRS, 24 did not provide consent, while 112 signed written consent. Mean age of 112 patients was 38.38 ± 13.32 (range, 16–66) with an equal frequency of sex. Mean duration of CRS was 13.77 ± 10.6 years (range, 2–40 years). Recalcitrant NP with repeated FESS (reFESS) was found in 44.6% patients with CRS. About 51.8% of patients had severe CRS estimated by rhinological score and 36.6% measured by CT score (Suppl. 1).

Relationship between clinical characteristics of CRS patients with and without positive moulds sIgE

Out of 112 CRS patients, 34 (30.4%) had positive moulds sIgE and represented sIgE+ group, while 78/112 (69.6%) patients represented sIgE− group. By comparing difference between sIgE− and sIgE+ groups, we found that patients with positive moulds sIgE had more often reFESS (67.6% vs. 34.6%, $P = .005$) and presence of NP (34.9% vs. 23.1%, probability value/$P = .025$), and more severe forms of CRS measured by Rhinological (76.5% vs. 41%, $P = .02$) and CT (47% vs. 32.1%, $P = .042$) indexes (Suppl. 1).

Relationship of clinical characteristics of CRS patients with positive moulds sIgE and positive fungal finding (group A) and with negative moulds sIgE and positive fungal finding (group B)

Mycological analyses revealed positive fungal finding (F+) in 28/112 ISNS-F/ISNS-A patients. Out of 28 F+ patients, 13 (46.4%) had positive moulds sIgE (**group A, AFRS**). Patients with F+ and negative moulds sIgE Ab (15/28, 53.6%) were considered as non-mould-allergic FRS (**group B, non-mould-allergic FRS**) (Table 1). Patients with an absence of fungi on nasal mucosa and without mould hypersensitivity were classified as group C (84/112, 75%). It was found that frequency of AFRS was 1.3%, while the frequency of non-mould-allergic FRS was 2.8%, in the group of patients with CRS. Group A had an almost equal distribution of sex with a male to female ratio 1.2:1 and mean age 32.23 ± 13.25. More patients from group A than from group B (76.9% vs. 33.3% respectively, $P = .021$) had

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Table 1. Relationship of clinical characteristics of patients with positive moulds sIgE Ab and positive fungal finding (A group, $n = 13$) and negative moulds sIgE Ab and positive fungal finding (B group, $n = 15$).

Variables		Group n (%) B $n = 15$ (53.6)	Group n (%) A $n = 13$ (46.4)	p
Sex				0.139
	M	12 (80)	7 (53.9)	
Age (mean ± SD)				0.535
		39.53 ± 13.06	32.23 ± 13.25	
Subjective improvement of symptoms after corticosteroid Th				*0.021
	Yes	5 (33.3)	10 (76.9)	
NP				0.705
	Yes	7 (46.7)	7 (53.9)	
Previous FESS > 1 (reFESS)				0.630
	Yes	13 (86.7)	12 (92.3)	
Asthma				*0.024
	Yes	4 (26.7)	9 (69.2)	
Duration of CRS (years)				*0.000
	<5	7 (46.7)	0 (0)	
	5-10	6 (40)	0 (0)	
	10-20	1 (6.7)	5 (38.5)	
	>20	1 (6.7)	**8 (61.5)**	
Rinoscopic score for objective assessment of CRS				0.637
	Mild	1 (6.7)	0 (0)	
	Moderate	1 (6.7)	1 (7.7)	
	Severe	13 (86.7)	12 (92.3)	
CT score for objective assessment of CRS				0.889
	Mild	1 (6.7)	1 (7.7)	
	Moderate	1 (6.7)	1 (7.7)	
	Severe	12 (80)	11 (84.6)	
Absolute eosinophils count (mm^3)				*0.006
	Positive (>350)	5 (33.3)	11 (84.6)	
Absolute eosinophils count (mean ± SD)				0.970
		442.40 ± 455.794	426.0 ± 228.125	
Total IgE Ab (kU/ml)				*0.021
	Positive (>100)	5 (33.3)	10 (76.9)	
Total IgE Ab (mean ± SD)				**0.05
		392.0 ± 589.803	685.38 ± 695.121	
SPT (inhalant allergens)				0.194
	Positive	7 (46.7)	3 (23.1)	
SPT dust mite				*0.008
	Positive	2 (13.3)	8 (61.5)	
SPT animal epithelia				0.750
	Positive	3 (20)	2 (15.4)	
SPT pollens				0.840
	Positive	11 (73.3)	9 (69.2)	
SPT moulds				
	Positive	7 (46.7)	3 (23.1)	0.194
	Alternaria alternata	0 (0)	3 (23.1)	*0.049
	Penicillium notatum	5 (33.3)	0 (0)	*0.022
	Cladosporium herbarum	2 (13.3)	1 (7.7)	0.630
	Aspergillus fumigatus	6 (40)	1 (7.7)	*0.049

* According to Chi-square test, $p < 0.05$, **According to Mann–Whitney U test, $P < 0.05$.
Abbreviations: Ab, antibody; CRS, chronic rhinosinusitis, CT, computerized tomography; Eo, eosinophiles; FESS, functional endoscopic surgery of sinuses; NP, nasal polyposis; SD, standard deviation; sIgE, specific Immunoglobulin E; SPT, skin prick test; Th, therapy.

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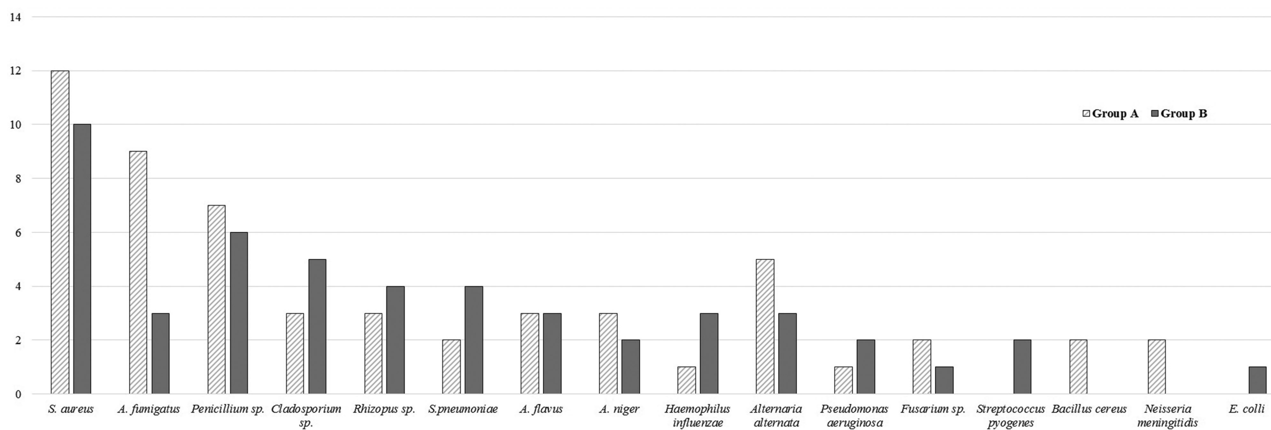

Figure 1. Distribution of fungal and bacteria species isolated from sinonasal mucosa of patients from groups A and B: predomination of *S. aureus*, *A. fumigatus*, *Penicillium* sp. and *A. alternat*e in the group A and *S. aureus*, *Penicillium* sp. and *Cladosporium* sp. in the group B.

improvement of symptoms after 3 months of local corticosteroid therapy. Asthma was detected more frequent in group A than in group B (69.2% vs. 26.7% respectively, $P = .024$). Duration of CRS more than 10 years, was more frequent in group A than in group B (100% vs. 13.3% respectively, $P = .000$) (Table 1).

Allergological parameters of patients

Patients from group A had more often eosinophils count ≥350 (84.6% vs. 33.3%, respectively, $P = .006$) and higher mean level of total IgE Ab than patients from group B (685.38 ± 695.121 vs. 392.0 ± 589.803, respectively, $P = .05$). There were no differences in the sum of results of SPT with inhalant allergens between groups, but individually, patients from group A were more often positive to dust mite allergens (*D. pteronyssinus* and *D. farinae*) than non-mould-allergic FRS patients (61.5% vs. 13.3%, respectively, $P = .008$). There was also statistically significant difference between groups in predominant mould SPT allergens: patients from group A had the most common hypersensitive reaction to *A. alternata* ($P = .049$), while patients from group B had the most common hypersensitive reaction to *P. notatum* ($P = .022$) and *A. fumigatus* ($P = .049$) (Table 1).

Distribution of fungal and bacteria species isolated from sinonasal mucosa of patients

The most common fungal species isolated from ISNS-F/ISNS-A of group A was *A. fumigatus* isolated in 28.6% (10/35) cases, followed by *Penicillium* sp. 20% (7/35); while *Penicillium* sp. (6/27; 22.2%) followed by *Cladosporium* sp. (5/27; 18.5%) were the most common isolates in group B. *Staphylococcus aureus* was commonly isolated from both groups; 60% in ISNS-F/ISNS-A of group A and 45.5% of group B (Fig. 1). There was no relationship between the most common sensitized fungi and the most common cultured fungi nor in group A neither in group B.

Biodiversity of fungi in air samples obtained from homes of patients

Out of all fungal isolates ($n = 225$) from the air samples obtained from the homes of patients with CRS, 41 isolates were from the home samples (HS) of group A, 24 from HS of group B, and 159 from the HS of non-FRS patients (Fig. 2). The most predominant species was *A. niger* isolated in 57% of HS, followed by *Penicillium* sp. isolated in 26% of HS (Fig. 2). There was an overlap in fungal isolates from ISNS-F/ISNS-A and air sample in the case of only one patient (Table 2).

Discussion

This extensive study provided a comparison of CRS cases due to fungal allergies compared to those without allergy. Our analyses found: (i) patients with CRS and positive mould sIgE (30.4%) had more often repeated FESS intervention ($P = .005$), presence of NP ($P = .025$), and more severe CRS form; (ii) in our study, frequency of AFRS was 1.3%, while frequency of non-mould-allergic FRS was 2.8%; (iii) in patients with AFRS following parameters were more frequently present: asthma ($P = .024$), longer duration of CRS (>10 years) ($P = 0$) and positive response to corticosteroid therapy ($P = .021$).

Given ubiquitous nature of fungi, exposure is unavoidable, but it is not yet known which fungi are able to

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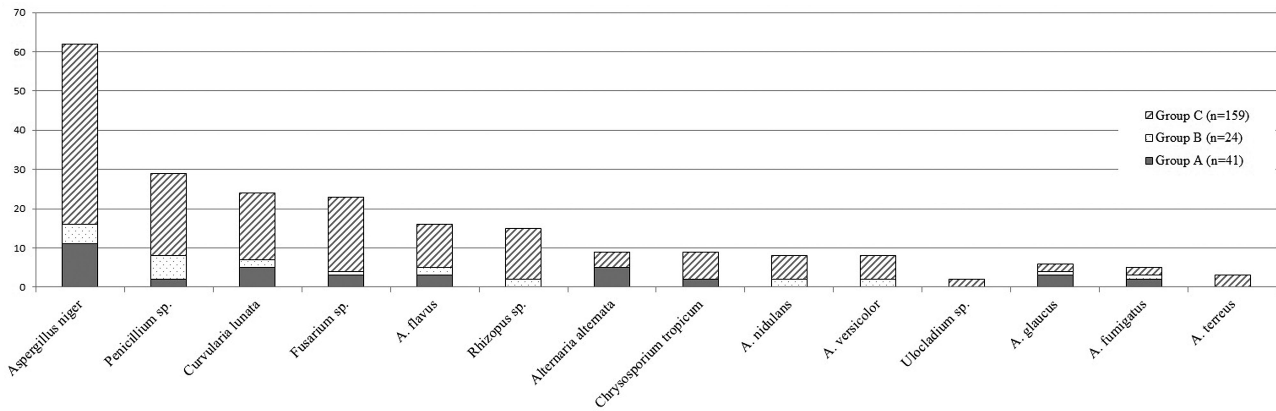

Figure 2. Fungal biodiversity in environment: *A. niger*, *Penicillium* sp. and *Curvularia* were the most common isolates obtained from the home's air samples from all groups.

cause allergies and chronic airway inflammation.[15] There is growing necessity to show which fungi are prevalent in patients' environments and to compare them with fungi prevalent at sinonasal mucosa of FRS patients.[15,16] Common fungal species isolated from the sinonasal cavity of AFRS patients in our study were *A. fumigatus* (28.6%) and *Penicillium* sp. (20%), while the most common isolated bacteria was *S. aureus* (60%). Few studies claim the strong association between *S. aureus* superantigens and AFRS.[17,18] Additionally, *S. aureus* colonization was significantly more prevalent in AFRS in comparison with nonallergic FRS.[19] *S. aureus* superantigen-induced immune response leads to modulation of a severity of eosinophils inflammation[20] and correlates with total IgE Ab levels in AFRS patients, intimating relationship between bacterial colonization and allergic hypersensitivity.[21] In meta-analyses of Jing Ou et al., it was claimed that *S. aureus* superantigen may be the risk factor for the chronicity and severity of CRS that leads to modulation of local immune response resulting in the development of AFRS.[17,21]

The presence of fungus in culture does not establish nor eliminate the diagnosis of AFRS, as there is the question if an isolated fungus is a causative agent or contaminant from air.[22] Buzina et al. showed that proportion of individuals positive for fungal growth was exactly the same (91.3%) by flushing the noses of CRS patients and healthy volunteers.[15] Study results of Ponikau et al., who used the same sampling technique, showed the slightly higher result (96% and 100% positive for patients and controls, respectively).[25] Other studies reported comparatively lower rate of isolation: 80% (Perez-Jaffe et al. 1997), 61.5%[22] and 48.6%.[26] Though we applied improved technique, fungal detection in the sinuses was only 25% of CRS. It is difficult to explain such low isolation rate in our study, but geographical location of study and environment factors may play an important role in fungal colonization of nasal mucosa.

As fungi are continually present as allergens on sinonasal mucosa, lavage with hypertonic NaCl may help to decrease fungal load and antigenic exposure. Our results showed that longer duration of CRS (>10 years) leads to the development of AFRS. This finding could be explained by modulated local immune response due to the long-lasting inflammation and continual presence of fungi as triggers on the sinonasal mucosa. We propose that duration of CRS should be crucial for progression from local to the systemic allergic reaction, which is manifested as the finding of positive moulds sIgE and the higher number of eosinophils. In related studies, authors found clusters of eosinophils grouped around fungal elements in nasal mucus and concluded that fungi act as triggers for an eosinophil-mediated immune response.[22,23] In our study, patients with AFRS had higher eosinophil count ($\geq$350 mm^3) compared to patients with non-mould-allergic FRS, which supports the hypothesis of eosinophil's important role in pathogenesis and diagnosis of AFRS.

Though type I hypersensitivity and presence of fungi in allergic mucin are considered as a hallmark of AFRS, Ponikau et al. showed that fungi in mucin may be present in many cases of CRS without the presence of type I hypersensitivity and coined term "eosinophilic fungal rhinosinusitis" (EFRS).[25] Their hypothesis was further supported by subsequent studies.[4,5,8] On the other hand, the presence of other allergological parameters, such as mould sIgE Ab, but without the presence of any fungal hyphae in mucin, has been noted in the group of patients with CRS.[27] In our study, 46.4% of patients with positive anti-moulds sIgE had the positive fungal finding on sinonasal mucosa and could be considered as AFRS. Differential diagnostic criteria for AFRS evolved from our study are: (i) presence of specific

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Table 2. Comparison of fungal isolates from sinonasal and air samples from patients from groups A and B.

	Patients (Pt) Group A	Sinonasal isolate $n = 35$	Air sample isolate $n = 41$
Group A	Pt 1	*Fusarium* sp.; *Penicillium* sp.	*A. niger; Curvularia lunata; A. alternata*
	Pt 2*	*A. niger; Rhizopus* sp.; *A. fumi gatus*	*Penicillium* sp.; *A. alternata; A. fumigatus; A. niger*
	Pt 3	*A. flavus; Penicillium* sp.; *A. fumigatus*	*A. niger; Curvularia lunata*
	Pt 4	*Cladosporium* sp.; *A. alternata; A. fumigatus*	*A. niger; A. glaucus; Curvularia lunata*
	Pt 5	*Fusarium sp.*	*A. niger; Penicillium* sp.
	Pt 6	*A. niger; Penicillium sp.; A. fumigatus*	*Curvularia lunata; A. alternata;*
	Pt 7	*A. flavus; Penicillium* sp.; *A. fumigatus*	*A. niger; Fusarium* sp.; *A. glaucus*
	Pt 8	*Rhizopus* sp.; *Penicillium* sp.; *A. fumigatus*	*A. niger; Fusarium* sp.
	Pt 9	*A. alternate*	*A. niger; A. flavus*
	Pt 10	*Cladosporium* sp.; *A. alternata; A. fumigatus*	*A. niger; Chrysosporium* sp.; *Curvularia lunata*
	Pt 11	*A. alternata; A. fumigatus*	*A. niger; A. flavus*
	Pt 12	*A. flavus; Penicillium* sp.; *A. fumigatus*	*A. niger; Curvularia lunata; A. alternata*
	Pt 13	*Cladosporium* sp.; *Penicillium* sp.; *A. fumigatus*	*A. niger; A. flavus*
	Pt 14	*Rhizopus* sp.	*Curvularia lunata; A. alternata A. fumigatus; A. niger*
	Pt 15	*A. alternate*	*Fusarium* sp.; *Chrysosporium* sp.; *A. glaucus; Curvularia lunata*
	Group B	$n = 27$	$n = 24$
Group B	Pt 1	*Fusarium sp.*	*A. niger; Penicillium* sp.
	Pt 2	*Rhizopus sp.*	*Curvularia lunata*
	Pt 3	*Cladosporium* sp.; *A. alternata*	*A. niger; Penicillium* sp.
	Pt 4	*A. niger; Rhizopus* sp.	*Penicillium* sp.
	Pt 5	*A. flavus; Cladosporium* sp.	*A. niger; Penicillium* sp.
	Pt 6	*Rhizopus* sp.; *Penicillium* sp.	*Curvularia lunata*
	Pt 7	*Cladosporium* sp.; *Penicillium* sp.; *A. fumigatus*	*A. niger; Rhyzopus* sp.
	Pt 8	*A. flavus; Penicillium* sp.	*Fusarium* sp.; *A. nidulans*
	Pt 9	*A. flavus; Cladosporium* sp.	*Penicillium* sp.; *A. versicolor*
	Pt 10	*Rhizopus* sp.; *Penicillium* sp	*A. niger; A. nidulans*
	Pt 11	*Cladosporium* sp.; *A. alternata; Penicillium* sp.	*A. flavus; Rhyzopus* sp.; *A. fumigatus*
	Pt 12	*A. alternata; A. fumigatus*	*Penicillium* sp.; *A. glaucus*
	Pt 13	*A. niger; Penicillium* sp.; *A. fumigatus*	*A. flavus; A. versicolor*

*Patient with same fungal isolate from sinonasal and air samples.

allergological indicators (sIgE, eosinophils, or SPT), (ii) presence of fungi in sinonasal aspirate, and (iii) positive response to corticosteroid therapy.

We postulated that pathophysiology of AFRS is similar to the allergic fungal bronchopulmonary disease. The atopic host is exposed to the fungi via normal nasal respiration, which provides initial antigenic stimulation.[22] Accumulation of allergic mucin obstructs involved sinuses and creates an ideal environment for proliferation of fungi inhaled from the air.[5,23] Predominant fungal species in air samples taken from homes of CRS patients was *A. niger* (57%), followed by *Penicillium* sp. (26%), and we found common positive reactions on SPT in AFRS group were to allergens of *Alternaria*, *Aspergillus,* and *Penicillium*. The presence of different species in outdoor air depends on geographic location as is shown previously, and discrepancies in our study, between isolated species in the sinuses and isolated species from the air samples, could be because of regional differences in the etiologic fungi, as well as because cross-reactivity of allergens derived from common airborne fungi that could also play important role in atopic patients.[5,23,28] Avoidance of places with the fungal overload of air may help patients with AFRS in the prevention of possible sharing of airborne fungal epitopes.

Investigators from the Mayo Clinic hypothesized that nearly all cases of CRS were driven by the host responses to common airborne fungal elements.[16,29,28] Using specialized detection techniques, fungal microorganisms were identified in 100% sinonasal cavities of both CRS and healthy control patients.[15] These findings were later combined with *in vitro* studies demonstrating hyperreactivity of peripheral blood mononuclear cells to antigens of *Alternaria*.[17] This response was obtained in CRS patients but was absent in controls and thus was proposed to reflect an immunologic sensitization to fungal antigens independent of atopy.[19] Later studies demonstrated that nasal mucus from

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patients with CRS triggered eosinophil migration[18] and that 60-kDa component of *Alternaria* elicited eosinophil degranulation.[29] In summary, *Alternaria* and possibly other fungi were proposed to serve a dual role: first, inhaled and processed fungal proteins were presented to sensitize T-cells triggering cytokine response that activated and attracted eosinophils to the mucosal surface. Second, these eosinophils then targeted fungi as an aberrant host defense response, with degranulation and collateral tissue damage mediating symptoms of CRS. Activated eosinophils, obtained from CRS patients, were shown on videos attacking fungi, providing persuasive visual evidence to support this hypothesis.[16,30] Lastly, fungal cell walls contain chitin, which has been shown to induce the Th2 response in some human and animal models, although any role in CRS remains unclear.[31–34] Fungi also likely play the key role in classic AFRS.[33]

The mechanism by which airborne fungi turn from 'normal flora' into triggers of inflammatory reactions, resulting in FRS, is currently under investigation at several research centers. It is shown in our study that patients with AFRS have similar symptoms as patients with non-mould-allergic FRS, but the statistically significant better response on corticosteroid therapy. This result would encourage in diagnosing of AFRS cases.

Fungal species are ubiquitously present in an environment, as well as in human sinonasal tract as normal content. We propose that persistent presence of fungi and prolonged duration of CRS should be the silent threat for progression of inflammation and development of FRS. As the fungi trigged enhancement of IgE Ab and eosinophils, lavage with hypertonic NaCl should be included in everyday hygiene routine in an effort to decrease fungal load and antigenic exposure. The presence of allergological parameters and better response to corticosteroid therapy in AFRS patients should be crucial diagnostic criteria for AFRS. Further studies are required to precise define and resolve diagnosis of non-mould-allergic FRS.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Supplementary material

Supplementary data are available at *MMYCOL* online.

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