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IDENTIFICATION AND INCIDENCE OF FUNGAL STRAINS IN CHRONIC RHINOSINUSITIS PATIENTS

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The fungal revolution taking place in otorhinology inspired us to study the frequency of occurrence of fungi in the nasal mucus of chronic rhinosinusitis (CRS) patients (with or without polyposis) in order to evaluate the incidence of eosinophilic fungal sinusitis in CRS patients. Ninety-six samples were examined from patients with CRS. In 74 cases mucus was collected non-invasively, and in 22 cases during operation. The Gram-stained direct smears of all samples were also evaluated. Bacteria and fungi colonizing in the mucus were detected by culturing method. The control group consisted of 50 healthy volunteers. Typical aerobic pathogenic bacteria could be isolated from 34 patients. Fifty-seven aerobic bacteria were isolated, i.e. 1.6 bacteria/positive patient with a maximum of 3 different bacteria/sample. The most frequently isolated bacteria were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Streptococcus pneumoniae,* and *Haemophilus influenzae*. Yeasts and moulds could be detected from 79 patients (83%): *Candida albicans, Candida* spp., *Aspergillus* spp., *Cladosporium* spp, and *Penicillium* spp. were isolated most frequently. Altogether 237 yeasts and moulds were isolated, i.e. 3.0 different fungi/positive patient, with a maximum of 5 different fungi/sample. In the control group aerobic pathogens were not isolated, only apathogenic species. Fungi were isolated from 22 healthy patients (44%). These data indicate that fungi are frequently involved in the aetiology of CRS. IgE-mediated hypersensitivity to fungal allergens could not be proven in our patients.

Keywords: chronic rhinosinusitis, fungi, fungal rhinosinusitis, polyposis

Introduction

Although fungal disease of the paranasal sinus was once considered as a rare disorder, fungi have been increasingly recognized as important pathogens in sinusitis over the last decade. Classification of fungal rhinosinusitis is important for accurate prognosis and therapy. This classification is based on the immunological relationship of the fungus to the host. The most important distinction is between invasive and noninvasive fungal rhinosinusitis. Within the invasive division we distinguish acute invasive and chronic invasive (granulomatous and nongranulomatous forms) rhinosinusitis. Within the noninvasive division saprophytic colonization, fungus balls, and allergic fungal rhinosinusitis can be found [1] (Table I).

Table I

Classification of fungal rhinosinusitis

Non-invasive	**Invasive**
Saprophytic colonization	Acute fulminant
Fungus ball (mycetoma)	Chronic
Allergic fungal infection	Immunocompetent
	Immunocompromised
	Sclerosing
	Granulomatous

Allergic fungal rhinosinusitis (AFRS) [2] is possibly a chronic fungal disease (not an infection) of the sinuses to which the body's immune system hyperreacts, thereby creating significant inflammation, oedema, obstruction, and polyposis [3]. Although AFRS (once called allergic aspergillus sinusitis) is now widely accepted as a distinct clinical problem, controversies regarding the causative organism, diagnosis and the best treatment continue. AFRS typically occurs in atopic immunocompetent adults, and is often associated with polyps, asthma or aspirin intolerance. Although no universally accepted standards were described for the diagnosis of AFRS, a hallmark of AFRS is the presence of allergic mucin characterized by eosinophilia and by the presence of Charcot–Leyden crystals, and fungal hyphae. Immunochemical and molecular techniques have also been developed for the diagnosis of AFRS [4–7].

The fungal revolution taking place in otorhinology [8–14] inspired us to examine chronic rhinosinusitis (CRS) patients (with or without polyposis). We determined the frequency of occurrence of bacteria and fungi in the nasal mucus of CRS patients. This prospective study proved the incidence of AFRS in CRS patients.

Material and methods

Samples were examined from 96 patients with a clinical diagnosis of eosinophilia and CRS. Mucus was collected non-invasively in 74 cases, and intraoperatively in 22 cases. The Gram-stained direct smears of all samples were evaluated.

Bacteria and fungi present in the mucus were detected by culture methods. Ten ml samples were diluted with Sputolysin (1:1). The mixtures were vortexed for 30 s, and centrifuged at 3000 g for 10 min. One and half ml of the prepared sediment was inoculated onto 2 different inhibitory mould agar plates containing brain-heart infusion agar with 5% sheep blood, ciprofloxacin (5μg ml^{-1}), gentamycin (5μg ml^{-1}), and chloramphenicol (125μg ml^{-1}). All samples were also cultured aerobically on 5% blood agar, chocolate agar and Sabouraud agar. The special mould plates were incubated at 30 ^{o}C and allowed to grow for 30 days. Blood agar and chocolate agar cultures were incubated at 37 ^{o}C for 48 h. Sabouraud agar plates were incubated at 37 ^{o}C for 48 h and allowed to grow at 30 ^{o}C for 5 days. The control group consisted of 50 healthy volunteers whose nasal secretion was cultured in the same way.

The total IgE levels were determined using standard techniques. The VIDAS (Vitek Immunodiagnostic Assay System) 30 assay (bioMerieux) was applied with a VIDAS Instrument using an automated enzyme-linked fluorescent immunoassay (ELFA) for the quantitative detection of total IgE in human serum [15].

Results

Chronic rhinosinusitis patients were selected for this study based on cytological and histological examinations. The presence of allergic mucin characterized by eosinophilia, fungal hyphae and Charcot–Leyden crystals were the criteria for selecting CRS patients to be involved. The numbers of males and females examined were the same. The age distribution of the CRS patients is shown in Figure 1. Most of the patients belonged in the age group 41–50 years.

Typical respiratory tract pathogenic bacteria were isolated from 34 CRS patients. Fifty-seven different aerobic bacteria were isolated, i.e. 1.6 bacteria/positive patient, with a maximum of 3 different bacteria/sample. The most frequently isolated bacteria were *Staphylococcus aureus, Escherichia coli, Klebsiella* spp., *Streptococcus pneumoniae*, and *Haemophilus influenzae* strains (Table II and Figure 2). In the control group, no typically aerobic respiratory tract pathogenic bacteria were isolated, only apathogens (Figure 2).

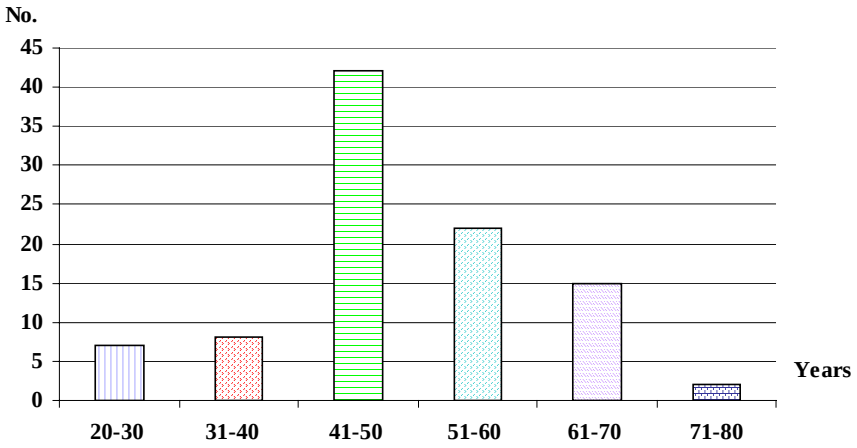

Figure 1. Age distribution of chronic rhinosinusitis patients (96)

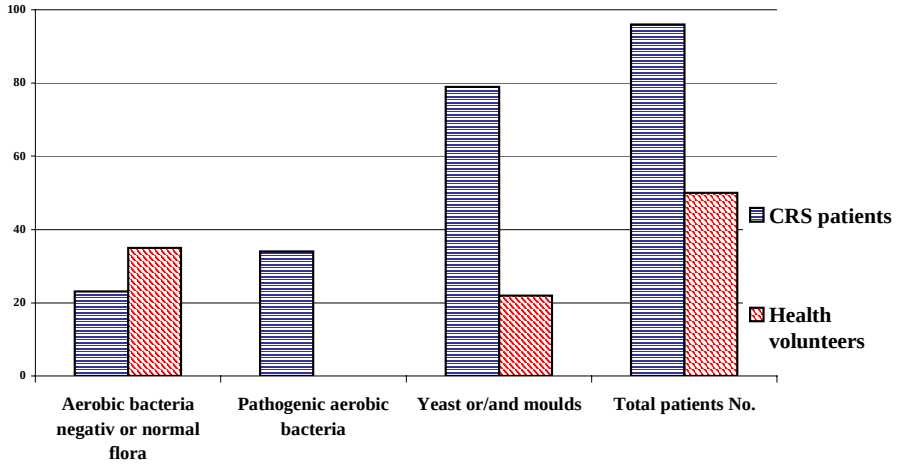

Figure 2. Culture results from healthy volunteers (50) and from chronic rhinosinusitis patients (96)

Yeasts and moulds could be detected in 79 (83%) of the 96 CRS patients. *Candida albicans, C. krusei, C. glabrata, C. parapsilosis, Aspergillus* spp., *Cladosporium* spp., *Geotrichum candidum, Penicillium* spp., *Scopulariopsis* spp. and Zygomycetes were isolated (Figure 3). Altogether 237 strains of different species of yeasts and moulds were identified (Table III), i.e. 3.0 different fungi/positive patient,

with a maximum of 5 different fungi/sample. Fungi were also isolated from nasal secretions of 22 of the examined 50 healthy volunteers (44%) (Figure 4).

IgE levels in most CRS patients were not significantly higher than those observed in the control group (data not shown).

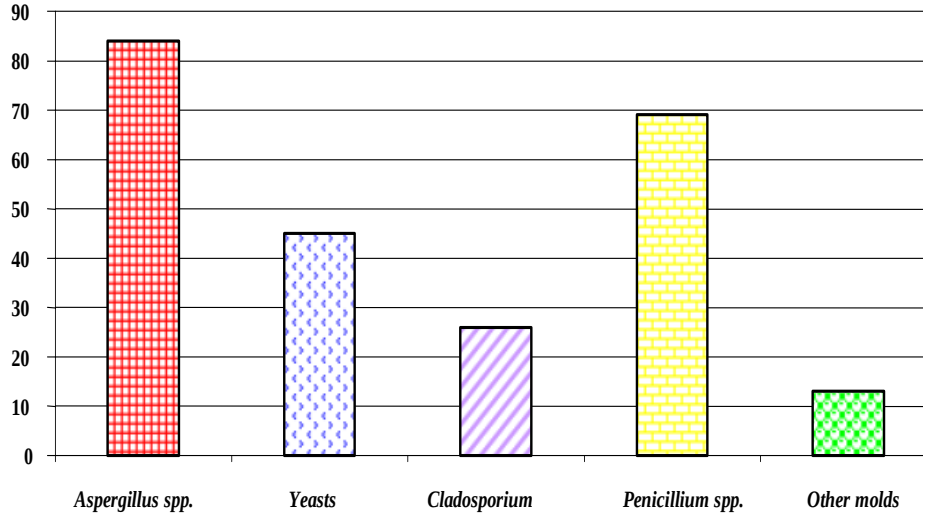

Figure 3. Distribution of fugni isolated from chronic rhinosinusitis patients (79 patients / 273 isolates)

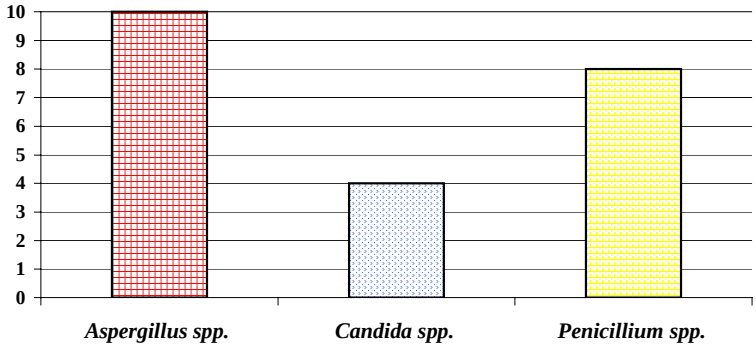

Figure 4. Distribution of fungi isolated from healthy volunteers (50 patients / 22 isolates)

Table II

Distribution of aerobic pathogenic bacteria from chronic rhinosinusitis patients

Species	No.
Gram-positive bacteria	
Staphylococcus aureus	17
Streptococcus pyogenes	3
Streptococcus pneumoniae	4
Streptococcus agalactiae	1
Enterococcus faecalis	2
Gram-negative bacteria	
Haemophilus influenzae	4
Moraxella catarrhalis	3
Escherichia coli	8
Klebsiella spp.	8
Proteus mirabilis	4
Pseudomonas aeruginosa	3
Total	57
Patients No	34
Total isolates	57
Bacteria/patient	1.6
Max. isolates/patient	3

Table III

Distribution of moulds from chronic rhinosinusitis patients (CRS) and healthy volunteers

Species	CRS patients	Healthy volunteers
Acremonium spp.	4	0
Alternaria spp.	2	0
Aspergillus candidus	6	0
Aspergillus clavatus	2	0
Aspergillus flavus	25	2
Aspergillus fumigatus	10	3
Aspergillus niger	6	0
Aspergillus versicolor	29	5
Aspergillus spp.	6	0
Candida albicans	17	2
Candida glabrata	12	2
Candida parapsilosis	10	0

Table III (continued)

Species	CRS patients	Healthy volunteers
Geotrichum candidum	6	0
Cladosporium spp.	26	0
Humicola spp.	1	0
Penicillium chrysogenum	21	0
Penicillium humuli	7	0
Penicillium roquefortii	12	2
Penicillium simplicissium	21	6
Penicillium spp.	8	0
Scopulariopsis spp.	1	0
Trichophyton spp.	2	0
Zygomycetes	3	0
Total	237	22

Discussion

Our prospective study assessed the incidence of eosinophilic fungal sinusitis in chronic rhinosinusitis patients. Samples were examined from 96 patients with a clinical diagnosis of CRS. Before culturing of nasal samples, microorganisms were released from the mucus by Sputolysin treatment. Inoculated plates were incubated at 30 ^{o}C, since this temperature is appropriate for most fungal organisms encountered in the clinical laboratory. Mucus was collected non-invasively in 74 cases, and intraoperatively in 22 cases. Bacteria and fungi present in the mucus were detected by direct microscopy, and by culture methods. The most frequently isolated bacteria were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* strains, while among fungi, *Candida albicans*, *Candida* spp., *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp. could be isolated most frequently. While bacteria were isolated from 34 patients, fungi

were present in the mucus of 79 of the examined 96 CRS patients. Significant differences were observed concerning the number and variety of yeasts and moulds recovered from CRS patients and the control group. IgE-mediated hypersensitivity to fungal allergens could not be proven in most of our patients.

The unrefuted diagnostic criteria of AFRS are: (1) CRS, (2) presence of allergic mucin, and (3) presence of fungal organisms in the mucin [11]. These criteria were met in 83% of the CRS patients examined in our study. Concerning the causative organism, AFRS was once thought to be caused by *Aspergillus* species partly because of its histopathological similarities to allergic bronchopulmonary aspergillosis [8]. However, subsequent studies implicated several other fungi [9, 10]. Although these studies found *Bipolaris (Drechslera) spicifera* as the most common fungus cultured from patient samples, strains representing other fungal genera have also been isolated (*Aspergillus, Curvularia, Alternaria, Cladosporium,* etc.). Recently, a more comprehensive study carried out at the Mayo Clinic examined the fungi isolated from nasal secretions of 210 patients [11]. Altogether 541 fungal cultures were obtained, representing 40 different genera. The most prevalent genera implicated were *Alternaria, Aspergillus, Candida, Cladosporium, Fusarium* and *Penicillium*. *Curvularia* and *Bipolaris* species were recovered in only 1% of cases. Our data are mostly in agreement with those of Ponikau et al. [11] regarding both the frequency and the range of fungi recovered from CRS patients. A similar correlation was observed between our findings and those of Hendolin et al. [6], Kaprovich-Tate et al. [5], Perez-Jaffe et al. [7] and Noble et al. [13] concerning the incidence of fungal species recovered from nasal secretions of CRS patients. In contrast, Catten et al. [4] used a PCR based method to detect fungi in nasal secretions, and did not observe significant differences between the incidence of fungi recovered in CRS and healthy patients. This result could be explained by the fact that PCR also detects non-viable cells, thus giving rise to false positive results.

All the fungal species identified in this study were previously implicated as possible etiological agents of AFRS [2, 6–13]. Noble et al. [13] observed that the predominant fungi isolated from patients with AFRS could also be recovered from air samples of the patients' residences. The lack of IgE mediated hypersensitivity reaction and involvement of Th2 cells in the response [14] indicate that these fungi possibly cause a local demarcated non-systematic allergy in the sinonasal region resulting in AFRS and frequently leading to polyps [13,14].

Although AFRS is considered as one of the most frequent form of nasal fungal disease in the USA, it was rarely encountered in Europe [16]. Our results indicate that AFRS is present in about the same proportion of CRS patients in Hungary as observed in the USA [11].

The effectiveness and required duration of the various medical treatments of AFRS are under active investigation. Both surgical intervention and the use of systemic steroids are recommended in the treatment of allergic fungal sinusitis. Some clinicians also used antifungal therapy [17–19]. Our previous results also indicated that treatment with antifungals could also improve the patients' status [20]. To clarify which antifungal compounds could be used most effectively antifungal susceptibility tests of the isolated fungi are in progress.

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