

# Mycology of the Nasal Cavity of Chronic Rhinosinusitis Patients with Nasal Polyps in the Island of Crete

Hajjiannou J.<sup>1,\*</sup>, Maraki S.<sup>2</sup>, Vlachaki E.<sup>3</sup>, Panagiotaki I.<sup>3</sup>, Lagoudianakis G.<sup>3</sup>, Skoulakis C.<sup>1</sup>, Bizakis J.<sup>1</sup>

<sup>1</sup>Department of Otolaryngology, university Hospital of Larissa, Thessaly, Greece

<sup>2</sup>Department of Microbiology, University Hospital of Heraklion, Crete, Greece

<sup>3</sup>Department of Otolaryngology, University Hospital of Heraklion, Crete, Greece

**Abstract** We evaluated the presence and microbiology of fungi in nasal secretions of patients suffering of chronic rhinosinusitis with nasal polyps on the population of Crete island. We tried to detect a correlation between the long term use of corticosteroid nasal sprays and fungus existence in nasal secretions. Nasal lavage specimens were collected and cultured for fungal growth. Twenty one different fungal species were isolated. Cultures were positive in 62.4% of chronic rhinosinusitis patients while they were positive in 18.8% of healthy controls ( $p < 0.001$ ). A comparison between nasal corticosteroid users vs. non users revealed fungal growth in 62% vs. 62.9%. Fungal growth was more frequent in chronic rhinosinusitis patients with polyps compared to healthy controls. This finding does not necessarily signify a causative role of fungi in chronic rhinosinusitis and might suggest that geographic and climate variations possibly influence nasal fungal colonization. Fungus species did not differ markedly to these reported by other investigators, however different prevalence rates of particular species may reflect the geographic and seasonal variations.

The long term use of local nasal corticosteroids does not seem to affect nasal fungal colonization in sufferers of chronic rhinosinusitis with nasal polyps.

**Keywords** Chronic, Rhinosinusitis, Nasal, Polyps, Corticosteroids, Mycology

## 1. Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nose and paranasal sinuses that is present for at least 12 weeks without complete resolution and is characterized by the presence of distinctive symptoms (e.g. nasal blockage, nasal discharge, facial pain and/or reduced sense of smell) and either endoscopic signs or computed tomography (CT) changes characteristic of the disease[1]. The cause and pathophysiology of CRS have been the field of extensive controversy. Several competing hypotheses exist regarding the underlying cause and the mechanism of the persistence of pathology and the categorization of patients into subgroups. Until recently bacterial invasion has been considered the main cause of CRS[2]. Several factors have been described as contributors to the development of CRS. Ostial blockage by oedema and inflammatory mucous, delayed recovery of mucociliary function, and mucous recirculation were described as mechanisms that directly lead to a transition from an acute to a chronic inflammatory process[3]. Certain anatomic abnormalities may not be as much of a factor as previously believed[2]. Despite the frequent co-occurrence

of bacteria with CRS, it is less certain that they have a vital role in the origin of the disease in all cases or are merely bystanders invading already inflamed tissue in some patients[4].

Molds are a subset of fungi that cover surfaces. They have been cultured from human sinuses with many different ramifications[5]. Their presence may be relatively benign, colonizing normal sinuses or forming saprophytic crusts. They also may cause a spectrum of pathology, ranging from non-invasive fungus balls to invasive, debilitating disease. There is increasing interest in the concept that the most common form of sinus disease induced by fungus may be caused by the inflammation stimulated by airborne fungal antigens[3].

The presence of non-invasive fungi in the nose and paranasal sinuses is required to adequately diagnose non-invasive fungal rhinosinusitis. Nevertheless, proving their presence is difficult. For many years, contradictory results have been published; prevalence rates range from 0% to 100% depending on different specimen collection and fungus detection techniques[6-10].

In recent studies using newer collection and culture techniques a broad spectrum of fungal species has been identified with more than one species isolated from individual patients and with several geographic variations [3].

\* Corresponding author:  
irakliotis@yahoo.com (Hajjiannou J.)

Published online at <http://journal.sapub.org/otolaryn>

Copyright © 2012 Scientific & Academic Publishing. All Rights Reserved

We sought to evaluate the presence of fungi in nasal secretions of patients suffering of CRS on the population of Crete and to assess the most common species.

Topically administered glucocorticoids comprise one of the first line treatments of CRS and their prolonged use is very common in patients suffering of this condition[1]. Therefore we tried to detect a potential effect of the long term use of corticosteroid nasal sprays in the existence of fungi on nasal secretions.

## 2. Material and Methods

This study was a prospective nonrandomized controlled trial conducted in the University hospital of Crete, a tertiary hospital who accepts referrals from all over the island of Crete. Ethical approval was obtained by the local ethics committee. All subjects gave written informed consent and underwent a complete medical history and examination. In this prospective study, one hundred seventy consecutive patients suffering of CRS with nasal polyps of different stages, divided in two groups, were enrolled. The first group consisted of 100 otherwise healthy individuals suffering of CRS with nasal polyps using intranasal corticosteroid sprays but not oral steroids for the past three months. The second group included 70 patients suffering of CRS with nasal polyps that had not been treated with nasal or oral corticosteroids for the past three months. Thirty-two healthy volunteers without any history of nasal pathology and healthy nasal mucosa, determined by nasal endoscopy, comprised a third group serving as controls. Clinical diagnosis of CRS with nasal polyps was established by means of nasal endoscopy. CRS patients enrolled in the study had CT scans without signs of invasive disease. Patients that had been treated with antibiotic therapy within 4 weeks, acute airway infection or acute bacterial exacerbation of their CRS and patients that had nasal surgery in the past six months were not included in the study.

### 2.1. Collection and Culture Technique

Specimens were collected using the nasal lavage method as described by Ponikau[6]. Two puffs of Oxymetazolin spray 0.1% were sprayed into each nostril to produce vasoconstriction. After approximately two minutes each nostril was flushed with 20ml of sterile saline using a sterile syringe with a sterile curved blunt needle. The patient was taking a deep inspiratory breath and hold it before the injection of the saline. The patient then forcefully exhaled through the nose during the flushing. The return was collected in a sterile pan, put in a sterile centrifuge 50 ml tube and sent directly to the mycology laboratory, where it was processed under a laminar flow hood to prevent contamination with airborne fungi. The fluid was suspended with an equal volume of diluted dithiothreitol (Sputasol<sup>®</sup>; Oxoid, Hampshire, England), vortexed for 30 seconds and incubated at room temperature for 15 minutes. Dithiothreitol breaks apart the disulfide bonds, liquefying the mucus and

releasing the fungal elements. The specimen was then centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the sediment was homogenized by vortexing for 30 seconds and inoculated eight petri agar plates (two with Sabouraud glucose agar supplemented with chloramphenicol (0.05 g/l), two with Sabouraud glucose agar supplemented with chloramphenicol (0.05 g/l) and cycloheximide (0.4 g/l), two with malt extract agar and two with Czapek-Dox agar); then one petri dish of each set was incubated at 30<sup>o</sup> C and 20<sup>o</sup> C, respectively for a 30-day period. Plates were examined at 3-day intervals. Fungal isolates were identified using standard methods[11].

### 2.2. Statistics

Pearson's Chi-Square test was used for comparing groups. Statistical analysis was performed with the use of SSPS 14.0 software (SPSS, Inc., Chicago, IL, U.S.A.)

## 3. Results

Positive cultures for fungus were obtained in 106 (62,4%) of 170 patients with CRS while there were positive in 6 (18,8%) of 32 healthy control subjects ( $p < 0.001$ ).

A total of 21 fungus species were identified. *Penicillium* species (28.1%), *Aspergillus niger* (21.9%), *Candida albicans* (9.4%) and *Alternaria alternata* (6.3%) were more frequently isolated from both, patients with CRS and healthy individuals. *Table 1*.

**Table 1.** Percentage of fungi species in culture positive specimens

Fungal species	Percentage
<i>Penicillium</i> species	28.1
<i>Aspergillus niger</i>	21.9
<i>Candida albicans</i>	9.4
<i>Alternaria alternata</i>	6.3
<i>Aspergillus fumigatus</i>	3.1
<i>Cladosporium</i> species	3.1
<i>Rhodotorula glutinis</i>	3.1
<i>Aspergillus flavus</i>	3.1
<i>Alternaria</i> species	3.1
<i>Scedosporium piospermum</i>	1.6
<i>Candida parapsilosis</i>	1.6
<i>Bipolaris</i> species	1.6
<i>Verticillium</i> species	1.6
<i>Curvularia</i> species	1.6
<i>Rhizopus</i> species	1.6
<i>Candida tropicalis</i>	1.6
<i>Candida</i> species	1.6
<i>Candida parapsilosis</i>	1.6
<i>Pseudoallescheria boydii</i>	1.6
<i>Fusarium</i> species	1.6
<i>Scopulariopsis brevicaulis</i>	1.6

There was an average of 1.3 organisms per CRS patient and healthy controls without marked differences between these two groups.

In the group of the CRS patients using nasal corticosteroids sprays positive fungal cultures were obtained in 62 (62%) of 100. Likewise, 44 (62.9%) of 70 patients that

did not use nasal corticosteroids sprays had positive cultures for fungi. This difference was not significant.

#### 4. Discussion

Although fungal organisms are known to be responsible for some forms of nasal disease especially in immunocompromised patients, their role in the pathogenesis of CRS has been a topic of research and debate by many investigators with non clear conclusions yet [3, 6, 9]. It seems that mere presence of fungi in the nasal cavity is insufficient to implicate them as a pathogen in CRS. Several authors have published controversial results based on the identification of fungi in nasal secretions specimens collected with various techniques and identified with different methods. In several studies, reported results of fungal detection vary from 0-100 % depending on the employed method [3, 6-12]. Of all the collection techniques, the nasal lavage technique is considered to be the best while the addition of polymerase chain reaction (PCR) to standard culture techniques is considered to increase the rate of fungus detection according to recently published data [7, 10, 13]. However even with the combined use of standard culture techniques and PCR a high rate of negative results has been reported [13].

This is the first nasal mycology study performed in the Greek population. We have used the nasal lavage technique for specimen collection. Mucus was liquefied by using a mucolytic agent and fungi were separated from the mucus by centrifugation. Cultures were performed in selective media for fungi in samples taken from 170 patients suffering of CRS with nasal polyps and from 32 healthy individuals. Positive cultures for fungi were obtained in 62.4% (106 out of 170) patients suffering of CRS, and only in 18.8% (6 out of 32) healthy control subjects. This difference reached statistical significance suggesting that fungi are isolated more commonly in CRS patients than in healthy individuals. This is in contrast with previous studies where no significant difference was noticed between CRS patients and healthy controls [6, 11, 12]. This finding does not necessarily reflect a causative relationship among fungi and CRS with nasal polyps. It could indicate that in CRS patients the host immunologic response might differ compared to healthy individuals, influencing the colonization of the degenerated polypoid nasal mucosa by fungi. Besides in patients with CRS, the mucociliary transport system is severely impaired and thus the chance to detect inhaled spores is increased [14, 15]. As a consequence, inhaled spores may stay longer within the nasal airways in patients with CRS than in the airways of healthy controls, increasing the chance of them being detected. This might explain the difference between the high fungus detection rates in patients with CRS compared to the healthy control group. Of course since no PCR was performed for the fungi detection in our study the actual percentage of the colonized subjects might be higher; whereas the possibility of specimen contamination or climate influence cannot be excluded as well [16-18].

Regarding the high rate of negative cultures in our study, in comparison to those reported by other investigators, a possible explanation might be the specific climate characteristics of the island of Crete with its seasonal variations. Other investigators have reported regional and seasonal variations in nasal fungal colonization, for chronic rhinosinusitis and for rhinosinusitis with history of allergic disease [17-20]. While geographic variability of fungal rhinosinusitis has already been proposed in the past as a possible explanation of different cultural fungus detection rates [13].

Crete straddles two climatic zones, the Mediterranean and the North African. The north coast, falls within the former while the south coast falls in the North African climatic zone. As such, the atmosphere in certain areas of the island can be quite humid, depending on the proximity to the sea, while winter is fairly mild. During the Cretan summer, average temperatures reach the high 20s-low 30s Celsius (mid 80s to mid 90s Fahrenheit), with maxima touching the upper 30s to mid 40s (above 110°F / 43.3°C). (World Meteorological Organization, Hong Kong Observatory). Since our patients were referrals from all over the island of Crete, the unexpected high percentage of negative results could suggest that different proportions of nasal fungi colonization exist in the population of different parts of the island due to the climate differences between the north and south part of the island. However since geographic origin of the patients was not recorded and statistically studied further investigation is necessary to support this theory.

In a total of 112 positive for fungi culture 21 species were identified. *Penicillium* species (28.1%), *Aspergillus niger* (21.9%), *Candida albicans* (9.4%) and *Alternaria alternata* (6.3%) were more frequently isolated from both, patients with CRS and healthy individuals with an average of 1.3 organisms per patient. No difference between patients and healthy controls was observed. These findings are similar to those previously reported by other investigators although there are differences in their frequency of appearance [6, 11, 17]. In Ponikau's study *Aspergillus* was the most frequently isolated species followed by *Penicillium*, *Cladosporium*, *Candida*, *Aureobasidium*, and *Alternaria* [6] while in another study published by Shin *Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium* were more frequently isolated from CRS patients and normal volunteers. These differences could be attributed to geographic and climate variations as well.

Topically administered glucocorticoids comprise one of the first line treatments of CRS [1]. The clinical efficacy of glucocorticoids may depend in part on their ability to reduce airway eosinophil infiltration by preventing their increased viability and activation [21]. Potential adverse events related to the administration of intranasal corticosteroids are effects on growth, ocular effects, effects on bone, and on the hypothalamic-pituitary-adrenal axis [21]. There are several reports regarding the effect of inhaled steroids in the fungal colonization of the oesophageal and airway mucosa [22-24]. However few are known about their impact in nasal fungal

colonization especially in patients suffering of CRS where their prolonged use constitutes a common practice.

We compared 100 patients using nasal corticosteroid sprays for a prolonged period of time with a group of 70 patients that had not done been using any of these sprays for at least six months. There were not any significant differences between these groups regarding positive fungal cultures or the specific fungal species that were cultured in these two groups. This finding suggests that nasal corticosteroid sprays do not influence nasal colonization by fungi. Of course this conclusion is not safe since several bias exist. The study was not randomized, different ways of sprays application exist, and different sprays containing different types of steroids are commonly used by the patients. Properly designed studies in the future are needed to extract safer conclusions.

## 5. Conclusions

Conclusively, in our study patients suffering of CRS with nasal polyps in the island of Crete appear to be more frequently colonized by fungi species than healthy controls in contrast to previous published studies. This finding alone is not enough to suggest a pathogenetic role of fungi in CRS and if detection techniques limitations are to be excluded it might reflect an increased susceptibility of this group to fungi colonization especially in certain geographic areas or a decreased rate of colonization in healthy individuals in these areas. Further investigation is necessary to elucidate the accurate role of fungi in CRS pathogenesis.

Nasal mycology of our study population does not seem to differ regarding the fungi species detected by other investigators and the frequency in the appearance of different species could be attributed to geographic and seasonal climate variations.

Nasal corticosteroid use does not seem to influence fungal nasal colonization in patients with CRS, although further studies are required for safe conclusions.

## REFERENCES

- [1] Fokkens W, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps 2007. *Rhinol Suppl.* 2007;1:1-136.
- [2] Hamilos DL. Chronic sinusitis. *J Allergy Clin Immunol.* 2000;106:213-27.
- [3] Davis LJ, Kita H. Pathogenesis of chronic rhinosinusitis: role of airborne fungi and bacteria. *Immunol Allergy Clin North Am.* 2004;24:59-73.
- [4] Subramanian HN, Schechtman KB, Hamilos DL. A retrospective analysis of treatment outcomes and time to relapse after intensive medical treatment for chronic sinusitis. *Am J Rhinol.* 2002;16:303-12.
- [5] Schubert MS. Fungal rhinosinusitis: diagnosis and therapy. *Curr Allergy Asthma Rep.* 2001;1:268-76.
- [6] Ponikau JU, Sherris DA, Kern EB, Homburger HA, Frigas E, Gaffey TA, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc.* 1999;74:877-84.
- [7] Ebbens FA, Fokkens WJ. The mold conundrum in chronic rhinosinusitis: where do we stand today? *Curr Allergy Asthma Rep.* 2008;8:93-101.
- [8] Jiang RS, Su MC, Lin JF. Nasal mycology of chronic rhinosinusitis. *Am J Rhinol.* 2005;19:131-3.
- [9] Ebbens FA, Georgalas C, Rinia AB, van Drunen CM, Lund VJ, Fokkens WJ. The fungal debate: where do we stand today? *Rhinology.* 2007;45:178-89.
- [10] Ebbens FA, Georgalas C, Fokkens WJ. Fungus as the cause of chronic rhinosinusitis: the case remains unproven. *Curr Opin Otolaryngol Head Neck Surg.* 2009;17:43-9.
- [11] Ragab A, Clement P, Vincken W, Nolard N, Simones F. Fungal cultures of different parts of the upper and lower airways in chronic rhinosinusitis. *Rhinology.* 2006;44:19-25.
- [12] Gosepath J, Mann WJ. Role of fungus in eosinophilic sinusitis. *Curr Opin Otolaryngol Head Neck Surg.* 2005;13:9-13.
- [13] Polzehl D, Weschta M, Podbielski A, Riechelmann H, Rimek D. Fungus culture and PCR in nasal lavage samples of patients with chronic rhinosinusitis. *J Med Microbiol.* 2005;54:31-7.
- [14] Bertrand B, Collet S, Eloy P, Rombaux P. Secondary ciliary dyskinesia in upper respiratory tract. *Acta Otorhinolaryngol Belg.* 2000;54:309-16.
- [15] Hafner B, Davris S, Riechelmann H, Mann WJ, Amedee RG. Endonasal sinus surgery improves mucociliary transport in severe chronic sinusitis. *Am J Rhinol.* 1997;11:271-4.
- [16] Faure O, Fricker-Hidalgo H, Lebeau B, Mallaret MR, Ambroise-Thomas P, Grillot R. Eight-year surveillance of environmental fungal contamination in hospital operating rooms and haematological units. *J Hosp Infect.* 2002;50:155-60.
- [17] Shin SH, Ye MK, Lee YH. Fungus culture of the nasal secretion of chronic rhinosinusitis patients: seasonal variations in Daegu, Korea. *Am J Rhinol.* 2007;21:556-9.
- [18] Ferguson BJ, Barnes L, Bernstein JM, Brown D, Clark CE, 3rd, Cook PR, et al. Geographic variation in allergic fungal rhinosinusitis. *Otolaryngol Clin North Am.* 2000;33:441-9.
- [19] Smith WM, Davidson TM, Murphy C. Regional variations in chronic rhinosinusitis, 2003-2006. *Otolaryngol Head Neck Surg.* 2009;141:347-52.
- [20] Ahsan SF, Jumans S, Nunez DA. Chronic rhinosinusitis: a comparative study of disease occurrence in North of Scotland and Southern Caribbean otolaryngology outpatient clinics over a two month period. *Scott Med J.* 2004;49:130-3.
- [21] Fokkens W, Lund V, Mullol J. EP3OS 2007: European position paper on rhinosinusitis and nasal polyps 2007. A summary for otorhinolaryngologists. *Rhinology.* 2007;45:97-101.
- [22] de Vries TW, Rottier BL, Visserman H, Wilffert B, Weel J. The influence of inhaled corticosteroids and spacer devices on the growth of respiratory pathogenic microorganisms. *Am*

J Infect Control. 2009;37:237-40.

[23] Mullaoglu S, Turktas H, Kokturk N, Tuncer C, Kalkanci A, Kustimur S. Esophageal candidiasis and *Candida* colonization in asthma patients on inhaled steroids. *Allergy Asthma Proc.* 2007;28:544-9.

[24] Aun MV, Ribeiro MR, Costa Garcia CL, Agondi RC, Kalil J, Giavina-Bianchi P. Esophageal candidiasis--an adverse effect of inhaled corticosteroids therapy. *J Asthma.* 2009;46:399-401.