



The role of fungi in the etiology of chronic rhinosinusitis: A prospective study[☆]

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Abstract

Objectives: Allergic fungal sinusitis (AFS) may represent an endpoint in a spectrum of sinonasal disease. Patients fulfilling criteria for a diagnosis of AFS have a poor prognosis often requiring multiple surgeries. The detection of ‘allergic mucin’ with mucus containing clusters of eosinophils and the detection of fungi by histological examination or culture is key criteria necessary for diagnosis.

This study was performed to assess the strength of the association of the presence of fungi with the presence of eosinophils in a group of patients with chronic rhinosinusitis and controls.

Methods: Thirty patients with a clinical diagnosis of chronic rhinosinusitis and 10 individuals as control group were selected for the study. Total serum IgE level testing, nasal mucus cytology and fungal culture were performed in all subjects.

Results: Fungal spores were present in the nasal mucus in 14 patients with rhinosinusitis, and in 7 controls. The detection of eosinophils on cytology correlated significantly both with a clinical diagnosis of chronic rhinosinusitis (95% confidence intervals (CI) 0.34–0.81) and with the presence of fungal elements on cytology ($p = 0.02$). The average serum IgE level was higher in patients with fungal spores ($p = 0.039$).

Conclusion: In certain susceptible individuals inhaled fungi may provoke an eosinophilic response. This response varies from simple eosinophilic inflammation to classic allergic fungal sinusitis.

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1. Introduction

In 1981 Millar et al. described what at that time appeared to be, either a completely new or else a previously unrecognized disease entity [1]. This disease entity was described in five patients, with symptoms and signs of chronic rhinosinusitis (CRS) and with histological features identical to the previously known condition of allergic bronchopulmonary aspergillosis (ABPA) [1]. All these cases demonstrated intra-sinus allergic mucin, comprising layers of degenerated

inflammatory cells, with scattered scarce septated fungal forms. A relationship between allergic fungal lung disease and sinus disease was proposed and the term ‘allergic aspergillosis’ of the paranasal sinuses was coined [1]. In 1983 Katzenstein and Greenberger reviewed 119 nasal mucus samples, reporting 9 which demonstrated histological features that were indistinguishable from those seen in the bronchi of patients with ABPA [2]. Seven showed scattered fungal hyphae. The morphology of the hyphae resembled that of aspergillus. As a result of this finding, they proposed the term ‘allergic aspergillus sinusitis’ [2]. Subsequent to these initial reports, there were several further reports of identical or similar findings in other patients [3–7].

There were however, discrepancies in the clinical and laboratory findings between different patients in these

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reports. Some had all the features originally described, i.e. so called allergic eosinophilic mucin with fungal hyphae [3,4], some had skin test reactivity to the fungus concerned [5], some had fungi other than aspergillus [6] and some had no fungi detected [7,8]. These findings led to a number of attempts to define the condition, with the description of a number of criteria for diagnosis. As a result, patients were then frequently described as having “allergic fungal sinusitis” (AFS) or “allergic fungal sinusitis-like syndrome”, with the major difference between the two being the presence or absence of fungi [6,7]. In 1999 Ponikau et al. from the Mayo clinic published a paper which not only reignited the controversy but threatened to change the way most cases of CRS is perceived [9]. Using certain techniques for collecting, handling and culturing nasal mucus they detected fungi in 96% of a series of 210 patients with chronic rhinosinusitis as well as 100% of healthy controls. They concluded that the diagnostic criteria for AFS are present in the majority of patients with CRS with or without polyposis. They proposed a change in the terminology from AFS to eosinophilic fungal rhinosinusitis based on the presence of eosinophils in the allergic mucin, and not a type 1 hypersensitivity, as likely the common denominator in the pathophysiology of AFS. In 2000, the controversy was further ignited when Ferguson suggested that ‘eosinophilic mucin sinusitis’ was a distinct clinical entity from AFS [8]. The debate continues with some suggesting that fungi have always been a factor in a significant proportion of patients with CRS but were not properly detected and others suggesting that the fungi only play a role in a small group of patients as represented by the original AFS patients.

This paper determines the prevalence of fungi in our patient population with chronic rhinosinusitis relative to controls. We also determine the strength of the association between the presence of fungal elements on cytology and the presence of eosinophils. We determine the variation of total serum IgE and eosinophils in patients with CRS relative to controls.

2. Material and methods

2.1. Patients and controls

The patients selected for this study were from a group of rhinology patients attending the ENT department at our institution who fulfilled the inclusion criteria: all patients greater than 16 years old, all should have clinical diagnosis of CRS based on the AAO-HNS definition of CRS [10]. However, as no skin test was done on these patients, we cannot rule out additional allergic component. Patients with immunocompromise or with diabetes mellitus were excluded. No patients should have received topical or systemic corticosteroids for at least 4 weeks prior to mucus collection. Based on these criteria 30 patients were selected for the study. Twenty-four of the patients underwent surgical

treatment for their CRS. The surgical procedures undertaken were simple nasal polypectomy, headlight assisted intranasal ethmoidectomy or functional endoscopic sinus surgery depending on the preference of the consultant surgeon responsible. The following demographic data and clinical characteristics were prospectively recorded for all patients: age, sex, occupation, and existence of an allergy, asthma, intolerance to aspirin and previous surgeries. Symptoms scores were decided on a visual analogue score (VAS) according to the ICSD system of scoring [11]. The VAS symptom questions include a single question in which the patient is asked to rate his/her overall discomfort due to chronic rhinosinusitis. In addition, patients are asked to evaluate separately the symptoms of nasal blockage/congestion, nasal discharge, olfactory disturbance, facial pain or pressure and headache.

Ten volunteers with no previous history of nasal or paranasal sinus disease, with no symptoms of allergy, and normal nasal mucosa confirmed by nasal examination served as controls. All patients and controls consented to participate.

Statistical analysis involved calculating proportions and their differences with calculation of 95% confidence intervals (CI) for these differences. Chi-squared analysis was used to determine association of several variables.

2.2. Collection and culture technique

Mucus was collected by two methods. If mucus was readily visible in the nose, it was collected and aspirated from middle meati or from maxillary sinuses into a suction trap. If there was no obvious mucus or only a scanty amount of mucus, each side of the nose was flushed with 10 ml of sterile normal saline using a sterile syringe connected to a sterile ear section tip. During this procedure the patient was asked to take a deep breath via the mouth and exhale forcefully through the nose, to a sterile dish, during the flushing. The sample was then collected in a sterile bottle.

All samples were sent without delay to the laboratory where the specimens were placed into centrifuge tubes. Strict measures were taken by the lab technicians to prevent contamination. To liquefy the mucus, the collected specimen was suspended with an equal volume of diluted dithiothreitol and centrifuged for 10 min. The supernatant was discarded, and the sediment was vortexed for 30 s. The sediment was then divided into two samples; one for fungal culture and the other one for direct cytological examination. The sample for fungal culture was inoculated into Sabouraud medium. The media was incubated at 30 °C and allowed to culture for 30 days. The specimens were checked at 2-day intervals.

2.3. Direct cytological and histological examination

As discussed above, the remaining part of the sediment was sent for direct cytological examination. The material

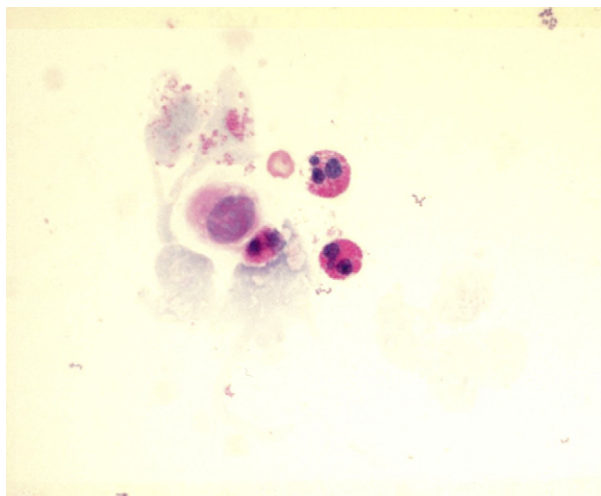


Fig. 1. Eosinophils as seen in nasal mucin on cytological examination.

was spread on three slides. The first slide was stained with haematoxylin and eosin (H&E) stain, the second with Gomori methenamine silver stain and the third slide was retained as a spare slide in case there was insufficient material on the other two. All specimens were examined by a same consultant pathologist (RL) looking for the presence of degenerated eosinophils (Fig. 1), Charcot-Leyden crystals and fungal elements such as spores and hyphae (Fig. 2). The patients were marked as positive for eosinophils in cytology when they have moderate or large amounts of clusters or sheets of eosinophils in their mucus. In the patients who underwent surgical treatment further material was available for histological examination.

All patients and controls also had serum IgE and eosinophils measured.

3. Results

Twenty-four of the 30 patients had bilateral nasal polypsis on nasal endoscopy; the remaining 6 patients had non-

polypoidal chronic rhinosinusitis. Seven patients (23.4%) had asthma. One patient had known aspirin intolerance. Twelve patients (40%) had undergone previous surgery including; antral lavage, head light assisted intranasal ethmoidectomy, simple polypectomy or endoscopic sinus surgery.

Patients' responses for a single VAS score relating to overall discomfort or severity of their sinonasal symptoms ranged from 0 to 10, with a median score of 8 (mean is 7.8, S.D. $\pm$ 2.2), see Table 1. If the five VAS symptom domains (nasal blockage/congestion, nasal discharge, disturbance of smell, facial pain/pressure and headache) were added together, score ranged from 21 to 50, with a median score of 42.5 (mean is 41.9, S.D. $\pm$ 6.1).

The average age for patients was 47.4 years and for controls was 43.3 years. The age range of patients was 17–73 years and of controls was 19–65 years. The sex distribution in patients was 19 males and 11 females; in controls 3 males and 7 females.

Fungi were detected by microscopy or culture in 19 patients. Of these, 14 patients grew fungi on culture (Table 2). Four of these patients grew two distinct species of fungi in their culture medias. The fungal species which were identified in this study were not different from those identified in previous studies. In seven (70%) of the 10 normal healthy controls, the culture were positive for fungi. Four of these grew two species of fungi. There was no significant difference in the type of fungal species cultured between the patients and controls. Two of the culture positive controls had microscopic evidence of fungi on cytological analysis of the mucus. Interestingly, one of the cultures in the control group was positive for mucormycosis (Table 2).

Table 3 shows a comparison of the patient groups and control groups with respect to total serum IgE, serum eosinophils, eosinophils on cytology, fungi on microscopy and fungal cultures.

The total serum IgE was measured for all patients and controls. The normal reference value in our lab is 0–100 IU/ml. It was elevated in 11 (36.7%) of the 30 CRS patients in this study. Only one of the controls had elevated total IgE.

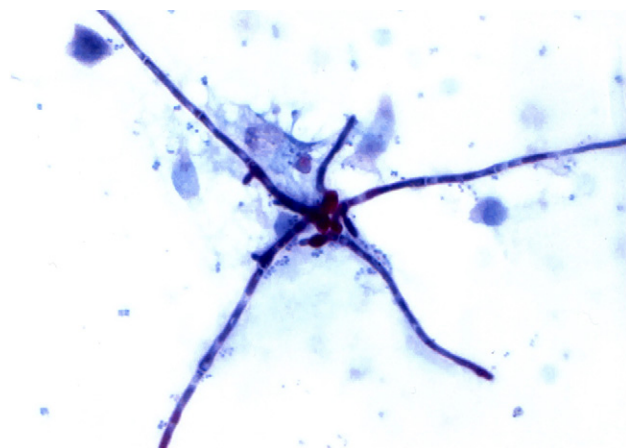


Fig. 2. Fungal hyphae surrounded by spores seen in nasal mucin.

Table 1
Presenting symptoms and average score in patients' group

symptoms	Average scores (scores 0-10) ICSD
Nasal obstruction	8.0
Nasal discharge	7.7
Disturbance of smell	6.8
Facial pain or pressure	6
Headache	5.6
Overall discomfort	7.8

Table 2

Number and percentage of patients and control group colonized with fungi on culture

Fungal species	Patients(n:30)	Control(n:10)
Penicillium	7(23.4%)	5(50%)
Candida albicans	4(13.4%)	1(10%)
Aspergillus flavis	2(6.7%)	-
Candida parasilosi	1(3.4%)	-
Alternaria	1(3.4%)	-
Paecilomyces variotii	1(3.4%)	-
Aspergillus niger	1(3.4%)	4(40%)
Candida krusei	1(3.4%)	-
Mucormycosis	-	1(10%)
Negative culture	16(53.4%)	3(30%)

The difference between mean serum IgE in patients and controls was not significantly different ($p = 0.16$), see Table 3. However, when the average serum IgE was calculated in those with fungal spores on cytology (mean serum IgE = 185) and those without fungal spores on cytology (mean serum IgE = 55), the difference of 130 IU/ml was statistically significant ($p = 0.039$) data not shown.

Nine (30%) of the 30 patients had elevated eosinophils percentage in the blood ($>0.44 \text{ ml}^{-1}$). None of the controls had elevated eosinophils in the blood. This was statistically significant ($p = 0.013$) (Table 3).

Degenerated eosinophils or their by-product were identified in 20 (67%) of the 30 patients by direct cytological

Table 4

Association of eosinophils and fungal elements

	Patients with eosinophils in cytology N=20	Patients without eosinophils in cytology N=10	P value
Spore/ hyphae cytology positive (n=12)	11	1	0.02
Spore / hyphae cytology negative (n=18)	9	9	

examination of the nasal mucus specimens using H&E and Gomori methenamine silver stain. None of the controls had eosinophils in their nasal mucus. There was a strong correlation between the presence of eosinophils on cytology and a diagnosis of chronic rhinosinusitis (95% CI 0.34–0.81) (Table 3).

There was not a significant association between a diagnosis of CRS and the presence of fungal elements in cytology ($p = 0.114$) or in culture ($p = 0.490$) (Table 3). However, there was a positive association between patients with eosinophils identified on cytology and those that also had fungal elements seen on cytology ($p = 0.02$) (Table 4).

In 7 of the 14 patients with positive culture, the presence of fungi in cultures was identical with the presence of spores on cytological examination of the nasal mucus. Sixteen (53.4%) of the 30 patients have eosinophils in the mucus and one of the following: fungal spores in the nasal mucus or positive fungal culture. Although the criteria in those 16 patients are filling the criteria of definition of 'eosinophilic fungal rhinosinusitis' or even the classic AFS, we cannot call them so as we have not done skin test or IgG levels.

Histological examination of all surgical material showed no evidence of invasive fungal infection.

Table 3

Findings in patients and controls

	Patients N=30	Controls N=10	P value
Average serum IgE	107	47	0.16
Average serum eosinophils	0.36	0.15	0.013
% of cases with eosinophils on cytology	67%	0%	0.67 (0.34 to 0.81)
% of cases with fungal spore on cytology	40%	20%	0.114
% of cases with positive fungal culture	47%	70%	0.490

4. Discussion

This study has outlined four key points. Firstly, fungi can be cultured from nasal mucous in healthy controls (70%) as well as in patients with CRS (47%). Secondly, when fungal elements are identified, there is an associated elevation of serum IgE. Thirdly, the presence of eosinophils in nasal mucous is significantly associated with CRS (95% CI 0.34–0.81). Fourthly, the presence of eosinophils in the nasal mucus is significantly associated with the presence of fungal elements on cytology ($p = 0.02$). This suggests that fungi do not cause sinonasal disease in the majority of patients. It suggests that eosinophilic inflammation is a component of CRS in a significant proportion of patients, and this process may be stimulated by fungal elements in a proportion of susceptible individuals. This analysis did not subdivide the patients with CRS in to those that fulfilled the classic criteria for AFS (53% of our patient group) and those that did not. Stammberger and coworkers has outlined that these criteria may be present in the majority of patients and has questioned the existence of AFS as an independent entity [12]. However, many questions remain unanswered. Why are some people susceptible to the fungi, whereas others are not? What drives the response (as it does not appear to be allergic in most cases)? Do the fungi play a role in the ongoing inflammation or do they simply incite the reaction that then becomes autonomous? This would obviously have implications for treatment with antifungal as antifungal may only prove effective at a particular point in the process perhaps before the process becomes autonomous. In spite of the above findings and those of Ponikau et al. [9], Cody et al. [4], Cory [3] and Manning and Holman [5], the evidence of a relationship between fungi and eosinophils and eosinophilic chronic rhinosinusitis is still circumstantial, however it is becoming sufficiently strong enough to warrant further investigation. Finally what then about the entity of allergic fungal sinusitis? We would agree with Stammberger that its strict definition with rigid criteria is perhaps unnecessary as it may not be a separate entity but rather one end of a spectrum of conditions of similar etiologies. If this is in fact the case then its description as a separate condition may serve no clinical purpose and be unnecessary.

5. Conclusion

Fungi exist in the most environments and are likely to be inhaled on a regular basis. In certain susceptible individuals their inhalation may provoke an eosinophilic inflammatory response. Optimal collection and thorough cytological examination of the sinonasal specimen are important measures for increasing the diagnostic yields of eosinophilic fungal rhinosinusitis. The association of eosinophils and fungal elements on cytology in patients with chronic rhinosinusitis has been shown in this study. Further studies will be required to determine causality.

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References

- [1] Millar JW, Johnston A, Lamb D. Allergic aspergillosis of the maxillary sinuses. *Prod Scot Thor Soc* 1981;36:710.
- [2] Katzenstein SR, Greenberger PA. Allergic aspergillus sinusitis: a newly recognized form of sinusitis. *Allergy Clin Immunol* 1983;72:89–93.
- [3] Cory JP. Allergic fungal sinusitis. *Otolaryngol Clin North Am* 1992; 25:225–30.
- [4] Cody T, Neel HB, Ferrerio JA. Allergic fungal sinusitis: the Mayo clinic experience. *Laryngoscope* 1994;104:1074–9.
- [5] Manning SC, Holman M. Further evidence for allergic pathophysiology in allergic fungal sinusitis. *Laryngoscope* 1998;108:1485–95.
- [6] Kinsella JB, Bradfield JJ, Gourley WK. Allergic fungal sinusitis. *Clin Otolaryngol* 1996;21:389–92.
- [7] Ramadan HH, Quraishi HA. Allergic mucin sinusitis without fungus. *Am J Rhinol* 1997;11:145–7.
- [8] Ferguson BJ. Eosinophilic mucin rhinosinusitis: a distinct clinicopathological entity. *Laryngoscope* 2000;110:799–813.
- [9] Ponikau JU, Sherris DA, Kern EB. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999;74:877–84.
- [10] Lanza DC, Kennedy DW. Adult rhinosinusitis defined. *Otolaryngol-Head Neck Surg* 1997;117(Suppl.):S1–7.
- [11] Lund VJ, Kennedy DW. Quantification for staging sinusitis. the staging and therapy group. *Ann Otol Rhinol Laryngol* 1995;167(Suppl.):17–21.
- [12] Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H. Eosinophilic fungal rhinosinusitis: a common disorder in Europe. *Laryngoscope* 2003;113:264–9.