Allergic fungal sinusitis. A naso-sinusal specific hyperreactivity for an infectious disease?

La sinusite allergica micotica. Iperreattività naso-sinusale specifica o infezione?

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Summary

Allergic fungal sinusitis (AFS) is a rare disease of naso-sinusal complex affecting mainly young, immunocompetent adults who complain of chronic rhinitis and/or recurrent nasal polyps despite medical and/or surgical treatment. Aim of the study is to analyse, from an allergological and otorhinolaryngological point of view, patients affected by the so-called “allergic fungal sinusitis” in order to better define the relationship between fungi present in naso-sinusal secretions and the host’s immunoreactivity. From February 2001 to January 2002, 24 selected patients (13 male 11 female) age range 25-65 years (mean 45), with chronic rhinosinusitis, with a positive fungal examination of nasal secretion, underwent allergological evaluation. All patients were positive for diagnostic criteria of allergic fungal sinusitis and, in all patients, nasal lavage was performed for microscopic examination by fluorescence. Samples were then cultured on Sabouraud growth media for identification of the fungus. Skin prick tests (SPT) were then performed with the 15 main inhalant allergens and twelve fungal allergens (Bracco). The total IgE serum level (PRIST), the specific fungal IgE and the eosinophilic cationic protein were then investigated by means of an immuno-fluorine enzymatic method. Finally, a nasal provocation test was carried out with diluted solutions (1/100, 1/10) and with a pure solution of fungal allergens, selected according to microbiological examination of nasal secretion of each subject. Prick tests were positive for seasonal and perennial allergens in 5 patients (21%), while prick tests with fungi were positive in only 4 patients (16.6%). Total IgE levels were higher than in normals (200 KU/l) in 6 patients (25%) (mean 364.74 KU/l). In another 18 patients, total IgE were normal. Specific IgE levels for the tested fungi and eosinophilic cationic protein levels were within normal range in all patients. Nasal provocation test was negative in all patients. Presence of fungi in nasal secretions of patients with AFS does not appear to be correlated with an allergic status to the isolated fungus. A role for IgE in either the aetiology or the pathophysiology of allergic fungal sinusitis is unlikely, and probably the diagnostic criteria for allergic fungal sinusitis should not include type I hypersensitivity, since no confirmed evidence exists that IgE-mediated type I hypersensitivity is involved in the pathophysiology of allergic fungal sinusitis.

Key words

Paranasal sinuses diseases • Nasal polyps • Mycosis • Allergic sinusitis

Parole chiave

Malattie dei seni paranasali • Poliposi nasale • Micosi • Sinusite allergica

Riassunto

La sinusite allergica micotica (SAM) è una rara affezione del complesso naso-sinusale che colpisce prevalentemente soggetti giovani, immunocompetenti, e che si presenta sotto forma di una rinosinusite cronica. Nella maggioranza dei casi è associata a polipi nasali, con alto tasso di recidiva nonostante i vari trattamenti medici e/o chirurgici instaurati. L’incidenza della sinusite allergica micotica nella rinosinusite cronica iperplastica trattata chirurgicamente oscilla, come riportato in letteratura, tra il 6% ed il 13% dei casi. I polipi nasali e l’asma incidono invece, rispettivamente, nel 75% e nel 65% dei casi riportati in letteratura. Scopo del presente lavoro è quello di analizzare dal punto di vista otorinolaringoiatrico ed allergologico un gruppo di pazienti selezionati sulla base di criteri diagnostici riportati in letteratura e suggestivi di sinusite allergica micotica, nel tentativo di meglio definire le relazioni etiopatogenetiche tra la presenza di micotiti nel liquido di lavaggio nasale e l’immunoreattività del paziente. Dal febbraio 2001 al gennaio 2002 abbiamo selezionato 24 pazienti (13 uomini ed 11 donne) di età compresa tra i 25 ed i 65 anni (età media 45 anni) affetti da rinosinusite cronica polipoidi micotica sulla base delle evidenze cliniche, endoscopiche e microbiologiche. Tutti i pazienti studiati sono stati sottoposti ad esame allergologico mediante “skin prick test” con un pannello di 15 allergeni inalanti e 12 estratti micotici. Sono state inoltre dosate in tutti i pazienti le IgE totali (PRIST), le IgE specifiche e la proteina cationica degli eosinofili. Infine i pazienti sono stati sottoposti a test di provocazione nasale con soluzioni contenenti l’estратto allergenico del micotita isolato nel liquido di lavaggio nasale. Le cutareazioni sono risultate positive per allergeni stagionali e perfem in 5 pazienti (21%), e per estratti micotici in 4 pazienti (16,6%). Il valore delle IgE totali è risultato superiore alla norma in 6 pazienti (25%), le IgE specifiche sono risultate assenti in tutti i casi studiati, così come la proteina cationica degli eosinofili ed il test di provocazione nasale micotita-relato. I risultati ottenuti dal nostro studio ci permettono di affermare che la presenza di funghi nelle secrezioni nasali di pazienti affetti da rinosinusite allergica micotica non sembra potersi correlare ad uno stato allergico/iperergico nei confronti del fungo isolato. Il ruolo di meccanismi IgE-mediatì nell’etiologia e nella fisiopatologia della sinusite allergica micotica non sembra ancora potersi confermare, anche se ulteriori studi sono necessari per la definizione di questo problema.
Introduction

Currently, most rhinologists recognise 4 types of fungal sinusitis: acute/fulminant (invasive), chronic/indolent (invasive), mycetoma (fungus ball) and allergic fungal sinusitis (AFS) (Tab. I). The first type is the only form of acute fungal sinusitis. It occurs exclusively in diabetic or immunosuppressed patients, most typically in oncological or transplanted patients. Fungal cultures usually reveal Phycomycetes (Mucor or Rhizopus), Candida or Aspergillus species. Chronic/indolent invasive fungal sinusitis occurs in immunocompetent individuals who usually have a long-standing history of rhinosinusitis. The disease progresses slowly, producing chronic granulomatous inflammation and extension beyond the sinus walls. Aspergillus species and members of the Dematiaceous family are the most frequent causative organisms. Mycetoma or fungus ball affects immunocompetent, non-atopic patients and the disease may involve any sinus, but usually occurs in a single sinus, most frequently the maxillary antrum. Bone erosion and mucosal invasion does not occur. The lack of sinus inflammation distinguishes this disorder from other forms of chronic fungal sinusitis. The etiologic organism is almost always Aspergillus fumigatus.

AFS was first described in the early 1980s when Millar et al.1 recognised immunologic and histologic similarities between the specimens obtained from the maxillary sinuses of 5 patients and those of Allergic Bronchopulmonary Aspergillosis (ABPA). Katzenstein et al.2 then retrospectively reviewed 119 histologic specimens obtained from patients who had previously undergone sinus surgery. They found 7 cases with mucin-containing eosinophils, Charcot-Leyden crystals and fungal hyphae, histologically resembling ABPA and called this entity Allergic Aspergillus Sinusitis. Since 1989, as it became apparent that Dematiaceous fungi and not Aspergillus (only 15% of cases), were the primary etiologic agents, the name was changed to AFS. Indeed, bipolaris spicifera was the most commonly isolated fungus, with a prevalence of 67%. Other species of the Dematiaceous family include drechslera, alternaria, curvularia, exserohilum, rhizopus, fusarium.

Although this entity is more frequently recognised, today, it is still presumably underdiagnosed. Warm humid climates seem to foster fungal proliferation. The prevalence in the population of patients requiring surgery for chronic sinusitis is currently estimated to range between 6% and 13%, with a slight prevalence in males (ratio 1.6), with no ethnic differences. The typical AFS patient is a young - 23-42-years-old - immunocompetent, atopic adult with chronic sinusitis. The incidence of asthmas ranges from 30% to 100% of cases. Aspirin intolerance is present in 27% of cases; nasal polyposis is a common feature, with an incidence ranging from 75% to 100% of cases. Even if nasal polyposis is not a specific marker of chronic nasal inflammation, the incidence of AFS ranges from 5% to 10% in patients submitted to surgery for sinonasal polyposis. Patients typically report a history of sinonasal polyposis, recurrent sinusitis and numerous surgical procedures. Sinusitis is usually refractory to antibacterial treatment. While the initial signs and symptoms are those typical of polypoid rhinosinusitis, orbital proptosis, malar deformities, mucoceles and diplopia are occasionally seen. Inflammation, usually, affects all paranasal sinuses, but may, at times, be asymmetric involving only one side. In 75% of cases, patients complain of the presence of a characteristic “so-called” allergic mucus, which is thick and viscous and often stained.

<table>
<thead>
<tr>
<th>Type</th>
<th>Immune status</th>
<th>Fungal role</th>
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<tbody>
<tr>
<td>Acute/fulminant</td>
<td>Compromised</td>
<td>Pathogen</td>
</tr>
<tr>
<td>Chronic/indolent</td>
<td>Competent, non atopic</td>
<td>Pathogen</td>
</tr>
<tr>
<td>Mycetoma (fungus ball)</td>
<td>Competent, atopic</td>
<td>Saprophyte</td>
</tr>
<tr>
<td>Allergic fungal sinusitis (AFS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue invasion</td>
<td>Sinusal involvement</td>
<td>Course</td>
</tr>
<tr>
<td>Yes</td>
<td>Single</td>
<td>Acute</td>
</tr>
<tr>
<td>No</td>
<td>Multiple, unilateral</td>
<td>Chronic</td>
</tr>
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Table I. Clinical and diagnostic features of fungal sinusitis.
brown, yellow or green due to bacterial superinfection or fungal material. Histologic observation of the surgical specimen reveals a triad of eosinophilia, Charcot-Leyden crystals and extramucosal hyphae. Charcot-Leyden crystals are simply a byproduct of necrotic eosinophils such as phospholipases. Hyphae can usually be seen with haematoxylin-eosin (HE) or potassium-hydroxide stains, with PAS technique and, if necessary, special stains such as Gomori methenamine silver (GMS). The presence of fungi in the mucin but not in the tissues of AFS patients differentiates AFS from chronic invasive fungal sinusitis. By definition fungal invasion does not occur in any case of AFS and for this reason, most Authors believe that the method of choice for identification of fungi in AFS is represented by the collection of nasal fluids by means of a correctly performed nasal lavage. With particular stains, such as “Fontana-Masson”, it is possible to reveal the presence of melanine, a typical element of the Dematiaceous fungi. Total serum IgE levels are generally elevated, although less than with ABPA. Sometimes, peripheral eosinophilia and/or fungal-specific IgE are present. In 60% of cases, skin test reactivity to a broad range of commercial fungal extracts can be demonstrated. According to some Authors, it is possible to reveal positivity to skin prick tests (SPT) and the presence of fungal-specific IgE for at least one commercial fungal extract of the Dematiaceous group in 100% of patients with AFS. On the contrary, this positivity can be found in only 5% to 20% of normal individuals. Our study does not support these conclusions, that were based upon a limited number of patients (8 cases) and, therefore, without statistical significance.

At computerised tomography (CT) fungi release ferromagnetic elements (magnesium and calcium) creating a serpiginous area of high attenuation, especially in the ethmoidal and maxillary sinuses. Bone thinning and erosions with dislocation of adjacent structures can be observed. As far as concerns Magnetic Resonance Imaging (MRI) the ferromagnetic elements show decreased signal intensity, leading to hypointense T1-weighted and markedly hypointense T2-weighted images with typical void signal.

### Materials and methods

From February 2001 to January 2002, 24 adult AFS patients (11 female, 13 male), aged between 25 and 65 years, were examined from an allergological point of view.

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<table>
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<th>Table II. Diagnostic features suggesting AFS.</th>
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<tr>
<td><strong>Major diagnostic criteria</strong></td>
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<tr>
<td>Positivity of allergological examination for fungi (skin prick tests and/or RAST and/or nasal provocation test). Identification of allergic mucin by rhinoscopy either at time of sinus surgery or later on histopathologic evaluation of material from sinus, containing fungal hyphae, dense accumulations of eosinophils with Charcot-Leyden crystals and necrotic cellular debris. Demonstration of fungal elements in nasal discharge or in material obtained by nasal lavage or at time of surgery by stain or culture.</td>
</tr>
<tr>
<td><strong>Minor diagnostic criteria</strong></td>
</tr>
<tr>
<td>Chronic rhino-sinusitis (endoscopic and/or peroperative demonstration). Presence at CT scan of serpiginous areas of high attenuation especially in ethmoidal and maxillary sinuses, with bone thinning and erosions with dislocation of adjacent structures. Presence at Magnetic Resonance images of areas showing decreased signal intensity leading to hypointense T1-weighted and markedly hypointense T2-weighted images with typical void signal.</td>
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</table>

From Bent and Khun 11, 1994 (modified).
Diagnosis was based upon medical history, clinical, endoscopic and imaging findings (Fig. 1). In this study population, 5 (20.8%) patients (4 M, 1 F) were affected by respiratory allergy, with seasonal (1 case), perennial (2 cases) and mixed perennial and seasonal (2 cases) allergic rhinitis; 5 (20.8%) patients (3 M, 2 F) presented an association of nasal polyposis with asthma and aspirin intolerance [Aspirin disease or ASA (Acetylsalicylic) Triad]. The clinical and endoscopic examination of the patients revealed the presence of bilateral nasal polyps with near total obstruction of the nose. CT scan confirmed the presence of a serpiginous area of high attenuation, especially in the ethmoidal region due to fungal release of ferromagnetic elements (magnesium and calcium).

MR scan confirmed the presence of micotic sinusitis with decreased signal intensity, leading to hypointense T1-weighted, and a markedly hypointense T2-weighted, images with a typical void signal (Figs. 2, 3). All patients underwent anteroposterior ethmoidectomy with maxillary antrostomy. During the operation, the typical viscous, yellow mucous was found,
containing eosinophils, Charcot-Leyden crystals and, in 12 patients, extramucosal hyphae (8 patients with Gomori stains, 4 with PAS technique). No fungal mucosal invasion was detected. Patients were enrolled in the study, based upon these findings and upon the major diagnostic criteria of AFS. The allergological evaluation started with SPT, using a needle in singleton, with sites spaced at least 5 cm apart. For SPT, a panel of 15 inhalant allergens (Bracco Pharmaceuticals, Allergy Division Dermatophagoides f. and pt., Graminaceae, Parietaria judaica and officinalis, Plantago i., Ambrosia, Olea, Cupressus, Alnus, Betulae, Corylus, Ragweed, Cat and Dog dandruff), histamine, glycerosaline controls (ALK-Abellò), and the following 12 fungal allergen extract (Bracco Pharmaceuticals, Allergy Division): 1) Stemphljum b., 2) Aspergillus f., 3) Mucor mix, 4) Penicillium n., 5) Chetomium o., 6) Epicoccum p., 7) Rhizopus n., 8) Botrytis s., 9) Candida a., 10) Alternaria f., 11) Cladosporium, 12) Helminthosporium s. were used. A positive test was defined as a wheal > 3 mm in diameter compared with the negative control and with surrounding erythema and oedema. The PRIST, the specific fungal IgE and the eosinophilic cationic protein (ECP) were then investigated by means of an immuno-fluorine enzymatic immuno assay (FEIA Cap System Pharmacia, Stockholm, Sweden). The normal values were considered < 200 KU/l for PRIST, values ranging from 0 to 0.75 KU/l for RAST and < 20 mcg/l for ECP. Finally, a nasal provocation test (NPT) was performed both with progressively diluted solutions (1/100, 1/10) and with a pure solution of fungal allergens, selected according to the results of the nasal secretion examination.

Nasal provocation test was carried out with allergenic extract containing 1250 SBE (Standardisierte Biologische Einheiten or BU, Biologic Unit). The test starts with the topical nasal administration of 0.04-0.05 ml of Chloruro-saline solution with diluted phenol acid. The first nasal response was recorded after 15, 30 and 45 minutes by means of active anterior rhinomanometry with analysis of nasal resistances. The absence of increased nasal resistance > 20% was considered a negative response to stimulation, excluding the presence of aspecific nasal hyperreactivity. Specific allergic stimulation was carried out using 0.04-0.05 ml of a mycotic allergic extract with 2 BU/ml, 4 BU/ml and 8 BU/ml concentrations at intervals of 15, 30 and 45 minutes. A ≥ 20% increase in nasal resistances was considered a positive response to stimulation.

Results

The collected nasal fluids showed, in all cases, mucin containing inflammatory cells such as basophils, eosinophils and mast cells, as well as Charcot-Leyden crystals. SPT was positive for seasonal and perennial allergens in 5 patients (20.8%), while SPT for fungi was positive in only 4 patients (16.6%), 2 for Alternaria (8.3%), 1 for Penicillium n. (4.1%) and 1 for Rhizopus (4.2%). Total IgE levels exceeded normal values (200 KU/l) in only 6 patients (25%) (mean value 364.74 KU/l). In the remaining 18 cases, the value of PRIST was normal (mean value 107.5 KU/l). No specific fungal IgE was found for the fungi tested. ECP levels were normal in 23 out of 24 patients (<20 mcg/l). Only in 1 patient was the ECP level 41.4 mcg/l. NPT was negative in all the patients studied.

The microbiological evaluation of nasal secretions revealed the presence of fungi in all enrolled patients; the following fungi have been identified: Aspergillus f. (no. 8), Penicillium n. (no. 7), Penicillium n. and Aspergillus f. (no. 2), Alternaria t. (no. 4), Mucor m. (no. 1), Rhizopus n. (no. 1), Cladosporium (no. 1).

Discussion and conclusions

In the present study, evidence of fungi was found, in the nasal fluid of all enrolled patients, but only 4 (16.6%) showed a positive SPT for fungi, even with negative RAST and NPT. The pathogenesis of AFS remains to be fully elucidated. Much controversy exists, in the literature, concerning the role that hypersensitivity (Gell and Coombs type I IgE-mediated and type III-immuno-complex-mediated responses) play in this disease. According to most Authors, fungi presumably become entrapped in the sinuses of allergic subjects resulting in an osteomeatal complex obstruction, extremely thick mucus and a mucociliary clearance disor-
The ensuing immune response exacerbates the disease. The diagnostic tool for AFS should be the microbiological examination of nasal secretions, especially in those cases with few fungal hyphae, not detected with routine histological techniques (H&E, PAS).

Another aspect worthy of attention is the immunoreactive role of fungi in the genesis of nasal polyposis. Albeit the small size of the tested group of patients (24), the even smaller group of patients who way positive at the mycophyte prick test (4) and the very small number of commercially available mycophytes in comparison to those theoretically responsible for hyperphonetic respiratory disorders, allows us only to hypothesise that the fungi, especially those that are not normally saprophytes of the nasosinusal region, could play a significant pathogenetic role in nasal polyposis.

In conclusion, in our opinion, although physiopathological mechanisms underlying AFS still remain to be fully elucidated, there is increasing evidence that fungi play mainly a saprophytic role and that they represent an important inflammatory stimulus rather than a clearcut allergenic effect. The allergenic role of many fungi is still uncertain and difficult to identify also due to the lack of availability of purified fungal extracts both for “in vivo” and “in vitro” tests, but the issue is still unsolved and further studies are necessary in order to better understand the real nature of AFS and the therapeutic implications. At present, even if the physiopathological mechanisms involved in AFS still remain to be defined and controversial theories exist regarding the allergenic or infectious nature of the disease, there is, in our opinion, mounting evidence demonstrating the saprophytic role of fungi, even if further research is required to define the exact nature of this disease and its implications from a therapeutic point of view.
References


Received July 2, 2002.
Accepted September 19, 2002.
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