

Impact of fungal load on diagnosis and outcome of allergic fungal rhinosinusitis

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Received: 25 January 2013 / Accepted: 20 March 2013 / Published online: 9 April 2013
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Abstract Fungal load colonization may modify the classic eosinophilic inflammation in allergic fungal rhinosinusitis (AFRS). We aimed to evaluate the impact of fungal load on diagnosis and outcome of AFRS. In the present cohort study fungal load differences were determined prospectively according to Gomori methenamine silver (GMS) fungal stained (histopathological and cytological examination) with the tenacious mucus, cheesy clay-like materials and sinus mucosa/polyps in 12 AFRS patients. Two groups with different fungal loads, AFRS with (six patients) and without (six patients) high fungal loads (HFL) were evaluated for nasal endoscopic score, paranasal sinuses CT score, histopathological and immunohistochemical changes. Endoscopic outcome scoring differences were evaluated for 1 year after endoscopic sinus surgery and 1 month oral corticosteroids treatment. No differences were observed between both groups (AFRS with/without HFL) concerning the total CT score and opacification features ($P > 0.05$). Eosinophils and CD3 + CD8 + T cell were dominant in both groups. More edema and less fibrosis were observed in HFL group. Gliotoxin producers *Aspergilli* were present in all HFL in comparison to 5/6 (83.3 %) in cases without HFL. Fewer

patients 1/6 (16.6 %) and less number of recurrences/year 0.1 ± 0.4 occurred in the AFRS with HFL compared to the AFRS without HFL [5/6 (83.3 %) and 1.16 ± 0.7] ($P = 0.021$ and 0.023 , respectively). In addition to mucus and mucosal tissues, cheesy clay-like materials must be assessed in AFRS cases. Although patients of AFRS with HFL had negligible clinical differences from ordinary AFRS without HFL, they had better outcome after treatment.

Keywords Fungal load · Allergic fungal rhinosinusitis · Diagnosis · Mycosis diagnosis · Nasal polyps diagnosis · Sinusitis diagnosis · Outcome · Nasal polyps outcome · Sinusitis outcome

Introduction

Since the initial classification of fungal-related sinonasal diseases by deShazo in the late 1990s, [1] there have been a number of studies, proposals, and revisions to this classification, culminating most recently in the international society for human and animal mycology working group consensus on terminology and disease classification. [2] These classifications divide the noninvasive diseases into saprophytic fungal infestation, fungal ball, and fungus-related eosinophilic fungal rhinosinusitis including allergic fungal rhinosinusitis (AFRS). [2] The proposed system has been useful in providing unifying terminology for classification of invasive forms of the disease, although it is not optimal for non-invasive forms and forms that may occur together with other components.

Together with its distinctive clinical features, complications (including bony erosion and facial dysmorphism) and recurrence, recent studies support the existence of AFRS as

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a separate, distinct entity of CRS. [3] The most commonly cited criteria used in the diagnosis of AFRS have been previously described [4] and depend on the presence of nasal polyposis, characteristic findings on CT scan, the presence of allergic mucin containing fungi on microscopic examination together with fungal allergy. Allergic mucin is generally identified at the time of surgery in such disease entity as thick, inspissated mucus that ranges in color from light tan to brown to dark green and contains degranulating eosinophils. Various factors may modify the classic eosinophilic inflammation in this setting, including genetic polymorphisms [5], tissue immune response [1] together with triggers from fungal load colonization [6]. Fungal hyphae are demonstrable dispersedly within the allergic mucin, which is consistent with fungal colonization rather than invasive fungal disease [1, 4, 6]. At the time of surgery some AFRS cases had also fungal concretions (cheesy clay-like material) which usually overlooked in its assessment. Some studies had reported such increase of fungal load as AFRS with fungal balls [7–9]. In AFRS the same fungal species were identified in the indoor air and patients' mucus [10]. Under conditions of high levels of indoor exposure (fungal concentrations; 90th percentile), an association was also found between in-home fungal concentrations and development of allergic rhinitis [11]. So in addition to the effect of high levels of fungal exposure; the sinus burden of fungal antigen in mucus may be increased by antibiotics [6] and corticosteroids use [9]. It can be speculated that increased sinus fungal burden may play a role in increasing fungal sensitization and development of the intense-related inflammatory response. Nevertheless, it is not known if such sinus fungal load differences will affect the AFRS disease process and subsequently its diagnosis and prognosis. So the aim of the present study is to assess the impact of fungal load on diagnosis and outcomes of AFRS cases after predetermined surgical and oral systemic corticosteroids treatment. This will provide an understanding of the disease spectrum and better outlining of the treatment plans.

Subjects and methods

Approval for this prospective study was obtained from the ethics committee of the hospital. Patients with criteria of AFRS were included in the present study [4]. Inclusion criteria included a diagnosis of AFRS using Bent and Kuhn criteria [4] and subsequent primary endoscopic sinus surgery (ESS) with confirmation of the diagnosis by pathologic examination of the sinus contents. Patients were excluded if they were diagnosed with cystic fibrosis, immunodeficiency status, or pregnancy. All of the patients had primary surgery without the administration of any

medications 1 month prior to the surgery, including corticosteroids. Before surgery, each patient had a complete medical history, examination, and endoscopic assessment with grading of polyps according to the Lund–Kennedy score [12]. In addition; a computed tomography (CT) scan was obtained and graded in line with the Lund–Mackay score [13]. The CT scan was also evaluated for opacity characteristics produced by the diseased tissue: inequality of the sinus scores from both sides (right and left), expansion of the involved sinuses, areas of bone erosion, heterogeneity (areas of increased attenuation in the soft tissue opacification) and its characteristics.

ESS was performed for all patients using the antero-posterior (Messerklinger) technique. The intra-operative findings were recorded. In all cases, the tenacious mucus, cheesy clay-like material and sinus mucosa/polyps were sent as separate specimens for mycological, cytological and histopathological assessment. Also immunohistochemical assessments were done for sinus mucosa/polyps.

Patients presenting with criteria of AFRS [4] were involved in the present study. Patients were categorized into AFRS with or without high fungal loads (HFL) based on the following definitions. AFRS without HFL cases had the presence of nasal polyposis, characteristic CT scans, the presence of allergic fungal mucin (thick tenacious-colored mucus at the time of surgery, viable and degranulating eosinophils with scattered fungal hyphae dispersed within eosinophilic mucin by cytological examination), and had an allergy to any fungi determined by a positive skin prick test. AFRS with HFL cases had additionally cheesy or clay-like materials at the time of surgery, and microscopically containing scattered fungal hyphae together with dense conglomerations of hyphae.

Histopathological and cytological assessment

Specimens were fixed in 10 % neutral-buffered formalin, sectioned, and stained with hematoxylin and eosin (H&E). Basement membranes thicknesses were judged as: mild = slight and localized thickening, marked = severe and diffuse thickness and moderate = any abnormality between both. Sub-epithelial edema and fibrosis were also assessed in a categorical fashion (0 = not present, 1 = focal/mild, 2 = patchy/moderate and 3 = extensive/ marked). Eosinophils were quantified in the foci of the densest cellular infiltrates. [12] Scoring was carried out using a wide-angle Olympus CH2 light microscope (Tokyo, Japan) (field size of 0.274 mm² and field diameter of 0.59 mm²). Counts were recorded in 10 fields per patient sample at ×400 magnification and reported as absolute numbers. Unintentional bias was prevented by coding tissue samples, so that histopathological study was done without knowledge of the used component characteristics.

Also Gomori methenamine silver (GMS) stains and Periodic acid Schief (PAS) were used for evaluation of the presence of fungi in the mucus and determination of their density.

Immunohistochemical staining procedures and assessment

Five μm thick sections were deparaffinized and rehydrated in xylene combined with a graded series of alcohol. Antigen retrieval was performed by boiling in 10 mM citrate buffer (pH 6.0) for 20 min, followed by cooling at room temperature. Six slides per/patient's tissue specimen were incubated overnight at room temperature with rabbit polyclonal anti-CD3 (Cat. No. 103A-78, Cell Marque Corporation, Rocklin, CA), monoclonal anti-CD 20 (Cat. No. 120 M-88, Cell Marque Corporation), mouse monoclonal anti-CD4 (Product Code: RTU-CD4-1F6, clone 1F6, NovocastraTM, Leica Biosystems Newcastle Ltd., UK), mouse monoclonal anti-CD8 (Product Code: RTU-CD8-295, clone 1A5). The ultra vision detection anti-polyvalent HRP/DAB was used to visualize staining (Lab Vision). The reaction was visualized using an appropriate substrate/chromogen (Diaminobenzidine, DAB) reagent with Mayer hematoxylin as a counter stain. Tonsil tissues were used as a positive control.

All lymphocytes with complete membranous staining were regarded as positivity stained, regardless of intensity. All samples were read in a blinded fashion. Scoring was performed for all resident lymphocytes in tissue sections and data were expressed as the absolute number of positive immunoreactive lymphocytes among the total number of lymphocytes.

Mycology workup

Mycological investigation of mucus samples was performed as described in our previous study [14]. Fungal colonies were taxonomically identified by macroscopic and microscopic morphological features, and the isolated fungi were evaluated in vitro for the ability to produce mycotoxins (i.e., Gliotoxin by the Mycology Research Unit, Genetic Engineering and Biotechnology Research Institute, Menoufia University).

Follow-up after surgery

The existence of AFRS criteria (with or without HFL) was an indication for postoperative oral corticosteroids (OCS) treatment. The contraindications for OCS were immunodeficiency, tissue fungal invasion and patients with contraindications for corticosteroids therapy. Within 48 h after surgery, oral prednisolone was administered for 1 month,

including 40 mg/day for 4 days, 30 mg/day for 4 days and 0.2 mg/kg/day, or 20 mg/day at whichever dose was greatest for 20 days. Also the patient continued over nasal corticosteroids (Budesonide) 64 μg tds for 1 year. Standard endoscopic staging during follow-up of the patients was performed at 1, 3, 6, and 12 months as previously described [15], where stage 0 was no evidence of disease, stage 1 was mucosal edema with or without allergic mucin, stage 2 was polypoid mucosa with or without allergic mucin, and stage 3 was polyposis with allergic mucin. Also assessment of recurrence of associated fungal concretions was observed. No systemic corticosteroids were administered after 1 month if the patient had stage 0. In cases of recurrence (stage ≥ 1) OCS was administered with same schedule as described after surgery.

Statistical analysis

Statistical analyses were performed using SPSS software, version 17.1 (SPSS Inc., Chicago, IL, USA). Mean, medians and SD were calculated. Fisher's exact test and Chi square test (χ^2) were used in analysis of the differences between the qualitative data. Mann–Whitney *U* tests were used to ascertain quantitative differences between groups. Results yielding a *P* value <0.05 are considered statistically significant. If the *P* value is under 0.001, results are considered highly statistically significant.

Results

Nine cases of AFRS without HFL and seven cases of AFRS with HFL had the criteria of enrollment in each group. Four cases of AFRs [(one patient) with HFL and (three patients) without HFL] were excluded from the study as they did not follow-up after surgery. Twelve patients continued the treatment and followed up after surgery (six patients of AFRS associated with HFL and six patients without HFL). The demographic data are shown in Table 1. The mean age of patients in both groups was less than 40 years of age. Patients ≤ 25 were 4 (66.7 %) and 1 (16.3 %) in AFRS with and without HFL groups, respectively. In both groups no patients had aspirin sensitivity. There was predominance of nasal polyposis scores in one side over the other in both groups. Diffuse heterogeneity (multiple, diffuse and central intra-sinus high-attenuation areas) was found to be the most common CT radiological difference between both groups but without significant differences ($P = 0.061$) (Table 1; Fig. 1). There was no difference in the total CT score, bone expansion and erosion, and inequality in sinus score between the two groups (Table 1). In AFRS with HFL, fungal mucin (green-black-brown mucus with a thick tenacious, glue-like consistency and fungal debris on the

Table 1 Demographic criteria, polyp scores, CT findings and, fungal culture results in AFRS patients with and without HFL

Parameters	AFRS with HFL No = 6 No (%)	AFRS without HFL No = 6 No (%)	Test of significance <i>P</i> value
Age			
Mean $\pm$ SD	29 $\pm$ 14	34 $\pm$ 3	0.422 ^a
$\leq$ 25 years	4 (66.7)	1 (16.7)	0.071 ^b
>25 years	2 (33.3)	5 (83.3)	
Gender			0.567 ^b
Male	2 (33.3)	4 (66.7)	
Female	4 (66.7)	2 (33.3)	
Male: Female	2:4	4:2	
Asthma			1.000 ^b
Present	2 (33.3)	1 (16.7)	
Absent	4 (66.7)	5 (83.3)	
Endoscopic nasal polyposis score			
Polyp score Lt			
Mean $\pm$ SD	2.3 $\pm$ 1.2	1.1 $\pm$ 0.7	0.057 ^a
Polyp score Rt			
Mean $\pm$ SD	0.6 $\pm$ 0.8	2.5 $\pm$ 0.8	0.010 ^a
CT scan findings			
Total CT scan score			
Mean $\pm$ SD	12.6 $\pm$ 4.8	11.5 $\pm$ 4.0	0.519 ^a
Score difference between sides			
Mean $\pm$ SD	7.8 $\pm$ 3	6.5 $\pm$ 3.7	0.686 ^a
Diffuse heterogeneity			0.061 ^b
Present	6 (100)	2 (33.3)	
Absent	0 (0)	4 (66.7)	
Expansion			0.455 ^b
Present	6 (100)	4 (66.7)	
Absent	0 (0)	2 (33.3)	
Erosion			1.000 ^b
Present	4 (66.7)	4 (66.7)	
Absent	2 (33.3)	2 (33.3)	
Fungal culture			
Fungal culture			
<i>Aspergillus fumigatus</i>	5 (83.3)	6 (100)	1.000 ^c
<i>Aspergillus flavus</i>	1 (16.7)	0 (0)	
<i>Bipolaris (Drechslera) oryzae</i>	1 (16.7)	0 (0)	
Glutotoxin			1.000 ^b
Producer	6 (100)	5 (83.3)	
Non producer	0 (0)	1 (16.7)	

^a Mann–Whitney *U* test^b Fisher's exact test^c Chi square (χ^2) test

surface) was observed together with fungal concretions with cheesy or clay-like characteristics at different sites of the paranasal sinuses. Pathologic features of patients from both groups are summarized in Table 2. The cytological examination of the allergic mucin showed a mixture of sloughed epithelial cells, eosinophils, and fungal hyphae. The more definitive branching septate nature of the hyphae was demonstrated dispersed more easily by fungal stains in both groups (Fig. 2a, b). In AFRS with HFL, there were

also extramucosal colonies with tightly packed hyphal arrays (Fig. 2c, d). The histopathological examination in such group demonstrated a marked degree of edema ($P = 0.002$), but without a significantly higher eosinophil counts than AFRS without HFL (Table 2). In AFRS without HFL patients, greater degree of fibrosis ($P = 0.002$) was observed. Both groups showed epithelial ulcerations in all the patients. Using immunohistochemical stains, both groups showed increased CD3 and CD8

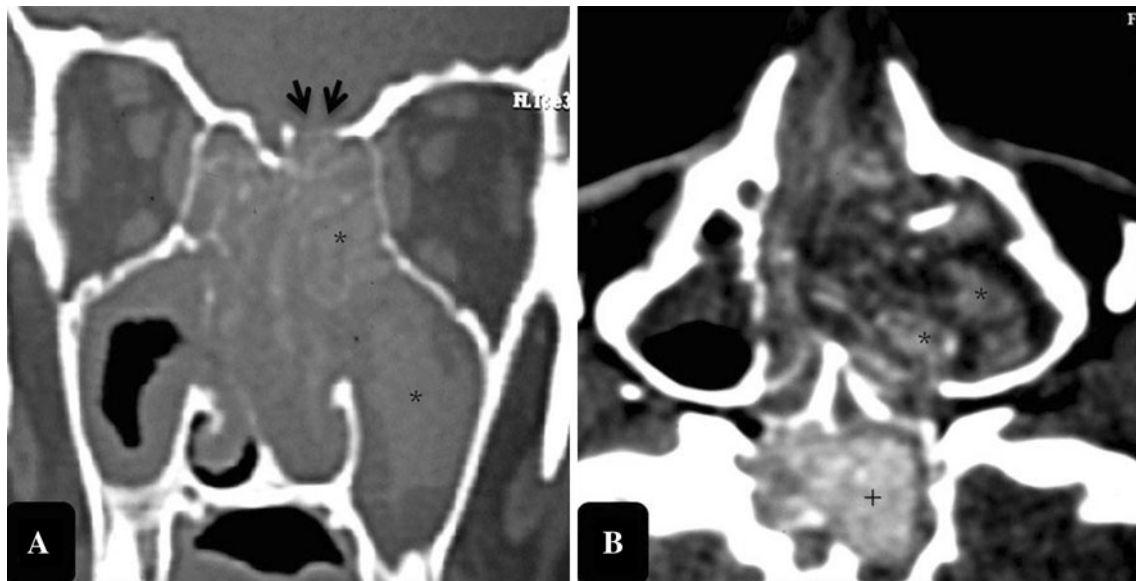


Fig. 1 Allergic fungal rhinosinusitis with high fungal load in a 16-year-old female patient: **a** coronal CT scan bone window, **b** axial CT scans soft tissue window showing characteristic expansile, hyperattenuating material (multiple, central and diffuse) in the ethmoid, and left maxillary sinuses (asterisk). More central hyperattenuation is

noted in the sphenoid sinus (cross). Extension into the nasal cavity from the bilateral ethmoid sinuses and left maxillary sinus is noted. There is also smooth erosion of the roof of the left ethmoid sinus (black arrows)

expression, without significant differences between groups. Fungal cultures results were all positive in both groups (Table 1). In the AFRS with HFL patients single fungal isolates were identified in 5/6 (83.3 %) cases (*Aspergillus fumigatus* 4/6 (66.7 %) and *Aspergillus flavus* 1/6 (16.7 %)) and double isolates of (*Aspergillus fumigatus* and *Bipolaris (Drechslera) oryzae*) in 1/6 (16.7 %) cases. All the *Aspergilli* in HFL group produced gliotoxin. While mucus cultures identified single isolates of *Aspergillus fumigatus* in all the patients without HFL and gliotoxin producers fungi were present in 5/6 (83.3 %) of the cases.

Follow-up endoscopic examinations were carried out for 1 year post-surgery while they received (1 month) corticosteroid therapy (Table 3). In AFRS with HFL group only one patient had one recurrence (stage 2 associated with HFL) at 9 months after surgery. Outpatient debridement was done and followed by OCS without recurrence. In AFRS without HFL group; 2 patients had two recurrences at 6 and 12 months with stages (3, 1) and (2, 1), respectively. Another three patients in the same group had single recurrence at 12 months follow-up (stage 1). Based on the results, significantly fewer patients had recurrences with less recurrence rate/year in the AFRS with HFL group compared to the AFRS without HFL group ($P = 0.021$, 0.023 , respectively). No differences in the endoscopic scoring between both groups during the first and second 6 months ($P > 0.05$). The only side effects developed after OCS treatments were: transient weight gain (all 12 patients) and moon face appearance (two patients in AFRS

without HFL group). Both side effects were reversed at the end 12 months follow-up period.

Discussion

The present clinical series represents an analysis of the effect of fungal load differences on diagnosis and prognosis of AFRS. Patients were uniformly evaluated, treated, and given follow-up examinations. The key determinant of the role of fungi, and the reason for the diversity of host outcomes in fungal-related sinonasal diseases, is hypothesized to be the nature of the immune response of the host [1, 3, 4, 6], and the diseases may be conceptualized as points along a spectrum of abnormal immune responses of the host [16]. The presence of the same cellular inflammatory and immunological responses (eosinophils and CD3 + CD8 + T cell) with different fungal loads in AFRS cases can highlight the presence of the same repertoire of cross-regulatory and antifungal host responses that allow for the development of AFRS. In AFRS with HFL, fungi have developed their own tactics to colonize the sinonasal mucus blanket and evade or overcome host defenses in the presence of special T cell responses. There are several possible explanations for the development of high fungal load in some AFRS cases. First, continuous CD3 + CD8 + T cell-dependent inflammation occurs in response to the inhaled fungal spores in AFRS patients. The resulting tissue inflammation, edema, and obstruction

Table 2 Histopathological and immunophenotyping characteristic differences between AFRS patients with and without HFL

Parameters	AFRS with HFL <i>N</i> = 6 <i>N</i> (%)	AFRS without HFL <i>N</i> = 6 <i>N</i> (%)	Test of significance <i>P</i> value
Squamous metaplasia			
Complete	0 (0)	1 (16.7)	1.00 ^a
Incomplete	6 (100)	5 (83.3)	
Basement membrane thickness			
Mild	0 (0)	0 (0)	0.182 ^a
Moderate	6 (100)	3 (50)	
Marked	0 (0)	3 (50)	
Edema			
Mild	0 (0)	2 (33.3)	0.002 ^b
Moderate	0 (0)	4 (66.7)	
Marked	6 (100)	0 (0)	
Mucous secreting glands status			
Normal	1 (16.7)	1 (16.7)	1.00 ^a
Proliferated	5 (83.3)	5 (83.3)	
Atrophy	0 (0)	0 (0)	
Fibrosis			
Mild	6 (100)	0 (0)	0.002 ^a
Moderate	0 (0)	6 (100)	
Eosinophils cells			
Count/10 HPF			
Mean ± SD	36.1 ± 7.7	30.0 ± 9.3	0.334 ^c
CD20			
Mean ± SD	11.3 ± 5.7	12.8 ± 5.6	0.411 ^c
CD3			
Mean ± SD	88.6 ± 5.7	87.1 ± 5.6	0.411 ^c
CD4			
Mean ± SD	18.0 ± 3.9	19.6 ± 5.8	0.936 ^c
CD8			
Mean ± SD	69.01 ± 7.6	67.0 ± 6.4	0.872 ^c

^a Fisher's exact test^b Chi square (χ^2) test^c Mann–Whitney *U* test

of the sinus ostia can lead to stasis of secretions within the sinuses. This creates an ideal environment for further fungal proliferation. Subsequently, this will increase the inflammatory response and expand to other sinuses, resulting in bony expansion and erosion. The marked degree of tissue edema provides additional support for this mechanism. Second, the presence of inhaled fungi with their secretion of gliotoxin and their growth can promote an altered T cell response toward the fungi. It has been demonstrated that gliotoxin increases the allergic immune response in mice by modulating the Th1/Th2 balance through direct effects on IL-12 secretion by dendritic cells and by inducing oxidative stress [17]. In addition, culture supernatants of *Aspergillus fumigatus* strains lacking the ability to produce gliotoxin showed a significantly lower cytotoxicity on macrophage-like cells and T cells in vitro [18]. After replication, a certain level of gliotoxin can trigger an immune response toward AFRS responses. Good

responses in patients to surgical and corticosteroid treatment can support this hypothesis.

The slow accumulation of allergic fungal mucin, the hallmark of AFRS, imparts unique and rather predictable characteristics of the disease. Allergic mucin is somewhat more thin and mucinous. In AFRS with and without HFL, the involved sinuses contain brown or greenish-black material that has the consistency of peanut butter or cottage cheese. This has been termed “allergic fungal mucin” and occurs in addition to the cheesy or clay-like characteristics, which represent the fungal-load concretion in HFL form of the disease. Such findings were clear in the cytology and histopathology examinations and were associated with higher degree of edema.

The combination of metal ions (e.g. iron and manganese), calcium salts, melanin and inspissated secretions in fungal aggregations should be more readily detected at CT [19]. The presence of central areas of increased attenuation

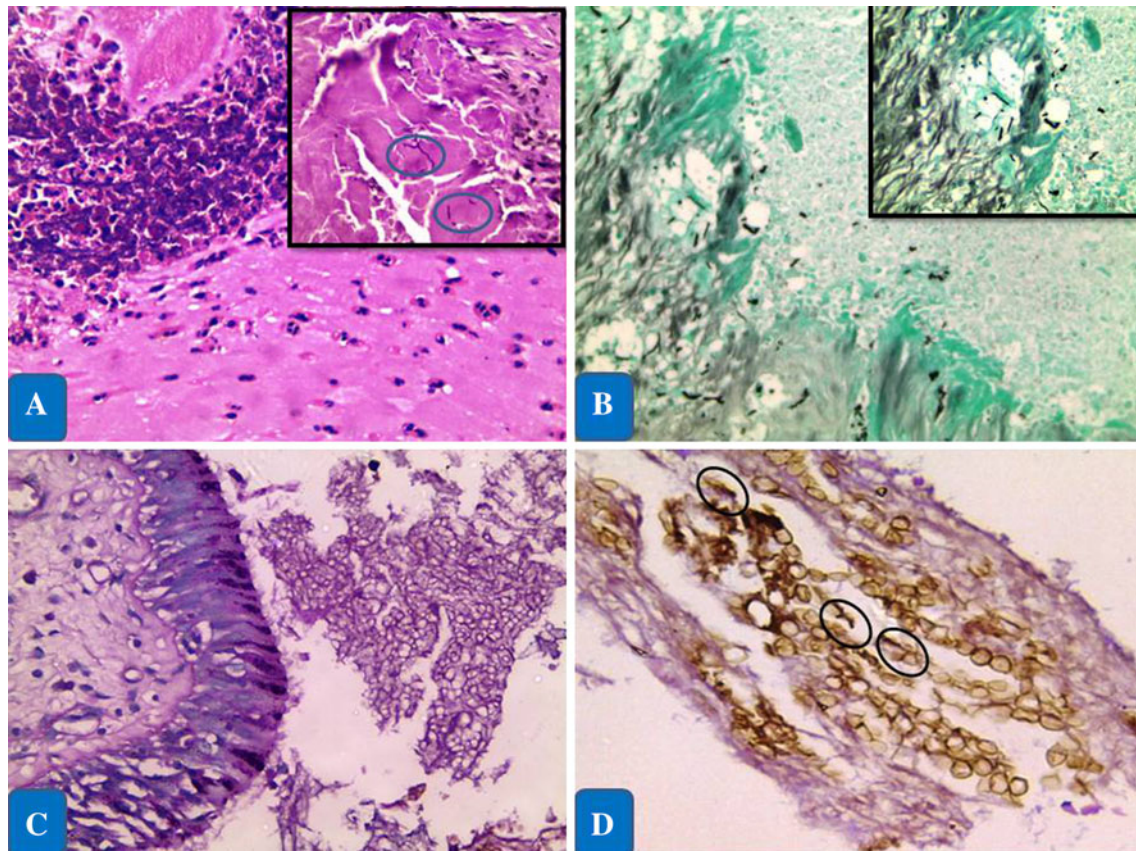


Fig. 2 Histopathology findings in an AFRS with HFL patient. **a** H and E stained allergic mucin shows viable and degranulating eosinophils and sloughed epithelial cells dispersed within eosinophilic mucin; *inset* PAS stained mucin displays fungal hyphae (*circles*). **b** GMS stained mucin demonstrates fungal hyphae; *inset* higher

power view. **c** PAS stained section displays dense compact aggregation of fungal spores and hyphae laying in adjacent sinus mucosa. **d** High power view of PAS stained extramucosal colonies shows tightly packed fungal spores and hyphal arrays (*circles*) with brown pigmentation ($\times 400$ for **a**, **b**, **d** and $\times 200$ for **c**)

(serpiginous opacity) in the paranasal sinuses did indeed correlate well with allergic fungal sinusitis with high fungal loads in the present study population. However, since some patients in the present study without HFL were diagnosed with this criterion and the presence of inspissated mucosal secretions within the sinus cavity or along the crevices of polyps can result in a markedly hypointense T2-weighted signal [20], CT findings alone are not conclusive.

Although cases of AFRS with HFL could be mistaken with fungal balls, there are enormous differences between both. Fungus ball is usually a mass of densely packed fungal hyphae layers often exhibiting alternating zones of dense and less dense growth of the fungus giving a concentric onion skin-like appearance where the fungi on the inside are dead and calcified and the superficial layers are still alive [21]. They do not show nasal polyposis and allergic fungal mucin [22]. They usually are in single sinuses in patients which do not mount any exaggerated immune responses, and elicit non-specific inflammations (foreign body reaction or irritation) [21]. Skin test for fungi and fungal specific IgE is

usually negative [22]. They show a characterized CT image due to the calcified core [23]. Recently we showed that levels of CD3+ and CD8+ cells were increased in the sinus mucosa of AFRS compared to the elevated number of CD3+ and CD4+ observed in FB [24].

ESS was performed for all cases in both groups. ESS decreases the antigen load by removing the fungus entrapped in allergic mucin and improving the drainage of the sinuses. It also allows for accessibility during follow-up for debridement and evaluation of the disease processes. As both categories of the disease (AFRS with and without HFL) are associated with unique eosinophilic inflammatory response and a high recurrence rate, postoperative corticosteroids were used in both groups. Shubert identified that the immediate postoperative period is the most critical time to use OCS [25]. Regimens using oral steroids for treating AFS have shown some degree of variation in the literature [26]. In this study, we used the treatment protocol described in Kupferberg et al. [15], as the benefit of oral steroids in AFRS patients was found to be statistically significant. We followed the same treatment regimen for both treatment groups.

Table 3 Postoperative endoscopic follow-up in AFRS patients with and without HFL

Parameters	AFRS with HFL No = 6 No (%)	AFRS without HFL No = 6 No (%)	Test of significance <i>P</i> value
Recurrence/year			
Mean number of recurrences per year			
Mean N/Y $\pm$ SD	0.16 $\pm$ 0.4	1.16 $\pm$ 0.7	0.023 ^a
Recurrence/year			
Present	1 (16.7)	5 (83.3)	0.021 ^b
Absent	5 (83.3)	1 (16.7)	
Number of recurrence/year			
One recurrence	1 (100)	3 (60)	0.059 ^b
Two recurrence	0 (0)	2 (40)	
Follow-up endoscopic staging			
First 6 months:			
Stage 0	6 (100)	4 (66.6)	0.135 ^c
Stage 1	0 (0)	0 (0)	
Stage 2	0 (0)	1 (16.7)	
Stage 3	0 (0)	1 (16.7)	
Second 6 months			
Stage 0	5 (83.3)	1 (16.7)	0.092 ^c
Stage 1	0 (0)	5 (83.3)	
Stage 2	1 (16.7)	0 (0)	
Stage 3	0 (0)	0 (0)	

^a Mann–Whitney *U* test^b Fisher's exact test^c Chi square (χ^2) test

Waxman et al. [27] described 15 adults with AFS that could be clinically divided into three groups: those patients who showed frequently recurring disease (within months), those in whom disease recurred slowly (within years), and those who responded to surgery alone. These observed differences in treatment responses may be related to the presence of different fungi loads. The category of patients having AFRS with HFL described in the present study may have been present in the Waxman series [27], but it could have been missed during diagnosis. In our patients, with higher fungal loads the surgical removal of the fungal load induced a significantly lower recurrence rate and higher response to corticosteroids. Diagnosis of such patients with high sinonasal fungal burden can allow an image for good response to treatment. In the other group without high-fungal load re-inoculation of fungi from the patient environment can be the triggers for recurrences. Noble et al. showed that [10] of the predominant fungi recovered from air samples of selected patient residences included the same species isolated from their mucin. In both groups no OCS-treated patients developed fungal invasive disease forms.

Conclusion

In addition to mucus and mucosal tissues, cheesy clay-like materials must be assessed in AFRS cases. AFRS with HFL can be considered as a part of disease spectrum of

AFRS with negligible diagnostic differences (more edema and less fibrosis) from ordinary AFRS without HFL cases, however, it had better outcome after treatment. The results of the present study should be validated in next studies with higher numbers of patients.

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