Pathologic Features of Non-Invasive Fungal Rhinosinusitis

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Abstract: The purposes of this study were to quanititate and correlate the findings of pathologic specimens and fungal culture in patients with a diagnosis of allergic fungal rhinosinusitis. Seventeen patients were identified between 2003 and 2005 with noninvasive fungal rhinosinusitis. Surgical specimens were analyzed *via* routine and special pathologic processing and compared to the microbiological results of fungal cultures of these same surgical specimens. Eleven patients (65%) had a positive fungal culture, and 10 patients (59%) had a positive fungal stain. The most characteristic pathologic feature was the presence of allergic fungal mucin. This consisted of inspissated basophilic mucin with alternating areas of large numbers of eosinophils. There is a broad spectrum of pathologic changes found in specimens suspected of Eosinophilic fungal rhinosinusitis. Fungal cultures do not always correspond to these pathologic changes. A high index of suspicion is necessary to make this diagnosis and provide adequate treatment.

Keywords: Allergic fungal rhinosinusitis, fungal culture, pathology, antifungal therapy.

INTRODUCTION

Fungal infection in the paranasal sinuses manifests in a variety of ways. Most types of infection are diagnosed on pathologic evaluation of surgical specimens. Immunocompromised patients are susceptible to invasive fungal infection, where fungal elements are seen in the submucosal or deeper layers of the sinonasal mucosa. In compromised hosts, infection is usually acute and fulmanent and typically involves fungi from the *Mucor* family. Invasive disease is rarely seen in immunocompetent hosts and typically follows a chronic though destructive course. Immunocompetent hosts are susceptible to fungal infection, however these infections do not involve tissue invasion. Fungal balls are dense concretions of matted fungal elements typically found within the maxillary sinuses. Allergic fungal rhinosinusitis can involve any of the paranasal sinuses and is characterized by thick allergic/Eosinophilic mucous.

Allergic/Eosinophilic fungal rhinosinusitis (AFS) was first described in 1981 by Millar and for the past 25 years has been the subject of intense study and debate [1]. AFS patients frequently present with headaches, nasal obstruction, green/black thick discharge and a history of allergy [2]. On endoscopic exam extensive nasal polyposis is evident but the diagnosis is not confirmed until allergic mucin is identified on pathologic investigation of a surgical specimen. Patients frequently have impressive findings on radiographic studies of the sinuses. It is not uncommon for patients to have large erosive lesions filling and expanding the involved sinus cavity. Facial deformity and ocular or intracranial extension is not uncommon [3].

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Several diagnostic algorithms exist, most notable those by Bent and Kuhn and by deShazo and Swain [4], but the specific criteria differ. The Kuhn criteria include evidence of type I hypersensitivity and a positive fungal smear [5]. Marple has argued that the diagnosis of AFS does not require all 5 Kuhn criteria, but patients should at least demonstrate allergy to fungal elements and presence of allergic/eosinophillic fungal mucin in the pathologic specimen [3].

Pathologic evaluation of surgical specimens is the key to confirmation of the AFS diagnosis. Allergic/Eosinophilic mucin is the *sin qua non* for the diagnosis of AFS. This substance is grossly thick, tenacious rubbery mucus that ranges in color from green to brown to black. The mucin is formed from characteristic "tide-lines" or "waves" of mucin with degenerated debris of eosinophils some epithelial cells. Charcot-Leyden crystals may be present and are derived from the degenerated eosinophils. They appear as long needle shaped or bipyramidal shaped crystals. Fungal hyphae may be sparse and are highlighted by Gomori's metanamine silver (GMS) or periodic acid schiff (PAS) staining and are used as adjuvants to standard hematoxilyn and eosin staining. Organism counts may vary but do not usually exhibit fruiting heads [6]. Although this mucin is diagnostic for AFS, a high index of suspicion is necessary because missed diagnosis can occur, particularly since fungal elements do not stain well on traditional hematoxilyn and eosin stained specimens.

Adjuvant to staining of the specimen is fungal culture. A variety of staining methods exist including GMS, PAS, immunofluorescence to chitin [15], and others. Reports of positive fungal cultures vary from 50-100 percent [2,3,7]. Culture results have demonstrated a variety of organisms responsible for AFS. Although the original reports of AFS described Aspergillus as the causative organism, further study has demonstrated the majority of cases are linked to members of the Dematiaceous fungal family, namely *bipo-*

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laris and *curvularia*. The specific species is dependent on geographic location. Pathologically these organisms appear similar, with thin, septate hyphae branching at 45-degree angles. Culture is required to differentiate the different species.

This study was designed to evaluate the correlation of culture results and pathologic evaluation.

MATERIALS AND METHODS

The UCLA Institutional Review Board approved this study prior to commencement. Patients for the study were identified from a tertiary University practice who had received a diagnosis of non-invasive fungal rhinosinusitis from 2003-2005. These patients were identified in a retrospective fashion. All patients had undergone surgery by the senior author (MW). Diagnostic criteria for involvement in the study included presence of Eosinophilic mucin at surgery, identification of Eosinophilic mucin on pathologic evaluation, presence of fungal elements on pathologic evaluation and fungal culture results. All surgical specimens were reviewed by one of the senior authors (SB). After routine processing, samples were stained with hematoxilyn and eosin. Fungal staining with histochemical stains such as GMS or PAS were preformed when routine H&E stains demonstrated suspicious mucin. Results for each patient regarding fungal staining, culture and pathologic description were tabulated (Table **1**).

RESULTS

Seventeen patients were identified between 2003 and 2005 that were diagnosed with noninvasive fungal rhinosinusitis. Patient demographics are listed in Table **2**.

Fourteen patients (82%) had allergic fungal mucin with polyps, while two patients had positive fungal cultures and chronic rhinosinusitis without polyps. One patient (5.8%) had a positive fungal stain, negative culture and evidence of chronic rhinosinusitis. Eleven patients (65%) had a positive fungal culture, and 10 patients (59%) had a positive fungal stain. Eight of the 10 (80%) patients with a positive fungal stain subsequently had positive fungal growth on culture. Three of 11 (27%) patients with a positive fungal culture had a negative fungal stain. Organisms included aspergillus, alternaria, bipolaris, curvularia and cladosporium. Fourteen patients were immunocompetent, while 3 had underlying immune suppression (HIV/AIDS, metastatic breast cancer, and post organ transplant). Pathological findings included edematous respiratory mucosa with numerous mononuclear inflammatory cells, eosinophils, and neutrophils. The most characteristic feature was the presence of allergic fungal mucin, which was grossly thick and dark with a consistency of peanut butter or wet clay. This fungal mucin consisted of inspissated basophilic, purple-colored mucous with large numbers of degenerated eosinophils (Fig. **1**).

Fungal stains (GMS and PAS) demonstrated that the fungal hyphae were sparse and not always found (Fig. **2**). There were never fungal hyphae within the mucosal tissue.

DISCUSSION

It is believed that approximately 5-7% of patients with chronic rhinosinusitis patients suffer from allergic fungal rhinosinusitis but this diagnosis can be elusive [6]. Classically, the diagnosis of AFS requires that 5 criteria are met as originally set forth by Bent and Kuhn: (1) IgE mediated hypersensitivity, (2) nasal polyposis, (3) characteristic findings on radiologic studies, (4) presence of allergic/Eosinophilic

Table 1. Pathologic and Fungal Culture Results

Patient Number	Fungal Stain	Fungal Culture	Pathologic Description
1	+	+ Aspergillus	Chronic rhinosinusitis
2	-	-	Chronic allergic rhinosinusitis
3	-	-	Chronic rhinosinusitis
4	+	+ Alternaria and Bipolaris	Chronic allergic rhinosinusitis
5	+	+ Alternaria	Allergic fungal rhinosinusitis
6	-	+ Curvularia	Chronic rhinosinusitis
7	-	+ Aspergillus	Chronic allergic rhinosinusitis
8	+	+ Curvularia	Allergic mucin with acute and chronic inflammation
9	+	+ Aspergillus	Allergic fungal rhinosinusitis
10	-	-	Chronic allergic rhinosinusitis
11	+	+ Aspergillus	Allergic fungal rhinosinusitis
12	+	-	Chronic rhinosinusitis and mycetoma
13	-	+ Bipolaris	Chronic rhinosinusitis
14	+	+ Cladosporium	Polypoid allergic chronic rhinosinusitis
15	-	-	Allergic fungal mucin
16	+	+ Aspergillus	Allergic fungal rhinosinusitis
17	+	-	Chronic rhinosinusitis

Table 2. Patient Demographics

Patient Number	Age	Sex	Symptoms	Allergy Testing	Treatment	Outcome
1	53	F	Facial pressure, headache, pulmonary aspirgillosis	N	Voriconazole, nasal irrigation, systemic immune suppression for transplants	(Heart-Lung transplant) Resolution of patient's sinus symptoms
2	34	F	Three previous sinus surgeries, recurrent acute sinusitis	N	Voriconazole, nasal irrigation	Lost to follow up 6 mo after surgery
3	60	F	Two previous sinus surgeries, right facial pain/pressure, NAO, mucus	N	Systemic immune suppression for RA	Lost to follow up 2 mo following procedure (Rheumatoid Arthritis)
4	50	F	"Chronic sinusitis" with failure to medical therapy	N	Records not available	N/A (Metastatic Breast Carcinoma)
5	15	M	Severe allergies, L proptosis, NAO	N	Sporanox, nasal irrigation, nasal steroids	Lost to follow up at 1year, proptosis resolved
6	63	F	Occasional eye pain and epistaxis	N	Nasal irrigation, nasal steroids	1 year f/u revealed mild polypoid change bilaterally. Pt lost to follow up thereafter
7	27	F	Thick nasal discharge, NAO, epistaxis,	N	Sporanox, nasal irrigation, nasal steroids, medrol for flair, Augmentin for acute sinusitis	Required reoperation 6 months after original surgery for recurrent polyposis.
8	48	F	4 previous surgeries, facial pain, nasal obstruction	Y	Sporanox and levaquin post op, nasal steroids, irrigation, Singulair, Allegra, Medrol for flair, Vfend/vanco/flagyl for severe flair, changed to Cancidas and Zyvox for ↑ LFTs	No further surgery but frequent exacerbations requiring antibiotics and antifungals
9	22	M	Six previous sinus surgeries, nasal discharge, nasal congestion	N	Sporanox, converted to Voriconazole, nasal irrigation, nasal steroids	Lost to follow up 2 mo after surgery
10	56	F	Facial pain/pressure, NAO, "sinus congestion"; 8 previous sinus surgeries	N	Irrigation, intranasal steroids, Astelin, Zrytec, Qvar, Medrol and Levaquin for flairs,	Symptoms improved, has not required reoperation in 2.5 years
11	34	M	Epistaxis, nasal congestion, facial pressure, post nasal drip and thick mucus	N	Sporanox, nasal irrigation	Lost to follow up 1 year from surgery (HIV+)
12	46	F	Headache, "chronic sinusitis", NAO	N	Records not available	N/A
13	30	M	Four previous sinus surgeries, facial pain, persistent drainage	N	2 week course of IV antibiotics, nasal irrigation	Lost to follow up
14	49	M	"Chronic sinusitis"	N	Keflex, Medrol	Radiographic evidence of recurrent disease but pt refused further surgery
15	31	F	Congestion, thick mucus, facial pain/pressure, asthma	Y	Nasal steroids, irrigation	Improved smell, lost to follow-up
16	15	F	Facial pain, NAO, discharge, one previous sinus surgery, R proptosis, R epiphora	N	Voriconazole, Nasonex, nasal irrigation	Lost to follow up
17	64	F	Retroorbital pain, nasal discharge	N	Records not available	N/A

NAO = nasal airway obstruction; N/A = not available.

mucin, (5) positive fungal stain or culture [5]. However, there is not a consensus in the literature regarding a uniform set of criteria. Many authors feel that lack of positive stains or fungal cultures does not rule out the diagnosis of AFS. In fact, the Bent and Kuhn criteria have been criticized. In the original Bent and Kuhn criteria there was selection bias as all of the patients presented with the 5 criteria but they did not evaluate others who did not have all 5 criteria [7].

In the current investigation, 17 patients were identified with non-invasive fungal rhinosinusitis. At the time of operation, they were noted to have polypoid changes or thick tenacious mucus within the nasal and sinus cavities that alerted the surgeon to the possibility of AFS. It is crucial that when the possibility of AFS exists, secretions from the sinus cavities be sent for pathologic evaluation. The pathologic description of findings within the sinuses may vary. Patients

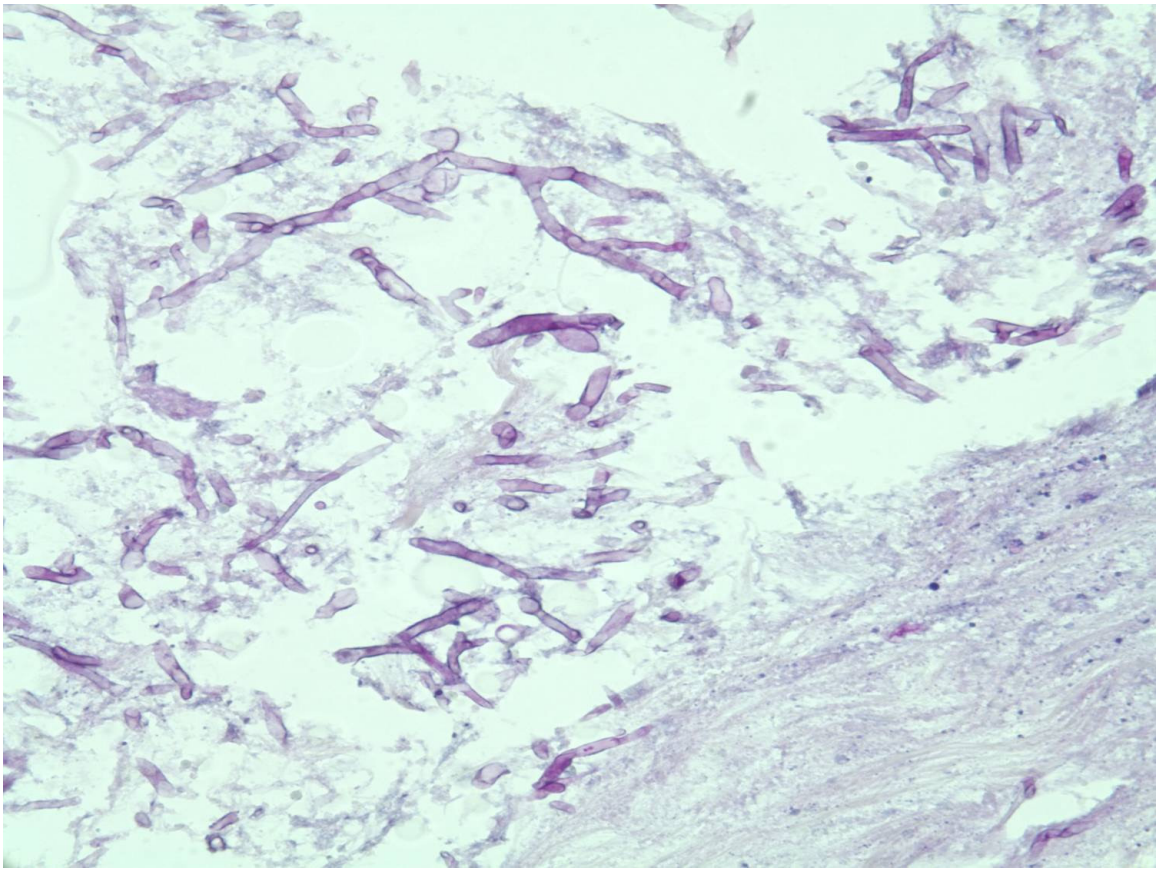

Fig. (1). Gomori's metanamine silver stain of allergic/Eosinophilic mucin. The fungal elements seen here are septated and branch acutely. Fungal elements were not always seen with in the allergic mucin but were never found invading the underlying mucosa. GMS staining, 10x magnification.

with chronic rhinosinusitis may demonstrate a ciliated psuedostratified columnar respiratory mucosa or squamous metaplasia. Submucosal changes include inflammatory infiltrate (usually lymphocytes and plasma cells), edema and mucus plugs. When the inflammatory infiltrate is comprised of primarily eosinophils, these changes are termed chronic allergic rhinosinusitis. Evaluation of sinus contents is also important in the diagnosis of rhinosinusitis. Allergie/Eosinophilic mucin is comprised of 4 parts: (1) dead or dying eosinophils, (2) sloughed epithelial cells, (3) Charcot-Leyden crystals, and (4) fungal hyphae [8]. However, up to one third of specimens may not contain fungal hyphae. This has lead to the recent description of Eosinophilic fugal rhinosinusitis-like syndrome. These patients clinically behave like AFS patients but do not demonstrate positive fungal stains [8]. It is the pathologic appearance of this mucin, rather than the sinus mucosa that is characteristic of the disease [6,7].

Several of the patients in this study demonstrate this point. The pathologic reports of 6 patients specifically mention the presence of characteristic allergic/Eosinophilic mucin within the pathologic specimen. These six patients, however, did not uniformly demonstrate positive fungal stains or fungal cultures. Furthermore, a number of other patients demonstrate both positive fungal cultures and stains but pathologically demonstrated changes consistent with chronic rhinosinusitis. A number of patients are also described as having allergic rhinosinusitis without positive fungal stains. All of the patients in the study were presumed to have AFS and were treated medically with intranasal steroids, oral steroids, nasal irrigation and close follow-up. A number of patients were also treated with oral antifungal therapy.

Granville *et al*. recently studied 34 cases of AFS and calculated the sensitivity and specificity of particular histologic features when compared to chronic rhinosinusitis. They found the presence of positive fungal staining to be 100% specific and sensitive for AFS. Furthermore, changes within the mucosa were not at all specific or sensitive for AFS. They concluded that the diagnosis of AFS remains a challenge but that certain pathologic features may raise the clinician's and pathologist's index of suspicion for this disorder [9]. In the current study, Granville's data are supported. Other authors have also described various combinations of pathologic findings [10]. Singh recent described a large series of AFS patients where 91% of patients demonstrated classic allergic/Eosinophilic mucin [11]. Taken together with fungal stain and fungal culture, these pathologic changes point to the fact that there is a spectrum of findings within the "allergic fungal rhinosinusitis" category of non-invasive fungal disease.

Allergic fungal rhinosinusitis requires aggressive multimodality treatment. However, the components of this therapy are constantly evolving as further research is conducted. While nearly all authors agree that therapy should include steroids and surgery, the role of antifungals and immuno-

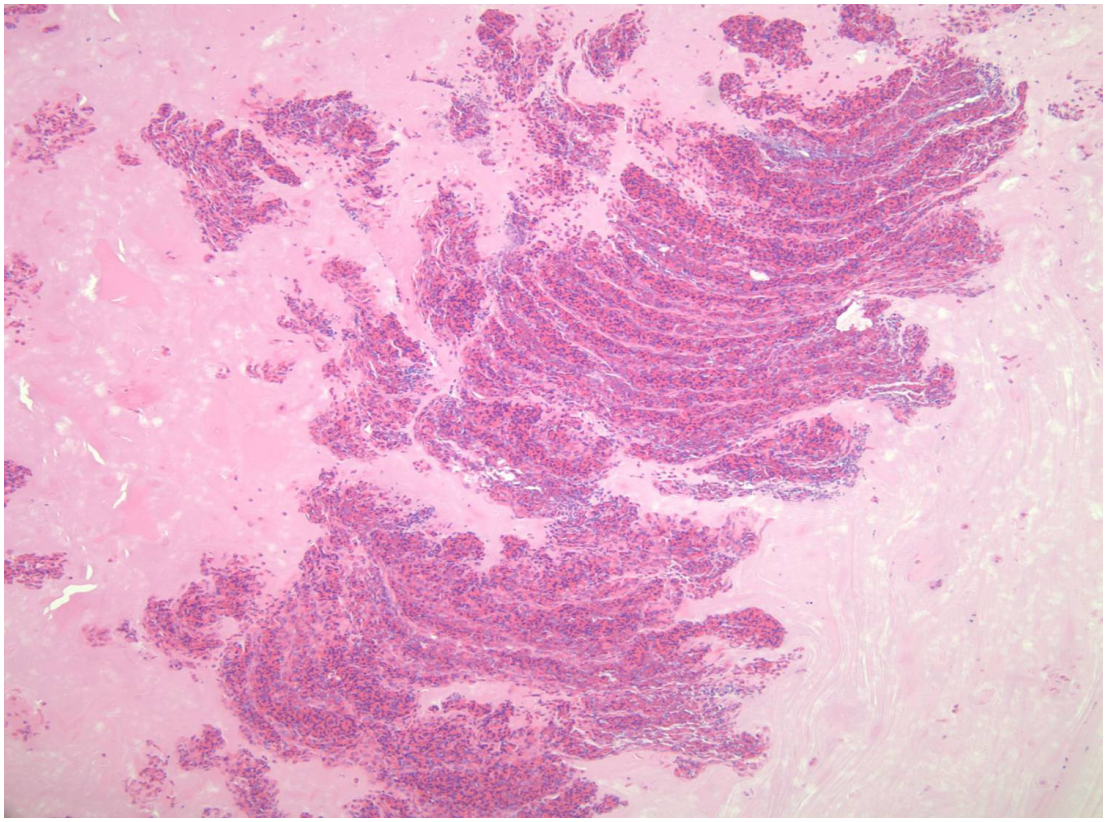

Fig. (2). Allergic/Eosinophilic mucin taken at surgery. The gross specimen was thick and dark with a consistency of peanut butter. This fungal mucin consisted of inspissated basophilic, purple-colored mucous with large numbers of degenerated eosinophils. The characteristic appearance of the clusters of eosinophils is referred to as "tide lines." Hematoxylin and eosin staining,10x magnification.

therapy is in flux. Early studies of oral antifungal therapy did not demonstrate significant improvement. Recent studies have been mixed with some demonstrating efficacy of both oral and topical antifungal therapy in decreasing recidivism [12,13], while others have not. A recent randomized control trial, failed to show benefit in patients with chronic rhinosinusitis and polyps when a 300 µg/ml of amphotericin B solution was used for nasal irrigation. In fact, patients reported worse nasal symptoms when using amphotericin B nasal irrigation [14]. However, in another recent randomized controlled trial of topical amphotericin B applied twice a day, 75% of patients reported improved rhinosinusitis symptoms and decreased pathologic findings on endoscopic exam [13]. Although the literature has conflicting reports regarding topical antifungal therapy,, we recommend antifungal therapy for selected patients. The role of immunotherapy may also be changing. Marple originally reported on a small group of patients that demonstrated improvement in symptoms following the induction of immunomodulating therapy. Unfortunately, recent studies have failed to demonstrate a long-term benefit for immunomodulation [15]. Fungal rhinosinusitis patients must be followed with serial endoscopic exams and flare-ups should be treated aggressively as recidivism is common.

There are several limitations of the current study. Only 65% of patients demonstrated a positive fungal culture. Ponikau *et al*. described nearly 100% of patients undergoing sinus surgery had detectable fungus in pathologic specimens [16,17]. This detection required special handling, however [17]. They argued this is not a surprising finding given the ubiquitous nature of fungus. We did not achieve such high results perhaps because the mucin is not digested prior to swabbing the culture plates. A further weakness in this study it the retrospective nature of the study design. As a result there is inherent selection bias. We chose patients who had already received the clinical diagnosis of AFS. This diagnosis was based on the findings a surgery and results of pathologic evaluation of the mucin recovered. These patients did not undergo allergic skin testing, however they demonstrated many of the other signs and symptoms of allergic/Eosinophilic fungal sinusitis. A prospective study of all patients treated for rhinosinusitis may have revealed different patterns of fungal disease.

Although the diagnosis of allergic fungal rhinosinusitis may be difficult, physicians should keep in mind the spectrum of pathologic changes in non-invasive fungal rhinosinusitis so that proper treatment may be instituted.

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