



Review Article

Current understanding of allergic fungal rhinosinusitis



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Abstract Studying the pathophysiology of allergic fungal rhinosinusitis (AFRS) has proved challenging. While this clinical entity is easily distinguishable based on the clinical criteria set forth by Bent and Kuhn twenty-five years ago, studies examining type 2 inflammatory profiles in AFRS can make it seem more alike other CRS subtypes than it is different. Still, evolving research seems to clearly delineate this subtype from others in CRS. This review will critically evaluate the evolution of research examining the pathophysiology of AFRS and will conclude with a summary of the special considerations in the management of this fascinating disease. Copyright © 2018 Chinese Medical Association. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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Introduction

It has been twenty-five years since Bent and Kuhn outlined the diagnostic criteria for allergic fungal rhinosinusitis (AFRS): (1) Type I hypersensitivity; (2) nasal polyposis; (3) characteristic computed tomography; (4) eosinophilic mucus; and (5) presence of non-invasive fungus in sinus contents. The dynamic interplay between sinonasal mucosa and fungus appears to culminate in a clinical presentation that is often striking - in severe cases, significant bony expansion can result in orbitocranial complications, making this disease easily distinguishable from other forms of chronic rhinosinusitis (CRS). Yet, in the past thirty years, describing pathophysiologic features that explain the presentation of this disease and separate it from other subtypes of CRS has prove elusive. As we further our pursuit towards more personalized medicine and disease endotyping in CRS, describing the drivers of disease in CRS subtypes, including AFRS, remains paramount. This review will focus on our current understanding of AFRS and the features that make it both alike, and different, from other subtypes of CRS. We then focus on specific clinical considerations as they relate to the AFRS subtype.

Epidemiology of AFRS

AFRS is almost exclusively a disease found in areas of high humidity, where mold counts are higher than other regions.¹ In these territories, AFRS can account for up to 32% of CRS cases undergoing functional endoscopic sinus surgery (FESS).^{2,3} AFRS presents at a younger age (mean 21–33 years), is more common in African Americans, and demonstrates a male predominance (1.5–2.6:1).^{4–10} Studies examining socioeconomic variations in CRS subtypes are controversial. Several retrospective investigations conducted in the southeastern United States have suggested that AFRS diagnosis is associated with lower socioeconomic status; the same studies have identified AFRS patients as presenting with advanced markers of disease, such as bone erosion and orbitocranial complications.^{3,11,12} Conversely, in a retrospective review of 54 patients with AFRS, Ghegan et al⁶ found no link between socioeconomic status and bone erosion in AFRS. Observations regarding the comorbidity of asthma in AFRS subtypes underscore an inherent biologic divergence from other subtypes of CRS. Several works from the group of the senior author have demonstrated the incidence of comorbid asthma to be nearly half of that of CRSwNP.^{10,13–15} For instance, in a prospective study of 410 patients, Promsopa et al¹⁴ recently used pulmonary function testing (PFT) to evaluate the prevalence of asthma CRS

subtypes. This study found 23.6% of AFRS patients as compared to 48.3% of CRSwNP patients with PFT-confirmed asthma. So clinically, AFRS has several unique features that sets it apart from other CRSwNP subtypes (Table 1).

Molecular and immune profiling in AFRS

The clinical description of AFRS evolved from an analogous clinical entity in the lower airway, allergic bronchopulmonary aspergillosis (ABPA), and described AFRS as an IgE-driven disease.^{16,17} Certainly, the sine qua non features of AFRS are the presence of fungus, IgE and systemic hypersensitivity to fungal antigen. Early studies investigating the etiology of AFRS supported this theory by identifying the presence of fungal-specific IgE and IgG in patients with AFRS.^{18–20} Increased scrutiny surfaced when follow up studies demonstrated the presence of positive fungal cultures in both non-AFRS CRS and healthy patients.^{21,22} Additionally, reactivity to fungal antigen and the presence of fungal-specific IgE and IgG could be demonstrated by both non-allergic and allergic fungal rhinosinusitis alike. Studies by Pant et al^{23–25} have demonstrated that fungal-specific IgE levels were not significantly different in patients with AFRS; and, fungal-specific peripheral blood lymphocyte proliferation could be observed in both AFRS patients and CRS patients without fungal allergy. Similarly, Porter et al²⁶ found a high recovery of positive fungal cultures from sinus lavages of both CRSwNP and AFRS undergoing surgery. In the same patient population, fungal-specific IL-4 production could be elicited in peripheral blood cells from both CRSwNP and AFRS.

Carney et al²⁷ performed immunohistochemistry studies using infundibulum tissue from patients with AFRS, non-allergic eosinophilic fungal sinusitis (NEFS) and CRS and found no difference in number of mast cells, eosinophils, and IgE⁺ cell numbers between AFRS and NEFS. Our group has performed large-scale microarray analysis with a specific attention to immune profiling in 130 patients with different subtypes of CRS.¹⁰ In this study, inflamed sinus mucosa from patients with AFRS and AERD demonstrated increased local IgE levels when compared to other subtypes, including CRSwNP. All subtypes, CRSwNP, AERD, AFRS, and CRSsNP, demonstrated increased levels canonical type 2 inflammatory markers, including IL-13 and IL-5, but only polyp subtypes AFRS and CRSwNP demonstrated increased IL-4 levels. Gene expression clusters associated with eosinophilic inflammation (CCL13, CCL18, CCL26, periostin) were overexpressed in all subtypes examined.

The aforementioned research regarding AFRS pathophysiology appear to highlight AFRS as more similar to other subtypes of CRS than different; however, this may be

Table 1 Comparison of clinical characteristics between AFRS and CRSwNP.

Characteristic	CRSwNP	AFRS
Nasal polyps	Present	Present
Age of presentation	No specific range	Often before age 30
Total serum IgE	Dependent on atopic status	Very high often more than 1000 U/ml
Peripheral eosinophil levels	Can be elevated	Typically within normal limits
Asthma	Up to 50% prevalence ¹⁴	Less than 25% ¹⁴

because few studies have systematically attempted to describe a divergence in the molecular profiles of AFRS from other subtypes. Orlandi et al²⁸ performed microarray gene expression analysis to identify gene expression variations in AFRS and EMCRS. The authors identified four genes uniquely overexpressed in EMCRS and not in AFRS, and 34 genes uniquely overexpressed in AFRS. The study was limited by small sample size (7 patients total) and the rationale for evaluating the target genes under study was not clear. Tyler et al¹⁵ recently sought to describe the gene expression variations in AFRS that make it unique when compared to CRSwNP. The study included a total of 86 patients (37 AFRS, 34 CRSwNP, 15 healthy control). Using whole-genome microarray analysis, the authors found that AFRS tissue demonstrated nearly 3000 unique gene expression variations, while CRSwNP only demonstrated 30; the two subtypes shared 405 gene expression alterations. Modeling these unique gene expression changes in a pathway analysis software revealed that unique gene expression variations in AFRS were strongly linked with T helper 2 (Th2) inflammation, co-stimulatory signaling, and T-cell receptor signaling. These findings implicate the adaptive immune arm as a key differentiating factor that separates AFRS from CRSwNP; and, that there is a clear molecular delineation between these two subtypes. These studies are supported by others which seem to similarly implicate an enhanced antigen-sensing and Th2-potentiating factor driving disease AFRS patients. For instance, Schubert et al²⁹ found that when compared to hypertrophic sinus disease, the frequency of HLA-DQB1*03 allelic variations significantly varied in patients with AFRS and positive *B. spicifera* cultures. Other investigations have also demonstrated increased levels of antigen-specific IgE in the subepithelium of sinus mucosa when compared to other subtypes of CRS.^{30,31} Furthermore, the antigen-specific IgE in these studies was not limited to fungal antigen. AFRS patients also demonstrate increased levels of circulating and sinonasal dendritic cells (DCs), DC chemoattractants and their receptors.^{32,33} Taken together, the above evidence in AFRS place this subtype along an extreme of type 2 inflammation, and it appears that the adaptive immune arm is a key driving force behind the observed inflammatory pattern in AFRS.

Innate immune dysregulation in AFRS

The challenge in separating clinical phenotypes based on type 2 inflammation is that oftentimes the characteristic metrics of disease, like eosinophilia and IgE expression, represent a common endpoint for a diverse milieu of inflammatory pathways. This may explain much of the overlap in the observed pathophysiology among different phenotypes of polyp subtypes of CRS. The epithelial barrier of the sinonasal mucosa provides the initial line of defense against microbial and allergen insult. It is comprised of a complex array of cells, peptides and proteins that are intricately linked with the adaptive immune response. As such, more recent studies have examined the pathophysiology of the innate immune arm within the context of CRS.

IL-33 is a member of the IL-1 family of cytokines with diverse cellular origins. It binds its receptor (IL1R, ST2) on immune cells and promotes Th2 polarization, eosinophilic and type 2 inflammation.^{34–37} In CRSwNP, IL-33 derived from sinonasal respiratory epithelium promote secretion of IL-13 via innate lymphoid cells (ILCs) expressing IL-33 receptor, ST2.³⁸ Of additional importance, IL-33 production can be stimulated by fungal antigen.³⁸ We have demonstrated via microarray analysis that IL-33 receptors are also elevated in AFRS patients, and further, that IL-33 receptor gene expression correlates with increases in eosinophilic and mast cell gene expression.¹⁰ Taken together, these findings implicate the importance of epithelial-associated cytokines in promoting type 2 inflammation observed in AFRS.

The “immune barrier hypothesis” in CRS posits that compromises in epithelial barrier function leads to chronic and overactive stimulation of adaptive immunity in response to external antigen that would normally be tolerated by the human host.³⁹ This is an intriguing concept, especially within the context of AFRS, as it may explain both the permissiveness of the sinonasal mucosa to mold growth, and also the type 2 inflammatory overdrive in these patients. Several studies have demonstrated decreased barrier function within the context of AFRS. Using air-liquid interface technique, Den et al⁴⁰ measured transepithelial resistance in cultured AFRS cells and found that the functional permeability of AFRS cells was significantly decreased compared to controls; and, AFRS cells demonstrated decreased tight junction proteins and increased expression of leaky tight junction proteins, like claudin-2. Using the same air-liquid interface model, Wise et al⁴¹ showed that IL-4 and IL-13 exposure decreased transepithelial resistance and altered the expression of epithelial junctional proteins in nasal polyp tissue.

Innate barrier proteins and peptides in the sinonasal mucosa constitute an important first line of defense against pathogens and mucosal injury in healthy tissue. These can be secreted or constitutively expressed. Studied examples in CRS include epithelial-derived proteins, such as defensins, cathelicidins, lysozyme, lactoferrin, and SPLUNC1.^{39,42,43} Psaltis et al⁴⁴ found that the antimicrobial peptide, lactoferrin, is decreased at the gene expression and protein level in AFRS. Ooi et al⁴⁵ showed that the antifungal protein surfactant protein (SP)-D was found in submucosa of patients with CRS, NAFES, NANFES, but not patients with AFRS. Our group has found that antifungal peptides, the histatins, normally expressed in the major salivary glands, are also expressed in the sinonasal mucosa in patients with CRSwNP, but not in AFRS tissue. The authors also reveal that the expression of histatins negatively correlates with the expression of several type 2 inflammatory mediators, including IL-13RA1, IL-4R, and periostin.¹⁵ Taken together, disruptions in innate immune signaling and epithelial barrier function appear to go hand-in-hand with the pronounced type 2 inflammation observed in AFRS. Future studies will elucidate the mechanisms surrounding decreased barrier protein expression in AFRS, whether this is the result of specific cytokine-mediated suppression, an inciting factor in Th2 inflammation, defect in activation of antifungal pathways, or a combination of mechanisms.

Fungal contributions to disease

There is evidence that fungus contributes to pathogenesis in AFRS, and this is supported by reports indicating that fungal antigen promotes a Th2 response and also that fungal components themselves are pathogenic. Few studies have elucidated a direct role for fungus in the pathogenesis of AFRS. Recent research in allergic asthma has posited an intriguing role of fungal proteases in the pathogenesis of allergic airway disease. Millien et al⁴⁶ intranasally stimulated mice with fungal protease from *Aspergillus oryzae* and observed categorical features of allergic airway disease marked by eosinophilia, increased mucin 5AC expression, and canonical type 2 cytokines IL-4, IL-5 and IL-13. The same experiments in TLR4 knockout mice showed attenuation of pulmonary hyper-reactivity, implicating TLR4 as instrumental in orchestrating allergic asthma in a mouse model. Fibrinogen cleavage products, derived from fibrinogen incubation with fungal protease, were also capable of inducing TLR4-dependent antifungal immunity gene expression (MUC5AC and IL-13Ra1).^{46,47} *Aspergillus* proteases have been shown to potentiate Th2 immune responses in mouse models of eosinophilic rhinosinusitis.⁴⁸ Ebert et al⁴⁹ used microarray gene assay to show that tissue obtained from AFRS patients undergoing ESS demonstrated increased expression of a Th2-potentiating receptor, protease-activated receptor 3, expression relative to healthy patients. There was no difference in PAR3 expression between AFRS and CRSwNP, however. These studies may provide a compelling argument for the direct role of fungal elements in promoting the exaggerated type-2 inflammatory response observed in patients with AFRS.

Taken together, we postulate that the phenotype observed in AFRS may be initiated by a barrier dysfunction and a defect in the TLR4 dependent innate antifungal response that is typically activated by the presence of fungi. As a consequence, fungal conidia can accumulate in the sinuses and germinate to fungal hyphae with significant protease activity which activate molecular pathways that lead to an exaggerated adaptive immune response to the fungal hyphae clinically resulting in an increased eosinophil presence, mucus production and local IgE levels. In addition, the defect in the TLR4 dependent innate pathway responsible for increased antifungal response would expectantly blunt the pathologic pathways leading to allergic asthma in this patient population, explaining the decreased prevalence of asthma in AFRS.

Clinical considerations

Diagnostic

Despite improving our understanding of the biological basis of AFRS, the diagnosis of this disease remains entirely clinical, as set forth by Bent and Kuhn nearly twenty-five years ago. Definitive diagnosis of AFRS is obtained at the time of surgery. There are no studies known to date which have attempted to confer a novel diagnostic tool that more specifically identifies this clinical entity. Thus, the diagnosis of AFRS remains largely clinical.

Surgery

Initial aggressive surgery to clear all involved sinuses of fungal debris and eosinophilic mucin is paramount in obtaining sustained clinical improvement. Failure to do so leads to early recurrence. Bony expansion of the sinuses opens natural sinus outflow tracts, but at the same time, demineralization and dehiscence of orbitocranial borders can make surgery more challenging. The use of image-guidance to aid surgical debridement is a necessity in these instances.

Medical therapy

Topical and systemic corticosteroids currently are the cornerstone of medical therapy for AFRS, as in CRSwNP. Suppressing inflammatory response in the sinuses is complementary to thorough surgical evacuation of eosinophilic material and fungal debris. Several randomized clinical trials have shown that oral corticosteroids improve olfaction, endoscopy scores, decrease polyp and blood eosinophilia, IgE, and IL-5 levels.^{50–54} In some instances, long-term, low dose systemic steroids are required to obtain a durable response; however, given the long-term and sometimes serious consequences of systemic corticosteroid use, practitioners should weigh the relative benefits and risks on an individual basis. Studies evaluating the efficacy of topical steroids in AFRS patients are limited; however, the available evidence in CRSwNP indicating both the safety and efficacy of standard and non-standard topical nasal steroids allows us to extrapolate that these drugs bear similar utility in AFRS.^{55,56}

Antifungal therapy

Several randomized controlled clinical trials and a pooled meta-analysis examining the utility of topical antifungals in patients with CRS and nasal polyps (without regard to AFRS status) have failed to show any benefit in terms of patient symptoms, markers of type 2 inflammation, or CT scores.^{57–62} There are currently two randomized controlled trials evaluating the efficacy of antifungal therapy in AFRS.^{63,64} Khalil et al⁶³ conducted a nonblinded prospective randomized controlled trial (not placebo-controlled) in 50 patients with AFRS diagnosed based on Bent and Kuhn criteria. The authors divided the study population into 5 groups: oral itraconazole (group A), fluconazole nasal spray (group B), combined oral itraconazole and fluconazole nasal spray (group C), fluconazole irrigation (group D), and conventional medical treatment (Group E). Forty-one patients were available for analysis at the maximum follow up of 9 months. Recurrence was not well defined, but reported as 66.7% (Group A), 10% (Group B), 14.3% (Group C), 28.6% (group D), and 75% (Group E). The research did not conduct statistical analysis to test for significant difference between groups.

Rojita et al⁶⁴ recently conducted a prospective randomized controlled clinical trial studying post-operative management of AFRS. The study included a total of sixty patients: thirty patients received systemic oral steroids for one month, followed by 6 months of steroids sprays (Group A);

thirty patients received 6 months of oral itraconazole (Group B) post-operatively. The authors noted symptomatic improvement and endoscopic clearance of disease in the itraconazole group (Group B); however, there was no difference between the two groups. In addition, there was no difference between groups when measuring absolute eosinophil count, serum IgE level, and mean SNOT scores. The authors conclude that oral itraconazole may be an effective alternative to systemic steroids post-operatively in patients with AFRS.

Seiberling conducted a retrospective chart review of 23 patients with AFRS and nonallergic eosinophilic fungal sinusitis treated with oral itraconazole for 6 months (100 mg BID) when recurrence developed after surgery.⁶⁵ Time to recurrence, oral steroid use, and outcomes were evaluated. Nineteen patients responded to the medication with a decrease in symptoms and fungal mucin/polyps on endoscopy. Sixteen patients noted a decrease in oral steroid use and eleven of those were disease-free at almost sixteen months follow up. There were no permanent complications; however, three patients had to stop itraconazole because of elevated liver enzymes. Chan et al⁶⁶ conducted a pilot study in thirty-two patients with refractory AFRS who failed systemic steroid and amphotericin B sprays. He evaluated the efficacy of oral itraconazole therapy for 3 months with regard to endoscopy, serum IgE and RSOM-31 scores. The study was inconclusive, with patients demonstrating higher mean IgE levels after treatment, 18 patients reporting (56%) moderate-significant improvement, and 12 patients with endoscopic improvement. Jen et al⁶⁷ performed a pilot study in patients with AFRS using itraconazole spray in addition to systemic steroids and itraconazole. They found that 75% of patients had disease stability; however, with no control group in the study, its findings were observational. Rains et al⁶⁸ conducted a retrospective chart review in 139 patients with AFRS and cited findings using their protocol including high dose itraconazole, low-dose oral steroids, and topical corticosteroids. The authors reported a 50.3% recurrence rate, with 20.5% of those patients requiring reoperation. They concluded that their regimen, with its use of itraconazole, was safe and effective.

Given the available evidence, which is mixed, it is challenging to make recommendations for or against the use of topical or systemic antifungals in AFRS.⁵⁶ Future, well-designed randomized clinical trials with robust outcomes measures in well-defined patient populations are needed to determine the efficacy of systemic or topical antifungals in AFRS.

Immunotherapy

AFRS is defined by a Type 1 hypersensitivity to fungus, so it stands to reason that immunotherapy (IT) could feasibly blunt the immune response to fungus and decrease disease burden. Gan et al⁵⁵ recently conducted a systematic review of the available literature regarding IT in AFRS. They found that the aggregate quality of evidence as grade C (2 level 3b studies and 3 level 4 studies). Again, it is difficult to recommend for or against IT in AFRS given the available evidence; however, it remains an option in recalcitrant AFRS, as some studies have reported decreased polyp burden, crusting, and need for corticosteroid use.^{20,69–71}

Biological therapy

Type 2 inflammation has long been a shared hallmark of disease in AFRS, CRSwNP and allergic asthma, a finding which has been bolstered by the theory of the unified airway. The introduction of biologic therapies targeting type 2 inflammatory mediators like IgE, IL-4, IL-5, and IL-13 in eosinophilic asthma has ushered in similar studies evaluating the safety and efficacy of these drugs in CRS.⁷² Bachert et al⁷³ performed a randomized, double-blind placebo-controlled clinical trial in one-hundred five patients with CRSwNP. In their study, 54 patients received the anti-IL-5 antibody mepolizumab, and 51 patients received placebo. Treatment with mepolizumab resulted in significant reduction in number of patients needing surgery at week 25, supported by significant improvement in nasal polyposis severity VAS score, endoscopic nasal polyp score, VAS symptom scores and SNOT scores. Rivero recently conducted a systematic review and meta-analysis of biologics in nasal polyposis.⁷⁴ The authors identified 8 studies that met their inclusion criteria, 5 were RCTs. Their meta-analysis showed that anti-IL-5 therapy reduced nasal polyp scores and concluded that biologics may be effective in certain CRS patient populations. Few have highlighted patient populations meeting diagnostic criteria for AFRS. Gan et al⁷⁵ conducted a retrospective chart review and identified seven patients with AFRS and moderate severe asthma who received subcutaneous injections with the anti-IgE antibody, omalizumab. The authors cite a 31% improvement in SNOT-22 scores and 61% improvement in endoscopy scores one year after omalizumab therapy. More prospective clinical trials are needed, and in these studies more clearly defined clinical subtypes, or endotypes, should be clarified in order to identify the patient populations that will benefit most from these novel therapies. Still, results from these initial studies hold promise for patients with AFRS and CRS, in general.

Conclusion

Clinically, AFRS can be differentiated from other polyp subtypes of CRS; however, research focusing on canonical markers of type 2 inflammation and fungal sensitivity seem to blur the lines separating this clinical entity from other subtypes of CRS. Nonetheless, emerging evidence involving molecular and immune profiling AFRS implicates the adaptive immune arm, innate barrier dysfunction, and possibly fungal proteases as key differentiating factors that make AFRS unique. Future research in AFRS should highlight the interplay between the innate and adaptive immune arm, and the role of fungus in promoting type 2 inflammation in AFRS. While it seems rational that immunotherapy and antifungal therapy should decrease disease burden, the quality of evidence supporting these therapies is low. As always, prospective randomized controlled trials with robust outcomes will better define the utility of therapies like IT, antifungals, and biologics in AFRS.

References

1. Ferguson BJ, Barnes L, Bernstein JM, et al. Geographic variation in allergic fungal rhinosinusitis. *Otolaryngol Clin North Am.* 2000;33:441–449.

2. Laury AM, Wise SK. Chapter 7: allergic fungal rhinosinusitis. *Am J Rhinol Allergy*. 2013;27:26–27.
3. Wise SK, Ghegan MD, Gorham E, Schlosser RJ. Socioeconomic factors in the diagnosis of allergic fungal rhinosinusitis. *Otolaryngol Head Neck Surg*. 2008;138:38–42.
4. Wise SK, Venkatraman G, Wise JC, DelGaudio JM. Ethnic and gender differences in bone erosion in allergic fungal sinusitis. *Am J Rhinol*. 2004;18:397–404.
5. Ghegan MD, Lee FS, Schlosser RJ. Incidence of skull base and orbital erosion in allergic fungal rhinosinusitis (AFRS) and non-AFRS. *Otolaryngol Head Neck Surg*. 2006;134:592–595.
6. Ghegan MD, Wise SK, Gorham E, Schlosser RJ. Socioeconomic factors in allergic fungal rhinosinusitis with bone erosion. *Am J Rhinol*. 2007;21:560–563.
7. Manning SC, Schaefer SD, Close LG, Vuitch F. Culture-positive allergic fungal sinusitis. *Arch Otolaryngol Head Neck Surg*. 1991;117:174–178.
8. Manning SC, Mabry RL, Schaefer SD, Close LG. Evidence of IgE-mediated hypersensitivity in allergic fungal sinusitis. *Laryngoscope*. 1993;103:717–721.
9. Thahim K, Jawaid MA, Marfani MS. Presentation and management of allergic fungal sinusitis. *J Coll Physicians Surg Pak*. 2007;17:23–27.
10. Tyler MA, Russell CB, Smith DE, et al. Large-scale gene expression profiling reveals distinct type 2 inflammatory patterns in chronic rhinosinusitis subtypes. *J Allergy Clin Immunol*. 2017;139:1061–1064.e4.
11. Miller JD, Deal AM, McKinney KA, et al. Markers of disease severity and socioeconomic factors in allergic fungal rhinosinusitis. *Int Forum Allergy Rhinol*. 2014;4:272–279.
12. Lu-Myers Y, Deal AM, Miller JD, et al. Comparison of socioeconomic and demographic factors in patients with chronic rhinosinusitis and allergic fungal rhinosinusitis. *Otolaryngol Head Neck Surg*. 2015;153:137–143.
13. Clark DW, Wenaas A, Luong A, Citardi MJ, Fakhri S. Staphylococcus aureus prevalence in allergic fungal rhinosinusitis vs other subsets of chronic rhinosinusitis with nasal polyps. *Int Forum Allergy Rhinol*. 2013;3:89–93.
14. Promsopa C, Kansara S, Citardi MJ, Fakhri S, Porter P, Luong A. Prevalence of confirmed asthma varies in chronic rhinosinusitis subtypes. *Int Forum Allergy Rhinol*. 2016;6:373–377.
15. Tyler MA, Padro DCJ, Russell CB, et al. Distinguishing molecular features of allergic fungal rhinosinusitis. *Otolaryngol Head Neck Surg*. 2018;159:185–193.
16. Jackson IT, Schmitt 3rd E, Carpenter HA. Allergic Aspergillus sinusitis. *Plast Reconstr Surg*. 1987;79:804–808.
17. Waxman JE, Spector JG, Sale SR, Katzenstein AL. Allergic Aspergillus sinusitis: concepts in diagnosis and treatment of a new clinical entity. *Laryngoscope*. 1987;97:261–266.
18. Mabry RL, Manning S. Radioallergosorbent microscreen and total immunoglobulin E in allergic fungal sinusitis. *Otolaryngol Head Neck Surg*. 1995;113:721–723.
19. Manning SC, Holman M. Further evidence for allergic pathophysiology in allergic fungal sinusitis. *Laryngoscope*. 1998;108:1485–1496.
20. Mabry RL, Marple BF, Folker RJ, Mabry CS. Immunotherapy for allergic fungal sinusitis: three years' experience. *Otolaryngol Head Neck Surg*. 1998;119:648–651.
21. JU Ponikau, Sherris DA, Kern EB, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc*. 1999;74:877–884.
22. Collins M, Nair S, Smith W, Kette F, Gillis D, Wormald PJ. Role of local immunoglobulin E production in the pathophysiology of noninvasive fungal sinusitis. *Laryngoscope*. 2004;114:1242–1246.
23. Pant H, Beroukas D, Kette FE, Smith WB, Wormald PJ, Macardle PJ. Nasal polyp cell populations and fungal-specific peripheral blood lymphocyte proliferation in allergic fungal sinusitis. *Am J Rhinol Allergy*. 2009;23:453–460.
24. Pant H, Kette FE, Smith WB, Macardle PJ, Wormald PJ. Eosinophilic mucus chronic rhinosinusitis: clinical subgroups or a homogeneous pathogenic entity. *Laryngoscope*. 2006;116:1241–1247.
25. Pant H, Kette FE, Smith WB, Wormald PJ, Macardle PJ. Fungal-specific humoral response in eosinophilic mucus chronic rhinosinusitis. *Laryngoscope*. 2005;115:601–606.
26. Porter PC, Lim DJ, Maskatia ZK, et al. Airway surface mycosis in chronic TH2-associated airway disease. *J Allergy Clin Immunol*. 2014;134:325–331.
27. Carney AS, Tan LW, Adams D, Varelias A, Ooi EH, Wormald PJ. Th2 immunological inflammation in allergic fungal sinusitis, nonallergic eosinophilic fungal sinusitis, and chronic rhinosinusitis. *Am J Rhinol*. 2006;20:145–149.
28. Orlandi RR, Thibeault SL, Ferguson BJ. Microarray analysis of allergic fungal sinusitis and eosinophilic mucin rhinosinusitis. *Otolaryngol Head Neck Surg*. 2007;136:707–713.
29. Schubert MS, Hutcheson PS, Graff RJ, Santiago L, Slavin RG. HLA-DQB1 *03 in allergic fungal sinusitis and other chronic hypertrophic rhinosinusitis disorders. *J Allergy Clin Immunol*. 2004;114:1376–1383.
30. Ahn CN, Wise SK, Lathers DM, Mulligan RM, Harvey RJ, Schlosser RJ. Local production of antigen-specific IgE in different anatomic subsites of allergic fungal rhinosinusitis patients. *Otolaryngol Head Neck Surg*. 2009;141:97–103.
31. Wise SK, Ahn CN, Lathers DM, Mulligan RM, Schlosser RJ. Antigen-specific IgE in sinus mucosa of allergic fungal rhinosinusitis patients. *Am J Rhinol*. 2008;22:451–456.
32. Mulligan JK, Bleier BS, O'Connell B, Mulligan RM, Wagner C, Schlosser RJ. Vitamin D3 correlates inversely with systemic dendritic cell numbers and bone erosion in chronic rhinosinusitis with nasal polyps and allergic fungal rhinosinusitis. *Clin Exp Immunol*. 2011;164:312–320.
33. Ayers CM, Schlosser RJ, O'Connell BP, et al. Increased presence of dendritic cells and dendritic cell chemokines in the sinus mucosa of chronic rhinosinusitis with nasal polyps and allergic fungal rhinosinusitis. *Int Forum Allergy Rhinol*. 2011;1:296–302.
34. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G. Cutting edge: the ST2 ligand IL-33 potentially activates and drives maturation of human mast cells. *J Immunol*. 2007;179:2051–2054.
35. Komai-Koma M, Xu D, Li Y, McKenzie AN, McInnes IB, Liew FY. IL-33 is a chemoattractant for human Th2 cells. *Eur J Immunol*. 2007;37:2779–2786.
36. Kurowska-Stolarska M, Kewin P, Murphy G, et al. IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol*. 2008;181:4780–4790.
37. Pecaric-Petkovic T, Didichenko SA, Kaempfer S, Spiegl N, Dahinden CA. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. *Blood*. 2009;113:1526–1534.
38. Shaw JL, Fakhri S, Citardi MJ, et al. IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps. *Am J Respir Crit Care Med*. 2013;188:432–439.
39. Tieu DD, Kern RC, Schleimer RP. Alterations in epithelial barrier function and host defense responses in chronic rhinosinusitis. *J Allergy Clin Immunol*. 2009;124:37–42.
40. Den BKA, Hoddeson EK, Parkos CA, Nusrat A, Wise SK. Epithelial permeability alterations in an in vitro air-liquid interface model of allergic fungal rhinosinusitis. *Int Forum Allergy Rhinol*. 2013;3:19–25.
41. Wise SK, Laury AM, Katz EH, Den BKA, Parkos CA, Nusrat A. Interleukin-4 and interleukin-13 compromise the sinonasal

- epithelial barrier and perturb intercellular junction protein expression. *Int Forum Allergy Rhinol.* 2014;4:361–370.
42. Wei Y, Xia W, Ye X, et al. The antimicrobial protein short palate, lung, and nasal epithelium clone 1 (SPLUNC1) is differentially modulated in eosinophilic and noneosinophilic chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol.* 2014;133:420–428.
 43. Lane AP. The role of innate immunity in the pathogenesis of chronic rhinosinusitis. *Curr Allergy Asthma Rep.* 2009;9:205–212.
 44. Psaltis AJ, Bruhn MA, Ooi EH, Tan LW, Wormald PJ. Nasal mucosa expression of lactoferrin in patients with chronic rhinosinusitis. *Laryngoscope.* 2007;117:2030–2035.
 45. Ooi EH, Wormald PJ, Carney AS, James CL, Tan LW. Surfactant protein d expression in chronic rhinosinusitis patients and immune responses in vitro to *Aspergillus* and *alternaria* in a nasal explant model. *Laryngoscope.* 2007;117:51–57.
 46. Millien VO, Lu W, Shaw J, et al. Cleavage of fibrinogen by proteinases elicits allergic responses through Toll-like receptor 4. *Science.* 2013;341:792–796.
 47. Leavy O. Asthma and allergy: a fibrinogen root to airway inflammation. *Nat Rev Immunol.* 2013;13:704.
 48. Kim JH, Yi JS, Gong CH, Jang YJ. Development of *Aspergillus* protease with ovalbumin-induced allergic chronic rhinosinusitis model in the mouse. *Am J Rhinol Allergy.* 2014;28:465–470.
 49. Ebert CS, McKinney KA, Urrutia G, et al. Expression of protease-activated receptors in allergic fungal rhinosinusitis. *Int Forum Allergy Rhinol.* 2014;4:266–271.
 50. Hissaria P, Smith W, Wormald PJ, et al. Short course of systemic corticosteroids in sinonasal polyposis: a double-blind, randomized, placebo-controlled trial with evaluation of outcome measures. *J Allergy Clin Immunol.* 2006;118:128–133.
 51. Kirtsreesakul V, Wongsritrang K, Ruttanaphol S. Clinical efficacy of a short course of systemic steroids in nasal polyposis. *Rhinology.* 2011;49:525–532.
 52. Vaidyanathan S, Barnes M, Williamson P, Hopkinson P, Donnan PT, Lipworth B. Treatment of chronic rhinosinusitis with nasal polyposis with oral steroids followed by topical steroids: a randomized trial. *Ann Intern Med.* 2011;154:293–302.
 53. Van Zele T, Gevaert P, Holtappels G, et al. Oral steroids and doxycycline: two different approaches to treat nasal polyps. *J Allergy Clin Immunol.* 2010;125:1069–1076.e4.
 54. Kroflic B, Coer A, Baudoin T, Kalogjera L. Topical furosemide versus oral steroid in preoperative management of nasal polyposis. *Eur Arch Otorhinolaryngol.* 2006;263:767–771.
 55. Gan EC, Thamboo A, Rudmik L, Hwang PH, Ferguson BJ, Javer AR. Medical management of allergic fungal rhinosinusitis following endoscopic sinus surgery: an evidence-based review and recommendations. *Int Forum Allergy Rhinol.* 2014;4:702–715.
 56. Orlandi RR, Kingdom TT, Hwang PH, et al. International consensus statement on allergy and rhinology: rhinosinusitis. *Int Forum Allergy Rhinol.* 2016;6(Suppl 1):S22–S209.
 57. Gerlinger I, Fittler A, Fónai F, Patzkó A, Mayer A, Botz L. Postoperative application of amphotericin B nasal spray in chronic rhinosinusitis with nasal polyposis, with a review of the antifungal therapy. *Eur Arch Otorhinolaryngol.* 2009;266:847–855.
 58. Ebbens FA, Georgalas C, Fokkens WJ. Fungus as the cause of chronic rhinosinusitis: the case remains unproven. *Curr Opin Otolaryngol Head Neck Surg.* 2009;17:43–49.
 59. Ebbens FA, Georgalas C, Luiten S, et al. The effect of topical amphotericin B on inflammatory markers in patients with chronic rhinosinusitis: a multicenter randomized controlled study. *Laryngoscope.* 2009;119:401–408.
 60. Weschta M, Rimek D, Formanek M, Podbielski A, Riechelmann H. Effect of nasal antifungal therapy on nasal cell activation markers in chronic rhinosinusitis. *Arch Otolaryngol Head Neck Surg.* 2006;132:743–747.
 61. Weschta M, Rimek D, Formanek M, Polzehl D, Podbielski A, Riechelmann H. Topical antifungal treatment of chronic rhinosinusitis with nasal polyps: a randomized, double-blind clinical trial. *J Allergy Clin Immunol.* 2004;113:1122–1128.
 62. Sacks PL, Harvey RJ, Rimmer J, Gallagher RM, Sacks R. Topical and systemic antifungal therapy for the symptomatic treatment of chronic rhinosinusitis. *Cochrane Database Syst Rev.* 2011:CD008263.
 63. Khalil Y, Tharwat A, Abdou AG, et al. The role of antifungal therapy in the prevention of recurrent allergic fungal rhinosinusitis after functional endoscopic sinus surgery: a randomized, controlled study. *Ear Nose Throat J.* 2011;90:E1–E7.
 64. Rojita M, Samal S, Pradhan P, Venkatachalam VP. Comparison of steroid and itraconazole for prevention of recurrence in allergic fungal rhinosinusitis: a randomized controlled trial. *J Clin Diagn Res.* 2017;11:MC01-01MC03.
 65. Seiberling K, Wormald PJ. The role of itraconazole in recalcitrant fungal sinusitis. *Am J Rhinol Allergy.* 2009;23:303–306.
 66. Chan KO, Genoway KA, Javer AR. Effectiveness of itraconazole in the management of refractory allergic fungal rhinosinusitis. *J Otolaryngol Head Neck Surg.* 2008;37:870–874.
 67. Jen A, Kacker A, Huang C, Anand V. Fluconazole nasal spray in the treatment of allergic fungal sinusitis: a pilot study. *Ear Nose Throat J.* 2004;83:692, 694–695.
 68. Rains BM, Mineck CW. Treatment of allergic fungal sinusitis with high-dose itraconazole. *Am J Rhinol.* 2003;17:1–8.
 69. Mabry RL, Marple BF, Mabry CS. Outcomes after discontinuing immunotherapy for allergic fungal sinusitis. *Otolaryngol Head Neck Surg.* 2000;122:104–106.
 70. Mabry RL, Mabry CS. Immunotherapy for allergic fungal sinusitis: the second year. *Otolaryngol Head Neck Surg.* 1997;117:367–371.
 71. Mabry RL, Mabry CS. Allergic fungal sinusitis: the role of immunotherapy. *Otolaryngol Clin North Am.* 2000;33:433–440.
 72. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med.* 2009;360:973–984.
 73. Bachert C, Sousa AR, Lund VJ, et al. Reduced need for surgery in severe nasal polyposis with mepolizumab: randomized trial. *J Allergy Clin Immunol.* 2017;140:1024–1031.e14.
 74. Rivero A, Liang J. Anti-IgE and Anti-IL5 Biologic Therapy in the treatment of nasal polyposis: a systematic review and meta-analysis. *Ann Otol Rhinol Laryngol.* 2017;126:739–747.
 75. Gan EC, Habib AR, Rajwani A, Javer AR. Omalizumab therapy for refractory allergic fungal rhinosinusitis patients with moderate or severe asthma. *Am J Otolaryngol.* 2015;36:672–677.