

REVIEW

Fungal rhinosinusitis: what every allergist should know

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Summary

The interaction between fungi and the sinonasal tract results in a diverse range of diseases with an equally broad spectrum of clinical severity. The classification of these interactions has become complex, and this review seeks to rationalize and simplify the approach to fungal diseases of the nose and paranasal sinuses. These conditions may be discussed under two major headings: non-invasive disease (localized fungal colonization, fungal ball and allergic fungal rhinosinusitis) and invasive disease (acute invasive rhinosinusitis, chronic invasive rhinosinusitis and granulomatous invasive rhinosinusitis). A diagnosis of fungal rhinosinusitis is established by combining findings on history, clinical examination, laboratory testing, imaging and histopathology. The immunocompetence of the patient is of great importance, as invasive fungal rhinosinusitis is uncommon in immunocompetent patients. With the exception of localized fungal colonization, treatment of all forms of fungal rhinosinusitis relies heavily on surgery. Systemic antifungal agents are a fundamental component in the treatment of invasive forms, but are not indicated for the treatment of the non-invasive forms. Antifungal drugs may have a role as adjuvant therapy in allergic fungal rhinosinusitis, but evidence is poor to support recommendations. Randomized controlled trials need to be performed to confirm the benefit of immunotherapy in the treatment of allergic fungal rhinosinusitis. In this article, we will summarize the current literature, addressing the controversies regarding the diagnosis and management of fungal rhinosinusitis, and focussing on those aspects which are important for clinical immunologists and allergists.

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Introduction

Fungi are ubiquitous and we inhale spores with every breath. Fortunately, however, only a minority develop a pathological inflammatory response to fungi in the airway, and invasive forms of fungal sinusitis are rare conditions. Fungal species are variably pathogenic in human hosts, resulting in a diverse range of diseases in the upper and lower airways.

Fungal rhinosinusitis is an umbrella term which includes both inflammatory and infectious conditions of the nose and paranasal sinuses caused by fungi. The spectrum of severity extends from innocuous colonization to potentially lethal invasive disease. It is the immune state of the patient which largely determines the nature of disease that fungus will produce in the sinonasal tract.

Fungal rhinosinusitis can be divided into non-invasive forms and invasive forms. The non-invasive conditions, which are generally manifested in immunocompe-

tent patients, include localized fungal colonization, fungal ball and allergic fungal rhinosinusitis (AFRS). Invasive forms, usually seen in immunocompromised patients, include acute and chronic invasive fungal rhinosinusitis. In addition, there is also a type of granulomatous invasive fungal rhinosinusitis that affects immunocompetent patients almost exclusively in the Middle East, Africa and India. Regional variations in the prevalence of the different forms of fungal rhinosinusitis have been reported [1–3]. In general, non-invasive forms are much more frequent than invasive ones and acute invasive fungal rhinosinusitis is the most frequent of the invasive forms. Table 1 summarizes the key features of the different forms of fungal rhinosinusitis.

Biology of fungi

Kingdom fungi constitute an independent group equal in rank to that of plants and animals. It encompasses an enormous diversity of organisms that have varied ecolo-

Table 1. Summary of key concepts in fungal rhinosinusitis

	Fungal ball	AFRS	AIFRS	CIFRS	GIFRS
Most common aetiological agent	<i>Aspergillus</i> spp	<i>Aspergillus</i> spp and dematiaceous moulds (<i>Alternaria</i> spp, <i>Bipolaris</i> spp and <i>Curvularia</i> spp)	<i>Aspergillus</i> spp and Mucorales (<i>Rhizopus</i> spp and <i>Mucor</i> spp)	<i>Aspergillus</i> spp and Mucorales	<i>Aspergillus flavus</i>
Immune state	Immunocompetent	Immunocompetent Atopic patient	Immunosuppression (Neutropaenia or neutrophil dysfunction) e.g. haematological malignancies, organ transplant or diabetic ketoacidosis	Immunosuppression (Subtle immune system abnormalities) e.g. diabetes mellitus or chronic low-dose corticosteroid	Immunocompetent
Demographics	Most commonly affects middle-aged and elderly females	Warm humid regions e.g. south of North America and India	Inpatient (Usually in critic care units)	Outpatient	Sudan, India, Pakistan and Saudi Arabia
Treatment	Surgery	Surgery + Ongoing medical therapy (Steroids, nasal douches) + Immunotherapy?	Surgical debridement + Systemic antifungals + Immune reconstitution	Surgical debridement + Systemic antifungals + Immune reconstitution	Surgical debridement + Systemic antifungals
Prognosis	Excellent cure rate	Chronic, but fairly good control of disease is achievable by means of surgery(ies) and ongoing medical therapy ± immunotherapy	High mortality rate, mostly related to absence of host immune reconstitution and the extent of involvement on recognition of the disease	Overall, better prognosis than AIFRS. Recurrence can occur.	Postoperative itraconazole seems to reduce the high relapse rate.

AFRS, allergic fungal rhinosinusitis; AIFRS, acute invasive fungal rhinosinusitis; CIFRS, chronic invasive fungal rhinosinusitis; GIFRS, granulomatous invasive fungal rhinosinusitis.

gies, life cycle strategies and morphologies ranging from single-celled yeasts to large mushrooms. The organisms of the fungal lineage include mushrooms, rusts, smuts, puffballs, truffles, morels, moulds and yeasts, as well as many less well-known organisms [4]. Research on DNA sequencing techniques has provided a multitude of new characters for analysis, thus a great number of new species have been recognized and fungal taxonomy continues to evolve. Estimations of the number of existing species are in excess of 1.5 million [5, 6].

Moulds and yeasts contain species that can produce human disease in immunocompromised and immunocompetent individuals [7]. The former are mainly responsible for causing disease in the sinonasal tract, although occasionally yeasts, mostly *Candida* species, are involved [1, 8]. Moulds are fungi that grow in the form of multicellular filaments called hyphae and their growth results in a mouldy appearance of objects [9]. Moulds and their airborne sexual spores or asexual conidia are ubiquitous in the environment and humans inhale them constantly [10–12]. In fact, the prevalence of fungi in the sinonasal tract of healthy individuals has been reported to be as high as 100% depending on the collection and the detection methods used (a com-

prehensive review on the subject was published by Ebens et al. [13] in 2007). However, clinical disease due to these fungi is uncommon [14–17]. Genetic predisposition is an important determinant of the immunological reaction to fungal exposure.

Aspergillus spp, Dematiaceous moulds, hyaline moulds causative of hyalohyphomycosis and members of the family *Mucoraceae* are the main moulds responsible of causing disease in the sinonasal tract. The genus *Aspergillus* shares a common asexual spore (conidia) forming structure, the aspergillum [18]. It comprises approximately 180 species, of which 34 have been associated with human disease. *A. fumigatus* and *A. flavus* are the most frequently involved in sinonasal tract disease, although *A. niger* and *A. terreus* are also variably implicated [1, 2, 14]. Dematiaceous moulds are naturally pigmented fungi whose hyphae and conidia are heavily melanized [19]. They belong to the phylum Ascomycota and are distributed along different classes, orders, families and genera. Around 60 genera and 100 species have shown to cause human disease [17, 19]. Melanin is considered an important virulence factor, since disruption of pigment production leads to reduced virulence in animal models [20]. Species of the genera

Alternaria, *Bipolaris* and *Curvularia* are among the most common dematiaceous moulds isolated in sinonasal tract disease [1, 19, 21]. Hyalohyphomycosis is an umbrella term proposed to cover a growing number of new mycoses caused by septate hyaline moulds other than the ones that cause the well established aspergillosis, penicilliosis and zygomycosis [17]. The Zygomycetes would also have been excluded on the ground that their hyphae are aseptate. These hyaline septate moulds are classified in the phyla Deuteromycota, Ascomycota or Basidiomycota. *Paecilomyces* spp, *Fusarium* spp and *Scedosporium* spp are the genera more frequently involved in sinonasal tract disease [1]. Members of the family Mucoraceae (which belong to the class Zygomycetes) are characterized by aseptate or pauciseptate ribbon-like hyphae [22]. Along with *Aspergillus* spp are the main species responsible of invasive fungal disease in the sinonasal tract [14, 23]. Although aerosolized sporangiospores (asexual spores) are the typical infective forms, angioinvasive hyphal forms are responsible for tissue invasion and dissemination [24]. The genera *Rhizopus* and *Mucor* are the most frequently involved [25].

Besides the difficulties in identifying the aetiological fungi, differentiating colonization from an active role in the disease process is a persistent problem. A combination of histopathology techniques (of tissue or sinus secretions), cultures and serological tests will help to clarify this issue. A comprehensive review on histopathological diagnosis of fungal infections was published recently by Guarner et al. [7].

Non-invasive disease

Localized fungal colonization

This condition refers to the asymptomatic macroscopically visible colonization of the sinonasal mucosa, in which fungal hyphae grow saprophytically within the mucus and mucous crusts of the airway [26]. Often seen in patients with the history of previous sinus surgery (Fig. 1), the colonizing fungi are thought to grow in areas of mucosa with impaired mucociliary clearance. Localized fungal colonization is diagnosed by endoscopy, usually in an asymptomatic patient; however, it can present with malodour. Treatment consists in simple mechanical removal on endoscopy and sinus rinses to avoid recurrence.

Fungal ball

A fungal ball is defined as the dense accumulation of fungal hyphae within the mucosal confines of a paranasal sinus, without tissue invasion [27]. The maxillary sinus is the most commonly affected and rarely more than one sinus is involved [1, 28]. This condition has



Fig. 1. Localized fungal colonization of a crust (arrow) in the posterior margin of a widely open right maxillary sinus in a patient with cystic fibrosis. C, choana, MT, remnant of middle turbinate, S, septum, A, maxillary antrum.

been given other names such as mycetoma, aspergilloma or chronic non-invasive granuloma; however, none of these is completely accurate and consensus recommendations have suggested avoiding their use [26]. For example, mycetoma actually refers to a chronic local invasion of subcutaneous tissue by bacteria or fungi with the formation of sinus tracts, swelling and granulomas. In the same way, not every fungal ball is due to *Aspergillus* species, thus aspergilloma is not always accurate. Rarely, progression to invasive fungal rhinosinusitis has been described in the context of substantial immunosuppression [29].

Fungal balls are caused mainly by *Aspergillus* species [1, 3, 28]. In contrast to other forms of aspergillosis, which are more common in men, fungal balls affect more commonly middle-aged and elderly females [28]. No clear specific risk factors for the development of fungal balls have been described. It has been proposed that overfilling of zinc oxide incorporated in dental filling paste plays a role in causing the affliction [30]. However, this is not a constant finding [28] and this theory does not explain the existence of fungal balls in other sinuses.

Presentation and diagnosis. The most common symptoms are purulent nasal discharge, facial pain or fullness, chronic nasal obstruction, fetid smell perception and postnasal discharge. However, it is not uncommon for these lesions to be recognized as an incidental radiological finding in an asymptomatic patient [28, 31]. Nasal endoscopy is generally not helpful in securing the diagnosis, with findings varying from normal aspect of the nasal passages to oedema of the mucosa or purulent discharge [28].

As the symptoms and clinical signs are non-specific, the diagnosis is usually suspected radiologically by CT

scan. CT images usually show heterogeneous opacities within the involved sinus cavity (Fig. 2a), most probably due to the accumulation of haemosiderin and metals such as iron and manganese (which are known to be essentials in fungal amino acid metabolism) within fungal hyphae [32]. Frequently, discrete and very dense areas (even metallic densities) are seen. These areas of increased density have been attributed to local calcification in the centre of the hyphae masses [32, 33]; however, these densities may also be found in non-fungal maxillary sinusitis [32, 34]. It appears that thick pus or thrombus can on occasion exhibit similar findings. In advanced cases, bone erosion and remodelling can be seen [28]. Although usually not essential, findings on magnetic resonance imaging are more specific than those of CT. Most typically, T1 scans show central hypointensity and T2 scans a signal void (most likely because of the presence of calcium, air and ferromagnetic elements) [32]. Both T1 and T2 series demonstrate peripheral enhancement.

The diagnosis is largely confirmed during surgery, when mucopurulent cheesy or clay-like materials within the sinus is found (Fig. 2b). Of note, cultures are negative in approximately 70% of cases [28, 35]. On histopathology, fungal hyphae are identified in more than 90% of cases [28, 31] with the aid of specific stains as Grocott's methenamine silver. The surrounding mucosa generally has an inflammatory infiltrate of mononuclear cells, including lymphocytes, plasma cells and/or mast cells [28]. There is no tissue invasion, and in contrast with other forms of fungal rhinosinusitis there is no predominance of eosinophils, granulomas or allergic mucin [27].

Treatment. Treatment consists of endoscopic removal of all the fungal content of the involved sinus. The tech-

nique to be used will depend on the involved sinus (e.g. wide middle meatal antrostomy, sphenoidotomy or ethmoidectomy). The success rate is high with a recurrence rate reported to be as low as 1% after surgical removal [1, 28]. Consequently, there is no need of topical or systemic antifungal therapy. Treatment of asymptomatic patients is generally recommended and widely accepted, although there is lack of research addressing this issue. This recommendation is tempered by the patient's age and fitness for anaesthesia.

Allergic fungal rhinosinusitis

Chronic rhinosinusitis (CRS) is defined as an inflammatory disease of the nose and paranasal sinuses that lasts three or more months without complete resolution of symptoms. The symptoms are nasal blockage, rhinorrhoea, facial pain or pressure and hyposmia. Symptoms must be associated with either endoscopic and/or CT evidence of disease [36]. CRS can be differentiated clinically according to the presence or absence of polyps, which consist of grossly oedematous prolapsing nasal and sinus mucosa.

Many cases of CRS are considered idiopathic, and almost certainly result from a complex interplay between the host mucosal immune system and the nasal and sinus microbiome. Fungi may be an important constituent of the nasal microbiome, and provide a range of antigens towards which hypersensitivity can develop.

Similarity to allergic bronchopulmonary aspergillosis. The concept of AFRS (a subtype of CRS) parallels allergic bronchopulmonary aspergillosis (ABPA), in which hypersensitivity reactions to *Aspergillus* species colonizing the lower respiratory tract result in significant pathology, which responds much more reliably to

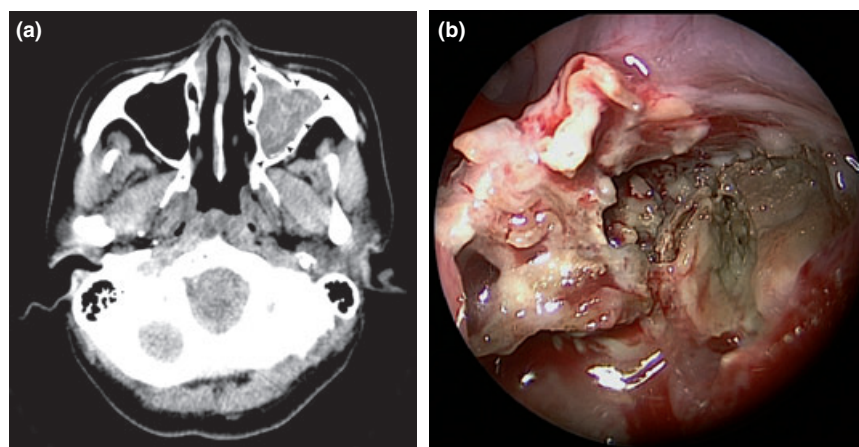


Fig. 2. (a) An axial CT scan demonstrating a fungal ball in the left maxillary sinus (black arrowheads). Note the heterogeneous radiodensity which is characteristic of this condition. (b) An intra-operative photograph of the same patient. Thick fungal debris is being removed through a middle meatal antrostomy.

corticosteroids than to antifungal agents. The sinonasal equivalent of this entity, AFRS, was described first in 1983 by Katzenstein et al. [37]. The initial understanding of AFRS was shaped by the previous understanding of ABPA, as it was felt to represent the sinonasal equivalent of it. Even more, the existence of concomitant AFRS and allergic bronchopulmonary mycosis in the same patient has been named sinobronchial allergic mycosis suggesting that the same process of fungal hypersensitivity occurs in the upper and lower airways [38]. Interestingly, however, the co-existence of both entities in the same patient is more an exception than a rule, and some differences exist between the two entities. The classic Bent and Kuhn diagnostic criteria are still widely accepted [39]. These are as follows: (1) nasal polyposis, (2) presence of fungi on direct microscopy or culture of sinus content, (3) eosinophilic mucin without fungal invasion into sinus tissue, (4) type I hypersensitivity to fungi demonstrated by skin testing or *in vitro* testing and (5) characteristic computed tomographic findings, including sinus expansion or heterogeneous opacification.

Presentation and diagnosis. Whether AFRS as defined by the Bent and Kuhn criteria represents a distinct condition remains controversial. Identical clinical forms of CRS, but without type I hypersensitivity to fungi have been described, prompting some authors on this subject to propose to remove type I hypersensitivity as a diagnostic criteria for AFRS [40]. Others have suggested a broader term to encompass both of these clinical entities – eosinophilic fungal rhinosinusitis (EFRS) [41]. In addition, the same disease phenotype, but without identifiable fungi or type I hypersensitivity to fungi has been described and named eosinophilic mucin rhinosinusitis (EMRS) [42]. The three entities have the common denominator of presenting with eosinophilic mucin, a term that describes the presence of eosinophils or eosinophil degraded products with or without Charcot-Leyden crystals in mucus. The mucin in this condition typically has a distinct macroscopic appearance, being thick, tenacious and coloured from tan to brown or black. Recently, the International Society for Human and Animal Mycology convened a working group to attempt build a consensus on terminology and disease classification of fungal rhinosinusitis [26]. They concluded that the three descriptions (AFRS, EFRS and EMRS) are imprecise and require better definition. Furthermore, Pant et al. [43] founded no difference in the levels of total IgE, ratio of mould-mix specific IgE to total IgE and absolute levels of IgE to specific fungi between AFRS patients and patients with allergic rhinitis and fungal allergy (without CRS). Challenging the central pathogenic importance of elevated fungal-specific IgE levels in AFRS, suggesting that immune mech-

anisms other than fungal allergy are involved and that elevated fungal-specific IgE may simply represent concurrent rhinosinusitis and fungal allergy. After in-depth examination of humoral immune response to fungal antigens, they founded that elevated fungal-specific IgG3 was the only marker that differentiated the group of patients with all forms of CRS that present with eosinophilic mucin from patients with other forms of CRS and from those with fungal allergy in the absence of CRS. In addition, Collins et al. [44] detected fungal-specific IgE in sinus mucin of some EFRS and EMRS patients, suggesting that a local type I hypersensitivity response against fungal antigens may be present even in those patients without evidence of systemic allergy and irrespective of the identification of fungi in eosinophilic mucin. However, in the absence of systemically detectable allergen-specific IgE or a positive prick test, the pathogenic importance of locally produced IgE in allergen-specific hypersensitivity responses is yet to be established. Despite this, a preponderance of evidence suggests that an IgE-mediated process is involved in the pathogenesis of AFRS. Whether this is the primary factor in the pathogenesis is still not certain. Notwithstanding this controversy, there is no doubt that this group of patients with tenacious, eosinophil laden mucin represents a subgroup of CRS patients who are more difficult to manage successfully.

Environmental factors and host genetics have shown to play a role in AFRS. The incidence of AFRS appears to have a geographic predisposition, with an increased incidence of allergic fungal rhinosinusitis seen in warm and humid areas like the southern US [45] and India [2]. Also, low socio-economic status has been associated with this condition [46–48]. Atopy to fungi is not enough to cause the disease and there must be host factors involved. A breached innate immune defence is required by fungal spores for their germination and establishment of fungal-mediated allergies as dormant conidia are immunologically inert [49]. Structural abnormalities in the airway epithelium may be responsible of the defective clearance of spores, allowing the germination of them into hyphae, as is the case with cystic fibrosis or asthma as predisposing factors for ABPA. An association between the class II MHC and AFRS has been recognized. HLA-DQB1*0301 and HLA-DQB1*0302 have shown to be significant risk factors for the development of AFRS, findings implicating innate immunity in the pathogenesis of this disease [50]. However, the precise role genetics plays in this condition is unknown and genetic analysis does not at present impact diagnosis or treatment.

AFRS typically affects young, atopic and immunocompetent patients. It presents as CRS with polyps, which are usually recalcitrant to conventional treatment. Patients may discharge green to black rubbery

nasal casts of eosinophilic mucin. Severe or neglected cases may present with facial deformity [51] hypertelorism and proptosis (Fig. 3). Visual loss occasionally complicates such cases [52, 53].

AFRS has some distinctive radiological findings both on CT and MRI scanning. Unilateral or asymmetric involvement of the sinuses is more frequent than in idiopathic CRS. Bone expansion and consequent thinning of the bony walls of the sinuses is a characteristic feature in CT and frequently affects the orbit and the skull base (Fig. 4a) [54, 55]. In contrast to idiopathic CRS, and similar to what is observed in fungal balls, the radiodensity of the soft tissues within the sinuses in AFRS is often heterogeneous. Metallic densities, however, are not a distinctive feature; this could be due to the sparse concentration of fungal hyphae within the eosinophilic mucin, which could prevent the deposits of calcium salts. There is usually an outer rind of less radiologically dense polypoid mucosa, surrounding the denser eosinophilic fungal debris. As in fungal balls, magnetic resonance imaging findings are more specific than those of CT. Commonly, T1 scans show central hypointensity and T2 scans a signal void. Both T1 and T2 series demonstrate peripheral enhancement (Fig. 4b).

A diagnosis of AFRS suspected on clinical and radiological grounds can be confirmed by allergy workup, culture and histopathology of surgically obtained mucus specimens. *Aspergillus* species and the dematiaceous moulds *Alternaria*, *Bipolaris* and *Curvularia* species are the most commonly isolated fungal species. Also, hyaline moulds causative of hyalohyphomycosis, mainly *Paecilomyces* spp, *Fusarium* spp and *Scedosporium* spp are isolated less frequently. Geographical variation in the preponderance of *Aspergillus* spp or dematiaceous moulds have been observed, with demati-



Fig. 3. Proptosis of the left eye in a patient with fungal rhinosinusitis.

aceous moulds been more prevalent in the south warm humid regions of North America and *Aspergillus* spp in India and the Middle East [1, 2, 21, 56].

Immunological studies are an important part of the diagnostic workup and can be helpful in the clinical follow-up of these patients. Patients with AFRS presents type I hypersensitivity to both fungal and non-fungal allergens [57, 58]. Skin tests are more sensitive than *in vitro* specific IgE detection to identify sensitization to moulds and are considered the gold standard [59–61]. However, *in vitro* tests as the modified RAST (mRAST) [62] or the ImmunoCAP (Pharmacia Diagnostics, Piscataway, NJ) have shown good correlation with skin testing [59, 60, 63]. Chamber et al. [59] compared both *in vitro* tests in the detection of allergy to *Alternaria* using skin testing as the gold standard and observed a specificity of 100% with both ImmunoCAP and mRAST; however, the sensitivity was 89% for ImmunoCAP and only 22% for mRAST. Type I hypersensitivity to multiple moulds have been reported consistently in patients with AFRS [58, 64, 65] and the existence of a fungal panallergen have been proposed [66]. AFRS presents in atopic patients and thus elevated total serum IgE is a characteristic feature. Total IgE elevation in AFRS does not reach such high levels as in ABPA. In AFRS, total IgE levels are usually raised between 500 and 1000 IU/ml, frequently above 1000 IU/ml, but infrequently above 4000 IU/mL [57, 64, 67]; in contrast, total IgE levels in ABPA are typically above 1000 IU/ml, frequently above 4000 IU/ml and often above 10 000 IU/ml in the acute phase [68, 69]. Total serum IgE levels have shown to correlate with the clinical rhinosinusitis severity and recurrence rates [47, 70], and monitoring of total serum IgE can be helpful in the clinical follow-up of these patients [65, 70].

In vitro detection of specific IgE (sIgE) against the involved fungi is positive in more than 80% of patients [64, 65, 71] usually to multiple moulds. Stewart et al. [64] proposed that detection of sIgE to multiple fungi combined with an elevated total IgE represents a good screening method to identify patients with AFRS within patients with CRS with polyps. Fungal-specific IgG (sIgG) is generally elevated [57, 64, 71], although the significance of this is not well understood. Even though uncommon, normal levels of total IgE, sIgE and sIgG do not exclude the diagnosis of AFRS. Fungal-specific precipitins and peripheral eosinophilia are present inconsistently [57, 71, 72] and are not a reliable tests to diagnose AFRS, although their presence demonstrates immune recognition of the involved moulds. The fact that most of these patients receive frequent courses of oral corticosteroids for the treatment of their disease may explain the inconsistency in the detection of peripheral eosinophilia. The presence or absence of delayed skin test reactions is seldom reported and usually absent [60, 73].

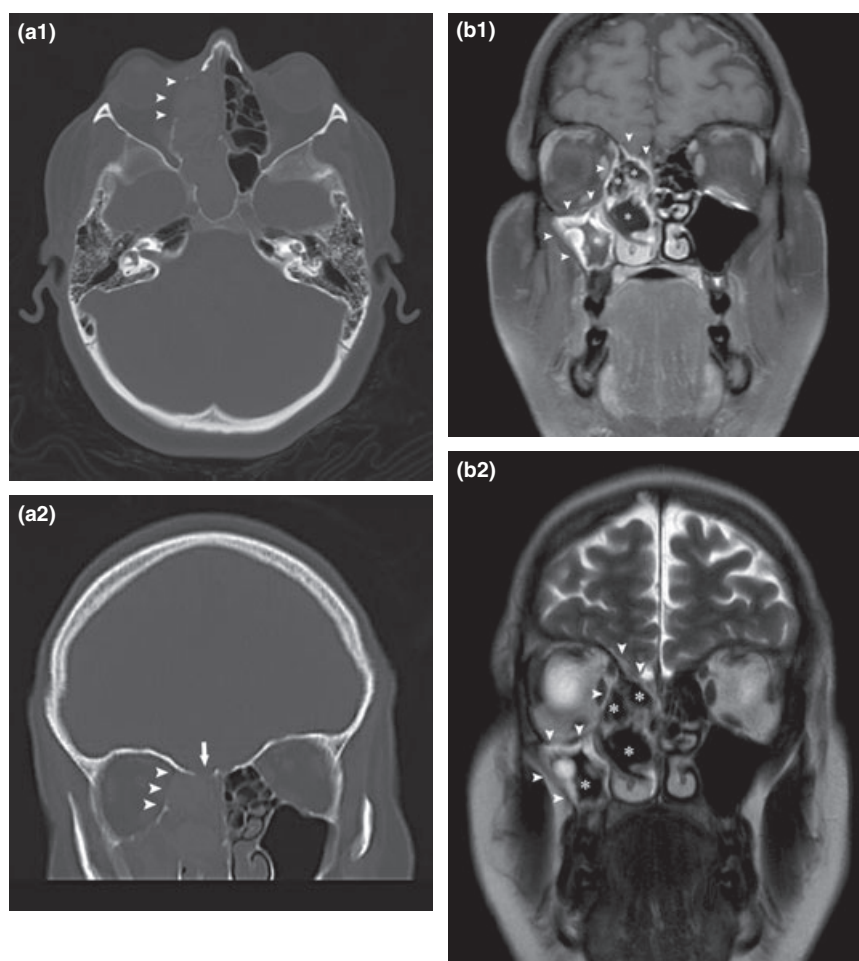


Fig. 4. (a) Sinus CT scans of a patient with allergic fungal rhinosinusitis (AFRS): 1. Medial orbital wall thinned and expanded laterally by the ethmoid sinus content (arrowheads), 2. Erosion of the skull base (arrow) and lateral expansion of the thinned medial orbital wall (arrowheads). (b) MRI of the same patient: 1. T1-weighted image showing central hypointensity (asterisks) and peripheral enhancement of right-side sinuses (arrowheads), 2. T2-weighted image showing central void signal (asterisks) and peripheral enhancement of right-side sinuses (arrowheads).

In summary, from the immunological point of view, patients with AFRS present (1) type I hypersensitivity to multiple moulds and non-fungal aeroallergens demonstrable by immediate skin test reactions and *in vitro* detection of sIgE, (2) increased total IgE, and (3) increased sIgG to multiple moulds. Fungal-specific precipitins and peripheral eosinophilia are presented inconsistently. Table 2 summarizes the similarities and differences between ABPA and AFRS.

The yield of positive fungal cultures from eosinophilic mucin in AFRS patients varies greatly (10% to 90%) [57, 65, 81]. These variations seem to depend to a great extent on the fungal culture method used rather than the presence of fungi in the allergic mucin [41, 81]. Also, in some cases, the fungal material within the mucin may be no longer viable. Novel methods, such as *in situ* hybridization [82, 83], polymerase chain reactions [84, 85] and fluorescein-labelled chitin-specific binding protein [86], have been used to try to overcome

some of these difficulties. Conversely, a positive fungal culture may simply represent saprophytic fungal growth, and therefore it does not in itself confirm the diagnosis of AFRS. In office cultures are not recommended as these often reveal saprophytic organisms within the nasal cavity rather than fungal material within the sinus itself. Culture of surgically obtained mucin is optimal.

Histopathology of the mucin is very helpful for the appropriate diagnosis to be made [39]. Maximal mucus preservation is important during the acquisition of surgical specimens to optimize the yield of eosinophilic mucin and fungal elements within the mucus [41]. On H&E staining, eosinophilic mucin is characterized by laminated accumulations of intact and degenerating eosinophils, frequently with the presence of Charcot-Leyden crystals, which represent eosinophilic breakdown products [37]. Fungal hyphae within the eosinophilic mucin are sparse and do not stain with H&E; however,

Table 2. Comparison between allergic fungal rhinosinusitis (AFRS) and allergic bronchopulmonary aspergillosis (ABPA)

	AFRS	ABPA
Affected population	Young, atopic, immunocompetent*	Asthma CF
Mucus	Thick, tenacious, casts	Thick, tenacious, plugs
HLA association	HLADQB1*0301 and HLA-DQB1*0302 [50]	HLA-DR2 and HLA-DR5 [74, 75]
Hypersensitivity reaction†	(I–III)‡	I–III [76, 77]
T cell activation	Predominantly Th2 response [78]	Predominantly Th2 response [79]
Serology		
Total IgE	Elevated (moderately) Helpful in the clinical follow-up	Elevated (highly) Helpful in the clinical follow-up
Specific IgE	Elevated	Elevated
Specific IgG	Elevated	Elevated
Precipitins	Inconsistently present	Frequent [80]
Peripheral eosinophilia	Inconsistently present	Generally greater than 1000/mL [80]
Treatment		
Systemic steroids	Useful	Useful
Systemic antifungals	Contradictory evidence	Adjunctive (corticoids sparing agents)
Immunotherapy	Low quality evidence in favour	Not used
Anti-IgE antibody	Lack of evidence	Low quality evidence in favour

*Asthma is frequent.

†Coombs and Gell classification.

‡Less strong evidence than for ABPA [64, 71].

their negative image can sometimes be appreciated. Fungal silver impregnation with Grocott's methenamine silver (GMS) is widely used as a screening for fungal organisms. Periodic acid-Schiff stain is also useful to detect fungi, however, this stain only shows viable fungi. Fontana-Masson stain, which is specific for melanin, can aid in the differentiation of dematiaceous moulds from others; however, caution should be exercised when interpreting the results as hyphae of *Aspergillus* spp, zygomycetes and *Trichosporon* spp (causatives of hyalohyphomycosis) can also show positive staining [87]. Nevertheless, none of these can make an organism-specific diagnosis, unlike cultures or non-culture dependent tests such as polymerase chain reactions or *in situ* hybridization. Microscopic examination of the mucosa with haematoxylin–eosin (H&E) staining shows mixed inflammatory infiltrate composed of eosinophils, lymphocytes and plasma cells. The mucosa is hyperplastic, but there is no evidence of fungal invasion, necrosis, giant cells or granulomas [35].

Treatment. Current recommendations regarding the treatment of AFRS are based largely on extrapolation of results achieved in patients with idiopathic CRS with polyps, retrospective case series and expert opinion. The initial treatment modality is medical and consists of combination therapy with nasal saline irrigations, topical steroids, and short courses of systemic corticosteroids and antibiotics (there is almost always an associated bacterial involvement). However, should maximal medical therapy fails to bring about a suffi-

cient improvement in symptoms, functional endoscopic sinus surgery (FESS) is then considered [88]. The aim of the surgery should be to remove as much antigenic and inflammatory load as possible and restore ventilation and drainage of the sinuses.

Surgery must be followed by aggressive medical therapy if a good long-term outcome is to be achieved. Many patients will have recurrence in spite of ongoing medical therapy [47, 89, 90] and will need multiple surgeries to achieve control of their disease [70, 91, 92]. With all of this in consideration, fairly good long-term control of the disease with ongoing medical care seems to be possible [92]. However, there is a lack of studies to help guide the medical therapy in terms of duration and dose of corticosteroids [89] and the type, dose and duration of antibiotics. Our regimen consists of several weeks of tapering prednisone and oral antibiotics, as there is usually a bacterial component, and ongoing treatment with high volume saline lavage and topical steroids.

In classical cases of AFRS, the inflammatory reaction reflects a hypersensitivity response to fungal antigens. Consequently, it has been thought that fungal immunotherapy (IT) may act beneficially by reducing the specific response to fungal antigens. There are some reports which suggest it may be helpful. Folker et al. [93] compared 22 patients with AFRS diagnosed using the Bent and Kuhn criteria. Eleven received IT after endoscopic sinus surgery and 11 did not. Immunotherapy consisted of subcutaneous administration of the relevant fungal and non-fungal antigens. All received similar treatment

consisting of endoscopic sinus surgery, corticosteroids and antibiotics as needed for complicating purulent rhinosinusitis. After a mean of 33 months' therapy, the group that received IT had significantly better outcomes as assessed by an endoscopic mucosal staging system ($P < 0.001$) and a sinusitis-specific quality of life scale ($P = 0.002$). In addition, IT was shown to reduce reliance on systemic ($P < 0.001$) and topical nasal ($P = 0.043$) corticosteroid therapy to control the disease. The same group compared retrospectively the re-operation rate between 36 patients with AFRS that received immunotherapy from 24 which did not [91]. After a mean follow-up time of 48.5 months, there was a difference in the re-operation rate between the group that received immunotherapy (11.1%) compared to the one that did not (33.3%, $P < 0.01$). A recent study compared the safety of high-dose subcutaneous fungal immunotherapy in 14 patients with AFRS and 14 controls with chronic sinusitis (non-AFRS) [94]. There were no differences between the two groups and only one patient in each group experienced a mild systemic urticarial reaction to immunotherapy. Conclusive evidence of the efficacy of immunotherapy for patients with AFRS in the form of a randomized controlled trial is currently lacking.

To date, there is paucity of studies addressing the efficacy of antifungal agents in the treatment of AFRS and evidence is poor to support recommendations. Several randomized controlled studies of topical or systemic antifungal therapy in the treatment of CRS have led to disappointing results [95], but studies addressing specifically AFRS patients are sparse. One non-controlled study reported a modest effect of fluconazole nasal spray used in 16 patients with AFRS and progression of symptoms in spite of treatment [96]. Twelve patients reported stabilization of symptoms or had a decrease in their mucosal oedema during treatment with fluconazole, and three patients without polyps developed them during the treatment. A recent randomized controlled trial evaluated the effect of systemic itraconazole (100 mg/day) and fluconazole nasal spray alone or in combination after FESS in 41 patients with AFRS [47]. All patients received conventional medical treatment postoperatively and were randomly assigned to 5 groups matched for sex, age and socio-economic status. The antifungal regimens included oral itraconazole (group A), fluconazole nasal spray (group B), combined oral itraconazole and nasal fluconazole (group C), and irrigation with a fluconazole solution through the nasal fossa (group D); the group of 10 controls (group E) received conventional medical treatment only. Recurrence rates in the 5 groups were 66.7%, 10.0%, 14.3%, 28.6% and 75.0% respectively (maximum follow-up was 9 months). These findings suggest that topical fluconazole may have a role in reducing the recurrence rate after FESS in

patients with AFRS, although show no benefit of itraconazole. However, recent retrospective case series and case reports have shown encouraging results with higher doses of oral itraconazole [97–100] or with the use of voriconazole [101]. Consistently, in patients with ABPA (the homologue of AFRS in the lower airway) treatment with total doses of 400 mg/day oral itraconazole has shown to allow a reduction in oral corticosteroid requirement in steroid dependent patients [102, 103] and to modify the immunological activation [104]. Similar effect of voriconazole in patients with ABPA has been suggested by retrospective case series [105–107]. More research to establish the therapeutics roles of anti-fungal agents as topical fluconazole and systemic itraconazole or voriconazole in AFRS patients is required.

Finally, monoclonal antibodies against IgE (omalizumab) have been used in few case reports and case series in the treatment of ABPA in patients with cystic fibrosis, suggesting a potential role [108–111]. There are case reports of omalizumab been successfully used to treat patients with recalcitrant CRS with polyps and concomitant asthma [112, 113]. However, a recent randomized, double-blind, placebo-controlled trial of anti-IgE for chronic rhinosinusitis, which included 14 patients that had undergone endoscopic sinus surgery (12 of them had polyps), showed only a small impact on CRS severity, possibly not of clinical relevance [114]. There are currently no reports of the use of omalizumab in the treatment of patients with AFRS specifically.

Invasive disease

Acute invasive fungal rhinosinusitis

Acute invasive fungal rhinosinusitis is an acute life threatening condition in which the fungal infection invades underlying mucosal tissue as a result of an impaired immune response. It tends to spread aggressively, and often has a fulminant course, progressing over hours. Eye, brain, palate and skin extension can occur.

This condition occurs almost exclusively in immunocompromised patients with neutrophil dysfunction or neutropaenia, such as those with haematological malignancies, aplastic anaemia and uncontrolled diabetes mellitus, or those undergoing anti-neoplastic chemotherapy or following transplantation. It is less frequently seen as a complication of HIV infection probably because the main affected cells are T lymphocytes and the granulocytes remain relatively intact [17]. Rarely, cases have been reported in apparently normal hosts [115, 116].

Aspergillus species and members of the order Mucorales (*Rhizopus* and *Mucor* species) are the most frequent types of fungi involved [1, 2, 23]. Typically, they

have an angio-invasive behaviour with subsequently necrosis [24]. Hyaline moulds causative of hyalohyphomycosis (e.g. *Fusarium* spp, *Scedosporium* spp and *Paecilomyces* spp) are much less common, but are emerging pathogens [117].

Presentation and diagnosis. Symptoms are non-specific and a high index of suspicion in an immunocompromised patient with sinonasal symptoms and/or fever is mandatory to allow diagnosis in early stages of the disease. When suspected, an endoscopic examination is indicated immediately so that biopsies of any ischaemic or necrotic tissue can be taken. Typically, the underlying tissue does not bleed when biopsied. Biopsies should be sent for microscopy, histopathology and culture.

Calcofluor-white, a fluorescent stain useful to detect hyphae by direct microscopy (under a fluorescent microscope), has advantages over conventionally prepared smears in KOH wet mount [118]. It is simpler, more sensitive and samples can be examined immediately. Direct microscopy also allows differentiating *Mucorales* seen as non-septate hyphae, from septate hyphae as *Aspergillus* species or other hyaline moulds.

The histopathology is characterized by hyphal invasion of blood vessels, vasculitis with thrombosis, haemorrhage, tissue infarction and necrosis, and acute neutrophilic infiltrates may be present [119]. Histologically, hyaline moulds causative of hyalohyphomycosis cannot be differentiated from each other or from *Aspergillus* spp; thus, in routine histological sections are often misidentified as *Aspergillus* [7]. It is important to make this distinction as it has implications in the treatment. Cultures are required to differentiate them, preferably of tissue since these moulds are rarely cultured from blood [7]. Galactomannans antigens are polysaccharides belonging to the cell wall of *Aspergillus* spp and their detection in serum is used as an aid in the diagnosis of invasive aspergillosis, although it can be positive in infections by other fungi (e.g. the hyaline mould *Paecilomyces* spp) [120].

The radiological imaging is typically also non-specific. CT scans usually show some degree of sinus involvement with soft-tissue opacification. The maxillary and ethmoid sinuses are the more frequently affected. More suggestive findings like osseous erosion, facial soft-tissue thickening, and periantral soft-tissue infiltration of the fat are late findings [121–123]. MR imaging is useful when orbital or intracranial involvement is suspected.

Treatment. The three principles of treatment are as follows: (1) immune reconstitution (if possible), (2) immediate initiation of systemic antifungal therapy and (3) urgent surgical debridement of all the necrotic tissue.

The use of topical amphotericin B has been documented in case reports [124] and case series [125]; however, there are no controlled studies establishing its efficacy. It is important to identify the fungal species involved as this determines the choice of antimycotic agent. Empiric antifungal therapy should start with intravenous amphotericin B, ideally liposomal amphotericin B preparations to decrease the risk of renal damage. Once the aetiological agent has been identified, amphotericin B is the treatment of choice for *Mucorales* and voriconazole for *Aspergillus* species [126, 127]. For serious infections, serum voriconazole trough levels should be monitored [128]. It is important to make the distinction between *Aspergillus* species and hyaline moulds causative of hyalohyphomycosis as some of the latter as *Fusarium* spp and *Scedosporium* spp frequently demonstrate resistance to azoles and echinocandins, and so require combination therapy [129, 130]. The duration of antifungal therapy remains unclear, and in the presence of ongoing immunosuppression, indefinitely maintenance of oral antifungal therapy should be considered.

Not unfrequently significant morbidity is associated with the treatment, requiring orbital exenteration or significant resections of the palate or the maxilla. The prognosis is extremely poor without improvement of the immune state of the patient. Mortality rates of 10% to 50% have been reported and depend mainly of the absence of host immune reconstitution and the extent of involvement on recognition of the disease (e.g. intracranial involvement is associated with poor prognosis) [25, 131–133].

Chronic invasive fungal rhinosinusitis

This condition tends to affect patients with subtle immune system abnormalities like diabetes mellitus or chronic low-dose corticosteroid, and is much less frequent than the acute invasive form [1]. It shares the same aetiological agents (*Aspergillus* species and *Mucorales*), although *Penicillium* species and *Candida* have also been reported [134].

It presents as an indolent infection with a prolonged clinical course and slow disease progression rather than hours as in the acute invasive form. Nonetheless, it is a severe and potentially lethal disease. The infection often affects the ethmoid and sphenoid sinuses, although any sinus can be involved. Orbital involvement is frequent and CT shows an image that resembles a soft-tissue mass.

Histopathology is characterized by a dense accumulation of hyphae (resembling a fungal ball), the occasional presence of vascular invasion and a sparse inflammatory reaction [26].

Treatment consists of surgery to debride all the involved tissue and systemic antifungal therapy accord-

ing to the aetiological agent. The overall prognosis is better than in acute invasive fungal rhinosinusitis and recurrence can occur [3, 134].

Granulomatous invasive fungal rhinosinusitis

This is a rare form of chronic invasive fungal rhinosinusitis that has been most commonly reported in Sudan, India, Pakistan and Saudi Arabia. It is not known if it is geographical or ethnic related. In contrast to the previous described forms of invasive fungal rhinosinusitis, this affects immunocompetent hosts. The usual causative organism is *Aspergillus flavus*.

Clinical presentation consists of a slow growing enlarging fibrous mass involving one or more sinuses, which may extent into the orbit or intracranially. CT imaging is similar to chronic invasive fungal rhinosinusitis, but histopathology differentiates them, characterized by non-caseating granulomas with dense fibrosis, Langerhans giant cells, occasional vasculitis and sparse hyphae [26, 135].

Treatment consists in surgical removal of all the involved tissue. Historically, a high relapse rate (up to 80%) and mortality have been described. A case series showed lower relapse rate with postoperative systemic itraconazole [136].

Conclusion

Fungi are ubiquitous and we inhale spores with every breath; however, only a minority develop a pathological inflammatory response to fungi in the airway, and

invasive forms of fungal rhinosinusitis are rare conditions.

Non-invasive forms of fungal rhinosinusitis are usually suspected on the grounds of imaging obtained in a patient with chronic sinonasal symptoms that does not respond to routine medical therapy; on the other hand, the detection of invasive forms require a high index of suspicion in immunocompromised patients and must be actively looked for when fever or sinonasal symptoms appear.

With the exception of localized fungal colonization, treatment of all forms of fungal rhinosinusitis relies heavily on surgery and thus close collaboration with the otorhinolaryngologist colleague is crucial. Systemic antifungal agents are a fundamental component in the treatment of invasive forms. Immunotherapy and antifungal drugs may have a role as adjuvant therapy in allergic fungal rhinosinusitis, but evidence is still poor to support recommendations.

Future research should focus on clarifying the concepts of AFRS, EFRS and EMRS. Adequately designed randomized controlled trials are required to determine the real benefit of immunotherapy and antifungal therapy in the treatment of AFRS.

Conflicts of interest

The authors declare no conflicts of interest.

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