Coping With Chronic Fungal Rhinosinusitis: Diagnosis to Therapy

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DOI: 10.18297/jri/vol2/iss2/7

Received Date: April 19, 2018

Accepted Date: June 29, 2018

Website: <https://ir.library.louisville.edu/jri>

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Introduction

Fungal infections are commonly neglected during differential diagnosis on clinical care. However, they should be considered in all patients with chronic sinusitis because they affect significant numbers of patients. Fungal infections of the sinuses have recently been attributed to most cases of chronic rhinosinusitis, although supporting evidence is still controversial. Most fungal sinus infections are benign or noninvasive, except when they occur in individuals who are immunocompromised [20, 21]. Invasive fungal infections in immunocompetent individuals do occur [1] and this subset of patients should not be overlooked. A review of the literature and presentation of 4 clinical cases of fungal rhinosinusitis (FRS) are presented in this article.

4 Case Reports

Case 1 – *Scopulariopsis*

A 54-year-old male smoker with a history of multiple traumatic events to the nose presented to the clinic with chronic facial pain with pressure and a series of 6 acute sinus exacerbations occurring over 11 months, requiring antibiotic therapy. The patient claimed right-sided facial pain/pressure and foul-smelling yellow discharge along with fever and chills. He also reported chronic itchy, watery eyes and intermittent rhinorrhea and current treatment with nasal steroid spray, saline nasal irrigation, and a decongestant. His rhinological exam demonstrated no external ecchymosis, diffuse mild erythema of the midline septum, which deviated slightly to the right and an exudate within the middle meatus, bilaterally, with thick secretions. The mucosa showed no polypoid degeneration. Radiologically, CT of the sinuses showed extensive chronic sinusitis involving primarily the right maxillary sinus but also extending into the right side of the ethmoids and frontal sinus towards the right side which was completely opacified, suggesting inflammation. There was only minimal mucosal thickening of the floor of the left maxillary sinus. The medial wall of the right maxillary sinus was absent but demonstrated an amorphous high attenuation material that was suspicious for mycetoma. The right ethmoid conchae were markedly attenuated to absent. A soft tissue

Figure 1. A. *Scopulariopsis* sp. in sinus biopsy, 400X, GMS stain. B. Flat sided, door-knob shaped echinulate spores (arrow), 1000X, Grocott's methenamine silver (GMS) stain. C-D *Scopulariopsis* grown on Sabouraud dextrose agar (SABDX); C. Colony at 5 days, incubated at 30°C. D. Lactophenol cotton blue stain (LPBC), 400X, showing chains of flat sided echinulate spores.

density was present that extended into the right frontal ethmoidal recess and slightly into the right side of the frontal sinus. Bilateral endoscopic ethmoidectomy was performed with removal of the right maxillary sinus contents which were sent to pathology. The patient's complete blood count was normal. Pathology revealed benign respiratory epithelium covered mucosa with marked acute and chronic nonspecific inflammation with focal erosion and numerous fungal microorganisms. A microbiologist consult revealed that the fungal morphology seen in the specimen was consistent with *Scopulariopsis* [2]. Numerous clusters of "door knob" shaped spiny conidia were evident (Fig. 1). Conidia were present singularly, and in chains, characteristic of this mold.

Scopulariopsis species are keratinophilic and are most commonly associated with onychomycosis; however, more

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Figure 2. A. Fungus ball in sinus biopsy, 100X, GMS stain.; B. *Cunninghamella* showing ribbon like, aseptate, hyphae (arrows), 1000X, GMS stain.; C. Columella and fruiting head of *Cunninghamella*, in sinus biopsy, 400X, GMS stain.; D. Round, echinulate spores of *Cunninghamella* (arrows), 1000X, GMS stain.; E. Typical fruiting head of *Cunninghamella* grown on SABDX, LPCB stain, 1000X.; F. Echinulate sporangia, LPCB stain, 2000X.

recently there have been some reports of invasive infections. Of these, about 90% are associated with one of the following risk factors: AIDS, organ transplantation, corticosteroid therapy, peritoneal dialysis, surgery, cardiac disease, and trauma. *Scopulariopsis* is a large genus of saprophytic organisms found mostly in soil, but frequently isolated from food, paper, and other materials [3]. Within the genus are members of both hyalohyphomycoses and phaeohyphomycoses. Of the hyaline species, *S. brevicaulis* is responsible for causing most of human infections while *S. candidum* and *S. acremonium* have also been reported to cause disease in humans [4]. The organism has been reported as a cause of rhinosinusitis [2,4,5,6,7]. The pathological diagnosis of *Scopulariopsis* can be made from the characteristic conidial morphology since there are few fungi that resemble these microorganisms. However, the organism is highly cultivable and grows on various mycology media commonly found in the clinical laboratory. In this case, removal of the infected material was sufficient to resolve the infection without antifungal therapy. The patient improved and in a 2-year follow-up, he was healthy without recurrence of symptoms or infection.

Case 2 – *Cunninghamella*

A 67-year-old male smoker with a history of insulin dependent diabetes mellitus and hypertension, presented to evaluation at the ENT clinics for nasal congestion. His symptoms began 25 years ago with right side nasal polyps requiring polypectomy at that time. He stated that recently the nasal congestion was worse to the point he could not use his CPAP machine. He was taking Fluticasone without any improvement. No facial pain or pressure was identified at physical examination. A CT of the sinus was performed with a descriptive polypoid soft tissue density involving the right nasal cavity adjacent to the midline turbinate, which was concerning for a nasal polyp. A significant soft tissue density involving the base of the left maxillary sinus with central hyper density including speckled calcified densities indicated that could represent fungal infection. After this finding he went to surgery and a bilateral anterior ethmoidectomy with right side polypectomy was performed. Pathology reported numerous of fungal hyphae morphologically consistent with *Zygomycetes*. A Microbiologist consult was obtained. The fungus was identified from the tissue sections as most consistent with *Cunninghamella*. The organism showed aseptate ribbon-like hyphae, with a vesicle supporting multiple denticles giving

rise to echinulate sporangia (spores) (Fig. 2).

Cunninghamella, is a saprobic fungus commonly found in the soil of temperate climates. It's a rare cause of zygomycosis in humans often associated with trauma and immunosuppression. [8]. The genus *Cunninghamella* in the order *Mucorales* encompasses filamentous fungi that are inhabitants of soil and other environments and are common laboratory contaminants. *C. bertholletiae* is the only member of the genus documented to cause human infections [9].

Case 3 – Blastomycosis

A 55-year-old male smoker with a history of diabetes mellitus and hypertension, presented with a right sided maxillary and ethmoid sinusitis and preseptal cellulitis. His symptoms began 3 months prior, and consisted of right orbital pain and pressure, headache, with a white to yellow drainage from the right nares. A CT of the chest was performed which showed a right hilar mass. He had an elevated white cell count of 19,400/mm³. A transbronchial lung biopsy and endobronchial ultrasound biopsy were performed but both showed only inflammatory tissue. A bronchial brush specimen subsequently yielded negative microbiology cultures. Treatment with nasal rinses, corticosteroid spray, and antibiotics were at first helpful but soon, earlier symptoms returned. Two months later, the patient returned with progressive fevers, chills, night sweats, posterior headache, and weight loss with continued right sided facial pain and drainage from the right nares. He denied any neck stiffness and had no other complaints. The patient underwent two right nasal septum biopsies which were sent to pathology and microbiology. He was initially started on fluconazole until laboratory results from the biopsy were completed. The right nasal septum biopsy showed yeasts, present in respiratory mucosa with severe-acute, chronic, and granulomatous inflammation, with necrosis. A right lateral nasal vestibule lesion biopsy showed squamous epithelium-lined mucosa with severe acute, chronic, and granulomatous inflammation, ulceration, and epithelial marked hyperplasia. Pathology obtained a microbiologist consult which identified the yeast as broad base budding yeast cells 10-20 µm in diameter, with thickened cell wall, and the absence of hyphae or pseudohyphae, most consistent with *Blastomyces dermatitidis* (Fig. 3). Microbiology fungal and mycobacterial stains were all negative

Figure 3. *Blastomyces dermatitidis*. **A.** *B. dermatitidis* in sinus biopsy (arrows), 100X, GMS stain, bar=200µm; **B.** *B. dermatitidis* in sinus biopsy, 1000X, GMS stain, bar= 20µm; **C.** *B. dermatitidis* in sinus biopsy showing broad base budding yeast cells, 1500X, hematoxylin and eosin (H&E) stain; **D.** *B. dermatitidis* in sinus biopsy showing thickened cell wall of two yeast cells, 2000X, H&E stain.

and cultures remained negative at five weeks. An infectious disease consult was immediately obtained, and the patient was started on itraconazole therapy for blastomycosis. Continued follow-up visits were scheduled along with CT of the head to rule out cerebral abscess.

Blastomyces dermatitidis is another fungus unique to different geographical areas which may find a focal niche in the sinuses and cause chronic invasive fungal rhinosinusitis. The dimorphic fungus typically exists in the warm, moist soil of wooden areas ricing in organ debris. Regions of Wisconsin and Tennessee are highly endemic for blastomycosis [10]. Mostly, patients infected with *B. dermatitidis* develop asymptomatic illness. This fungus has the potential to affect bones. A three-decade survey of skeletal blastomycosis at a United States Medical Center revealed 31 cases; of those, 7(23%) showed skull and facial bone involvement [10].

Case 4 – *A. fumigatus*

A 53-year-old, immunocompetent, white male with a 35-year history of allergic rhinitis, sinusitis, and septoplasty performed 26 years ago for a deviated septum, presented to the emergency department with increased nasal drainage over several weeks, and a feeling of sinus pressure and regular headaches on the right side, with no fevers but intermittent chills. He was given two weeks of amoxicillin-clavulanic acid along with a nasal decongestant and discharged. The patient returned twelve days later with continued symptoms of right-sinus pain and nasal discharge. A CT scan revealed complete opacification of the right maxillary sinus with a soft tissue mass extending in to the right side of the ethmoid air cells and towards the right frontoethmoidal recess. There were also findings to suggest changes of chronic right maxillary sinusitis with osteitis of the other walls of the right maxillary sinus. Endoscopic sinus surgery was performed and a biopsy of a suspected “fungal ball” from the right sinus was sent to microbiology for culture. Bacteriology recovered *Escherichia coli*, ampicillin resistant. Mycology recovered on the sixth day of incubation, a white-to-light-green mold from which vesicles were produced from single, broad, conidiophores (lacking rounded vesicles) along septate hyphae (Fig. 4). MALDI-TOF was performed on the isolate by the method of De Carolis et. al. [11] using the Bruker Daltonics filamentous fungi library v.1.0, which yielded *Aspergillus fumigatus*, log scores of 2.09, 1.79, and 1.73. The mold grew on brain heart infusion -5% sheep blood agar at 30°C, and 45°C, but

not at 55°C. Because microscopic morphology was inconsistent with *Aspergillus*, the isolate was referred to the University of Texas Health, San Antonio Department of Pathology and Laboratory Medicine, Fungus Testing Laboratory, San Antonio, Texas. Phenotypic characterization and DNA sequencing of the targets, ITS, D1/D2, TUB and CAL, identified the isolate as *A. fumigatus*. Their lab also reported susceptibility testing results of amphotericin B (MIC =0.5 µg/ml), fluconazole (MIC >64 µg/ml), itraconazole (MIC =0.5 µg/ml), and isavuconazole (MIC =0.5 µg/ml) in accordance with CLSI M38-A2 document [12].

Due to this atypical morphology for *A. fumigatus*, the possibility that this isolate was its anamorph, *Neosartorya*, we reviewed the previously published morphological characteristics of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya* [13]. The closest matching microscopic morphological features were similar with *A. unilateralis*. Microscopically, our isolate failed to demonstrate the characteristic rounded “aspergilli vesicle” but instead showed a segmented conidiophore with a central elongated vesicle (Fig. 4, C-D) with a central enlarged phialide and secondary smaller phialides produced on metulae. We noted that single-opposing conidiophores were sometimes produced at right angles along the hyphae, similar with those shown in Fig. 4C. The colonial morphology of our isolate was dissimilar to *A. unilateralis*. The colony surface remained white for several days, developing only light mint green coloration toward the center of the colony whereas *A. unilateralis* is fully green. The reverse morphology remained white as opposed to a dark, black reverse for *A. unilateralis*. Balajee et. al. [14] reported that the genus *Aspergillus* presents numerous difficulties for morphological identification. Some species exhibit slow sporulation and aberrant conidiophore formation, thus requiring DNA sequence-based identification. Thus, multiple genes such as found in ribosomal DNA regions ITS and the ribosomal subunit D1-D2, as well as structural genes of β-tubulin (TUB) and the calmodulin (CAL) genes must be used to segregate species within aspergilli. Despite the value of gene sequencing, there is no consensus as to the genes that can be used to specifically identify the aspergilli to species. Growth studies at various temperatures and electron microscopic analyses of conidia morphology are still needed to fully characterize these fungi. Since our clinical laboratory still relies primarily on morphological/physiological identification, we could not identify this isolate without the aid of MALDI-TOF and gene sequencing analysis. Unfortunately, this process took 10 weeks to complete. This demonstrates the complexity involved in the final diagnosis and subsequent reporting of important clinical information related to the treatment of FRS.

Clinical definitions, manifestations and epidemiology overview

The burden of FRS in the United States is not clearly defined. However, there is a growing consensus that fungal infections worldwide are increasing as certain at-risk populations are demonstrating increased survival time [15]. Generally, at risk populations for invasive fungal disease are those who are immunosuppressed for some reason like age, multiple underlying comorbidities, diabetes, malignancy, HIV, or receipt of immunosuppressive agents [16]. However, reports highlighting outbreaks following natural disasters or antecedent trauma in healthy adult populations demonstrate the wide array of potentially affected hosts [17].

Figure 4. *A. fumigatus* grown on SABDX, at 30°C. **A.** 14-day old colony; **B.** LPCB stain, 400X, bar = 50µm; **C.** LPCB stain, 1,000X, showing multi-septate, broad conidiophores ending in a central elongated vesicle with an enlarged phialide with cup shaped collaret. Secondary conidiophores were often produced from the vesicle; **D-F.** LPCB stain, 1,500X, showing multi-septate, broad conidiophores ending in a central elongated vesicle with an enlarged phialide having a cup shaped collaret and release of a terminal conidium (flat at the base), and primary/secondary metulae terminating in phialides (arrows) with dark collarets and chains of round conidia.

Fungi are ubiquitous in the environment and can colonize the upper respiratory tract mucosa when fungal spores are inhaled. In people with normal immune function, once inhaled the fungal growth is kept in check. With impaired host immunity, fungi can invade host mucosa and cause invasive disease [18].

FRS comprises a spectrum on disease process, which varies in clinical presentation, histologic appearance, and biological significance. FRS can be acute (aggressive; symptoms <30 days), subacute (symptoms 30-90 days), and chronic (indolent; symptoms >90 days) [19].

Clinically it could be classified as non-invasive or invasive. Assessing the viability of the host immune system is central to correctly differentiating and managing FRS. Immunity is the key predisposing factor for fungal invasion of the sinuses and must be considered for all FRS patients. Presumably, fungi are unable to penetrate the epithelial layer when the immune system is functioning normally. Fungal protease activation of epithelial cells can elicit increased cytokine production and the subsequent migration of inflammatory cells, including eosinophils which migrate through the barrier epithelial cells in response to fungi present [20]. Suppression of the immune system, such as from diabetes mellitus, chemotherapy, or corticosteroids, creates a condition in which fungi can penetrate normal mucosa barriers and invade host tissues [21]. Non-invasive rhinosinusitis includes: saprophytic fungal infection, fungal ball (FB), and allergic fungal rhinosinusitis (AFRS). The invasive forms include acute, chronic, and chronic granulomatous.

Non-invasive rhinosinusitis

AFRS represents a hypersensitive response of a competent immune system to fungal elements. The pathogenesis could be explained in part through IgE-mediated-(type 1) reaction to fungal species trapped in sinonasal mucus. It has been shown that fungi induce the production of eosinophil attracting T_H2 cytokines hence, large amount of mucin is produced due allergic inflammation [19]. The clinical presentation described includes nasal congestion, facial pain and pressure, nasal discharge, and diminished olfaction. Epidemiologically patients are younger, more likely male, living in warm and humid climates such as

southern and southeastern United States, India and Middle East. Most individuals have a history of atopy including allergic rhinitis and/or asthma [19].

The FB is described as a dense accumulation of fungal elements within a single sinus. The sinus most common affected is the maxillary sinus, followed by the sphenoid sinus and unlikely with the ethmoid of frontal sinus. These are more common in normally functioning immune system, and more common in middle-age women. Previously in the literature the FB was described as “mycetoma” or “aspergilloma”. FB rarely yield positive fungal cultures; however, *Aspergillus spp.* are the most commonly isolated pathogen [19].

Saprophytic fungal infestation describes fungal colonization of the sinonasal tract usually after a surgical procedure or a traumatic event. Subsequent inflammation of the sinonasal mucosa follows, and infection of the surface mucosa occurs without tissue invasion [19]. Nasal crust can become colonized by an extensive collection of fungi. This provides an environment for fungal replication and nearby mucosa is usually unaffected. Hoggard et.al. [20], reported that from one neonatal study where nasal mucus specimens were examined, 94% of infants yielded positive fungal cultures within the first 4 month of life. This led them to suggest that fungi are normal constituents of the sinonasal tract. This poses the basis of our case 1 (above) which may have initially been colonizing sinonasal flora. The genus of *Scopulariopsis* is one of the most common saprophytic filamentous fungi [2]. Invasive disease evolution is very rare; when present it's associated mainly with immunocompromised patients.

Categories of Invasive FRS

Acute invasive FRS should be suspected in immunocompromised, particularly those on chemotherapy or bone marrow transplant, or uncontrolled diabetic patients with acute sinusitis. It may also occur in individuals on therapy with chronic oral corticosteroids. The presentation of the symptoms occurs in less than four weeks. It has indolent clinical presentation like nasal congestion, drainage, facial pain/pressure. As the disease advances, the patient can develop fever, epistaxis, and extension

into the orbit causing proptosis, ophthalmoplegia, and a decreased visual acuity. With eventual dissemination of the disease bone erosion may occur. Often diabetics, especially those with diabetic ketoacidosis experience invasive fungal infection of this type. This is frequently caused by the fungi belonging to the order of *Zygomycetes*, such as *Rhizopus*, *Rhizomucor*, *Absidia*, and *Mucor*. In patients with severe neutropenia, *Aspergillus* species are responsible for up to 80% of infections [22]. Overall, the causal agents in invasive fungal sinusitis are the filamentous fungi, including *Apergillus*, *Mucor*, *Rhizozopus*, *Fusarium*, *Pseudallescheria boydii*, and the dematiaceous fungi such as *Alternaria*, *Bipolaris*, *Cladophialophora*, and *Curvularia* spp. [23].

Clinically invasive FRS is characterized by a painless, necrotic, nasal septal ulcer (eschar), sinusitis, and rapid orbital and intracranial spread, leading to death. Angioinvasion and hematogenous dissemination are common. Microscopic evaluation reveals hemorrhage, necrosis, and mixed acute and chronic inflammation that may have a granulomatous component. Given the urgent nature of acute fulminant invasive FRS, intraoperative evaluation of frozen sections may be requested, and antibiotic therapy should be initiated before culture results are available [24].

Chronic invasive FRS is usually seen among diabetics. The definition describes a sinusitis of at least more than twelve weeks of duration compatible with radiologic findings and histologic evidence of tissue invasion by fungal hyphal forms. Histologically it's characterized by vascular, bone and soft tissue invasion; most commonly occurring is *Aspergillus fumigatus* [24]. Fungal-specific cultures can grow a variety of species including dematiaceous molds (*Bipolaris*, *Curvularia*, and *Alternaria*) [21]. An important key that may play a role in paranasal sinus aspergillosis are secretions of a toxic substance by fungi that penetrates tissues under appropriate conditions and induces tissue necrosis via an immune mechanism. Most common symptoms described are diplopia, headache, loss and impairment of smelling and nasal stuffiness. Fever is most often absent [25].

Granulomatous FRS is a sub-type of chronic invasive FRS. The time course is more than twelve weeks. This is described more frequently in immunocompetent individuals. The cases are more common in countries such as India, Pakistan and the Sudan, but there are some cases reported in the United States. The presentation is slow progression of the sinusitis with headache and unilateral proptosis. *Aspergillus fumigatus* is the responsible agent in most of the cases. The prognosis is better than other invasive forms, and patients usually respond to debridement, aeration, and antifungal therapy, with recurrence being common [24].

Diagnosis of Rhinosinusitis

The diagnosis of invasive FRS is challenging. Often, cultures from non-sterile fluid may be difficult to interpret and unable to differentiate a pathogen from other flora. Invasive diagnostic procedures relying on tissue biopsy or histopathological specimens are preferred to make the diagnosis. The timing between the diagnosis of FRS and the administration of antifungal therapy will often influence the outcome of therapy. This phase can be prolonged by diagnostic difficulties, including diagnostic markers, such as fungal nucleic acids, antigens, antibodies or assays for cell wall components. Detection of

fungal cell wall components is a rapid and attractive tool for diagnosis. Beta-(1,3)-D-glucan (BDG) is one of those components and is present in most pathogenic fungi such as *Candida* spp., *Fusarium* spp., *Aspergillus* spp., *B. dermatitidis*, and *Pneumocystis* [26]. Fungi for which BDG is of no value are the *Zygomycetes* and cryptococci which have none to very low concentrations of BDG in their cell wall, below that of detection in human serum [27].

Galactomannan (GM) as another important cell wall component of *Aspergillus* spp., but it does occur in some fungi such as *Fusarium* spp. During invasive disease, as opposed to colonization, the GM is released into the blood-stream during intravascular hyphal growth. For this reason, in the immunocompromised patient, the serum GM assay has shown promise in the diagnosis of invasive aspergillosis. The association of elevated serum GM levels are thus indicative (70-95% specificity) of invasive aspergillosis. For immunocompromised patients with high GM levels, we can tailor the diagnosis to aspergillosis when used in combination with elevated C-reactive protein and low procalcitonin levels [27]. Low procalcitonin often rules-out bacterial infections, but it may still rise in some yeast infections, but not so with aspergillosis. However, false-positives for GM (45-55% specificity) are well noted, but in conjunction with pathology and microbiology results, false-positives can often be discounted.

Summary of Histopathologic and Microbiologic testing for FRS

Histopathology

Histopathology of FRS varies depending on whether it is non-invasive or invasive as discussed above. Montone, et al. [19] recently classified the various histopathological criteria of FRS. Histologic examination of saprophytic fungal infection reveals inflamed and ulcerated sinonasal mucosa with the presence of surface fungal organisms without tissue invasion. FB are characterized by numerous fungal organisms embedded in fibrinous and necrotic exudate with absent to minimal mucosal inflammatory reaction [19, 28]. For AFRS, hematoxylin and eosin (H&E)-stained histological sections show lamellated mucinous material with the presence of eosinophils, eosinophilic debris, Charcot-Leyden crystals and rare noninvasive fungal hyphae [29]. Schneiderian mucosa reveals thickened basement membrane with goblet cell hyperplasia, and numerous inflammatory cells with prominent eosinophils. Invasive FRS is classified into acute invasive FRS, chronic FRS and chronic granulomatous FRS. In acute invasive FRS, histology demonstrates rare inflammatory cells, with angioinvasion of fungal forms causing thrombosis of the luminal surface, often with infarction. H&E stains, Grocott's methenamine silver (GMS) and periodic acid-Schiff (PAS) stains provide good contrast of the organisms which allows differentiation from other material found in the vascular walls and lumens that are often fibrin encompassed (**Fig. 5**). However, fungal morphology is frequently elusive [19]. The histopathology of chronic invasive granulomatous FRS is mainly characterized by submucosal granulomatous inflammation, a small number of fungi, fibrosis, and sometimes reactive epithelial hyperplasia. It may also show florid granulomatous inflammation with tissue invasion by fungal hyphae. Noncaseating granuloma with giant cells and occasional central eosinophilic microgranulomata, as well as fibrinoid necrosis, vasculitis, vascular proliferation, and perivascular fibrosis are often seen [23]. In contrast, in cases

Figure 5. Various stains used in Pathology. **Left:** H&E stain showing *Aspergillus* hyphae. **Center:** GMS stain showing a dematiaceous mold with septate hyphae. **Right:** H&E lightly stained, showing dematiaceous mold with unstained brown hyphae (arrows).

of chronic invasive FRS, histopathology often shows more numerous fungal organisms with sparse inflammatory infiltrate and occasional angioinvasion and without the presence of granulomas [19]. When dematiaceous fungi are involved, a lightly stained, H&E stain can be performed; this will show the brown colored hyphae of the fungal cell walls (**Fig. 5, right**) when viewed by direct light microscopy.

Microbiology

Most serious fungal infections of the sinus are due to the *Zygomycetes*, *Mucor*, *Rhizopus*, *Rhizomucor*, *Absidia*, and *Cunninghamella* but reports exist of sinusitis also caused by *Apophysomyces*, *Syncephalstrum*, *Saksenaea*, *Cokeromyces*, *Entomophthora*, *Conidiobolus*, and *Basidiobolus* [30]. *Aspergillus* sp. are the second known leading cause of chronic invasive sino-orbital and sino-pulmonary disease as well as non-invasive FRS and AFRS [31]. In addition, several hyaline molds, dematiaceous molds, *Candida*, and *Basidiomycetes* may be involved in FRS of immunocompromised patients [32]. Therefore, the clinical microbiology of FRS is complex and requires lengthy and laborious microbiological techniques to recover and identify these microorganisms.

The diagnostic gold standard is to demonstrate the organism in pathological stains followed by isolation of the mold in culture of clinical specimens. Realistically this is not always the case. In fact, only rarely is a clinical biopsy sent to microbiology for recovery of the fungus in culture. Although pathologists can accurately recognize fungi in clinical specimens, they are not so familiar with morphological characteristics that they can identify the organism to fungal family or genus. Although molecular technology may change our ability to identify pathogens directly from specimens, it is unlikely that this will occur any time soon. Therefore, it is extremely important that microbiologists be consulted when a specimen is submitted for a diagnosis of FRS.

The basic microbiology technique is the microscopic examination of the fungal stain. Fungal stains commonly used in microbiology are potassium hydroxide (10%, KOH), Calcofluor white, lacto-phenol-cotton blue (LPCB), LPCB with KOH, and to a lesser extent, PAS stain, Fontana-Masson, and Giemsa stain. The Gram stain sometimes can reveal fungal elements, but often the stain is over or under decolorized which makes it difficult to interpret. Culture of the fungus is still the primary means of recovery and identification. Polymerase chain reaction (PCR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF), and DNA sequencing techniques have been applied directly to clinical samples for the initial identification of fungi, however there is still much to be accomplished in these areas before any of these will become available as standardized, FDA approved techniques in the clinical laboratory. Once the fungal isolate has been recovered and grown in pure culture, both MALDI-TOF and DNA

sequencing can be primarily used for identification. Many clinical laboratories now send isolates to reference laboratories for DNA sequencing analysis which is extremely accurate, but also costly (average of \$300/isolate). Both technologies show great promise for the future of clinical mycology.

The outcome of the microbiological workup is dependent upon the quality and site and specimen collection. The swab is never an optimal sample. Instead, surgically collected tissue including exudates, necrotic areas, and tissue biopsies, are optimal. Purulent drainage may be submitted to the laboratory, but due to its polymicrobial nature, it seldom yields a significant pathogen. The transportation of specimens to the laboratory should be immediate and at room temperature. If a biopsy specimen is sent and zygomycosis is suspected, the laboratory should be alerted so that a touch-plate preparation can be performed prior to grinding the tissue. The grinding process will often destroy hyphae rendering the isolate uncultivable. Microbiology laboratories often maintain many different culture media, both selective and non-selective for the recovery of fungi [33]. Fungi are grown on Sabouraud dextrose agar-Emmon's modification (SABDX), potato dextrose agar, potato flake agar, corn meal agar, brain heart infusion agar (BHI) both with and without 5% sheep blood and antibiotics, Littman oxgall agar, malt extract agar, and Czapek's agar. Cultures are incubated at 27°C, 30°C, 45°C, and >50°C, to achieve optimal growth and culture morphology. Increasing the spectrum of media planted and incubation temperatures may increase the % recovery and lead to faster identifications. Some fungi will develop full growth by 3 days, but others may take up to 2 to 4 weeks to fully develop.

To date, due to cost containment measures, morphological growth characteristics are still the most used method of fungal identification. Both macroscopic and microscopic observations are combined for final identification. Identification involves recognition of sexual and asexual structures, color, size, shape, ornamentation, septation, yeast forms, conidia production, hyphae production, and spore germination. Several new texts are available that describe and depict morphological characteristics of the various clinically important fungi [33-36]. Morphological identification requires extensive training and experience in the field of mycology. The fungi are a diverse and complex group of microorganisms that may have both sexual and asexual stages of growth. For instance, there are 339 known species of *Aspergillus* with nine teleomorph (sexual stage) genera. Recent phylogenetic studies indicate that this number may soon increase [37]. Of these, there are only seven species that are commonly considered clinically significant [14]. However, Chen et al., recently reported that as many as 48 other uncommon *Aspergillus* species may be pathogenic [38].

Many of the past clinically insignificant fungi have become important considerations in FRS for the reasons discussed above. The clinical laboratory formerly considered many fungal isolates

Table 1. Common Antifungal Agents and Considerations when Treating Systemic Infections [49, 51-55]

Antifungal Agent	Class	Mechanism of Action	Normal Dose	Dosage Form	Common Side Effects/ Specific Antifungal Considerations
Fluconazole	Triazole	Ergosterol inhibitor	800 mg times 1 dose, then 400 mg daily	IV/Oral	Hepatic effects, QTc prolongation, skin reactions, drug-drug interactions
Isavuconazole	Triazole		372 mg (isavuconazole 200 mg) every 8 hours for 6 doses, then 372 mg (200 mg isavuconazole) daily	IV/Oral	Hepatic effects, GI effects, QTc narrowing, CNS effects, skin reactions, drug-drug interactions, high cost (no generic available), risk of dosing errors due to prodrug dosing
Itraconazole	Triazole		200 mg 3 times daily for 3 days, then 200 mg twice daily	Oral	Hepatic effects, cardiomyopathy, GI effects, QTc prolongation, skin reactions, type of formulation, drug-drug interaction, patient tolerability, high cost dependent on product
Posaconazole	Triazole		Suspension: 200 mg 3 times daily with high fat meal preferred IV/Delayed Release oral: 300 mg twice daily, then 300 mg daily	IV/Oral	Hepatic effects, QTc prolongation, CNS effects, GI effects, skin reactions, type of formulation, absorption, potential for drug monitoring depending on formulation, patient tolerability, avoid IV use in renal insufficiency, high cost (no generic available)
Voriconazole	Triazole		6 mg/kg every 12 hours for 2 doses then 4 mg/kg every 12 hours	IV/Oral	Hepatic effects, CNS effects, visual disturbances, QTc prolongation, skin reactions, drug-drug interactions, avoid IV use in renal insufficiency
Anidulafungin	Echinocandin	β -1,3-D-glucan synthesis inhibitor	200 mg once, then 100 mg daily	IV	Hepatic effects, cardiovascular effects, insomnia, infusion reactions, only available IV, high cost
Caspofungin	Echinocandin		70 mg once, then 50 mg daily	IV	Hepatic effects, cardiovascular effects, CNS effects, localized phlebitis, infusion reactions, only available IV, high cost (no generic available)
Micafungin	Echinocandin		100-150 mg daily	IV	Hepatic effects, cardiovascular effects, CNS effects, GI effects, hemolytic anemia/hemoglobinuria, only available IV, high cost (no generic available)
Amphotericin B (Liposomal)	Polyene	Ergosterol binding	3-5 mg/kg/day	IV	Hepatic effects, nephrotoxicity, electrolyte abnormalities infusion reactions, anemia, only available IV, patient tolerability, high cost (no generic available)

GI=Gastrointestinal, CNS=Central Nervous System

as normal flora or contaminants and no further work-up was done. Now with more immunocompromised patients, clinical laboratories are reporting most isolates that occur in culture. This has created an enigma in clinical medicine and one must be current with the literature to determine if certain fungal isolates are pathogenic. The greatest problem herein is that some fungal names (genera and species) change with great frequency (every fourth year), and a literature search for the isolate reported by the laboratory may not yield adequate information to support serious consideration. Secondly, the sexual and asexual stages of the fungi have different names albeit they are the same microorganism. As gene sequencing becomes widely available, this conundrum of name changes due to re-classification of genera and species based on DNA phylogeny is sure to increase in the upcoming years. It is important that anytime an unusual fungus is reported on a specimen, that a microbiologist be consulted as to determine the clinical significance of the isolate. This includes yeast, yeast-like, and filamentous fungi. Genetic technology has revealed that many fungi that were previously reported to genus and species were indeed misidentified, leading mycologists to re-examine past cases to determine the prevalence of certain fungi in certain disease states [37-43].

Antifungal testing is a delicate, difficult, and often disappointing endeavor. Although the Clinical and Laboratory Standards Institute (CLSI) has taken great strides to publish both techniques and interpretive standards for the susceptibility testing of yeasts [44-47] and filamentous fungi [12], only a few laboratories offer this technology. The difficulty with minimum inhibitory concentration (MIC) interpretations is that there are very few outcome studies performed whereby MIC standardized susceptibilities were available on fungal isolates. This has limited

our efforts to establish reliable standard interpretive criteria and much is still unresolved toward improvements this area.

Antibiotic Stewardship of FRS

Treatment of FRS can be very challenging. The patient may have colonization or invasive infection with chronic or acute FRS and may require aggressive surgical debridement with antifungal treatment. Invasive FRS needing treatment is even more complex. The host is typically immunocompromised, the diagnosis elusive, and the identification prolonged. Because of these facts, patients considered having invasive FRS, based on risk factors and clinical presentation should be empirically started on broad-spectrum intravenous (IV) antifungal therapy, while identification is being done. In most cases, IV amphotericin B products should be selected. Once identification of the isolate is known, or if specific fungi can be ruled out, then other antifungal agents could be considered to optimize coverage, route of administration, toxicities, efficacy, or cost [48-53].

Antifungal agents discussed here target the fungal cell membrane (azoles and polyenes) or cell wall (echinocandins) [49]. There are other sites targeted by antifungal agents, but these agents are typically not used as monotherapy or in treatment of FRS. **Table 1** includes commonly used systemic antifungal agents in the hospital or outpatient setting. Some of these antifungal classes share similar side effects or considerations, whereas others have their own specific concerns. For example, all triazoles can affect QTc measurements (all prolong, except isavuconazole, which shortens) and have many drug-drug interactions. Whereas, caution must be displayed when deciphering itraconazole

capsules or tablets versus the suspension, since gastric acid affects the absorption (capsules/tablets should be taken with food, but the suspension is preferred and should be taken on an empty stomach) [49, 50].

Good antifungal stewardship practices should be utilized when possible. Once the fungus has been identified, the antifungal may be narrowed. However, many considerations need to be made and a multi-disciplinary approach should be used for medication transitions. Susceptibilities should always be checked since resistance by many fungi has been observed [49, 50, 56]. A clinical pharmacist, specifically trained in infectious disease if available, can assist the team in selecting the best option based on drug-drug interactions, common side effects or toxicities, absorption concerns, dosing (using pharmacokinetic and pharmacodynamics parameters), cost, and potentially therapeutic antifungal monitoring goals. Good antifungal stewardship with a multi-disciplinary approach is important because it helps ensure an optimized antifungal regimen that is both safe and efficacious.

Summary

We presented four human case reports of fungi that cause FRS. All four cases originated at our Veterans' hospital within a two-year period. The diagnosis and therapy in all four cases required a full spectrum of professional contributions: Infectious Disease Specialists, Pathologists, Microbiologist, and Clinical Pharmacists. It is important for ENT specialists and other disciplines of medicine to recognize that the fungi of FRS are complex microorganisms that encompass a wide variety of species (pathogenic and formerly non-pathogenic), and they are very difficult to recognize and identify. The current technology to identify fungi is lengthy, and optimum patient therapy is often delayed. Likewise, the host range of immunocompetent and immunocompromised individuals further complicates the clinical presentation of their various disease states. Antifungal therapy for FRS is vague in the literature, thus antimicrobial stewardship is important for patient care. The toxicity of antifungals is of significant concern and requires the skills of both Infectious Disease specialists and Pharmacists to establish the best dosing regimen. We briefly reviewed and summarized the contributions of many other authors to show the extensive amount of work already done in this area. There is yet much to be accomplished before FRS can be fully appreciated as a cause of significant morbidity and mortality. We attribute our success in this area to the willingness of our professional staff to work closely together and overcome the many challenges that are faced when a patient presents with FRS.

Acknowledgement: Much of this work was performed at the Robley Rex VA Medical Center, Louisville, KY.

Funding Source: No funding source was reported.

Conflict of Interest: All authors declared no conflict of interest in relation to the main objective of this work.

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