An Update on Fungal Rhinosinusitis
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Outline
- Evaluation of sinonasal ethmoidectomy specimens.
- Overview of Sinonasal fungal diseases
  - Fungal ball
  - Allergic fungal sinusitis
  - Acute invasive fungal sinusitis including methods for fungal detection by in situ hybridization

Sinonasal Endoscopic Surgery
Fungal Rhinosinusitis: University of Pennsylvania Experience

- 400 total patients
  - Invasive
    - Acute invasive: 44 patients
    - Chronic invasive: 4 patients
    - Chronic Granulomatosis: 1 patient
    - Mixed CIGFRS/FB: 1 patient
  - Non-invasive
    - Fungal Ball: 161 patients
    - Mixed AFS/FB: 8 patients
    - Allergic fungal rhinosinusitis: 180 patients
    - Eosinophilic mucin without histologic and/or culture evidence of fungus: an additional 128 patients
  - Total: 521 patients

Grossing Sinoethmoid Contents

- Measurement in all dimensions
  - Color, consistency, shape
  - Is there bone/cartilage? - May require DCAL
  - Are any special stains needed?
  - All tissue should be submitted for evaluation
    - Why?
      - Tumors can be focal
      - Infections can be focal
      - Benign lesions may harbor malignancies or be a result of a malignant sinonasal process
Histologic Examination of Sinonasal Specimens

- Non-neoplastic versus neoplastic
- If non-neoplastic – what kind of inflammation?
  - Acute
  - Chronic – eos/no – eos
  - Is there vasculitis?
  - Are there organisms?
  - Other: amyloid etc?
- If neoplastic
  - Benign versus malignant versus uncertain
  - What type of malignancy?
    - Carcinoma, melanoma, lymphoma, sarcoma

SINONASAL FUNGAL DISEASE UPDATE

What is Sinonasal Fungal Disease?

- A heterogeneous group of disease entities caused by fungi affecting the nasal cavities and the sinuses
- Allergic type is a significant but often under recognized form of chronic sinonasal disease.
- May be unsuspected clinically – therefore pathologist plays an important role in diagnosis
**Fungal Rhinosinusitis: History**

- 1791: Plaignaud
- 1897: Oppe
  - Aspergillus sinusitis with invasion into brain
- 1965: Hora
  - Noninvasive
  - Invasive
- 1976: Safirstein
  - Miller/Katzenstein
  - AFRS
- 1980: McGill
  - Fulminant

**2009 ISHAM Classification: Fungal Rhinosinusitis: Terminology**

- Invasive
  - Acute invasive (fulminant)
  - Granulomatous invasive
  - Chronic Invasive
- Non-invasive
  - Localized fungal colonization of nasal or paranasal sinus mucosa (Saprophytic fungal infestation)
  - Fungus Ball
  - AFRS

**FRS: Geography**

- US
  - Non-invasive disease most common (large studies from here, Chicago, Texas) with AFRS and FB varying with geography
  - Invasive disease most commonly AIFRS
- India: Non-invasive still most common (but frequent than US)
  - FB rare
  - Invasive disease about 40% of cases with CIGFRS and CIFRS most common
- France:
  - Almost 80-90% of FRS
FRS: Geography

- Type of organism isolated varies
- FB:
  - Aspergillus most common worldwide
- AFRS:
  - Dematiaceous species most common in Southwest and South
  - Almost equal incidence of Aspergillus and Dematiaceous fungi here and in midwest
  - Aspergillus elsewhere
- CIFRS: A. flavus
- CIFRS: Aspergillus most common
- AIFRS: Aspergillus and Rhizopus most common

Fungal Ball

- AKA: Mycetoma (not a desirable term)
- Clinical:
  - 3-4% of sinusitis requiring surgery
  - Middle age to elderly females
  - Maxillary sinus
  - Risks: previous surgery, oral-sinus fistula, previous chemotherapy
- Pathology:
  - Dense accumulation of hyphae
  - Non-invasive
  - Cultures often negative – most common Aspergillus sp.

Fungal Ball: Microbiology

Single Isolate (45 patients (88%))

- Aspergillus sp.
  - A. fumigatus 48%
  - A. flavus 14%
  - A. NOS 7%
- Dematiaceous species
  - Alternaria sp. 5%
  - Fusarium sp. 5%
  - Trichophyton sp. 2%
- Other 17%
  - Candida albicans 2%
  - Mucor sp. 2%
  - Non-spore forming mold 3%
  - Paecilomyces sp. 5%
  - Trichophyton sp. 3%

- Comparable to other studies (Willinger et al: J Clin Microbiol)
Allergic Fungal Rhinosinusitis (AFRS)

- Non-invasive fungal sinus representing an allergic/immunologic response to fungus in the sinonasal tract.
- Not a true fungal infection but an allergic reaction to fungal antigens

AFRS

- Clinical
  - 5-10% of patients with chronic rhinosinusitis M:F = 1.6:1
  - Young (23-42 yo) immunocompetent, atopic/asthmatic adult with CS, particularly unilateral, refractory to medical therapy including antibiotics
  - Other symptoms: nasal polyposis, orbital proptosis, mucocele, diplopia
  - Elevated IgE to fungal antigens (90%)
  - Asthma (50%)
  - Geography: variable locations; high humidity - ? Increase in Mississippi basin, Southeast and Southwest

AFRS: Historical Perspectives

- Safirstein – 1976: polyposis, crust formation, Aspergillus
- Millar - 1981
- Katzenstein – 1983
  - Allergic Aspergillus Sinusitis
  - ABPA
  - Eosinophilic mucin
    - Triad of eosinophils, Charcot Leyden crystals, fungus
  - Associations: nasal polyposis, asthma/astopic sx, sinus involvement, poor response to medical management, multiple surgeries
  - Other organisms such as dematiaceous fungi implicated over the years
- Robson - 1989 - coined "AFS"
Pathologic Criteria: Shubert, *Drugs* 64(4): 363, 2004
- Eosinophilic (allergic) mucin
- Either histologic evidence of fungus in mucin or fungal culture positive
- No other fungal disease
- CS with eos

What is Allergic Mucin?
- AKA: Eosinophilic Mucin
- Gross:
  - Thick, inspissated mucous
  - Variable gross appearance: white, tan, yellow, brown-green, green-black
  - Consistency of clay/peanut butter, cottage cheese
- May be seen in patients without AFRS (?atopic, aspirin, aspirin intolerance
Allergic Mucin: Microscopic

- Eosinophilic to basophilic
- Lamellated/layering
- Mucin containing degenerated cells: eosinophils, neutrophils, epithelial cells
- Charcot-Leyden crystals
- By definition, allergic mucin is required for a diagnosis of AFS
- Fungal forms
Acute Invasive Fungal Rhinosinusitis

- AKA fulminant/necrotizing fungal rhinosinusitis
- Clinical
  - < 4 weeks duration
  - Usually immunocompromised especially bone marrow transplant patients
  - Symptoms: paranasal anesthesia/fever
  - Small lesion – rapidly progresses to involve sinus/face/orbit
- Pathology
  - Aggressive, tissue and vascular invasive fungal disease.
  - Zygomycetes/Aspergillus; rarely others
  - Frozen sections often required
Olfactory Nerve

CULTURES ARE NEGATIVE >30% OF THE TIME AND MORPHOLOGY IS NOT ALWAYS PERFECT – IS THERE ANY THING WE CAN DO? – LOOK AT rRNA

rRNA Definition
- Central component of ribosomes involved in protein (polypeptide) synthesis
- All Prokaryotes and Eukaryotes contain rRNA.
- rRNA are the most conserved (least variable) nucleic acid sequences
  - Genes encoding rRNA (rDNA) are sequenced to identify an organism’s taxonomic group
  - rRNA analysis is used for infectious pathogen detection
- RNA’s role in the ribosome may support the idea that life on Earth began with RNA.
Why have an in situ method for fungi?

- Cultures may grow slowly/or not at all especially for fungal and mycobacterial diseases
- An infectious agent may be histologically seen in tissue but cultures are negative or were not performed at the time of surgery.
  - Treatment may be significantly different for different pathogens
  - Amount of material obtained in a procedure may be limited prohibiting further molecular testing

Routine histochemical stains cannot speciate

Can we identify rRNA Sequences in Routinely Processed Tissues?

- Human (primate) 28S rRNA sequences
Can ISH for rRNA be Useful for Diagnostic Pathology?
Brain Biopsy with Aspergillus sp.
(Culture positive for A. fumigatus)
Coccidiomycosis

Candida sp. – culture negative

Zygomycetes sp. – Culture negative
Fusarium sp.

Zygomycetes sp.

Aspergillus sp.
ISH can confirm mixed fungal infections

Brain Biopsy with mixed infection of A. fumigatus and Scedosporium apiospermum
Acute Necrotizing Fungal Rhinosinusitis: ISH Study

- 25 patients (60 specimens)
  - 6 Rhizopus sp
  - 9 Aspergillus sp
  - 1 Fusarium sp,
  - 1 Alternaria sp
  - 1 Paecilomyces sp
  - 7 negative cultures.
- Rapid (<3 hours) ISH with species specific biotin-labeled oligonucleotide DNA probes targeting rRNA.
- Preservation of fungal rRNA was determined using a pan-fungal rRNA probe.
Rhizopus

Aspergillus

Aspergillus sp.: Culture negative
Culture negative
ANIFRS:
Dematiaceous
species +; *Aspergillus
sp. -

Fungal Rhinosinusitis: Summary
- Most cases (90%) of fungal rhinosinusitis are non-invasive in US.
- Acute (fulminant) fungal rhinosinusitis is the most common type of invasive fungal rhinosinusitis in US
- *Aspergillus sp.* is a major cause of non-invasive and invasive fungal sinusitis
- Rapid ISH techniques can improve fungal detection in IFS patients