Are fungi responsible for chronic sinusitis?

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Definition of chronic rhinosinusitis

- **Inflammatory disease** of nose & paranasal sinuses
- Inflammatory changes detected on endoscopy / CT images
- **Duration** – at least 12 **weeks** without complete resolution
- Symptoms – nasal blockage, nasal discharge, facial pain, ± reduced sense of smell

Fokkens et al., Rhinology suppl, 2007; 45: 1-139
Etiology of CRS

Till one decade back
- Bacteria implicated as pathogen in most form of CRS
- Fungi may be responsible for few specific forms

- Hell broke!
- Claimed that fungi are responsible for nearly all cases of CRS
- Demonstrated the presence of fungi & eosinophils from nose & PNS from ~100% cases of CRS
- Coined the term ‘Eosinophilic fungal rhinosinusitis (EFRS)’
- Created intense debate about the role of fungi
Etiology of CRS

• Several types of sinus diseases have been attributed to the presence fungal organisms in nasal & sinus cavities
  – Invasive: acute invasive, chronic invasive, granulomatous invasive
  – Non-invasive: localized fungal colonization, fungal ball, fungus related eosinophil rhinosinusitis (AFRS)
# Fungus related eosinophilic CRS

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>AAS</td>
<td>AFS</td>
<td>AFS</td>
<td>EFRS</td>
<td>EMRS</td>
</tr>
<tr>
<td>Presence of fungi</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Type I hypersensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic or eosinophilic mucin</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Imaging consistent with CRS</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal polyposis</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
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</tr>
</tbody>
</table>
More new terms coined

Depending on presence of fungal allergy or fungus

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAFES</td>
<td>Non Allergic Fungal Eosinophilic Sinusitis (Ferguson, 2004)</td>
</tr>
<tr>
<td>CFS</td>
<td>Chronic Fungal Sinusitis (Collins et al 2003, 2004)</td>
</tr>
<tr>
<td>AFS like</td>
<td>Absence of fungi in mucin, but have fungal allergy (Collins et al 2003, 2004)</td>
</tr>
<tr>
<td>NANFES (CES)</td>
<td>Non Allergic, Non Fungal, Eosinophilic Sinusitis (Chronic eosinophilic sinusitis)</td>
</tr>
<tr>
<td>EMCRS</td>
<td>Eosinophilic Mucus Chronic Rhinosinusitis (Pant et al, 2005)</td>
</tr>
</tbody>
</table>
## Fungus rhinosinusitis – our experience

<table>
<thead>
<tr>
<th></th>
<th>1990-91 (2)</th>
<th>1992-96 (5) excluding AFRS</th>
<th>1997-98 (2)</th>
<th>2006-07 (1.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of cases</strong></td>
<td>50</td>
<td>176</td>
<td>25</td>
<td>105</td>
</tr>
<tr>
<td><strong>AFRS /EFRS (%)</strong></td>
<td>4</td>
<td>7</td>
<td>-</td>
<td>61</td>
</tr>
<tr>
<td><strong>Fungal ball (%)</strong></td>
<td>62 (classified as non-invasive)</td>
<td>46</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td><strong>Chronic invasive / granulomatous (%)</strong></td>
<td>30</td>
<td>31</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td><strong>Acute invasive (%)</strong></td>
<td>4</td>
<td>7</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td><strong>Destructive- non-invasive (%)</strong></td>
<td>Not known</td>
<td>9</td>
<td>48</td>
<td>-</td>
</tr>
<tr>
<td><strong>Mixed (AFRS + Granulomatous) (%)</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>
Possible mechanism of CRS

- Alteration of certain aspects of acquired &/ or innate immunity
- ↓ Mucociliary clearance
- Factors promoting mucous stasis & tissue edema
- Concurrent or preceding viral or bacterial infection
- Immune response elicited by superantigens
- Biofilm formation
- Allergy
- Antibiotic therapy &/ or topical steroid therapy
- Concentration of ambient mold
Examination of the fungus case to cause CRS

- Prevalence & distribution of fungi in CRS
- Allergy to fungi
- Fungal specific humoral response
- Cellular immune response – cytokine response
- Innate immunity
- Response to antifungal treatment
## Prevalence of fungi – CRS patients vs. healthy control

<table>
<thead>
<tr>
<th>Factor</th>
<th>Any significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection of fungi (PCR, culture, fluorescent labeled chitinase stain)</td>
<td>No significant difference (plenty of studies)</td>
</tr>
<tr>
<td>Fungal species &amp; fungal load</td>
<td>No significant difference (Ponikau, 1999; Buzina, 2003; Kim, 2005; Murr, 2006)</td>
</tr>
<tr>
<td>Fungal DNA level</td>
<td>No significant difference (Scheuller, 2004)</td>
</tr>
<tr>
<td>Allergen content</td>
<td>Not elucidated yet (in respiratory tract germination of spore in presence of mucus produce more allergen – Mitakakis, 2001)</td>
</tr>
</tbody>
</table>

Ebbens et al., 2007 & 2009
Geographical distribution of fungi in AFRS

PGI, India study 2006-08

- A. flavus: 90%
- Other Aspergillus sp.: 6%
- Dematiaceous fungi: 3%
- Other fungi: 1%

USA study

- Other fungi: 87%
- A. flavus: 13%

Manning & Holman, Laryngoscope 1998; 108: 1485
## Allergy to fungi & other factors in CRS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PCR +ve (%)</th>
<th>PCR –ve (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchial asthma</td>
<td>44</td>
<td>22</td>
<td>.121</td>
</tr>
<tr>
<td>Aspirin hypersensitivity</td>
<td>33</td>
<td>17</td>
<td>.238</td>
</tr>
<tr>
<td>Nasal polyposis</td>
<td>89</td>
<td>87</td>
<td>.851</td>
</tr>
<tr>
<td>Skin test for fungi</td>
<td>56</td>
<td>75</td>
<td>.399</td>
</tr>
<tr>
<td>Eosinophilia in nasal smear</td>
<td>73</td>
<td>56</td>
<td>.290</td>
</tr>
<tr>
<td>Serum eosinophilia</td>
<td>43</td>
<td>30</td>
<td>.440</td>
</tr>
<tr>
<td>High level of total IgE</td>
<td>44</td>
<td>47</td>
<td>.916</td>
</tr>
<tr>
<td>Fungal specific IgE positivity</td>
<td>10</td>
<td>31</td>
<td>.190</td>
</tr>
<tr>
<td>Sneezing</td>
<td>94</td>
<td>87</td>
<td>.410</td>
</tr>
</tbody>
</table>

Fungal allergy in support of CRS

- High proportion of AFRS patients have allergies to common environmental mold & serum↑IgE
  (Laryngoscope, 1994; Otolaryngol Head Neck Surg, 1997)
- Allergen-specific ↑IgE locally in polyp tissue that could not be detected systematically (Ann Allergy, 1985)
- Local fungal specific IgE in sinus mucin (Laryngoscope, 2004)
- Two small studies suggest immunotherapy is beneficial in AFRS (Otolaryngol Head Neck Surg, 1997; 2001)
- Rabbit model – allergic sensitivity & sinus obstruction - additive effect (Annals Otol Rhinol Laryngol, 2006)
Fungal-specific humoral response

A. alternata specific IgE
A. alternata specific IgG3

Pant et al. Laryngoscope 2005; 115, 601

- Specific IgE present in 18-75% of CRS from various studies
- Most likely, the presence of type I hypersensitivity to fungi represents concurrent fungal allergy in majority of CRS
Enhanced cytokine response to fungi

Shin et al.
J Allergy Clin Immunol 2004; 114: 1369
Enhanced immune response to fungi

Shin et al.
J Allergy Clin Immunol 2004; 114: 1369
Innate immunity vs fungi

- Proteases from fungi bind PAR on epithelial, airway cell, blood vessels etc. → release of cytokine, chemokine, eicosanoids, metalloproteinases → disruption of epithelial tight junction (J Allergy Clin Immunol 2004)
- Not clear whether genotypic difference in PAR expression can explain the difference of CRS patients & healthy controls
- Fungi induce production of inflammatory cytokines IL-6, IL-8 from primary nasal epithelial cells (J Allergy Clin Immunol 2000)
- Fungi directly interacts with eosinophils to produce pro-inflammatory mediators (J Immunol 2008)
Surfactant Protein D in CRS patients

- Low/absence of SP-D cause failure to clear fungi, leads to disease
- SP-D is known to shift cytokine response from Th2 to Th1
- Absence of SP-D in AFRS may explain allergic response

Ooi et al. Laryngoscope 2007; 117: 51
Lactoferrin level in CRS patients

Psaltis et al.
Laryngoscope 2007; 117: 2030
Is fungus a bystander?

• In AFRS, it is proposed that fungi produce Ag that stimulates IgE, IgG, & IgA production

• It is known that in AFRS (like ABPA) – a Th2 mediated eosinophilic reaction

• Once initiated, Ag independent permanent phase (Clin Rev Allergy Immunol, 2006)

• But what triggers its pathway?
  – role of allergen, fungus derived Ag, bacteria, bacterial super antigens are proposed
  – specific IgE to Staphylococcal enterotoxin present in 60% nasal polyp & 80% nasal polyp with asthma (J Allergy Clin Immunol, 2001)

• To prove the role of fungi the requirements are:
  – definite evidence of T cells in sinus responds to fungal Ag
  – removal of fungal Ag ameliorates the disease
Biofilm in CRS

- CRS is polymicrobial infection, which includes planktonic & biofilm formation with bacterial & fungal elements
- Biofilms are integral part of CRS pathology, most notably because of the inherent resistant (both antibiotic & host defense) phenotype associated with biofilm
- Lower incidence of biofilms, more successful outcome
- No correlation between the bacteria in the biofilms and the bacteria isolated in culture (molecular probe detected *H. influenzae* in 80% of CRS patients)
- Fungal element exists in bacterial biofilm, but which fungi – not clear yet
- Detail understanding would help to control CRS

Local & oral antifungal in CRS

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Patients /control (n)</th>
<th>Drug</th>
<th>Method</th>
<th>Study (center)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ponikau et al.</td>
<td>2002</td>
<td>51/0</td>
<td>Amp B</td>
<td>Lavage</td>
<td>Single</td>
<td>Positive</td>
</tr>
<tr>
<td>Ricchettti et al.</td>
<td>2002</td>
<td>74/0</td>
<td>Amp B</td>
<td>Lavage</td>
<td>Single</td>
<td>Positive</td>
</tr>
<tr>
<td>Weschta et al.</td>
<td>2004</td>
<td>28/32</td>
<td>Amp B</td>
<td>Spray</td>
<td>Single</td>
<td>Negative</td>
</tr>
<tr>
<td>Ponikau et al.</td>
<td>2005</td>
<td>10/14</td>
<td>Amp B</td>
<td>Lavage</td>
<td>Single</td>
<td>Positive (CT)</td>
</tr>
<tr>
<td>Kennedy et al.</td>
<td>2005</td>
<td>25/28</td>
<td>Terbinafine</td>
<td>Oral</td>
<td>Single</td>
<td>Negative</td>
</tr>
<tr>
<td>Helbling et al.</td>
<td>2006</td>
<td>21/0</td>
<td>Amp B</td>
<td>Spray</td>
<td>Single</td>
<td>Negative</td>
</tr>
<tr>
<td>Ebbens et al.</td>
<td>2006</td>
<td>59/57</td>
<td>Amp B</td>
<td>Lavage</td>
<td>Multicenter</td>
<td>Negative</td>
</tr>
<tr>
<td>Liang et al.</td>
<td>2008</td>
<td>32/32</td>
<td>Amp B</td>
<td>Lavage</td>
<td>Single</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Problems in antifungal therapy

- Reports showed systemic & topical antifungal therapies give temporary relief in AFRS of sinus inflammation & polyps (Am J Rhinol, 2003)
- However, no report of long term cure of CRS, nasal polyposis or AFRS with antifungal therapy

- **Systemic therapy**? whether therapeutic levels maintained in nasal secretion (Laryngoscope, 2005)
- **Local therapy** (especially amphotericin B) – the drug disrupts the integrity of epithelial monolayer, resulting in cell death, ↓ transepithelial resistance, & loss of tight junction (Rhinology, 2004)
Conclusions
The case for fungus – unproven (more questions than answers)

- Fungus can cause a variety of conditions in the nose & paranasal sinuses, partly competency of host’s immune system determines severity
- Fungi & eosinophil can be detected in nearly all CRS patients (However, fungi also present in healthy controls)
- Many mechanisms may be involved for the fungi to cause disease in those individuals (more research required!)
- Definite geographical variation exists in fungi causing CRS & allergy
- Antifungal therapy appears to be beneficial in selected group of patients like AFRS (but the effect is not permanent)
What future holds for us?

• More research required
• Most likely there are multiple pathways involved including the effects of fungi, viruses, & bacteria
• The role of fungi –
  – Which fungi?
  – Which component of fungi?
  – Which individuals are susceptible?
  – What immunological response to fungi?
• Antifungal therapy? Beneficial
  – Controlled clinical trials are required