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

Since 1999 (Ponikau *et al*. Mayo Clinic Proc 1999; 74: 877)

- 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

Criteria	Katzenstein, 1983	Bent & Kuhn, 1994	DeShazo, 1997	Ponikau, 1999	Ferguson, 2000
	AAS	AFS	AFS	EFRS	EMRS
Presence of fungi	√	√	√	√	
Type I hypersensitivity		√			
Allergic or eosinophilic mucin	√	√	√	√	√
Imaging consistent with CRS	√	√	√	√	√
Nasal polyposis	√	√	√		√

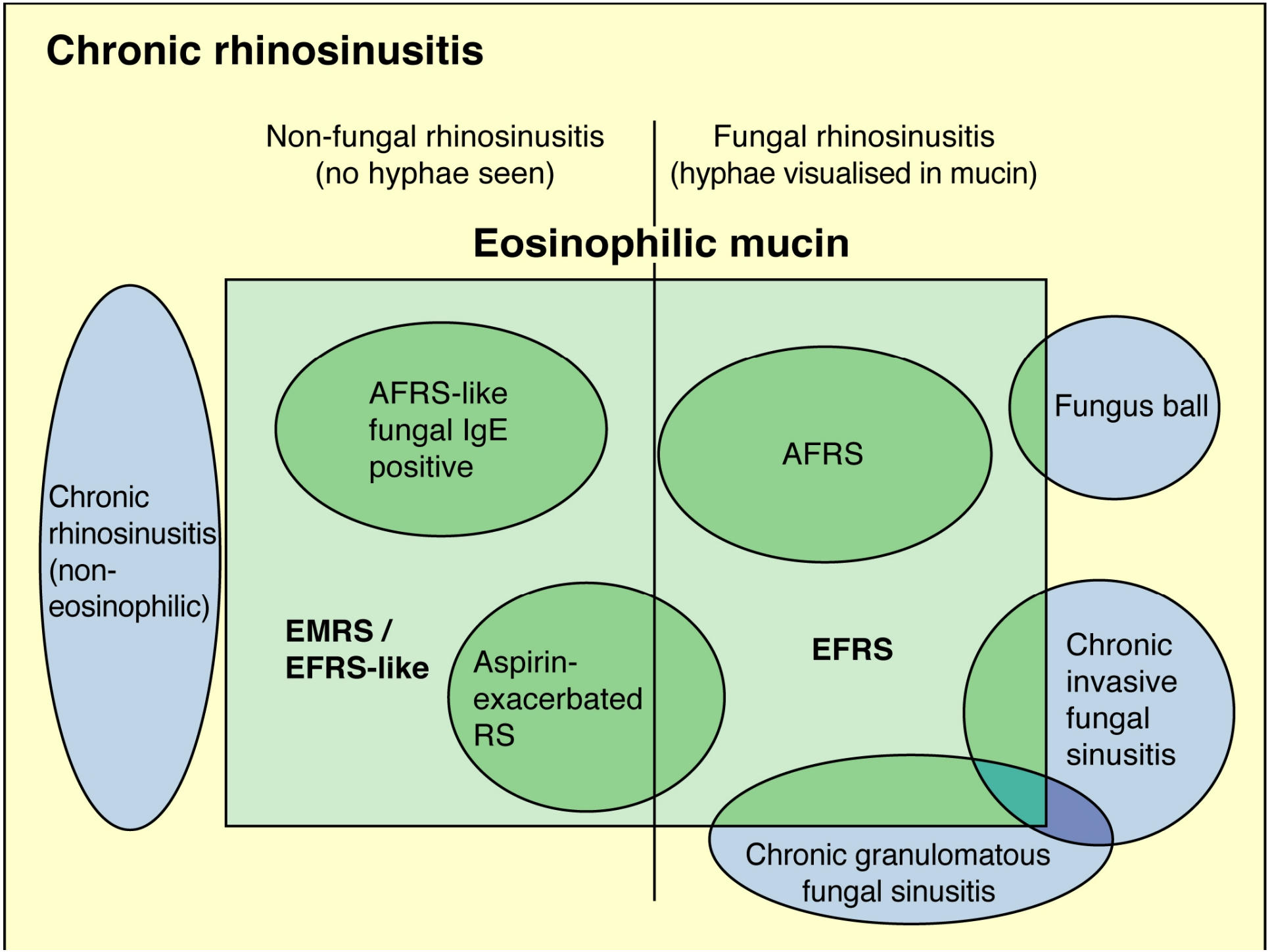
More new terms coined

Depending on presence of fungal allergy or fungus

NAFES	Non Allergic Fungal Eosinophilic Sinusitis (Ferguson, 2004)
CFS	Chronic Fungal Sinusitis (Collins et al 2003, 2004)
AFS like	Absence of fungi in mucin, but have fungal allergy (Collins et al 2003, 2004)
NANFES (CES)	Non Allergic, Non Fungal, Eosinophilic Sinusitis (Chronic eosinophilic sinusitis)
EMCRS	Eosinophilic Mucus Chronic Rhinosinusitis (Pant et al, 2005)

Fungus rhinosinusitis – our experience

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


Chronic rhinosinusitis
Non-fungal rhinosinusitis
(no hyphae seen)
Fungal rhinosinusitis
(hyphae visualised in mucin)
Eosinophilic mucin
Chronic rhinosinusitis (non-eosinophilic)
AFRS-like fungal IgE positive
AFRS
Fungus ball
EMRS / EFRS-like
Aspirin-exacerbated RS
EFRS
Chronic invasive fungal sinusitis
Chronic granulomatous fungal sinusitis

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

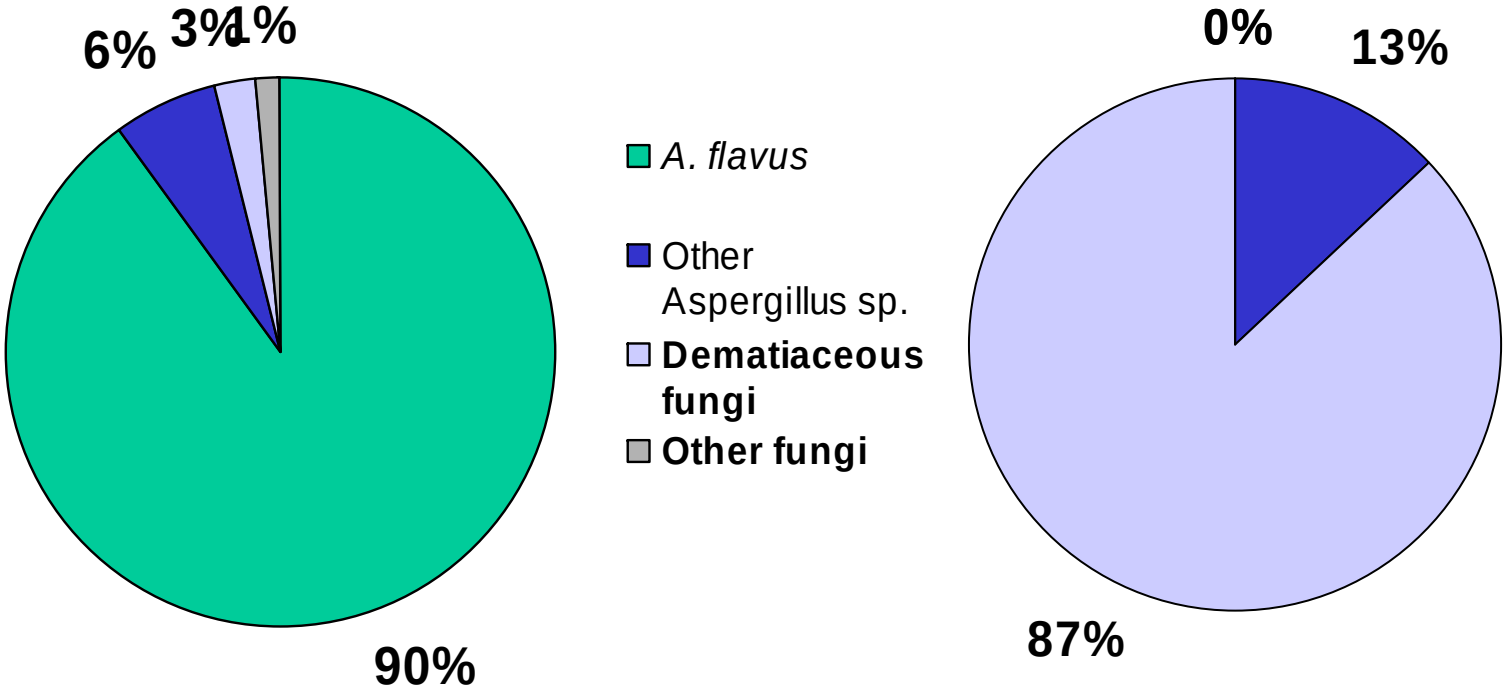
- Prevalence & distribution of fungi in CRS
- Allergy to fungi
- Fungal specific humoral response
- Cellular immune response – cytokine response
- Innate immunity
- Response to antifungal treatment

Prevalence of fungi – CRS patients vs. healthy control

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

Ebbens *et al.*, 2007 & 2009

Geographical distribution of fungi in AFRS



PGI, India study 2006-08

USA study

Manning & Holman, Laryngoscope 1998; 108: 1485

Allergy to fungi & other factors in CRS

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

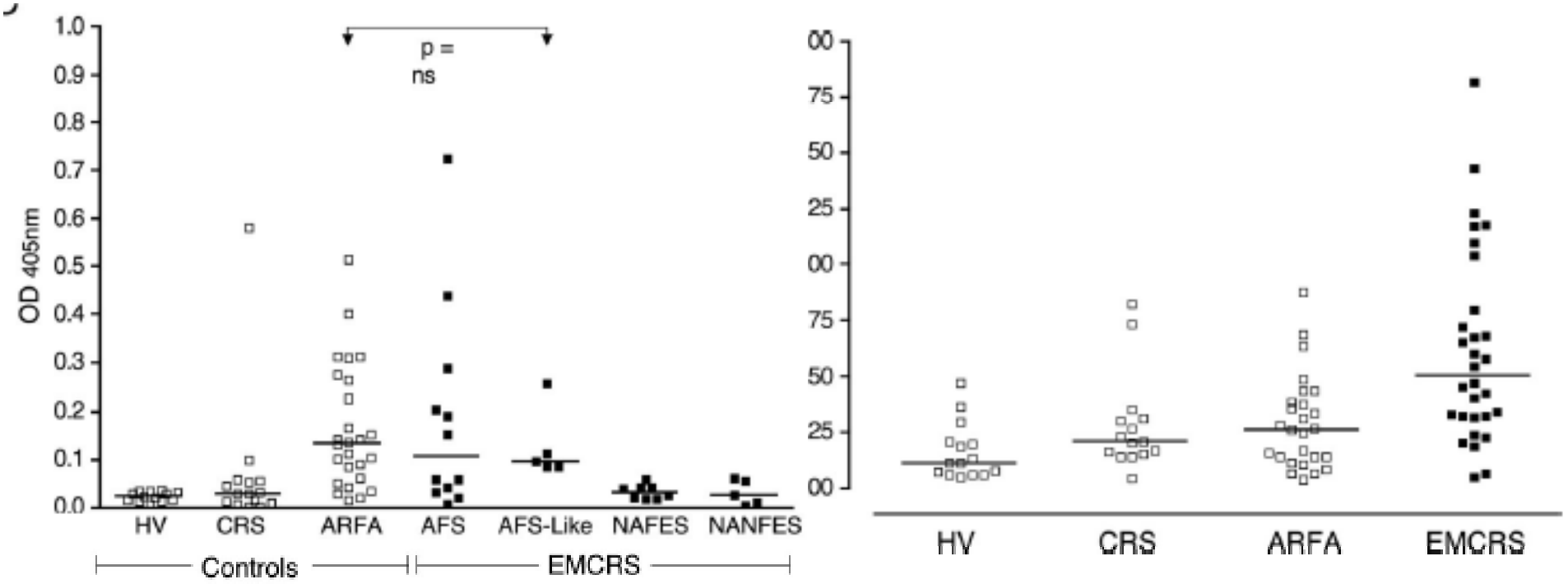
Tosun *et al*. Annals Otol Rhinol Laryngol 2007; 116: 425

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



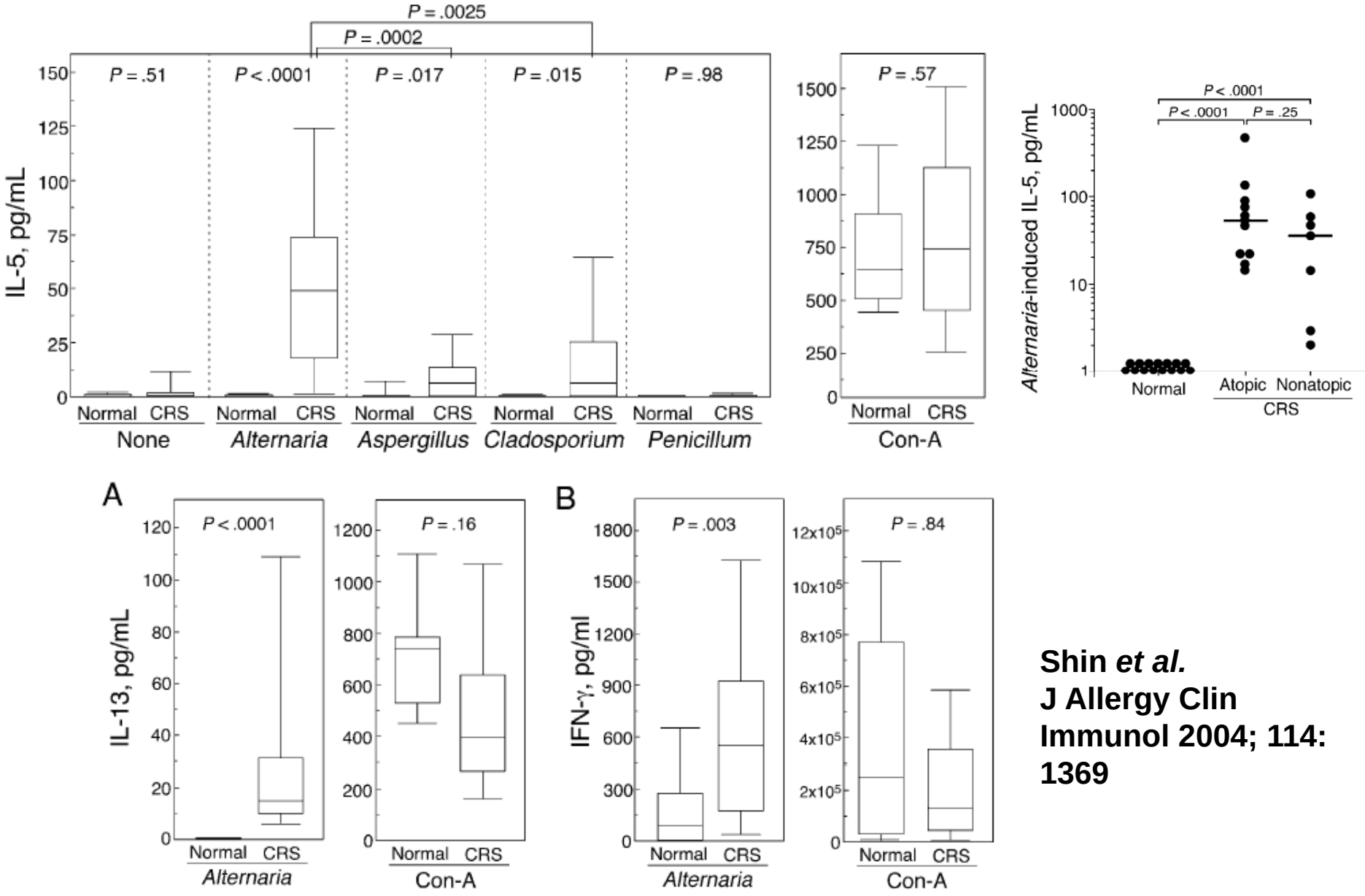
A. alternata specific IgE

A. alternata specific IgG3

Pant *et al*. Laryngoscope 2005; 115, 601

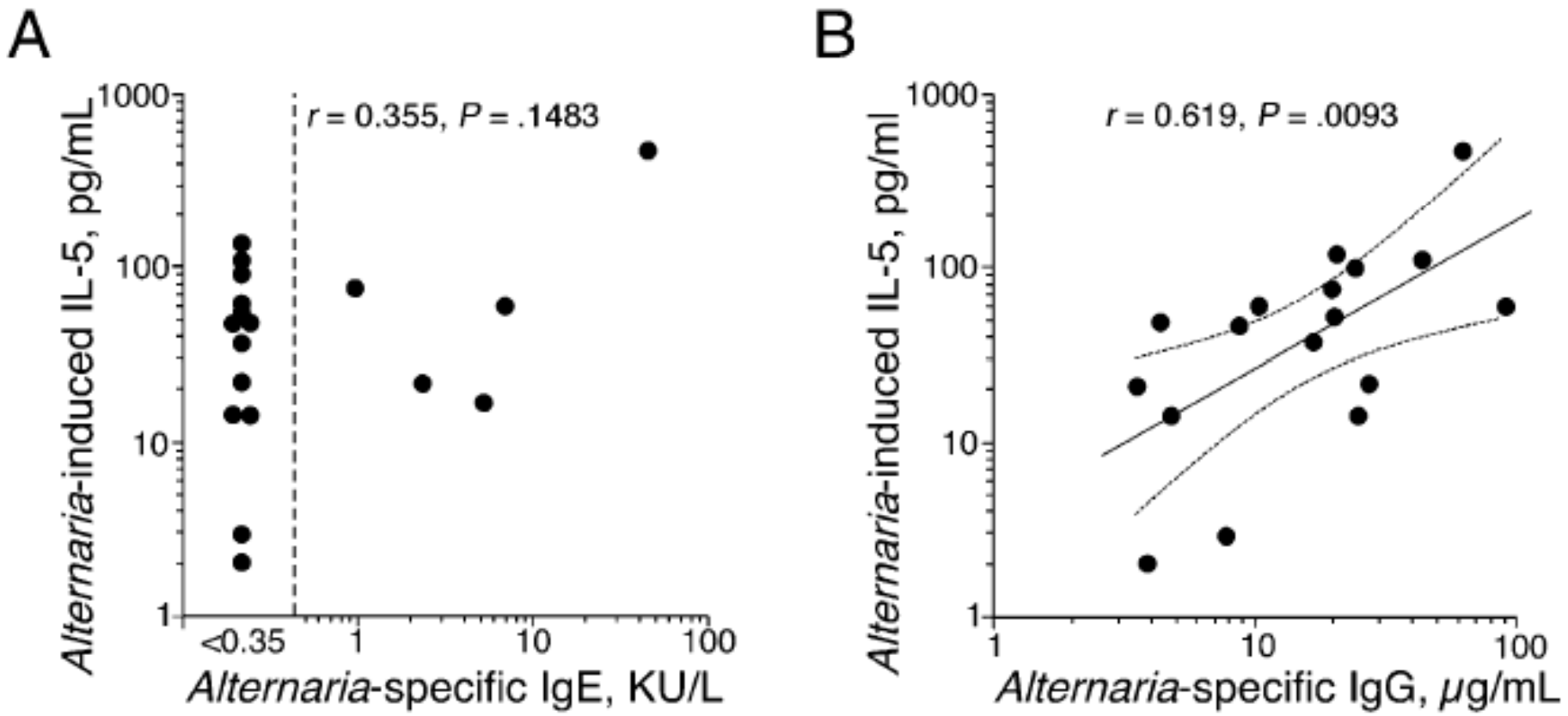
- Specific IgE present in 18-75% of CRS from various studies
- Most likely, the presence of type I hypersensitivity to fungi represents concurrent fungal allergy in majority of CRS

Enhanced cytokine response to fungi



Shin *et al.*
J Allergy Clin Immunol 2004; 114: 1369

Enhanced immune response to fungi

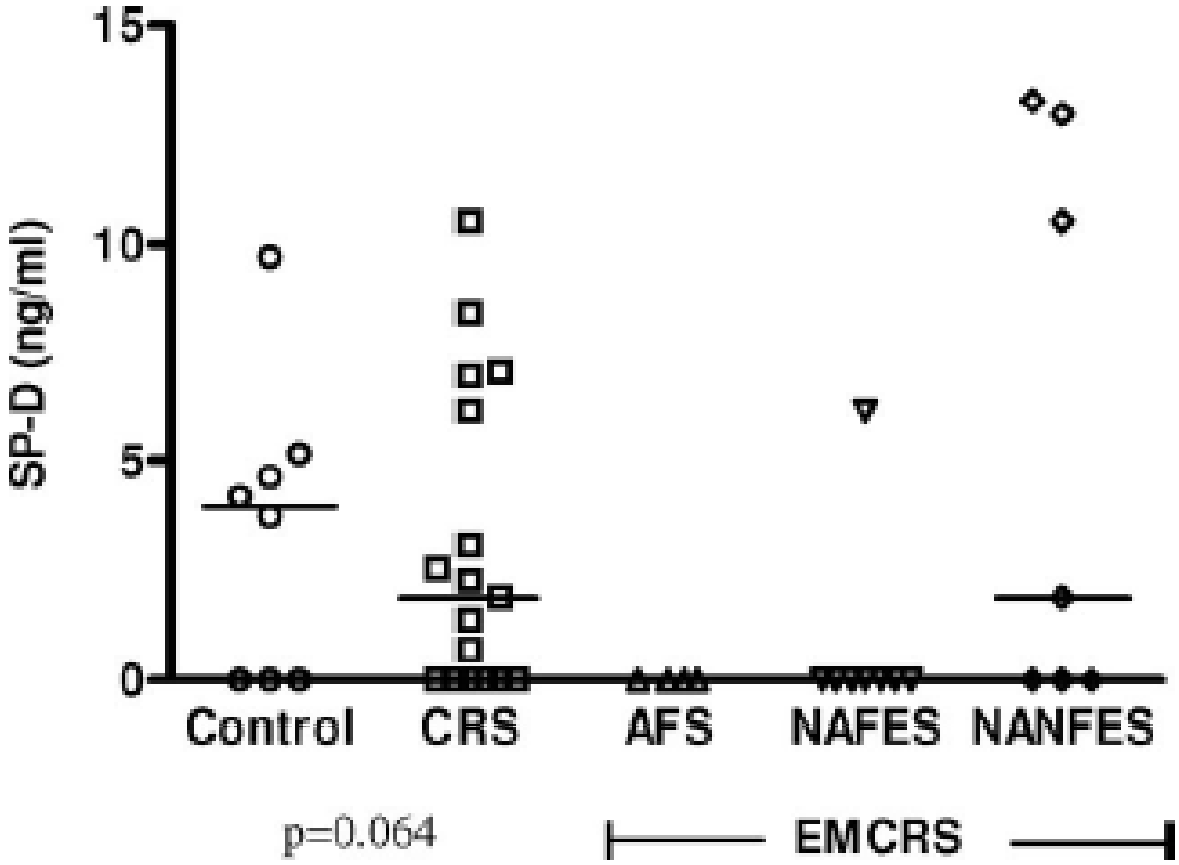


Shin *et al.*
J Allergy Clin Immunol 2004; 114: 1369

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)

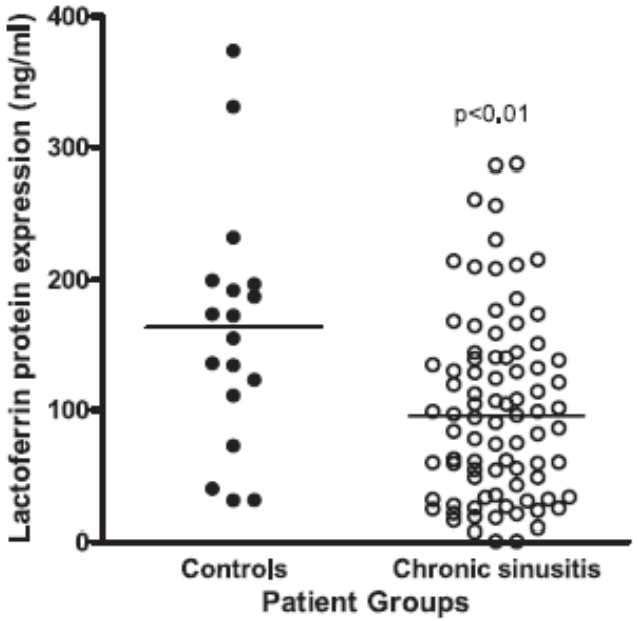
Surfactant Protein D in CRS patients

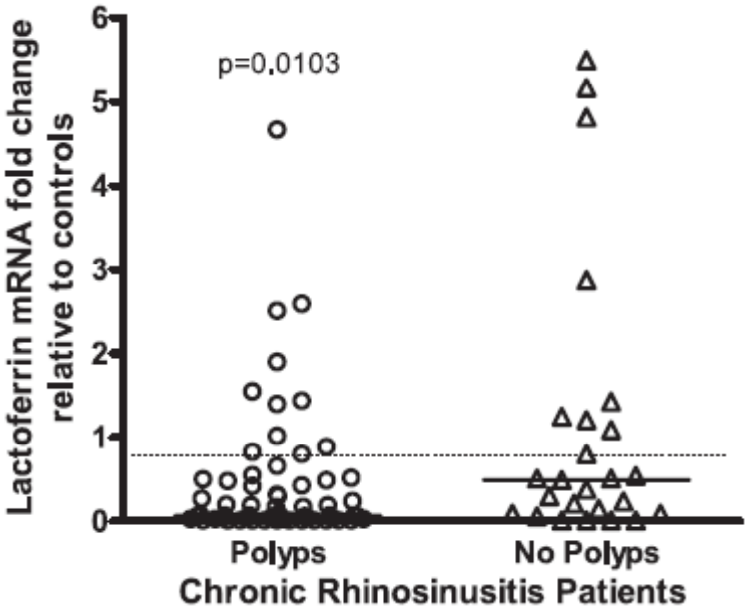


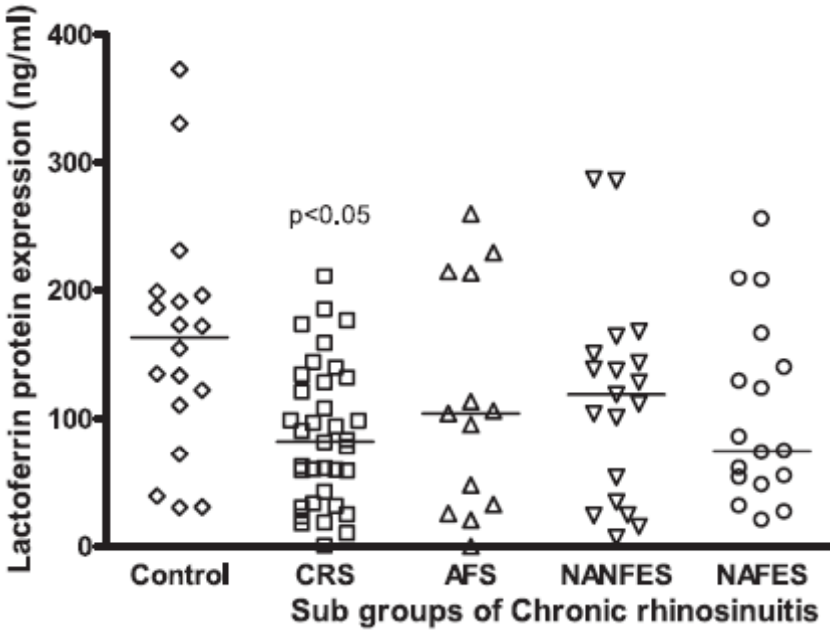
- Low/absence of SP-D cause failure to clear fungi, leads to disease
- SP-D is known to shift cytokine response from Th2 to Th1
- Absence of SP-D in AFRS may explain allergic response

Ooi *et al.* Laryngoscope 2007; 117: 51

Lactoferrin level in CRS patients





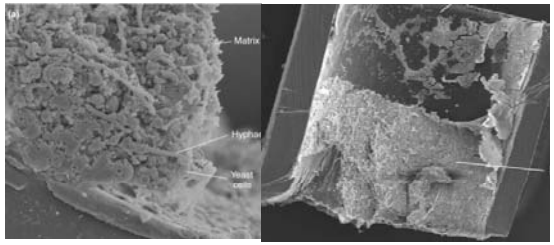


Psaltis *et al.*
Laryngoscope 2007; 117: 2030

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

Hunsaker & Leid. Curr Opin Otolaryngol Head Neck Surg 2008; 16: 237

Local & oral antifungal in CRS

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

Ebbens *et al*. Curr Opin Otolaryngol Head Neck Surg 2009; 17; 43

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

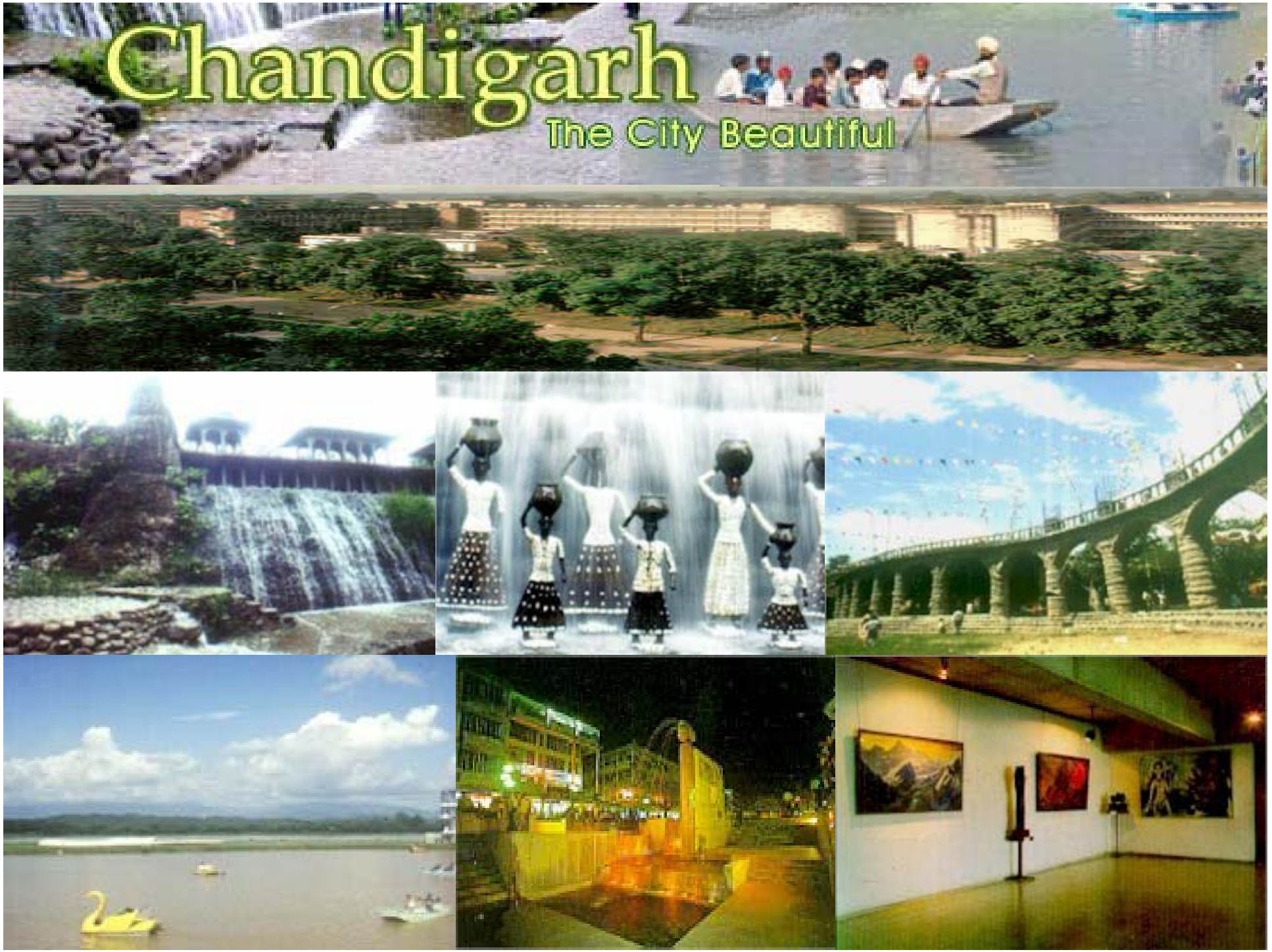
- Systemic therapy ? whether therapeutic levels maintained in nasal secretion (Laryngoscope, 2005)
- Local therapy (especially amphotericin B) – the drug disrupts the integrity of epithelial monolayer, resulting in cell death, ↓transepithelial resistance, & loss of tight junction (Rhinology, 2004)

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


Chandigarh
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