ANTIBODIES OF INTEREST IN MYCOLOGY

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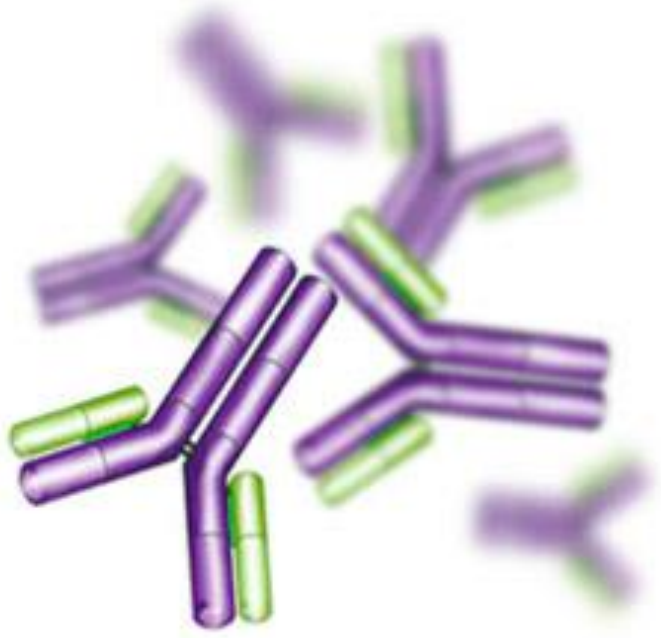
Antibodies of interest in mycology

- Introduction
- Specific IgG
  - Precipitins
  - ImmunoCAP
  - Meaning
  - Specific IgG in diagnosing fungal disease
    - Aspergillosis
    - Hypersensitivity pneumonitis
  - Conclusion
- Specific IgE
  - SPT
  - ImmunoCAP
  - Meaning
  - Specific IgE in diagnosing fungal disease
  - Conclusion
- General remarks



Introduction

- Antibodies = secreted immunoglobulins
- Humoral, adaptive immune system
- Isotypes: IgA, IgD, IgE, IgG and IgM
- Minor role in antifungal immunity…
- … but potential in diagnosing fungal disease?!

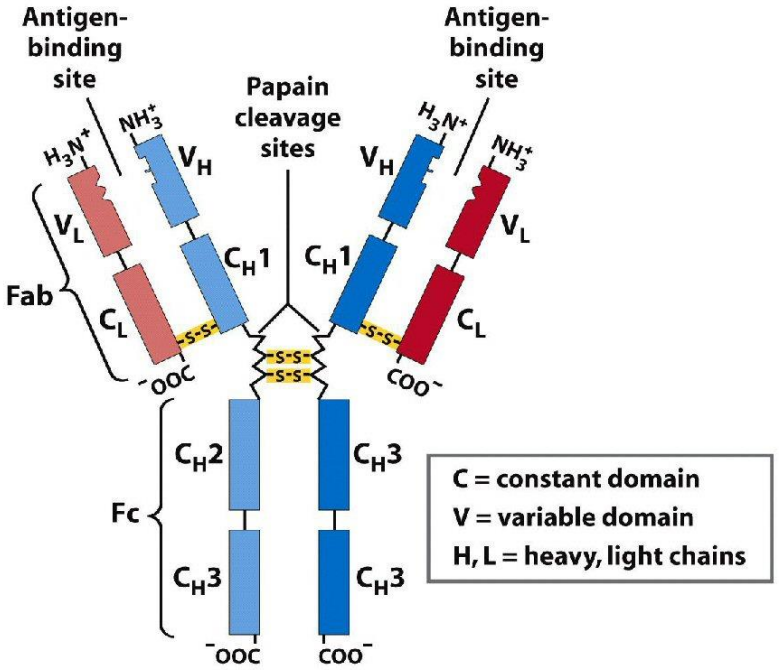
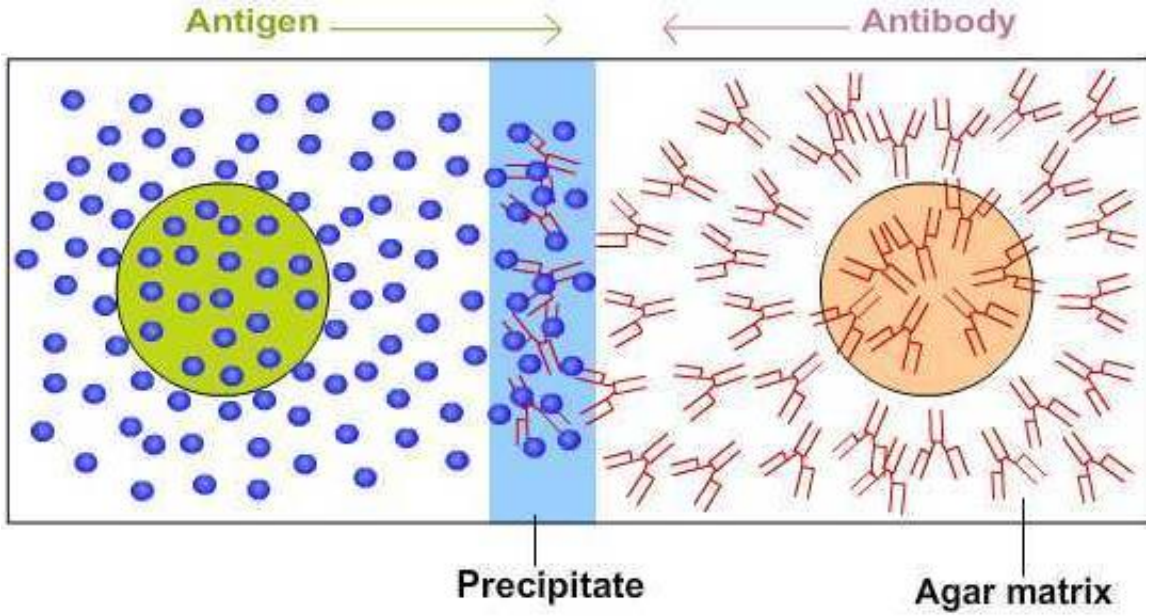


Figure 5-21a
Lehninger Principles of Biochemistry, Fifth Edition

Precipitins

- Passive double diffusion (Ouchterlony)

  All antibodies precipitate



  - Qualitative, time consuming (5d), labour intensive, human interpretation

  \+ no complex/expensive equipment needed

- Counter immunoelectrophoresis

Specific IgG - immunoassay

- Radioimmunoassay RIA

→ 1975 Enzyme linked immunosorbent assay ELISA

Anti-human antibodies

Colour change-optical density

- Specificity low, sensitivity high, cost

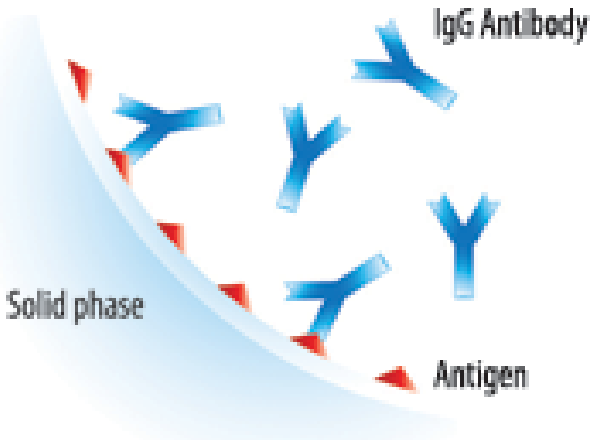
+ quantitative, comparisons

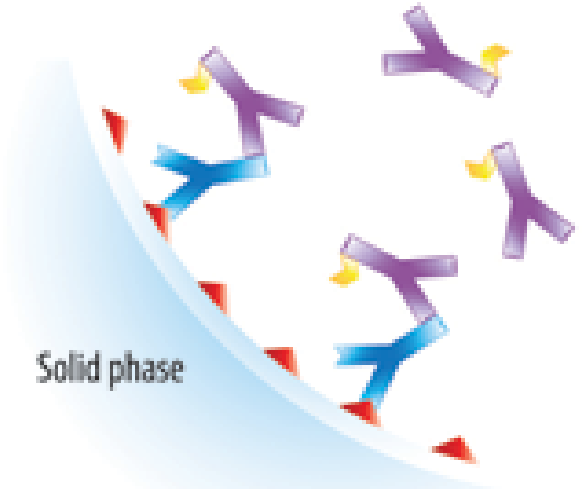
- Automated systems

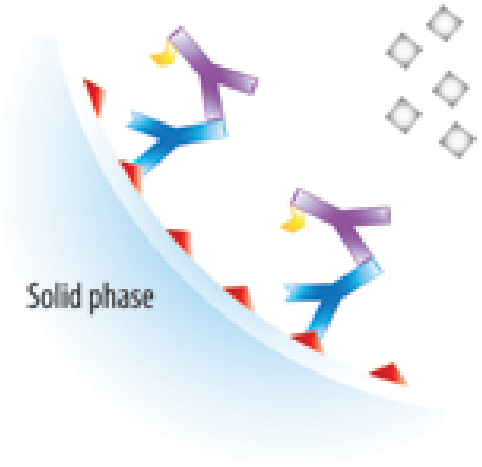
→ Fluorenzyme Immunoassay FEIA: ImmunoCAP (Phadia, Uppsala Sweden)

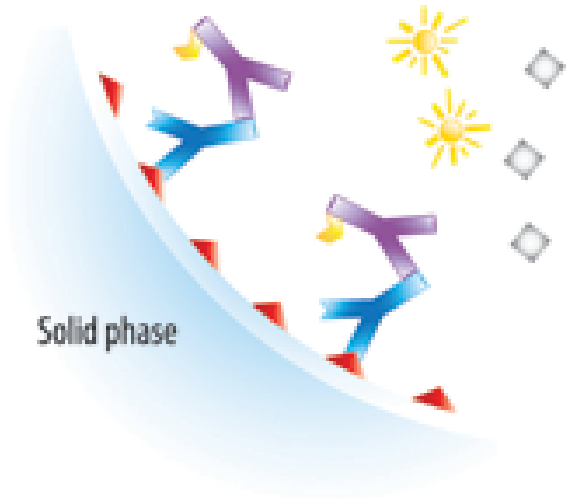
+ Absolute concentrations, reproducibility

ImmunoCAP


IgG Antibody
Solid phase
Antigen


Solid phase


Solid phase


Solid phase

Specific IgG - ImmunoCAP

	Specific IgG
Frequency	1x/w
Time	+- 3h
Sample	Serum/plasma
Procedure	FEIA
Machine	ImmunoCAP 250
	No accreditation
Units	Mg/L
Cut-off	Determined by lab Range 2-200 mg/L
Cost	16 €
Refund	Non-ZIV/INAMI

Specific IgG: meaning?

- Immunocompetent
- Exposure versus infection/disease
  - Healthy persons
    - cfr pigeon breeders disease
  - Cut-offs: what is significant?
    - Based on levels of healthy volunteers
    - ! Importance of underlying condition

	A. fumigatus sIgG							
	Healthy controls	Diseased controls					Patients	
		AB	CF	CF + ABPA	BRECT	OPD	ASP	ABPA
Number	42	20	112	11	8	48	10	10
Median	13.75	18.15	25.75	33.40	25	24.6	103	70.1
Percentile								
2.5th	2.01		2			2		
25th	7.09	11.01	8.34	24.35	18	14.25	90.95	41.83
75th	24.20	36.9	55.25	53.55	62.08	42.8	147	113.45
97.5th	70.10		139.53			684.4		
Cutoff, 35 mg_A/L								
Number above	6	5	44	5	2	13	9	9
Number below	36	15	68	6	6	35	1	1
Cutoff, 70 mg_A/L								
Number above	1	2	20	1	2	3	9	5
Number below	41	18	92	10	6	45	1	5
Sensitivity								
Cutoff, 35 mg_A/L (%)							90 (95% CI: 68.3–98.8)	
Cutoff, 70 mg_A/L (%)							70 (95% CI: 45.7–88.1)	
Specificity								
Cutoff, 35 mg_A/L	85.7% (95% CI: 71.5–94.6)			65.3% (95% CI: 58.3–71.9)				
Cutoff; 70 mg_A/L	97.6% (95% CI: 87.4–99.9)			85.9% (95% CI: 80.3–90.4)				

Van Hoeyveld E, Dupont L, Bossuyt X. Clin Chem 2006, 52:1785-93

Specific IgG in diagnosing fungal disease

SPECIFIEK IgG		
6212 ❑	Schimmelmengsel (m 1-Gm 2-m 4-Gm 6)	Gm x6
6214 ❑	Actinomycetenmengsel (Gm 22-Gm 23)	Gm x7
6215 ❑	Alternaria alternata	Gm 6
6181 ❑	Aspergillus fumigatus	Gm 3
6196 ❑	Aspergillus flavus	Gm 228
6183 ❑	Candida albicans	Gm 5
6186 ❑	Cladosporium herbarum	Gm 2
6191 ❑	Micropolyspora faeni	Gm 22
6197 ❑	Penicillium chrysogenum	Gm 1
6187 ❑	Stachybotrys atra	Gm 24
6211 ❑	Thermoact. vulgaris	Gm 23
Serum, pluimen, faeces van		
6208 ❑	duif	Ge 91
6195 ❑	parkiet	Ge 90
6209 ❑	papegaai	Ge 92
Faeces van		
6226 ❑	kanarie	Ge 200

Aspergillus fumigatus related disease

Specific IgG in aspergillosis

	Proven invasive [48]	Probable invasive [48]
Clinical criteria	NOT REQUIRED	neutropaenia OR stem cell transplant OR high dose corticosteroids for >3 weeks OR immunosuppressant drugs OR CGD OR SCID
Radiological criteria on CXR or CT scan	NOT REQUIRED	dense lesions +/− halo sign OR air-crescent sign OR one or more cavities
Laboratory criteria	culture from a sample from a normally sterile site OR histology (hyphae plus tissue damage on biopsy consistent with invasive mould infection but may not be able to differentiate *Aspergillus* from other fungi)	culture from sputum or BAL OR [illegible] in blood or BAL OR [illegible]-D-glucan in blood

Page ID, Richardson M, Denning DW. Medical Mycology 2015; 53: 417-39.

Specific IgG in invasive aspergillosis?

Biol Blood Marrow Transplant. 2012 Dec;18(12):1927-34. doi: 10.1016/j.bbmt.2012.07.013. Epub 2012 Jul 21.

Serum IgG responses against Aspergillus proteins before hematopoietic stem cell transplantation or chemotherapy identify patients who develop invasive aspergillosis.

Du C[1], Wingard JR, Cheng S, Nguyen MH, Clancy CJ.

Specific IgG in aspergillosis

	Sub-acute invasive (aka CNPA) [6]	CCPA [5,7,8,21]
Clinical criteria	>1 MONTH SYMPTOMS; weight loss OR productive cough OR haemoptysis AND *absence* of host factors for acute invasive disease	>3 MONTHS SYMPTOMS; weight loss OR productive cough OR haemoptysis AND *absence* of host factors for invasive disease
Radiological criteria on CXR or CT scan	new cavitation OR expanding cavity OR paracavitary infiltrates	new cavitation OR expanding cavity OR paracavitary infiltrates
Laboratory criteria	culture from sputum or BAL OR GM in blood or BAL OR ß(1,3)-D-glucan in blood OR raised *Aspergillus*-specific IgG OR histology	raised *Aspergillus*-specific IgG OR culture from sputum or BAL OR GM in blood or BAL* OR ß(1,3)-D-glucan in blood*

Page ID, Richardson M, Denning DW. Medical Mycology 2015; 53: 417-39.

Specific IgG in aspergillosis

	Aspergillus bronchitis [39]	ABPA [4]
Clinical criteria	persistent productive cough OR recurrent chest infections AND does not meet diagnostic criteria for chronic or allergic aspergillosis	asthma OR cystic fibrosis
Radiological criteria on CXR or CT scan	*absence* of changes consistent with CPA or ABPA	transient opacifications or permanent evidence of bronchiectasis of pleuropulmonary fibrosis (see other criteria below)
Laboratory criteria	raised *Aspergillus*-specific IgG AND EITHER recurrent culture growth from sputum or BAL OR persistently positive PCR from sputum or BAL	Obligatory criteria total IgE > 1000 IU/ml AND raised *Aspergillus*-specific IgE (or positive skin prick test) Other criteria (2 of 3 needed) raised eosinophil count OR raised *Aspergillus*-specific IgG precipitins OR radiological changes as above

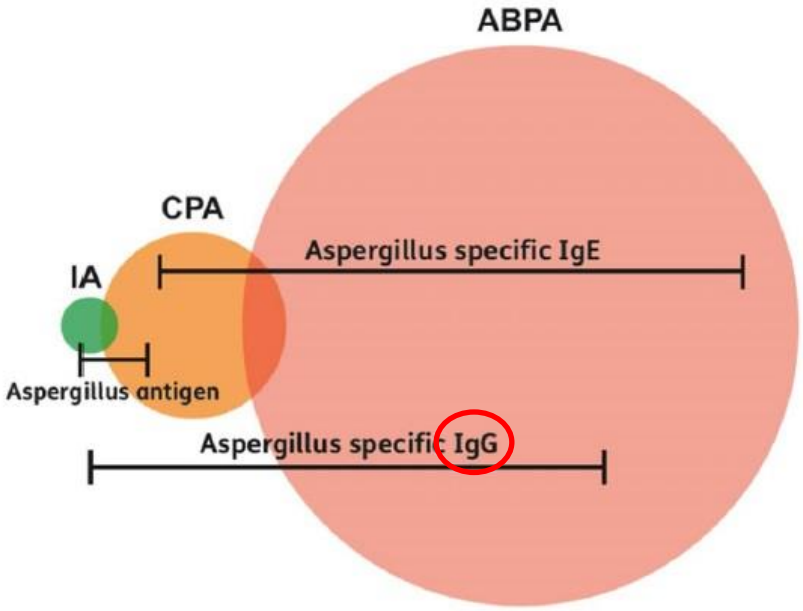
Page ID, Richardson M, Denning DW. Medical Mycology 2015; 53: 417-39.

Novel immunologic classification of aspergillosis in adult cystic fibrosis

	non-diseased	sensitized	ABPA	Aspergillus bronchitis
RT-PCR sputum	+ or -	+ or -	+	+
GM sputum	-	-	+	+
AsFu IgG	-	-	+	+
AsFu IgE	-	+	+	-

Baxter CG et al. J Allergy Clin Immunol. 2013; 132: 560-566

Specific IgG in aspergillosis



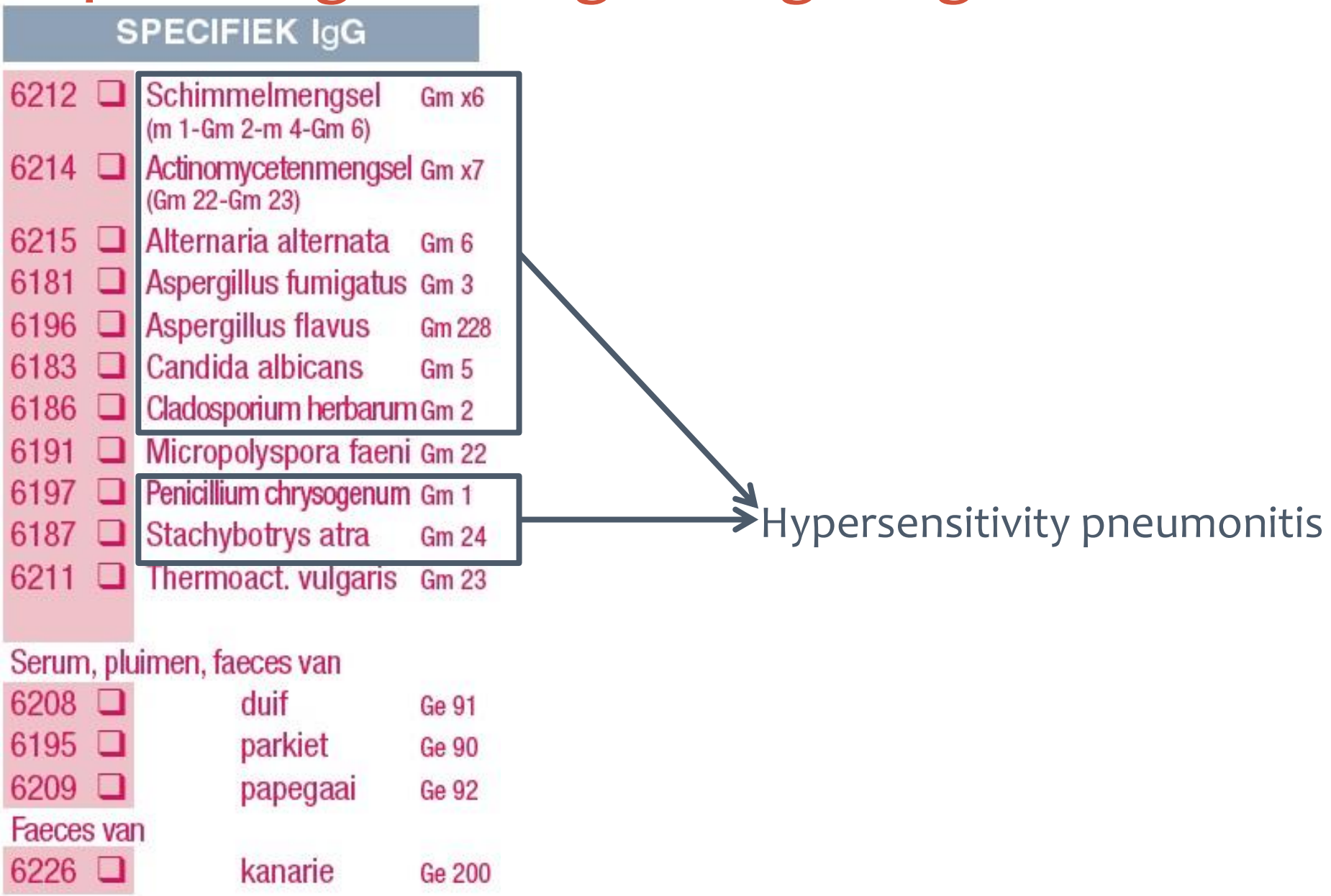
Page ID, Richardson M, Denning DW. Medical Mycology 2015; 53: 417-39.

- Monitor treatment response

→ Falling Aspergillus-specific IgG

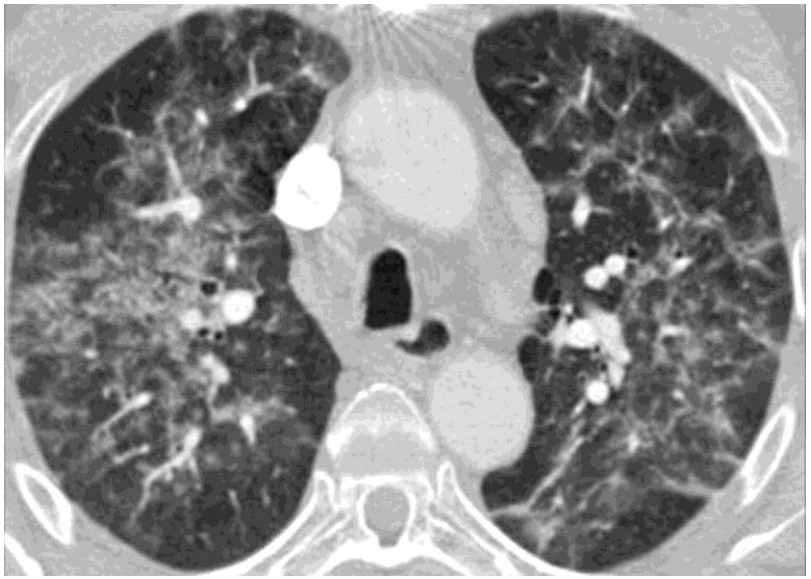
  - in acute invasive = poor prognosis
  - In CPA = good response

Specific IgG in diagnosing fungal disease



Specific IgG in hypersensitivity pneumonitis

- HP = extrinsic allergic alveolitis (EAA)
  - Inhalation of antigen to which previously sensitized
  - Dyspnea and cough
  - Acute – subacute – chronic



- Agricultural dusts, bioaerosols, microorganisms and chemicals

→ occupational and home exposure

Fungal related HP

Farmer's lung

Suberosis/corkworker's lung

Wood pulp/dust lung

Salami lung

Ventilator/humidifier lung

Indoor air contamination

Specific IgG in hypersensitivity pneumonitis

Diagnostic criteria: not validated, accurancy not known…

- History, physical findings, pulmonary function
- Radiology
- Exposure
- BAL, biopsy
- **“Precipitins” to that antigen**

Lacasse Y et al. Am J Respir Crit Care Med 2003; 168:952-958

TABLE 3. SIGNIFICANT PREDICTORS OF HYPERSENSITIVITY PNEUMONITIS

Variables	Coefficient	Odds Ratio	Confidence Interval
Intercept	−6.57	–	–
Exposure to a known offending antigen	3.66	38.8	11.6–129.6
Positive precipitating antibodies	1.68	5.3	2.7–10.4

TABLE 4. PROBABILITY OF HAVING HYPERSENSITIVITY PNEUMONITIS

Exposure to a Known Offending Antigen	Recurrent Episodes of Symptoms	Symptoms 4–8 h After Exposure	Weight Loss	Crackles, % + Serum Precipitins +	Crackles, % + Serum Precipitins −	Crackles, % − Serum Precipitins +	Crackles, % − Serum Precipitins −
+	+	+	+	98	92	93	72
+	+	+	−	97	85	87	56
+	+	−	+	90	62	66	27
+	+	−	−	81	45	49	15
+	−	+	+	95	78	81	44
+	−	+	−	90	64	68	28
+	−	−	+	73	33	37	10
+	−	−	−	57	20	22	5
−	+	+	+	62	23	26	6
−	+	+	−	45	13	15	3
−	+	−	+	18	4	5	1
−	+	−	−	10	2	2	0
−	−	+	+	33	8	10	2
−	−	+	−	20	4	5	1
−	−	−	+	6	1	1	0
−	−	−	−	3	1	1	0

All the predictors are dichotomous variables: '−' indicates absent; '+' indicates present.

Lacasse Y et al. Am J Respir Crit Care Med 2003; 168:952-958

Specific IgG in hypersensitivity pneumonitis

- False + / false –
- Suggestive NOT diagnostic
  - Acute > subacute > chronic
- Importance of history and exposure
- Monitoring of antigen-avoidance

Conclusion specific IgG

- Precipitins vs specific IgG
- ~~Precipitins~~ not in UZLeuven since 2006 → FEIA, ImmunoCAP
- Measure of **exposure**
  - CAVE cut-offs, underlying condition
- Use:
  - Aspergillosis: sub-acute and **chronic, bronchitis**, ABPA, monitoring of treatment
  - HP: not diagnostic, false+/-, limited test panel, but can be helpfull…
  - NOT: superficial infection, invasive aspergillosis, asthma,…

Antibodies of interest in mycology

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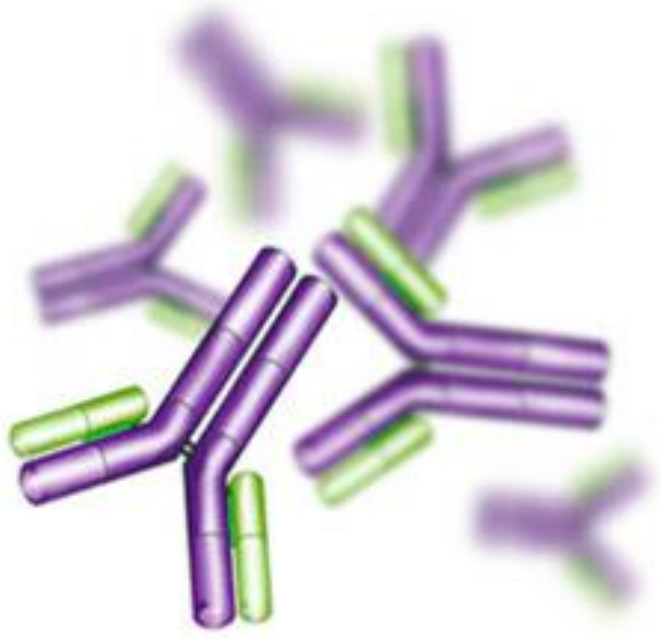
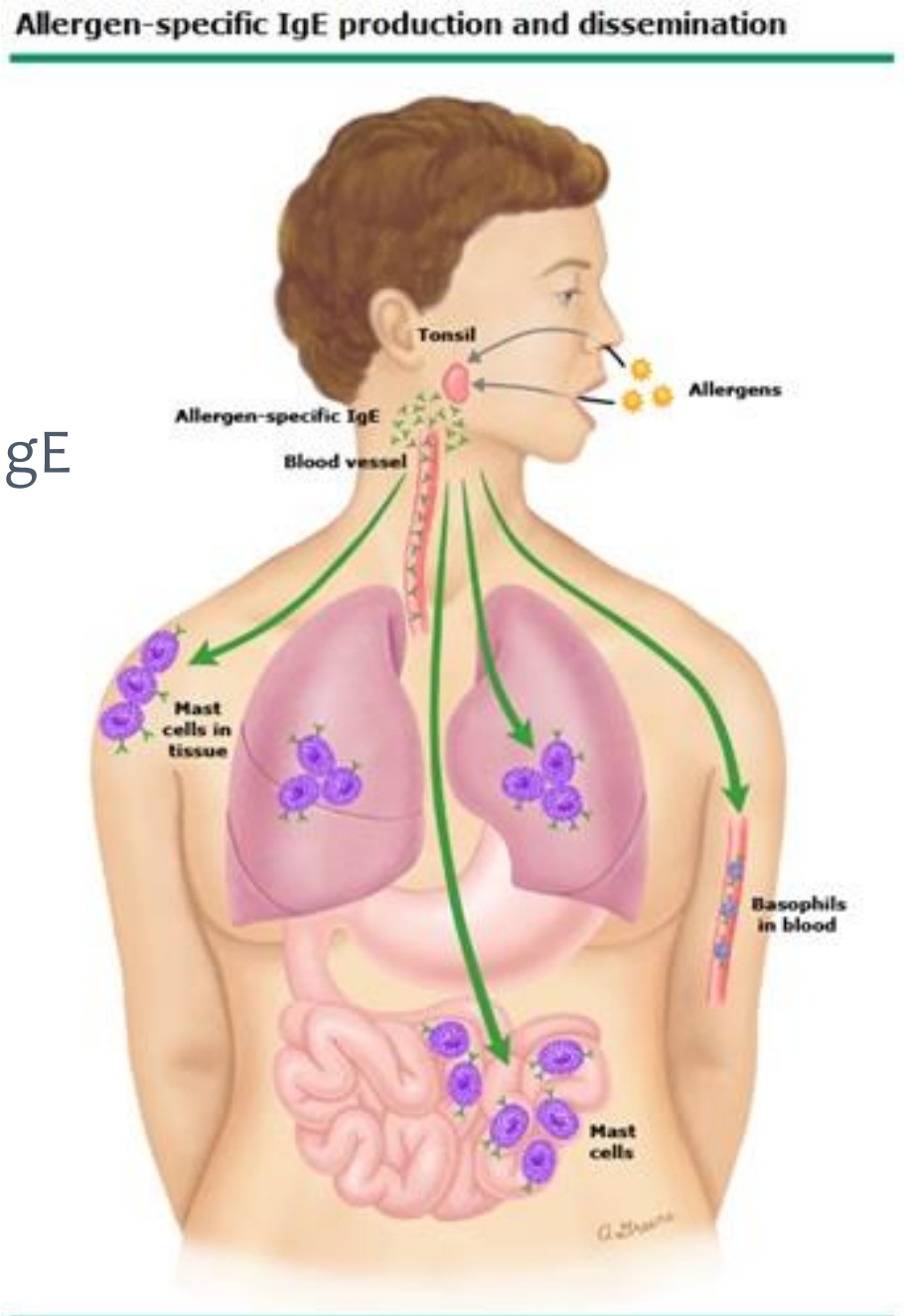
- General remarks



Specific IgE

- Parasitic and allergic diseases
- Production of allergen-specific IgE



Specific IgE – in vivo

- Skin prick tests (SPT)

  - skin problems, <12 mths, severe reactions, influence of therapy, not quantitative

  + fast (20') , low cost

  Sensitivity and specificity? → **Highly dependent on allergens used!**

Specific IgE - in vitro

- RAST = radioallergosorbent test → ELISA

- Automated fluoroenzyme immunoassay: ImmunoCAP
  - Developed in 1989
  - cfr IgG
  - FDA approval 2004
  - >90% sensitive and specific with allergens of common grasses, trees, dustmites and cat

  LESS with mold antigens → **! dependent on allergens**

Specific IgE: SPT or ImmunoCAP?

Clin Exp Allergy. 2009 Nov;39(11):1677-83. doi: 10.1111/j.1365-2222.2009.03339.x. Epub 2009 Aug 18.

Comparison of skin prick tests with specific serum immunoglobulin E in the diagnosis of fungal sensitization in patients with severe asthma.

O'Driscoll BR[1], Powell G, Chew F, Niven RM, Miles JF, Vyas A, Denning DW.

Table 2. Skin prick test (SPT) and specific serum IgE results for individual fungi

	SPT or specific IgE test positive	SPT and specific IgE both positive	SPT positive but specific IgE negative	Specific serum IgE positive but SPT negative	Concordance per species among positive results
Aspergillus	54 (45%)	29 (24%)	10 (8%)	15 (12%)	54% (29/54)
Candida	43 (36%)	22 (18%)	9 (7%)	12 (10%)	51% (22/43)
Penicillium	35 (29%)	10 (8%)	10 (8%)	15 (12%)	29% (10/35)
Cladosporium	29 (24%)	10 (8%)	11 (9%)	8 (7%)	35% (10/29)
Alternaria	27 (22%)	15 (12%)	4 (3%)	8 (7%)	56% (15/27)
Botrytis	22 (18%)	3 (2%)	8 (7%)	11 (9%)	14% (3/22)
Mean	35 (30%)	15 (12%)	9 (7%)	12 (10%)	40%

Concordance overall 77%, only 14-56% for individual fungi

→Use of both SPT en FEIA

Specific IgE - ImmunoCAP

	Specific IgG	Specific IgE
Frequency	1x/w	1x/d
Time	+- 3h	+- 3h
Sample	Serum/plasma	Serum/plasma
Procedure	FEIA	FEIA
Machine	ImmunoCAP 250	ImmunoCAP 1000
	No accreditation	Accreditation
Units	Mg/L	kUA/L
Cut-off	Determined by lab Range 2-200 mg/L	Determined by machine Range 0.1-100 kUA/L
Cost	16 €	8 €
Refund	Non-ZIV/INAMI	ZIV/INAMI: 6

Specific IgE – Meaning?

- Sensitization ≠ allergy

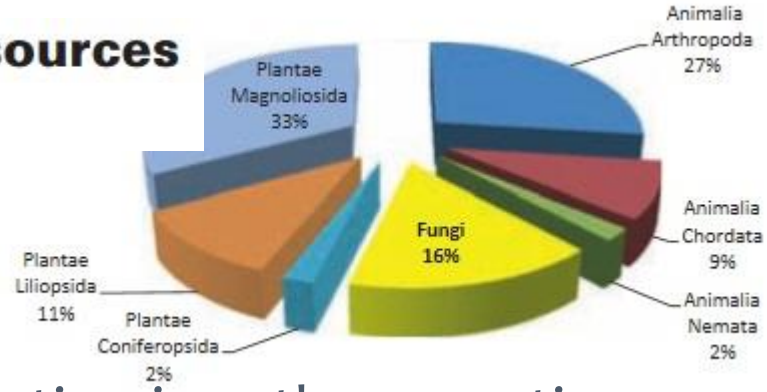
IgE-mediated allergy =

1. History
2. Specific IgE
3. Exposure resulting in symptoms

- Fungal sensitization 3-10% of the population?

Fungi: the neglected allergenic sources

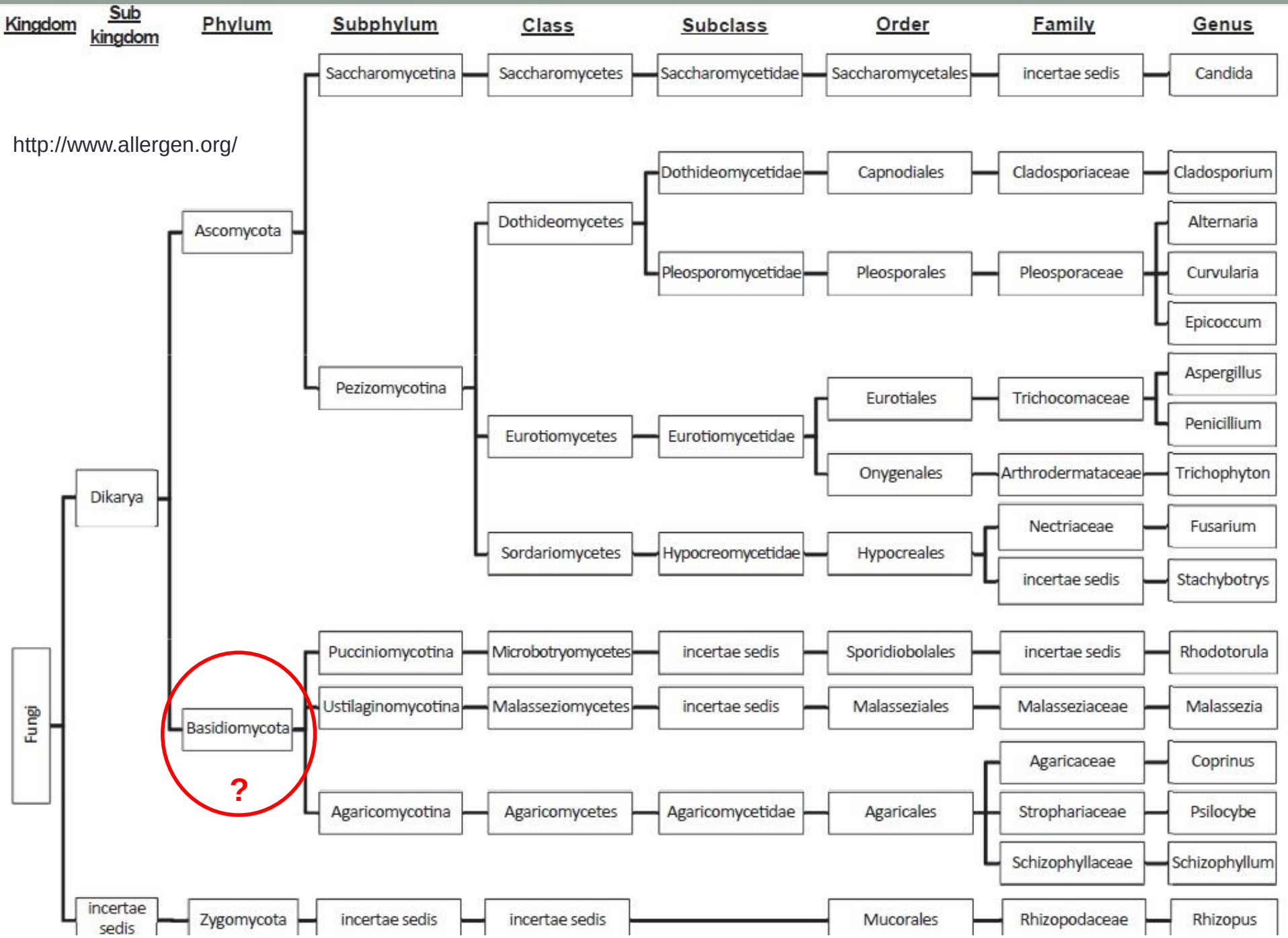
R. Crameri, M. Garbani, C. Rhyner & C. Huitema



- Sensitization to AsFu and lung function in asthma, cystic fibrosis and COPD… Pashley CH. Mycopathologia 2014; 178: 457-63.

schimmels en gisten		
10175 ❑	Schimmelmengsel (m1-m2-m3-m6)	mx1
10163 ❑	Alternaria alternata	m6
10712 ❑	rAlt a 1	m229
10160 ❑	Aspergillus fumigatus	m3
10628 ❑	rAsp f1	m218
10629 ❑	rAsp f2	m219
10630 ❑	rAsp f3	m220
10631 ❑	rAsp f4	m221
10632 ❑	rAsp f6	m222
10169 ❑	Aureobasidium pullulans	m12
10164 ❑	Botrytis cinerea	m7
10162 ❑	Candida albicans	m5
10159 ❑	Cladosporium herbarum	m2
10171 ❑	Epicoccum purpurascens	m14
10166 ❑	Fusarium moniliforme	m9
10165 ❑	Helminthosporium halodes	m8
10650 ❑	Malassezia spp.	m227
10161 ❑	Mucor racemosus	m4
10158 ❑	Penicillium notatum	m1
10170 ❑	Phoma betae	m13
10642 ❑	Stafylokokken enterotoxine A	m80
10643 ❑	Stafylokokken enterotoxine B	m81
10172 ❑	Trichoderma viride	m15
10454 ❑	Trichophyton rubrum	m205

Specific IgE in diagnosing fungal disease


Kingdom
Sub kingdom
Phylum
Subphylum
Class
Subclass
Order
Family
Genus
http://www.allergen.org/
Fungi
Dikarya
incertae sedis
Ascomycota
Basidiomycota
Zygomycota
?
Saccharomycetina
Pezizomycotina
Pucciniomycotina
Ustilaginomycotina
Agaricomycotina
incertae sedis
Saccharomycetes
Dothideomycetes
Eurotiomycetes
Sordariomycetes
Microbotryomycetes
Malasseziomycetes
Agaricomycetes
incertae sedis
Saccharomycetidae
Dothideomycetidae
Pleosporomycetidae
Eurotiomycetidae
Hypocreomycetidae
incertae sedis
Malasseziomycetidae
Agaricomycetidae
incertae sedis
Saccharomycetales
Capnodiales
Pleosporales
Eurotiales
Onygenales
Hypocreales
Sporidiobolales
Malasseziales
Agaricales
Mucorales
incertae sedis
Cladosporiaceae
Pleosporaceae
Trichocomaceae
Arthrodermataceae
Nectriaceae
incertae sedis
incertae sedis
Agaricaceae
Strophariaceae
Schizophyllaceae
Rhizopodaceae
Candida
Cladosporium
Alternaria
Curvularia
Epicoccum
Aspergillus
Penicillium
Trichophyton
Fusarium
Stachybotrys
Rhodotorula
Malassezia
Coprinus
Psilocybe
Schizophyllum
Rhizopus

Specific IgE in diagnosing fungal disease

- Allergic rhinitis
  - Not required but can be helpful

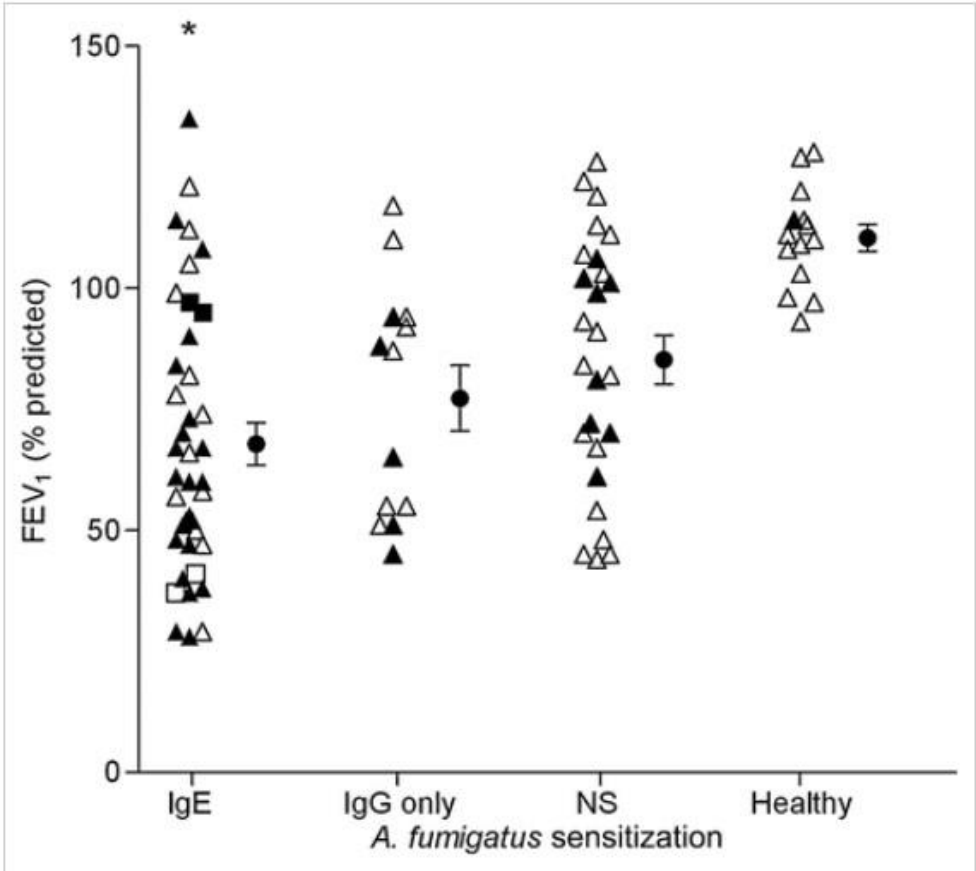
- Allergic fungal rhinosinusitis
  - Localized allergic reaction to noninvasive fungal growth in areas of compromised mucus drainage
  - **Specific IgE is a diagnostic criterium**
  - Bipolaris, alternaria, cladosporium, curvularia, aspergillus, exserohilum, Dreschslera species…

Specific IgE in diagnosing fungal disease

- Asthma
  - Development, persistence and severity
  - Severe asthma with fungal sensitisation **SAFS**

    75% sensitized in subgroup of severe asthma
  - Associated with worse lung function and disease
  - Therapeutic implication?

Conflicting results in clinical trials



Fairs A et al. Am J Respir Crit Care Med. 2010; 182:1362-1368,

Specific IgE in diagnosing fungal disease

- Allergic bronchopulmonary aspergillosis/mycosis

ABPA/ABPM

  - Complication of fungal colonisation in asthma and cystic fibrosis

  - Obligatory criteria (2/2)
    - *Aspergillus* skin test positive or elevated IgE levels against *A. Fumigatus*
    - Elevated total IgE levels (>1000 IU·mL^{-1})
  - Other criteria (≥2/3)
    - Precipitating or IgG antibodies against *A. Fumigatus*
    - Radiographic pulmonary opacities
    - Total eosinophil count >500 cells·µL−1 in steroid naïve patients

- Atopic dermatitis
  - Malassezia sympodalis, Saccharomyces cerevisiae
  - Specific IgE not recommended, but may be helpful

Conclusions Specific IgE

- ~~RAST~~ = anachronism → 2004 FEIA, ImmunoCAP in UZLeuven
- Don't forget SPT
- Sensitization ≠ allergy
- Limited test panel
- Use
  - Allergic fungal rhinosinusitis
  - Asthma
  - ABPA/ABPM

General remarks

- Fungal antigens/allergens

→ **Lack of standardization**

  - Crude extract
    - Extraction from cultures vs commercial available
    - Instability
    - Difference between strains, within strains, growth conditions, batch to batch variation, growth cycle
  - **Cross-reactions**
  - Recombinant antigens – pure extract
    - Produced in a microbial strain carrying a cloned cDNA
    - High specificity, but not as sensitive as total fungal extracts

General remarks

- IgA en IgM?
  - Limited diagnostic value, poor specificity (for CPA), no published data

- Why use of Ab?

Low sensitivity of culture

Easier to detect than finding organism directly

Produced in large quantities and found in body fluids

References

- Page ID, Richardson M, Denning DW. Antibody testing in apsergillosis – quo vadis? Medical Mycology 2015; 53: 417-39.
- Aspergillus precipitins and serology. Barton RC. Aspergillosis: From diagnosis to Prevention p159-169.
- Baxter et al. Performance of two Aspergillus IgG EIA assays compared with the precipitn test in chronic and allergic aspergillosis. Clin Microbiol Infect 2013; 19:197-204,
- Fenoglio CM, Reboux G, Sudre B et al. Diagnostic value of serum precipitins to mould antigens in active hypersensitivity pneumonitis. Eur Respir J 2007; 29: 706-712
- Lacasse Y, Selman M, Costabel U et al. Clinical diagnosis of hypersensitivity pneumonitis. Am J Respir Crit Care Med 2003; 168: 952-958.
- Asthma in the workplace, 4th edition CRCpress.
- Heinzerling L et al. The skin prick test – European standards. Clin Transl All 2013;3:3.
- O'Driscoll BR et al. Comparison of SPT with specific IgE in the diagnosis of fungal sensitization in patients with severe asthma. Clin Exp All 2009; 39: 1677-1683.
- Kespohl S et al. Biochemical and immunolgical analysis of mould skin prick test solution: current status of standardization. Clin Exp All 2013; 43: 1286-1296.
- Fukutomi Y, Taniguchi M. Sensitization to fungal allergens: resolved and unresolved issues. All int 2015; 64: 321-331.
- Crameri R et al. Fungi: the neglected allergenic sources. Allergy 2014; 69: 176-185.
- Fairs A et al. IgE sensitization to AsFu is associated with reduced lung function in asthma. Am J Respir Crit Care Med 2010; 182; 1362-1368.
- Shah A, Panjabi C. Allergic aspergillosis of the respiratory tract. Eur Respir Rev 2014; 23: 8-29.
- Simon-Nobble B et al. The spectrum of fungal allergy. Int Arch Allergy Immunol 2008; 145: 58-86.
- Pashley CH. Fungal culture and sensitisation in asthma, cystic fibrosis and COPD: What does it tell us? Mycopathologia 2014.
- R. Crameri. The problem of cross-reactivity in the diagnosis of fungal allergy. Clin Exp All 2011; 41: 302-304.
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