

Pulmonary aspergillosis: Pathogenesis and treatment

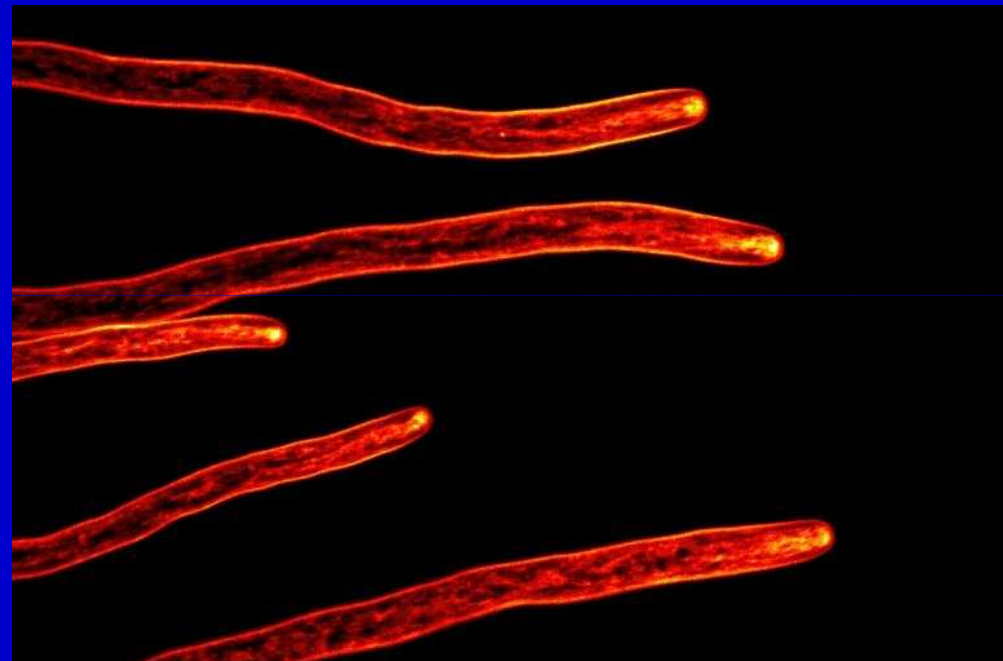
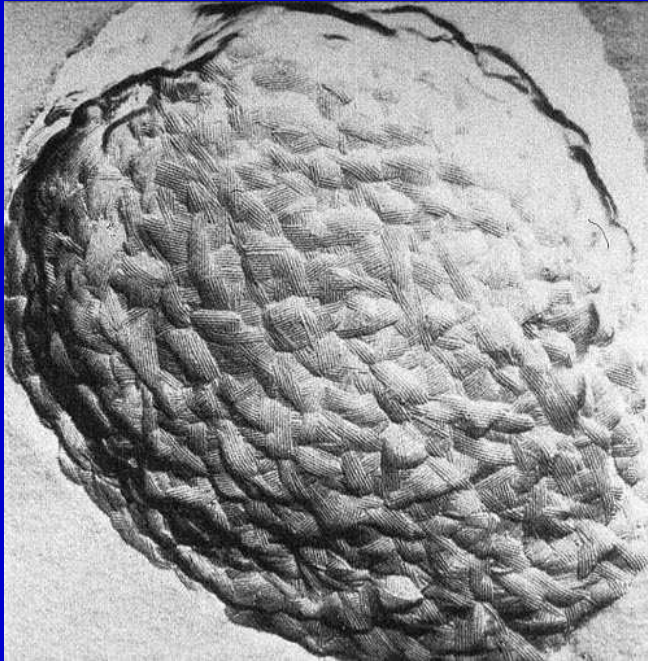
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University Hospital of South Manchester

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Aspergillus fumigatus

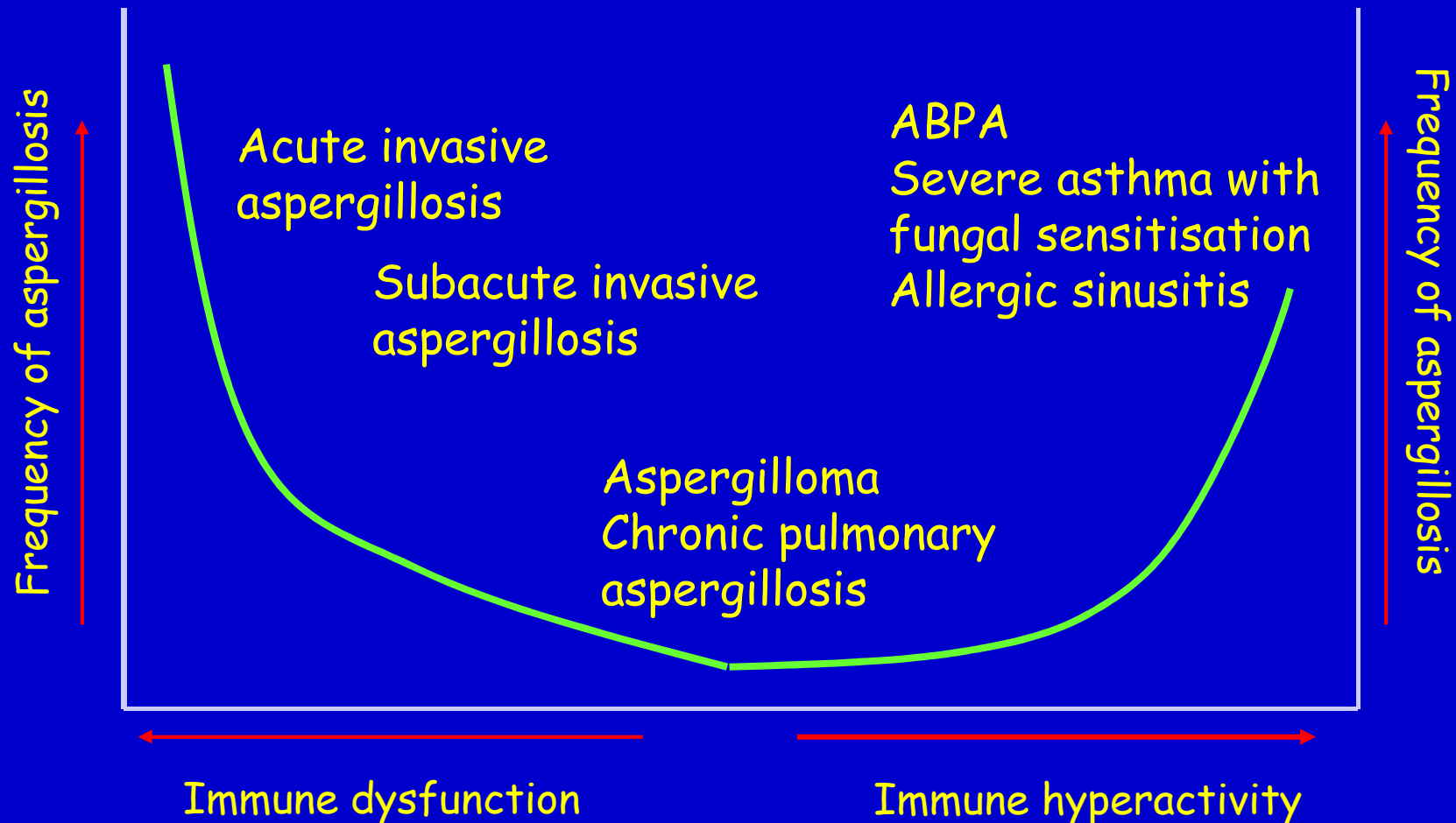


Species of *Aspergillus* causing IA

Species	Voriconazole RCT (MITT)	TransNet (surveillance)	MSG multicentre study
<i>A. fumigatus</i>	85 (77%)	136 (74%)	171 (67%)
<i>A. flavus</i>	7	16	41
<i>A. niger</i>	9	13	14
<i>A. terreus</i>	6	10	8
Other	3	8	4
Not speciated	167	16	18
Multiple		28	

Interaction of *Aspergillus* with the host

A unique microbial-host interaction

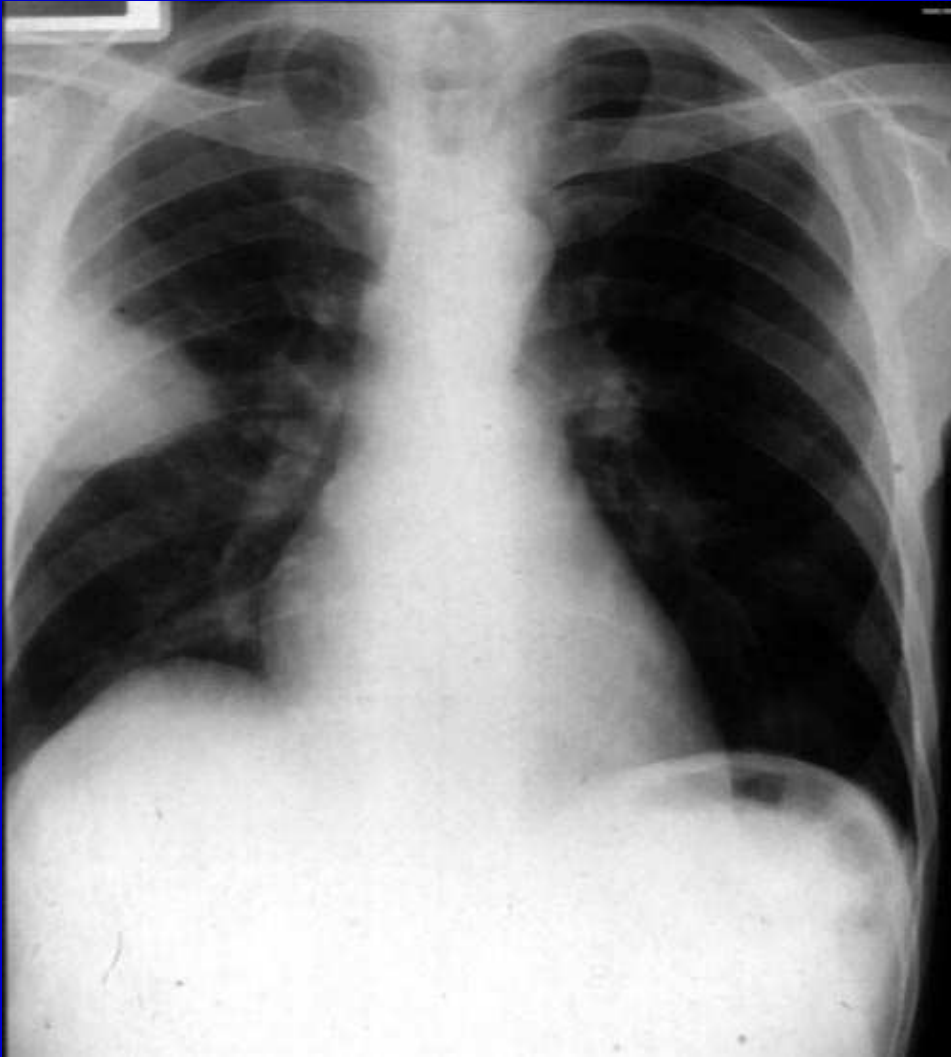


Size of Aspergillus disease problem globally

1. Over 350,000 patients develop IA including ~10% of acute leukaemia (30,000) and stem cell and other transplants (7,500) and 1.2% of COPD patients admitted to (216,000 IA cases)- ~50% mortality
2. Chronic pulmonary aspergillosis after TB - 1.1M cases prevalence
3. Chronic pulmonary aspergillosis total - ~3M
4. Asthma 197M in adults, of which ~10-20% severe, UK and USA have very high prevalence rates
5. Allergic bronchopulmonary aspergillosis in asthma - ~4M worldwide (2.1% of adults referred with asthma)
6. Severe asthma with fungal sensitisation - ~6M worldwide (33% of 10% (severe only))

Invasive aspergillosis

Invasive aspergillosis - 100% mortality, unless treated

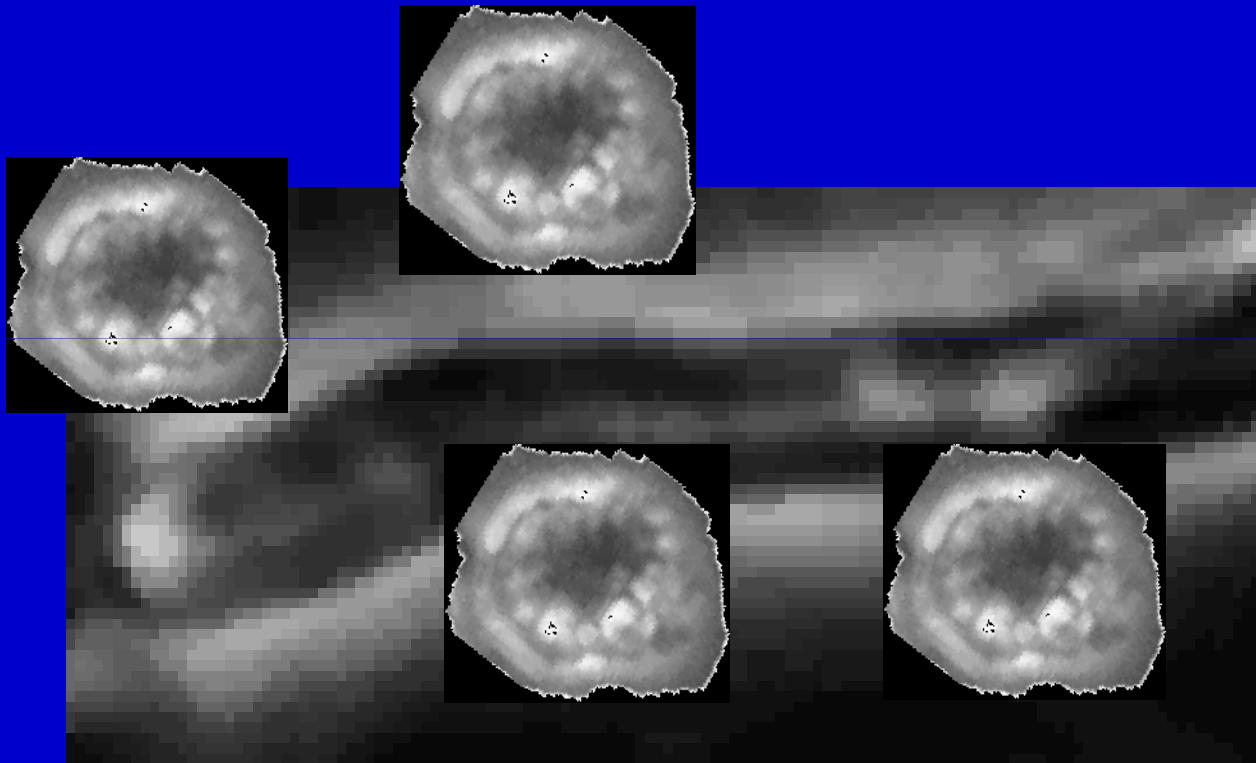


Risk factors for invasive aspergillosis

Major

- Neutropenia (+ monocytopenia)
- Corticosteroid treatment

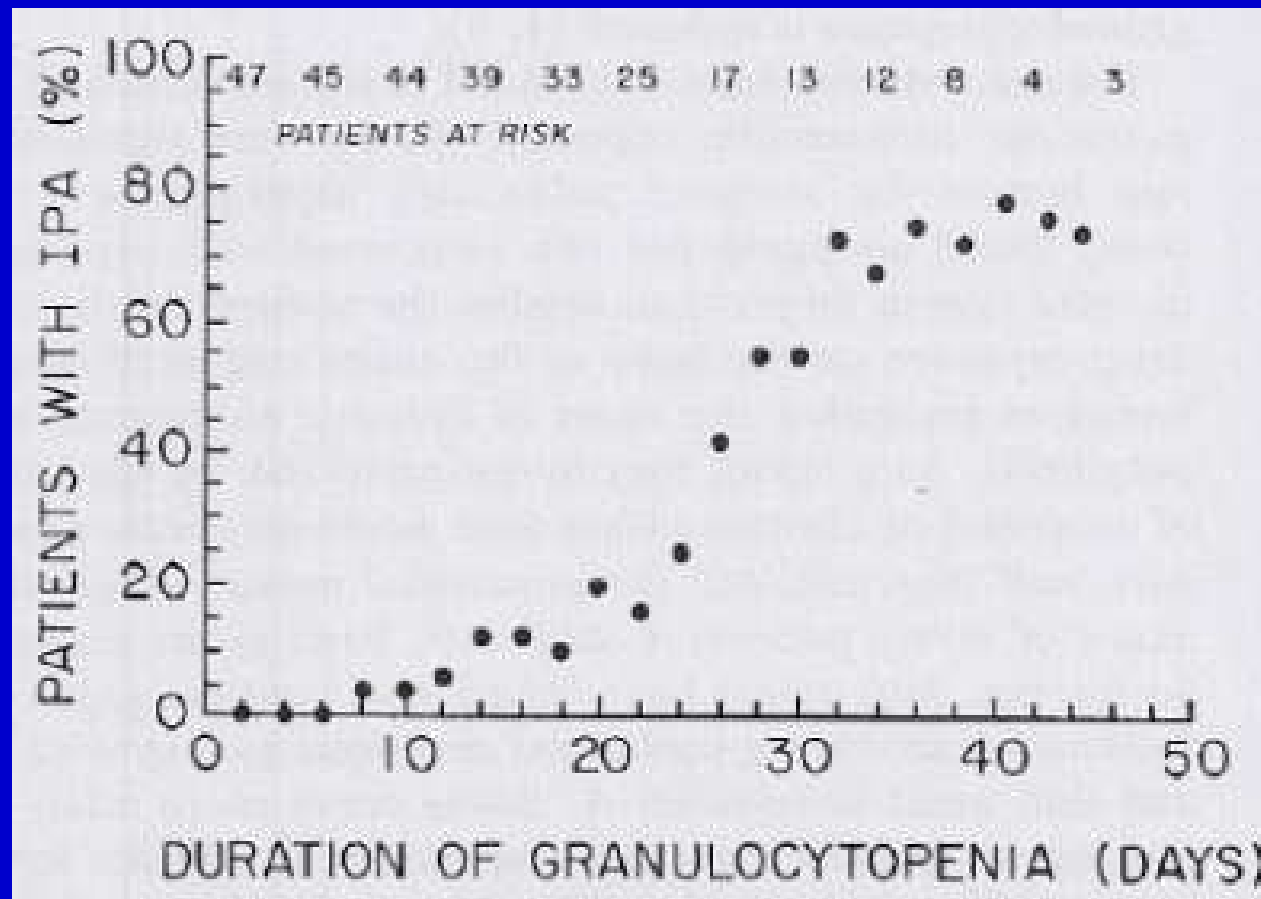
Neutrophils and monocytes key to damaging fungal hyphae



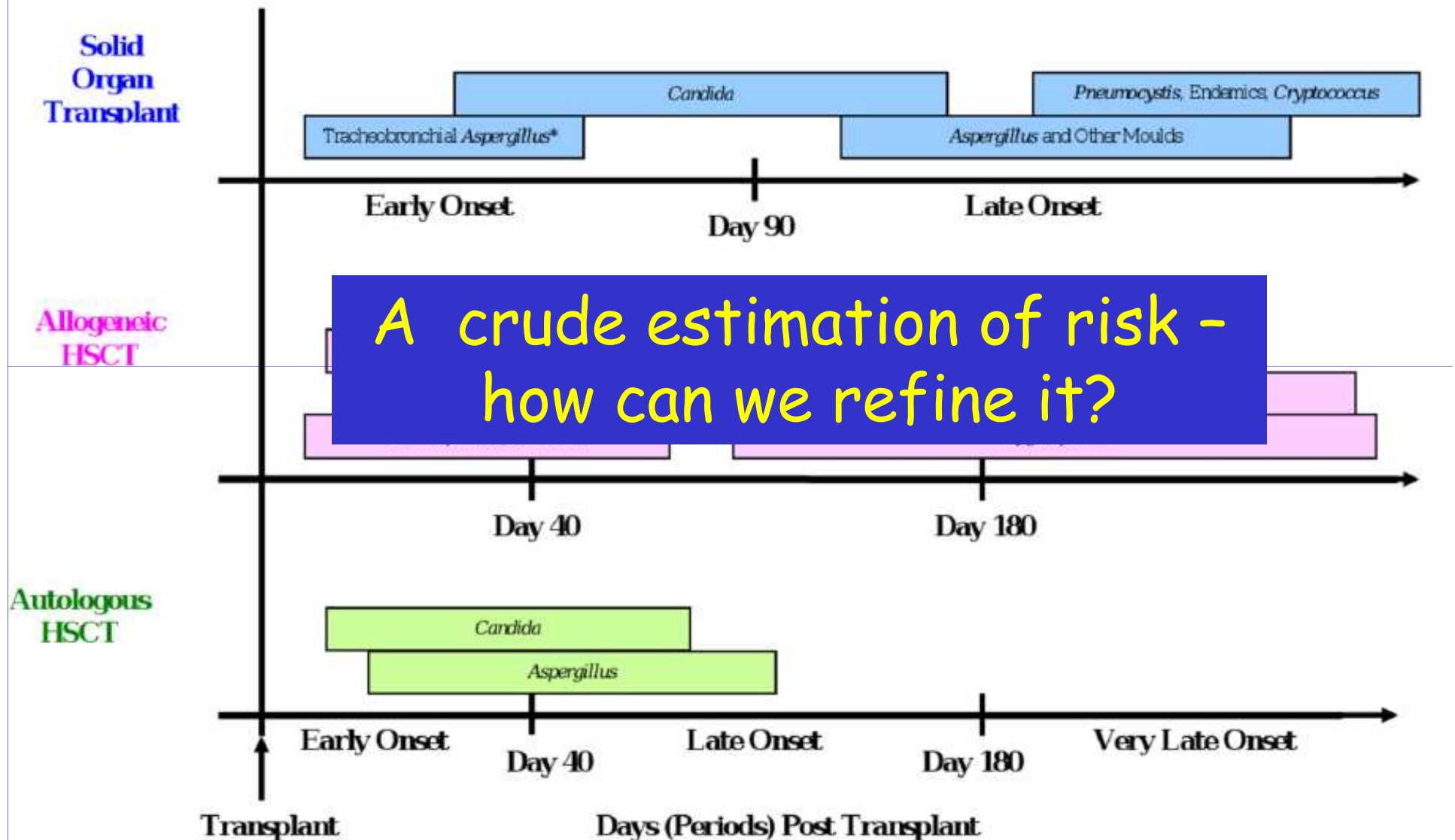
Invasive aspergillosis: Time of diagnosis

A single centre case control study :

- IA based on radiology (CXR) and clinical features



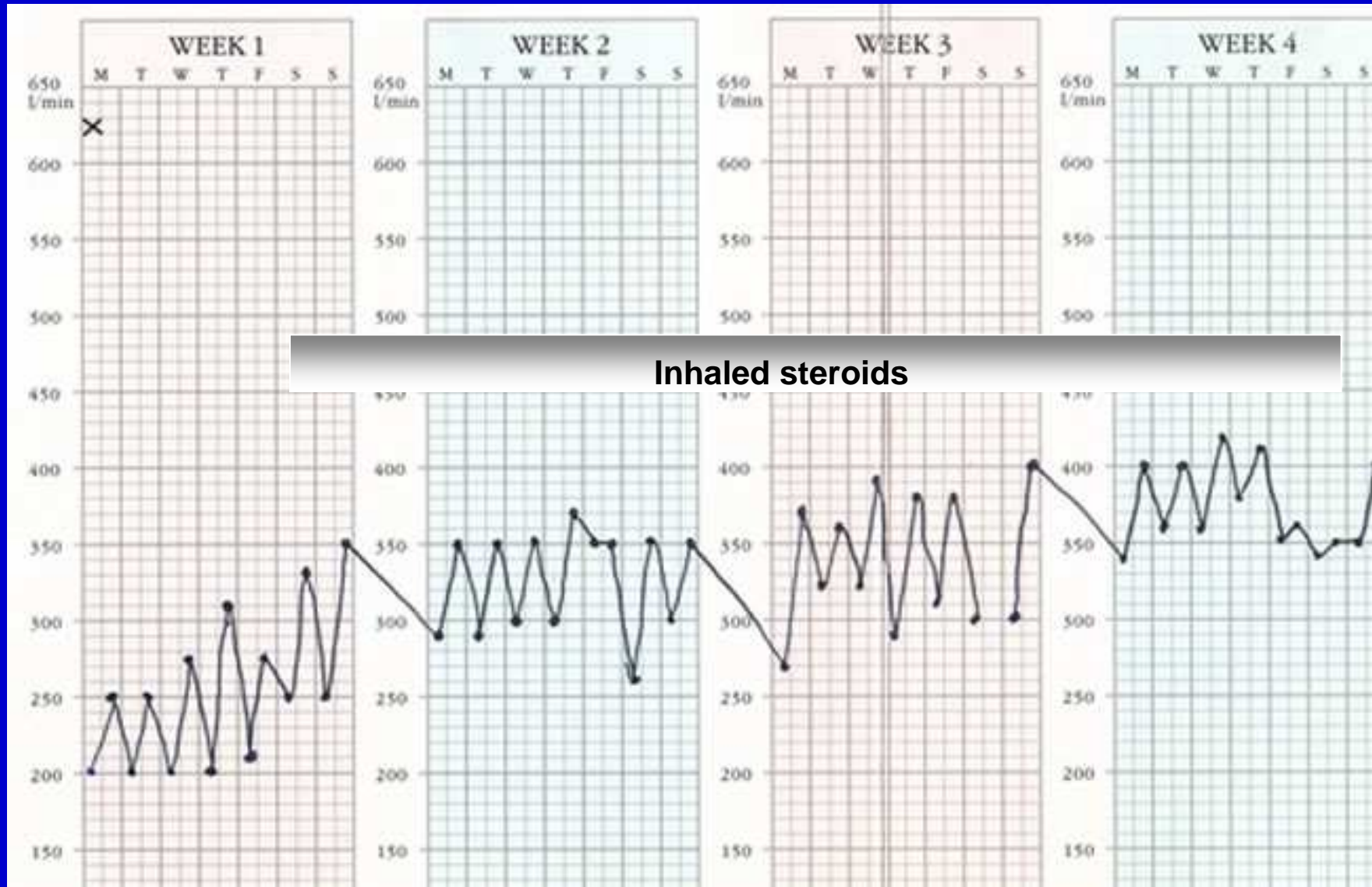
Risk period of fungal disease



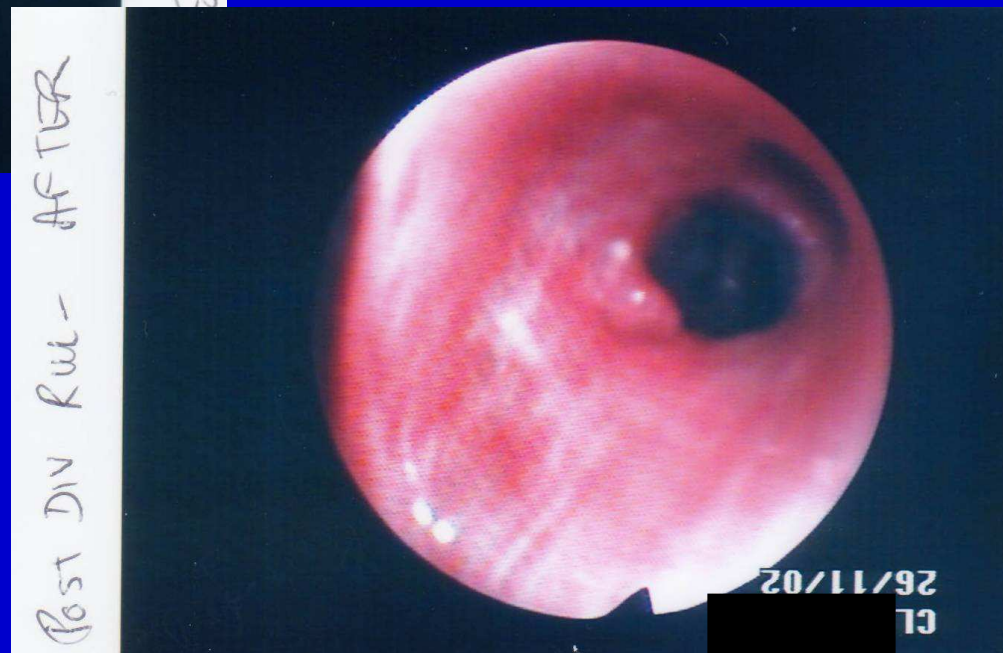
ABPA and severe asthma



Asthma - variable airflow obstruction



ABPA - bronchoscopy views showing mucus plugging



Fungal exposure in asthmatics is related to:

- Life-threatening asthmatic attacks (ie thunderstorm asthma)
- Severe asthma and hospital admission

Severe asthma with fungal sensitisation (SAFS)

Criteria for diagnosis

- Severe asthma (BTS step 4 or 5)
AND
- RAST (IgE) positive for any fungus
OR
- Skin prick test positive for any fungus
AND
- Exclude ABPA (ie total IgE <1,000 iu/mL)

Skin prick testing - example of SAFS result

Cladosporium +ve



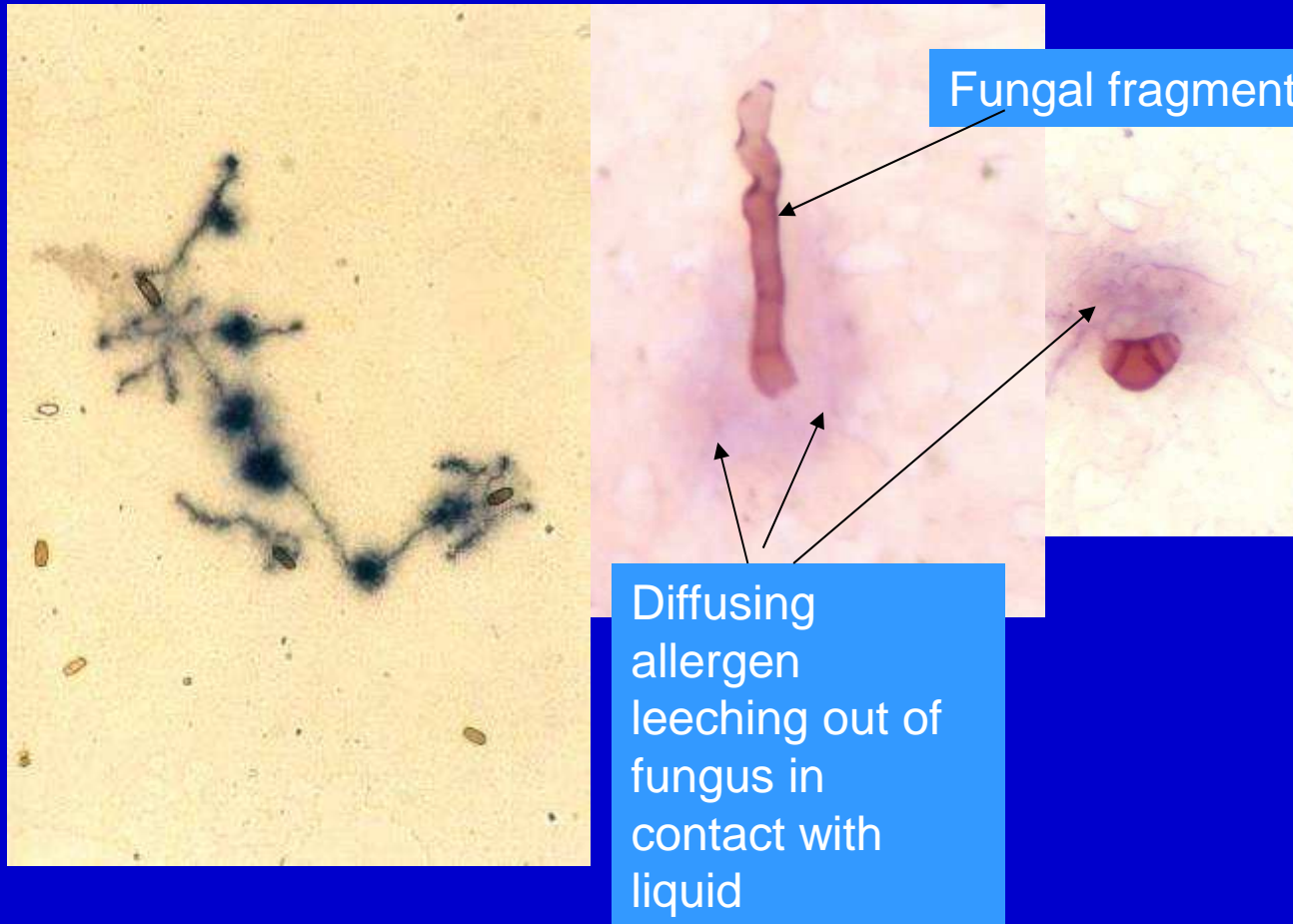
Hospital admission and sensitisation

<u>Allergen</u>	<u>Asthma, no admission (n=82)</u>	<u>Asthma, 2+ admissions (n=46)</u>
House dust mite	56 %	67 %
Grass pollen	46 %	63 %
Cat	37 %	59 %
Dog	18 %	48 %
Any non fungal allergen	70%	74%

Hospital admission and mould allergy

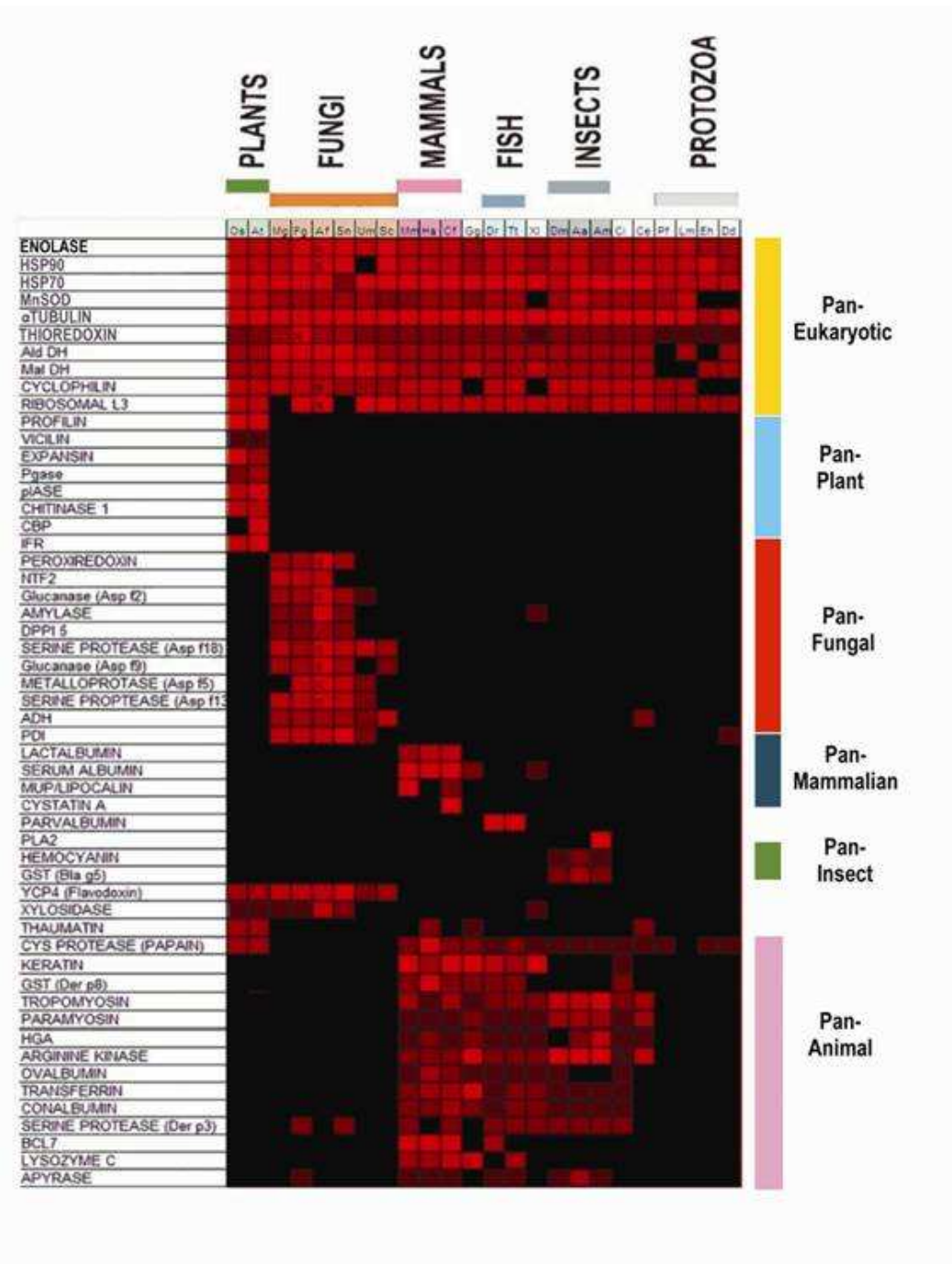
<u>Allergen</u>	<u>Asthma, no admission (n=82)</u>	<u>Asthma, 2+ admissions (n=46)</u>
<i>Aspergillus</i>	7 %	37 %
<i>Alternaria</i>	5 %	26 %
<i>Cladosporium</i>	1 %	41 %
<i>Penicillium</i>	2 %	30 %
<i>Candida</i>	10 %	33 %
<hr/> Any fungal allergen	16%	76%

Airborne fungal fragments

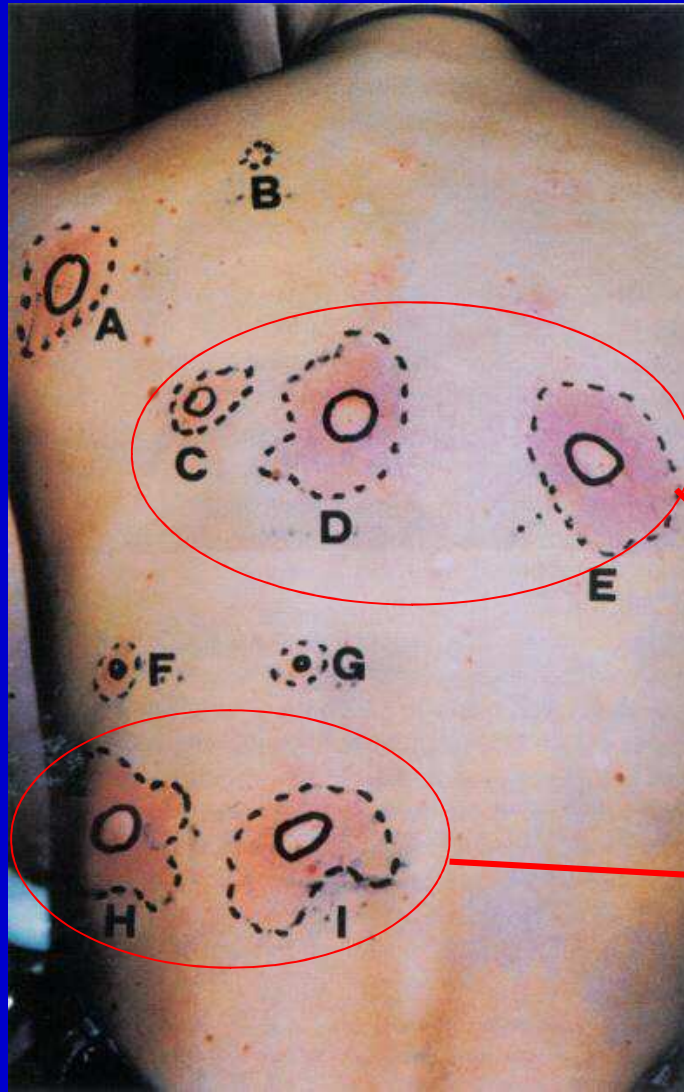


Genomic analysis of allergens

(allergen = IgE binding protein)



Skin test reactivity to recombinant *A. fumigatus* and human proteins in a patient sensitized to MnSOD and P₂ protein.



Intradermal skin tests, 100 μ l of the protein solutions (10^{-1} μ g/ml) injected.

(A) 0.01% histamine

(B) 0.9% saline

(C) human P₂ protein 1ng

(D) fungal P₂ protein 10ng

(E) human P₂ protein 10ng

(F) rAsp f 3

(G) rAsp f 11

(H) fungal MnSOD 10ng

(I) human MnSOD 10ng

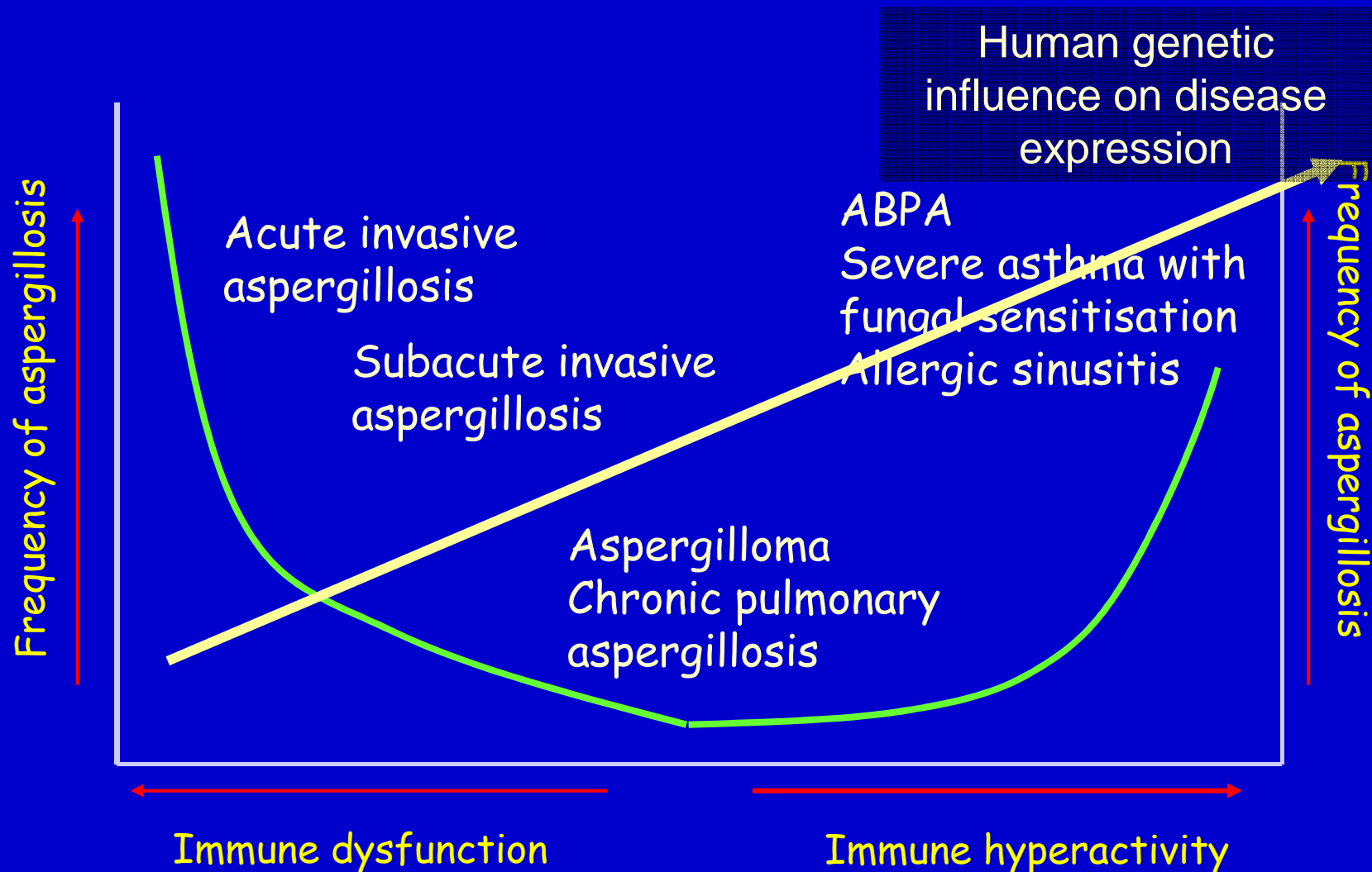
Allergens in the sequenced *Aspergilli*

<u>Accession</u>	<u>Allergen</u>	<u>Protein name</u>	<u>Asp-core</u>
AFUA_5G02330	Asp f 1	major allergen and cytotoxin	No
AFUA_4G09580	Asp f 2	major allergen	No
AFUA_6G02280	Asp f 3	allergen	<u>Yes</u>
AFUA_2G03830	Asp f 4	allergen	No
AFUA_8G07080	Asp f 5	elastinolytic metalloproteinase Mep	No
AFUA_1G14550	Asp f 6	Mn superoxide dismutase MnSOD	No
AFUA_4G06670	Asp f 7	allergen	No
AFUA_2G10100	Asp f 8	60S acidic ribosomal protein P2	<u>Yes</u>
AFUA_1G16190	Asp f 9	cell wall glucanase Crf1	<u>Yes</u>
AFUA_5G13300	Asp f 10	aspartic endopeptidase Pep1/aspergillopepsin F	<u>Yes</u>
AFUA_2G03720	Asp f 11	peptidyl-prolyl cis-trans isomerase	<u>Yes</u>
AFUA_5G04170	Asp f 12	molecular chaperone/allergen Mod-E/Hsp90/Hsp1	<u>Yes</u>
AFUA_2G12630	Asp f 13	allergenic cerato-platanin	<u>Yes</u>
AFUA_4G03240	Asp f 17	cell wall galactomannoprotein Mp1	No
AFUA_5G09210	Asp f 18	autophagic serine protease Alp2	<u>Yes</u>
AFUA_6G06770	Asp f 22	enolase	<u>Yes</u>
AFUA_2G11850	Asp f 23	60S ribosomal protein L3	<u>Yes</u>
AFUA_3G07430	Asp f 27	peptidyl-prolyl cis-trans isomerase/cyclophilin	No
AFUA_6G10300	Asp f 28	thioredoxin, putative	<u>Yes</u>

Human genetics

Interaction of *Aspergillus* with the host

A unique microbial-host interaction



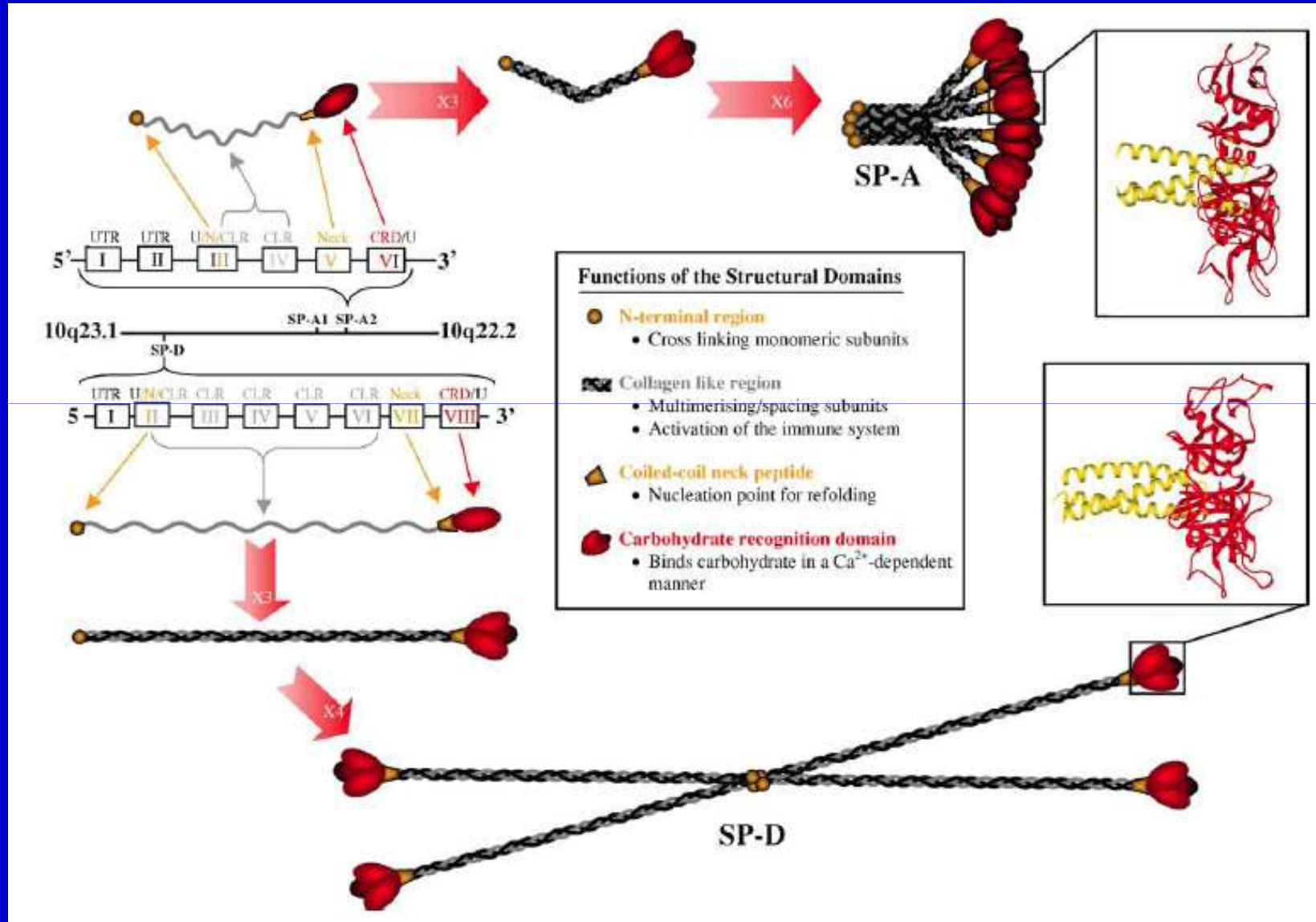
Genetic risks

Table 1

Association between defined genetic polymorphisms and an increased risk to suffer from diseases caused by *A. fumigatus*.

Gene	dbSNP number	SNP position	Asp pos.	Asp neg.	Statistics	Population	Disease	Reference
CXCL10 (4q21)	rs1554013	11101 C/T ^a [Downstream]	51	49	$p=0.007$ <u>OR= 2.2</u> CI= 1.2– 3.8	Caucasian (retrospective)	IA after HSCT [EORTC/IFCG]	Mezger et al. (2008)
	rs3921	1642 C/G ^a [3' UTR]	39	46	$p=0.003$ <u>OR= 2.6</u> CI= 1.4– 5.0			
	rs4257674	-1101 A/G ^a [Promotor]	52	44	$p=0.001$ <u>OR= 2.8</u> CI= 1.6– 5.2			
IFN-γ (12q14)	rs2069705	-1616 C/T ^a [Promotor]	69	56	$p=0.010$ <u>OR= 2.0</u> CI= 1.2– 3.4	Caucasian (retrospective)	colonization with <i>A.</i> <i>fumigatus</i> or ABPA after CF	Brouard et al. (2005)
	rs1800896	-1082 A/G [Promotor]	58	55	$p=0.046$ <u>OR= 1.7</u> CI= 1.0– 2.9			
IL-10 (1q31-q32)	rs1878672	2068 C/G ^a [Intron]	67	57	$p=0.025$ <u>OR= 1.8</u> CI= 1.1– 2.9	Caucasian (retrospective)	IPA after HSCT [EORTC/MSG]	Seo et al. (2005)
	rs1800896	-1082 A/G [Promotor]	119 Af col, 27 ABPA	232	$p=0.020$ <u>OR= 1.7</u> CI= 1.1– 2.5			
	rs1800896 rs1800871 rs1800872 (haplotype)	-1082 A/G -819 C/T -592 A/C [Promotor]	9	96	* $p=0.012$ <u>OR= 9.3</u> CI= 1.6– 52.8			
	rs1800896	-1082 A/G [Promotor]	59	61	$p=0.052$ <u>OR= 1.7</u> CI= 1.0– 2.9			
IL-1β (2q14)	rs1143627	-511 C/T [Promotor]	59	51	$p=0.095$ <u>OR= 1.7</u> CI= 0.9– 3.0	Caucasian (retrospective)	IPA in haematological patients [EORTC/IFCG]	Sainz et al. (2008a)
IL-4Rα (16p12.1-	rs1805010	4679 A/C/G/T [75 I/L/F/V]	40	56	$p=0.008$	Caucasian	ABPA	Knutsen et al. (2006)

Surfactant A and D



CPA or ABPA and surfactant defects

ABPA patients have a higher frequency of SP-A2 A1660G SNP compared with with control subjects (OR = 4.78). In combination with SP-A2 G1649C more significant (OR = 10.4) (P=0.016 and p=0.008)

CPA patients had 32% and 22% frequency of 2 SP-A2 mutations, compared with normals (18% and 11%) (p=0.021 and p=0.044)

SP-B - single ORF polymorphism (Thr131Ile) associated with ARDS

Exogenous SP-D protective against murine IA

TLR SNPs and ABPA vs. SAFS

SNP	Study groups (n)	Genotype			Allele		X ²	p value	OR (95% CI)
		A/A	A/G	G/G	A	G			
TLR2									
Arg753Gln	Controls (80)	0 (0.0)	5 (6.2)	75 (93.8)	5 (3.1)	155 (96.9)			
	ABPA (22)	0 (0.0)	0 (0.0)	22 (100)	0 (0.0)	44 (100.0)	1.41	0.587	1.032 (1.004-1.061)
	SAFS (14)	0 (0.0)	2 (14.3)	12 (85.7)	2 (7.1)	26 (92.9)	1.07	0.280	2.385 (0.439-12.944)
	CCPA (40)	0 (0.0)	2 (5.0)	38 (95.0)	2 (2.5)	78 (97.5)	0.07	1.000	0.795 (0.151-4.190)
TLR4									
Asp299Gly	Controls (80)	70 (87.5)	10 (12.5)	0 (0.0)	150 (93.8)	10 (6.3)			
	ABPA (22)	21 (95.5)	1 (4.5)	0 (0.0)	43 (97.7)	1 (2.3)	1.07	0.463	0.349 (0.043-2.802)
	SAFS (14)	12 (85.7)	2 (14.3)	0 (0.0)	26 (92.9)	2 (7.1)	0.03	0.695	1.154 (0.239-5.570)
	CCPA (40)	25 (62.5)	15 (37.5)	0 (0.0)	65 (81.3)	15 (18.8)	8.93	0.003	3.462 (1.477-8.110)
TLR9									
T-1237C	Controls (80)	65 (81.2)	15 (18.8)	0 (0.0)	145 (90.6)	15 (9.4)			
	<u>ABPA (22)</u>	14 (63.6)	7 (31.8)	1 (4.54)	35 (79.5)	9 (20.5)	4.08	0.043	2.486 (1.005-6.145)
	<u>SAFS (14)</u>	13 (92.9)	1 (7.1)	0 (0.0)	27 (96.4)	1 (3.6)	0.03	0.474	0.358 (0.045-2.825)
	CCPA (40)	33 (82.5)	7 (17.5)	0 (0.0)	73 (91.3)	7 (8.8)	0.02	0.874	0.927 (0.362-2.373)

Other genetic associations with ABPA

- HLA-DR2/DR5 restriction
 - DRB1*1501, 1502, 1503, 1601
 - HLA-DR5: DRB1*1101, 1103, 1104, 1202
 - HLA-DQ2 is protective (DQB1*0201)
- IL-4Ra polymorphism
- IL-13 polymorphism
- IL-10 polymorphism
- CFTR heterozygote

Making genetics work for patient care

1. Larger studies, across ethnic boundaries
2. Complex statistics (opportunity for many false or non-significant associations)
3. Needs a strong reproducible phenotype
4. Could be used for risk prediction (ie pre-transplant) or prognostication or drug disposition/toxicity
5. Will require integration with other parameters (ie CMV status)
6. Will require expert AI systems to optimise clinical utility

Treatment

Choice of antifungal for invasive aspergillosis

Priority sequence

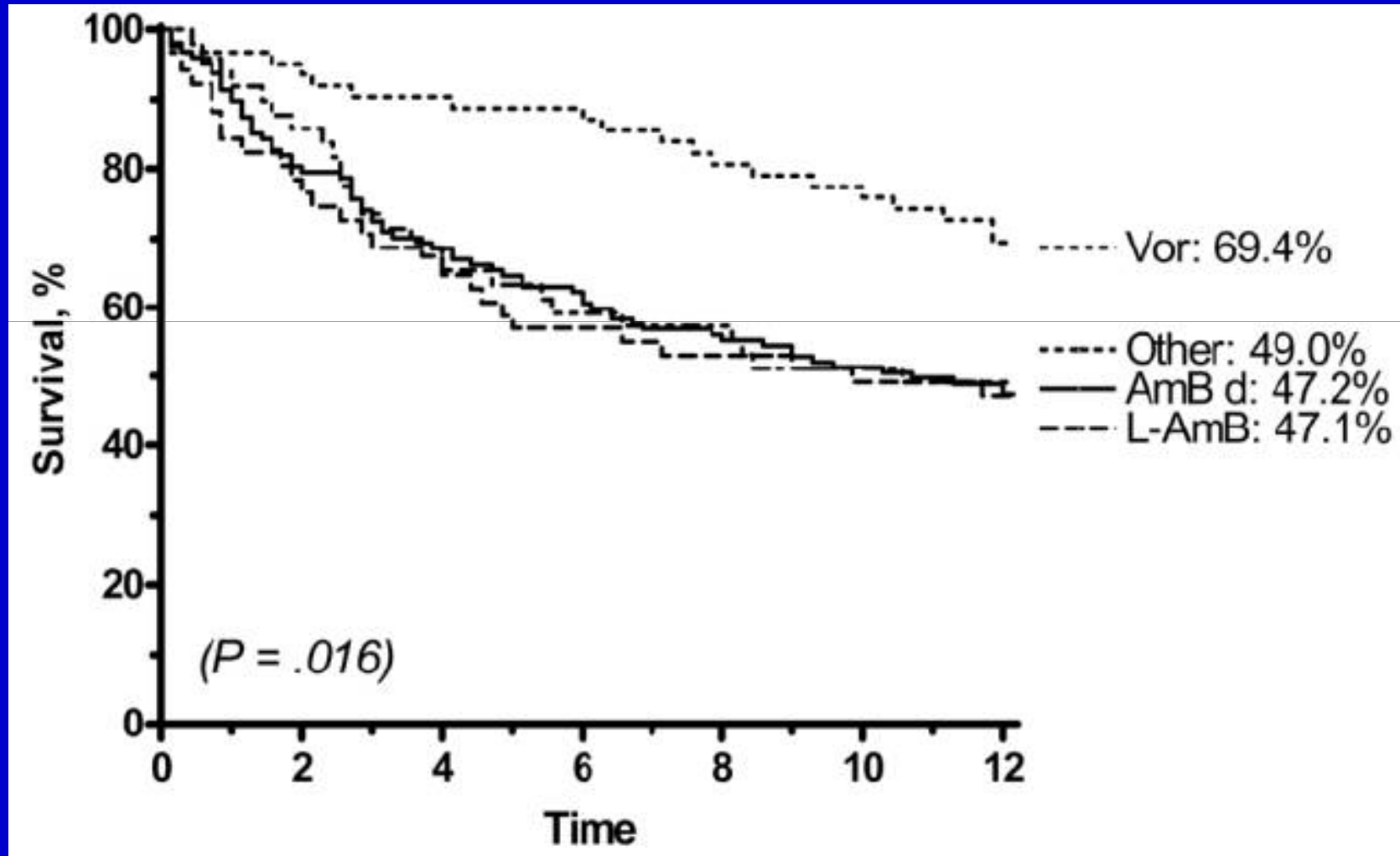
- Voriconazole (unless drug interaction)
- AmBisome 3mg/Kg (if not 'nephro-critical')

OR

micafungin/caspofungin (if not neutropenic)

3. Posaconazole (oral only, if no drug interactions)
4. Itraconazole

Impact of voriconazole in real life



Management of ABPA and SAFS

- Optimise inhaler regimen with inhaled steroids and long acting bronchodilator therapy
- Consider leukotriene antagonists
- Antifungal therapy
- Consider omalizumab for SAFS
- If bronchiectasis, consider long term macrolide therapy

Randomised studies of antifungals and ABPA and/or asthma

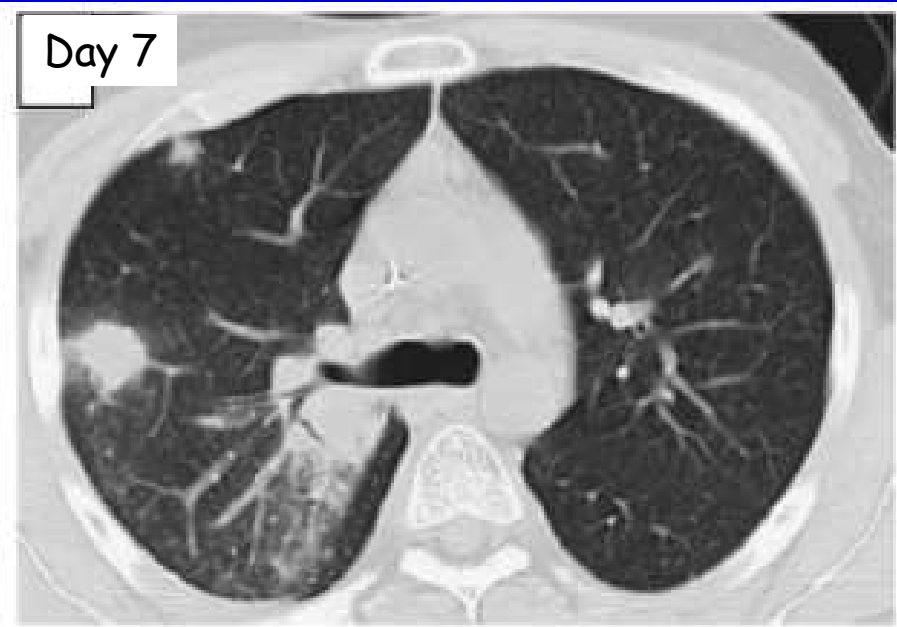
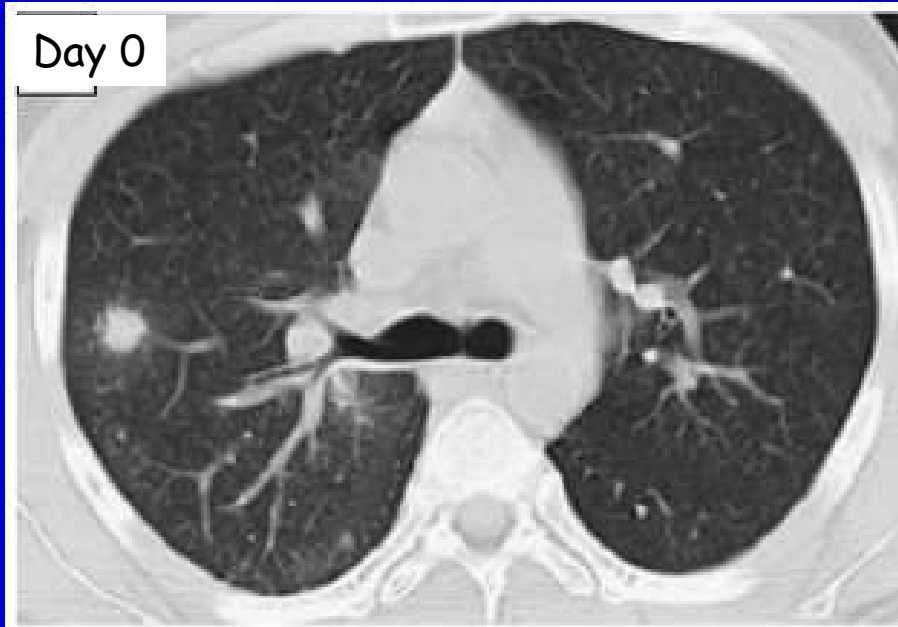
Disease	Antifungal, duration	Benefit?	Author, year
ABPA	Natamycin inh, 52 wks	No	Currie, 1990
ABPA	Itraconazole, 32 wks	Yes	Stevens, 2000
ABPA	Itraconazole, 16 wks	Yes	Wark, 2003
"Trichophyton" asthma	Fluconazole, 20 wks	Yes	Ward, 1999
SAFS	Itraconazole, 32 wks	Yes	Denning, 2009

Another challenge - immune reconstitution

Immune Reconstitution Inflammatory Syndrome in Cancer Patients With Pulmonary Aspergillosis Recovering From Neutropenia: Proof of Principle, Description, and Clinical and Research Implications

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BACKGROUND. Assessing the outcome of patients with invasive pulmonary aspergillosis by using conventional criteria is difficult, particularly when clinical and radiologic worsening coincides with neutrophil recovery. Usually, it is assumed that this deterioration is related to progressive aspergillosis, prompting changes in patient management. However, its temporal relation with neutrophil recovery suggests that it may be caused by an immune reconstitution syndrome (IRIS). Galactomannan is an *Aspergillus*-specific polysaccharide that is released during aspergillosis and is detected by the serum galactomannan test, which has been



Immune reconstitution in invasive pulmonary aspergillosis, in AIDS



Patient HB
Day +14, CD4 cells 84/uL



Patient HB
Day +42, after AmB and ITZ

Immune reconstitution in invasive pulmonary aspergillosis, in AIDS



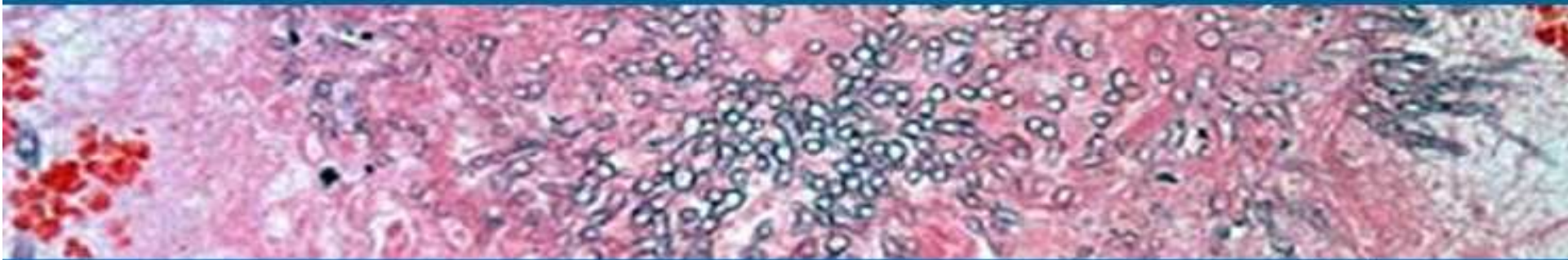
Patient HB
Day +64, CD4 cells 340/uL, on VRC



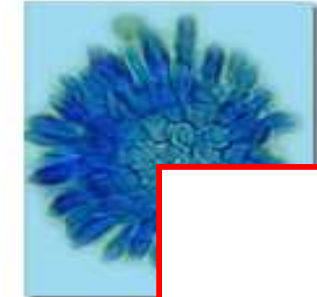
Patient HB
Day +87, day of death

Conclusions

- Invasive aspergillosis is primarily related to immune suppression, especially corticosteroid use
- ABPA and SAFS are primarily genetic diseases involving several genes
- Current treatments are partially successful but more oral therapies are needed
- Immune reconstitution poorly understood, but probably important
- Opportunities for immune therapies going forward



The Aspergillus Website



The **Aspergillus website** is a worldwide comprehensive resource providing a wide range of information about the **fungus Aspergillus** and the diseases - such as **Aspergillosis** that it can cause. This site is free to use and provides an encyclopaedia of Aspergillus for doctors, scientists, patients and their relatives. Some parts of the site for example the image bank require free registration.

13 years and counting
Over 2M pages read monthly in >125 countries
Supported by the Fungal Research Trust – 20 year anniversary in 2011

Aspergillosis is a group of diseases which can result from aspergillus infection and includes invasive aspergillosis, ABPA, CPA and aspergilloma. Some asthma patients with very severe asthma may also be sensitised to fungi like aspergillus (SAFS). There is a section devoted to the needs of patients, friends and family suffering from the effects of Aspergillosis.

The UK's first National Aspergillosis Centre opened on May 1st 2009 - the opening meeting can be viewed. The centre is supported by the Regional Mycology Lab which also provides both air sampling and mould identification services for domestic and working environments.

Aspergillosis may affect patients whose immune system may be compromised - including those with leukaemia, chemotherapy patients or those on steroids, transplant patients, cystic fibrosis, HIV or AIDS, chronic obstructive pulmonary disease (COPD), chronic granulomatous disease (CGD), severe asthma with fungal sensitivity (SAFS) and many others.

Aspergillus does not solely affect humans; birds and animals can also develop aspergillosis, and some plant

search

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